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# Synthesis and Evaluation of Novel Functionalised Indoles as Anticancer Agents




**Hannah Winfield, B.Sc.**

A Thesis Presented for the Degree of

Doctor of Philosophy

to

THE NATIONAL UNIVERSITY OF IRELAND

Analytical & Biological Chemistry Research Facility,

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University College Cork

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December 2015

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Finally, I would like to thank my family for their constant love, support and encouragement. Mum and Dad, I can't even begin to tell you how grateful I am - thank you so very much for everything!

## Declaration

I hereby confirm that the body of work described within this book, for the degree of Doctor of Philosophy, is the result of research carried out by me, within University College Cork, under the supervision of Dr. Florence McCarthy, and that this thesis has not been previously reproduced elsewhere, to the best knowledge of the author, in whole or in part, prior to presentation for this degree.

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*For Mum and Dad*

## Abstract

This thesis outlines the design and application of new routes towards a range of novel bisindolylmaleimide and indolo[2,3-*a*]carbazole derivatives, and evaluation of their biological effects and their chemotherapeutic potential.

A key part of this work focussed on utilising a hydroxymaleimide as a replacement for the prevalent lactam/maleimide functionality and forming a series of novel derivatives through substitution on the indole nitrogens. To achieve this, a robust synthetic strategy was developed which allowed access to key maleic anhydride intermediates using Perkin-type methodology. These hydroxymaleimides were further modified *via* a Lossen rearrangement to furnish a series of analogues containing a 6-membered F-ring.

The theme of F-ring modulation was further expanded through the utilisation of a second route involving the design and synthesis of β-keto ester intermediates, which afforded novel derivatives containing pyrazolone and isocytosine headgroups, and various *N*-substituents. Work on a further route involving a dione intermediate resulted in the isolation of a bisindolyl derivative with a novel imidazole F-ring.

Following the synthesis of 42 novel compounds, extensive screening was undertaken using the NCI-60 cell line screen, with twelve candidates progressing to evaluation *via* the five dose assay. This led to the identification of several lead compounds with high cytotoxicity and excellent selectivity profiles, which included derivatives with low nanomolar $GI_{50}$ values against specific cancer cell lines, and also derivatives with selective cytotoxicity. Preliminary results from a kinase screen indicated noteworthy selectivity towards GSK3α/β and PIM1 kinases, with low micromolar $IC_{50}$ values being observed for these enzymes.

# Abbreviations

| | |
|---|---|
| AcOH | Acetic acid |
| AIBN | Azobisisobutyronitrile |
| ASR | Age standardised rate |
| ATP | Adenosine triphosphate |
| bd | Broad doublet |
| BIM | Bisindolylmaleimide |
| Bn | Benzyl |
| Boc | *tert*-Butyloxycarbonyl |
| bs | Broad singlet |
| $CDCl_3$ | Deuterated chloroform |
| CDI | *N*-*N'* Carbonyldiimidazole |
| CDK1 | Cyclin-dependent kinase 1 |
| CHK1 | Checkpoint kinase 1 |
| CNS | Central nervous system |
| COSY | Correlation spectroscopy |
| CPT | Camptothecin |
| CSA | Camphorsulfonic acid |
| d | Doublet |
| DCM | Dichloromethane |
| dd | Doublet of doublets |
| DDQ | 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone |
| DEPT | Distortionless Enhancement by Polarisation Transfer |
| DIAD | Diisopropyl azodicarboxylate |
| DMAP | 4-Dimethylaminopyridine |
| DMF | *N*,*N*-Dimethylformamide |
| DMSO | Dimethylsulfoxide |
| DNA | Deoxyribonuleic acid |
| DTP | Developmental Therapeutics Program |
| EtOH | Ethanol |
| g | Gram |
| $GI_{50}$ | 50% Growth inhibition concentration |

| | |
|---|---|
| GSK3 | Glycogen synthase kinase |
| HCl | Hydrochloric acid |
| HMBC | Heteronuclear Multiple Bond Correlation |
| HMDS | Hexamethyldisilazane |
| HRMS | High-resolution mass spectrometry |
| HSQC | Heteronuclear Single Quantum Coherence Spectroscopy |
| Hz | Hertz |
| $IC_{50}$ | 50% Inhibition concentration |
| ICZ | Indolocarbazole |
| IR | Infrared |
| *J* | Coupling constant |
| JAK3 | Janus kinase 3 |
| KHMDS | Potassium hexamethyldisilazide |
| $K_i$ | Inhibitory constant |
| KISSf | Kinase Inhibitor Specialised Screening Facility |
| $LC_{50}$ | 50% Lethal concentration |
| LDA | Lithium diisopropyl amide |
| LiHMDS | Lithium hexamethyldisilazide |
| lit. | Literature |
| m | Multiplet |
| m.p. | Melting point |
| MeOH | Methanol |
| mg | Milligram |
| MHz | Megahertz |
| mL | Millilitre |
| mmol | Millimole |
| mol | Mole |
| μM | Micromolar |
| NaHMDS | Sodium hexamethyldisilazide |
| NBS | *N*-Bromosuccinimide |
| *n*-BuLi | *n*-Butyllithium |
| NCI | National Cancer Institute |
| nM | Nanomolar |

| | |
|---|---|
| NMR | Nuclear magnetic resonance |
| NSCLC | Non-small cell lung cancer |
| PDB | Protein Data Bank |
| PDK1 | Phosphoinositide-dependent kinase 1 |
| Phth | Phthalimide |
| PKA | Protein kinase A |
| PKC | Protein kinase C |
| PMB | *p*-Methoxybenzyl |
| PPSE | Polyphosphoric acid trimethylsilyl ester |
| *p*-TsOH | *p*-Toluenesulfonic acid |
| q | Quartet |
| quin | Quintet |
| r.t. | Room temperature |
| s | Singlet |
| SAR | Structure-activity relationship |
| SEM | 2-(Trimethylsilyl)ethyloxymethyl |
| sept | Septet |
| t | Triplet |
| TBAF | Tetrabutylammonium fluoride |
| TBDMS | *tert*-Butyldimethylsilyl |
| TBDPS | *tert*-Butyldiphenylsilyl |
| TFA | Trifluoroacetic acid |
| TGI | Total growth inhibition |
| THF | Tetrahydrofuran |
| TLC | Thin layer chromatography |
| TMS | Trimethylsilyl |
| Topo I | Topoisomerase I |
| Tr | Triphenylmethyl |
| U.V. | Ultraviolet |
| VEGF | Vascular endothelial growth factor |

# 1 Biological Introduction

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## 1.1 Cancer

One of the leading causes of morbidity and mortality worldwide, cancer causes about 13% of all human deaths and at least one fifth of all deaths in Europe and North America.[1,2] Although commonly referred to as a singular illness, cancer is in fact a class of related diseases in which a group of cells displays uncontrolled growth, invasion and progresses to metastasis. Cancer can start almost anywhere in the body and affects men and women of all ages and ethnicities (Figure 1.1.1) although certain factors can increase an individual's risk of developing cancer; such as increasing age, differences in exposure to carcinogens or radiation, or genetic susceptibility.

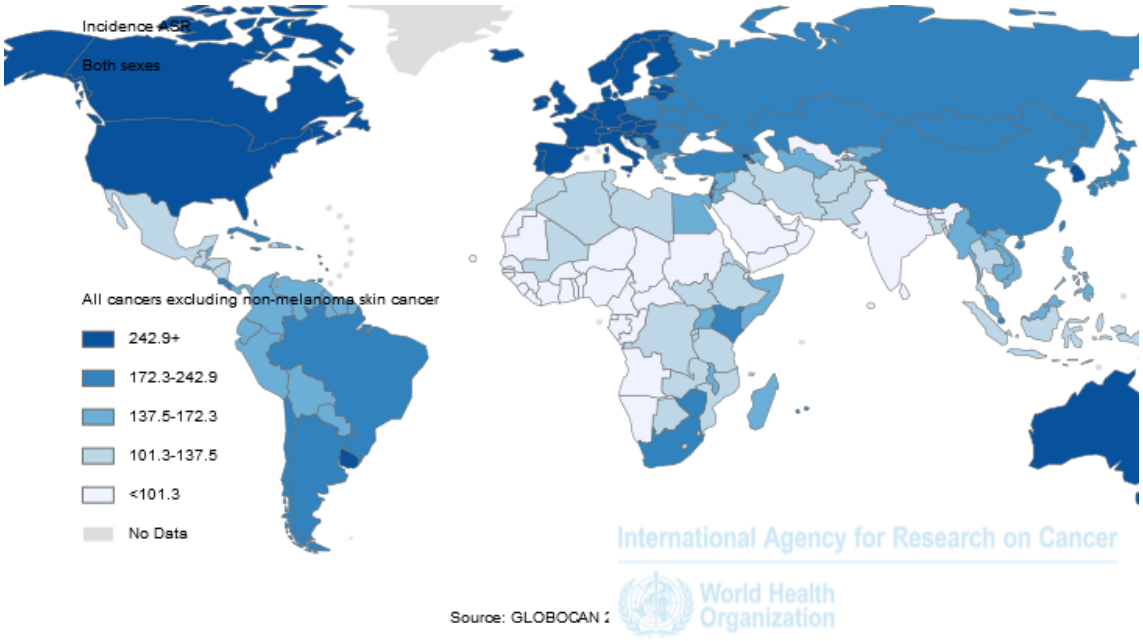



**Figure 1.1.1: Age standardised rate (ASR) of estimated incidence for all cancers excluding non-melanoma skin cancer[2]**

There are three main approaches for the treatment of cancer: surgery, radiotherapy and chemotherapy the usefulness of each of these approaches depends on the type of tumour and the stage of its development. Surgery is the oldest known treatment for cancer and can often initially be effective if the cancerous tissue is entirely removed by surgical excision, but this is not possible if the cancer has metastasized to other sites in the body. In such instances, radiotherapy and chemotherapy are usually required and are often used in combination with surgery or with each other. Chemotherapy is increasingly being utilised and traditional chemotherapy predominantly works by interfering with the

cell division of rapidly dividing cells. However, as this approach also affects all rapidly dividing normal cells, it can cause a range of side effects, such as myelosuppression, impaired wound healing and alopecia among others.[3] Newer treatments comprise of targeted therapies which are currently the focus of much anticancer drug development. Targeted cancer therapies involve drugs that block the growth and spread of cancer by interfering with specific molecular targets that are involved in the growth, progression, and spread of cancer. Current chemotherapy drugs can be classified into general categories based on their main biological mechanism and are summarised in Table 1.1.1.[4]

| Type | Mode of Action | Examples |
|---|---|---|
| Alkylating agents | Effect DNA covalently | Cyclophosphamide, lomustine, cisplatin |
| Antimetabolites | Substitute for normal cell building blocks to cause damage during DNA synthesis | Gemcitabine, methotrexate, fludarabine |
| Anthracyclins | DNA damage | Doxorubicin, idarubicin |
| Hormones/ antagonists | Disrupt growth of hormone dependant tumours | Diethylstilbestrol, tamoxifen |
| Topoisomerase Inhibitors | Complex DNA and topoisomerase together (see Section 1.3.2) | Irinotecan, topotecan |
| Taxanes | Prevent disassociation of microtubules | Paclitaxel, docetaxol |
| Vinca Alkaloids | Bind to tubulin, interferes with mitosis | Vincristine, vinblastine |
| Kinase Inhibitors | Block the action of kinases which signal for proliferation (see Section 1.3.1) | Imatinib, gefitinib, dasatinib |
| Monoclonal Antibodies | Attach to cancer cells and mark for destruction by the immune system | Rituximab, trastuzumab |

**Table 1.1.1: Current chemotherapy drugs**

Cancer types have traditionally been classified according to the site of origin, cell type and stage of progression; however, it is now clear that many significant differences can occur even between tumours derived from the same organ. In order to successfully treat the vast majority of cancers, it has become evident that the most effective combination of therapeutic agents needs to be chosen specifically for each individual patient and

cancer type.[5] Therefore, it is vital to continue the search for and development of novel drug templates with which to pursue these targeted cancer therapies.

## 1.2 Indolocarbazoles

The indolocarbazole family of compounds, including the biosynthetically related bisindolylmaleimides, are a class of compounds that are currently under study due to their potential as targeted anticancer drugs. Since their discovery, much work has been undertaken on the elucidation of their mechanism of action and on the development of analogues with improved biological properties, and these topics are discussed in detail in the remainder of this chapter.

### 1.2.1 Discovery

The first isolation of an indolocarbazole from a natural source was reported in 1977. This compound, later named staurosporine **1**, was discovered in a culture of an actinomycete, *Streptomyces staurosporeus*, from a Japanese soil sample while screening for microbial alkaloids.[6] The X-ray crystal structure was determined shortly after but this original structure **2** was found to be assigned as the incorrect enantiomer.[7] The correct relative stereochemistry was established in 1994 and was later confirmed by total synthesis by Link *et al.* in 1996 (Figure 1.2.1).[8,9]

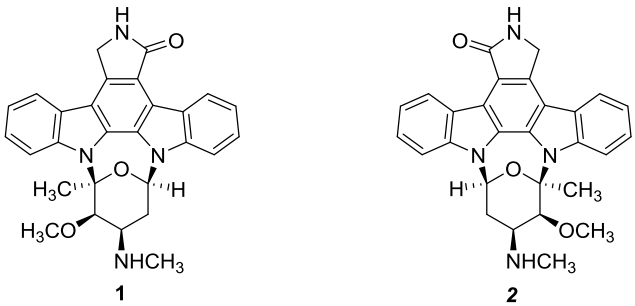



**Figure 1.2.1: Structure of staurosporine 1 and *ent*-staurosporine 2**

Early biological studies showed that staurosporine **1** displayed inhibitory action against fungi, possessed strong hypotensive activity and also inhibited platelet aggregation.[6,10] Several years later, in 1986, staurosporine began to attract much greater attention when

it was found to be a nanomolar inhibitor of protein kinase C ($IC_{50}$ = 2.7 nM) and also strongly inhibited the growth of a number of cancer cell lines.[11] Unfortunately, staurosporine itself was not suitable as a pharmaceutical candidate as it was soon found to inhibit a broad range of protein kinases and display a variety of biological activities.[12]

### 1.2.2 Isolation

Staurosporine **1** and very close derivatives have been isolated from a wide variety of different environments.[13] In addition to their widespread production among actinomycetes, a bacterial group commonly found in soil samples, these compounds have been isolated from diverse groups of organisms from prokaryotes (actinomycetes, cyanobacteria, β-proteobacteria) to eukaryotes (myxomycetes, basidiomycetes, marine invertebrates).

Of the five different isomeric ring systems available for indolocarbazoles only the indolo[2,3-*a*]carbazole isomer **3** has been isolated from nature and interest has mostly focused on this structure (Figure 1.2.2). In addition, the vast majority of these biologically active compounds also possess a sixth ring which is fused to the central benzene ring **4**.




**Figure 1.2.2: Structure of indolo[2,3-*a*]carbazole 3 and general structure of biologically active indolocarbazoles 4**

In the 1980s, staurosporine **1** along with new analogues containing a hydroxy lactam (UCN-01 **5** and UCN-02 **6**, Figure 1.2.3) were isolated from several *Streptomyces*, and these 7-hydroxy staurosporines were also found to be potent inhibitors of protein kinase C and possess significant antitumour activity.[14] In 1985, a new indolocarbazole was isolated from cultures of *Lechevalieria aerocolonigens* and named rebeccamycin **7** after the daughter of the scientist who isolated the compound.[15] The structure and absolute stereochemistry were determined by X-ray crystallography and total synthesis and this analogue was found to have one *N*-glycosidic linkage instead of two.[15,16] This

compound was subsequently found to be active against leukaemia and melanoma in mice and inhibited the growth of human adenocarcinoma cells, and produced single strand breaks in the DNA of these.[17]

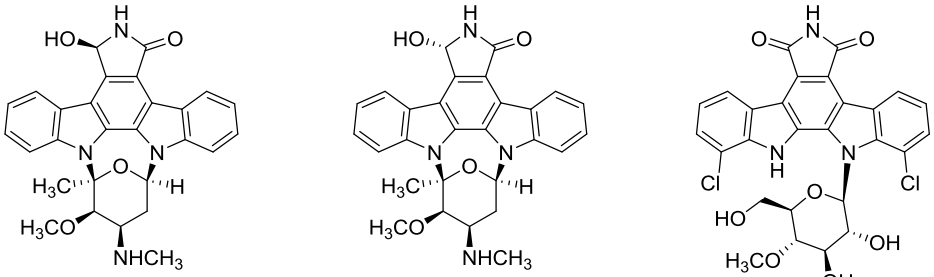



**Figure 1.2.3: Structure of UCN compounds** 5 **and** 6**, and rebeccamycin** 7

Many aglycone derivatives have also been isolated from various sources. The aglycone of staurosporine itself **8** (Figure 1.2.4) was first isolated from another actinomycete strain *Nocardiopsis* sp. K-290 and related arcyriaflavin A **9**, arcyriaflavin B **10** and arcyriaflavin C **11** were found to be contained in *Arcyria denudata* and *Arcyria nutans* slime moulds by Steglich *et al.*[18] Interestingly, a group of bisindolylmaleimides – arcyriarubin A **12**, arcyriarubin B **13** and arcyriarubin C **14**, were also isolated from the slime moulds in the same study. These non-planar analogues are considered to be part of the broader indolocarbazole family due to close structural and biosynthetic relationships.

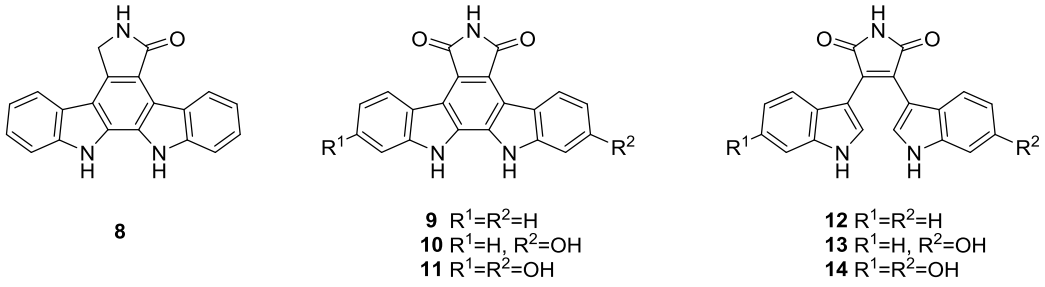
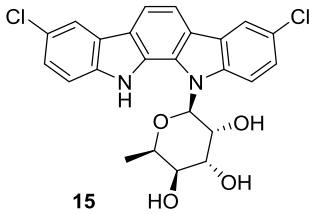



**Figure 1.2.4: Structure of staurosporine aglygone** 8**, arcyriaflavins A** 9**, B** 10 **and C** 11**, and arcyriarubins A** 12**, B** 13 **and C** 14

In 1991, the tjipanazoles, such as tjipanazole A **15** (Figure 1.2.5), were isolated from culture extracts of *Tolypothrix tjipanasensis*.[19] Unlike most other natural indolocarbazoles, these compounds generally do not possess an F-ring. Tjipanazoles

exhibited only weak cytotoxicity against tumour cells and did not inhibit protein kinase C, demonstrating the importance of the F-ring for kinase inhibition.




15

**Figure 1.2.5: Structure of tjipanazole A** 15

The indolocarbazoles isolated to date are a structurally diverse family of natural products. The various types of aglycones can be divided into four major groups: 1) the parent indolo[2,3-*a*]carbazole nucleus, such as that found in the tjipanazoles **15**; 2) an imide, as in rebeccamycin **7**; 3) hydroxy lactams, as in the UCN compounds **5** and **6**; and 4) simple lactams, such as those found in staurosporine **1**. In all of these aglycone types, substitution (i.e., halides, ethers, phenols) at various positions on the aromatic heterocycle has been observed.

### 1.2.3 Biosynthesis

In the 1980s and 1990s biosynthetic studies were carried out on staurosporine **1** and rebeccamycin **7** using isotope labelled precursors. From these it was determined that the indolocarbazole portion of the molecule was formed from two tryptophan molecules and the carbohydrate moiety is derived from glucose and methionine (Figure 1.2.6).[20,21,22] However, it was not possible to determine the origin of the nitrogen of the lactam or maleimide moiety in these studies.

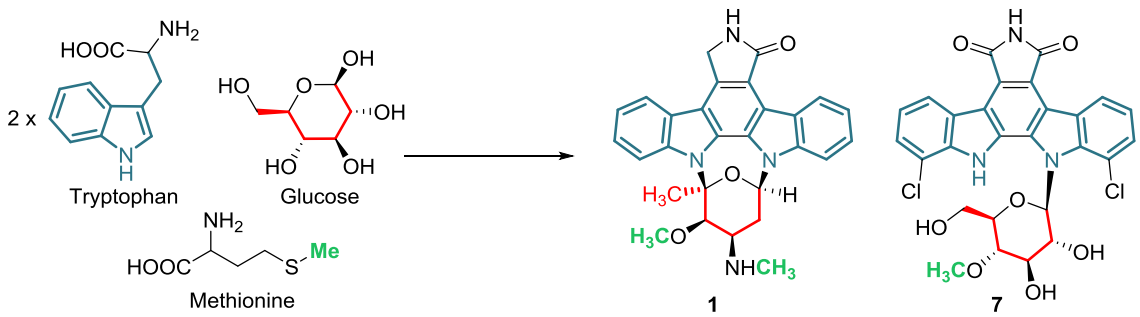



**Figure 1.2.6: Metabolic precursors of staurosporine** 1 **and rebeccamycin** 7

### 1.2.4 Biological activity

Anticancer activity has been by far the most extensively researched and reported aspect of the biological effects of indolocarbazoles, with a wide range of both natural and synthetic analogues displaying cytotoxic or antitumour properties.[13,23] This aspect of their biological action will also be the focus of this thesis. In addition to their direct action on tumour cells, some derivatives were also found to increase the effect of other anticancer therapies such as DNA-damaging agents or radiotherapy.[24,25]

Several analogues have also shown antibacterial activity.[17,18,26] This action was generally only observed against Gram positive bacteria, although there are a few exceptions. Antifungal and antiviral activities have also been observed.[6,18,26,27]

Indolocarbazoles have also displayed many other biological activities including hypotensive properties, inhibition of platelet aggregation and inhibition of smooth muscle contraction among others.[28]

## 1.3 Modes of biological action

A significant number of both natural and synthetic indolo[2,3-*a*]carbazoles and bisindolylmaleimides have shown excellent antitumour activity and their modes of biological action can be divided into two main types: inhibition of protein kinases and interaction with DNA.

### 1.3.1 Kinase inhibition

Protein kinases are enzymes which modify other proteins by transferring phosphate groups from a high-energy donor molecule, such as adenosine triphosphate (ATP), to a specific substrate amino acid on the target protein. This phosphorylation results in a conformational change in the protein, affecting its function in areas such as enzyme activity, association with other proteins or cellular location. The majority of known protein kinases are tyrosine kinases, which phosphorylate the phenolic hydroxyl group of tyrosine (Figure 1.3.1). This affects the target protein as the hydroxyl group can no

longer participate in hydrogen bonding and the newly added negatively charged oxygens can form strong ionic bonds to suitably positioned positively charged groups in the protein, resulting in a change in the protein's tertiary structure to optimize these new interactions.[29]

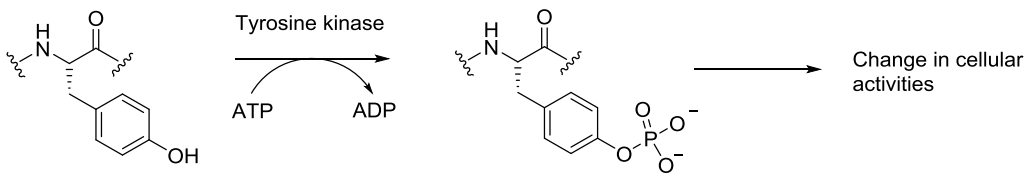



**Figure 1.3.1: Phosphorylation of tyrosine residue**

In this way kinases regulate other proteins and the activities of cells, playing an important role in many intracellular signalling pathways including those that control cell growth and cell division. Up to 518 different kinases have so far been identified in humans and this enormous diversity, as well as their role in signalling, makes them an object of study for drug design.[30,31] In addition to the treatment of disease, kinase inhibitors are also important as molecular probes to give us a better insight into the physiological roles of kinases. Figure 1.3.2 illustrates kinases of note for indolocarbazoles highlighted in red, and in particular demonstrates their functional diversity.

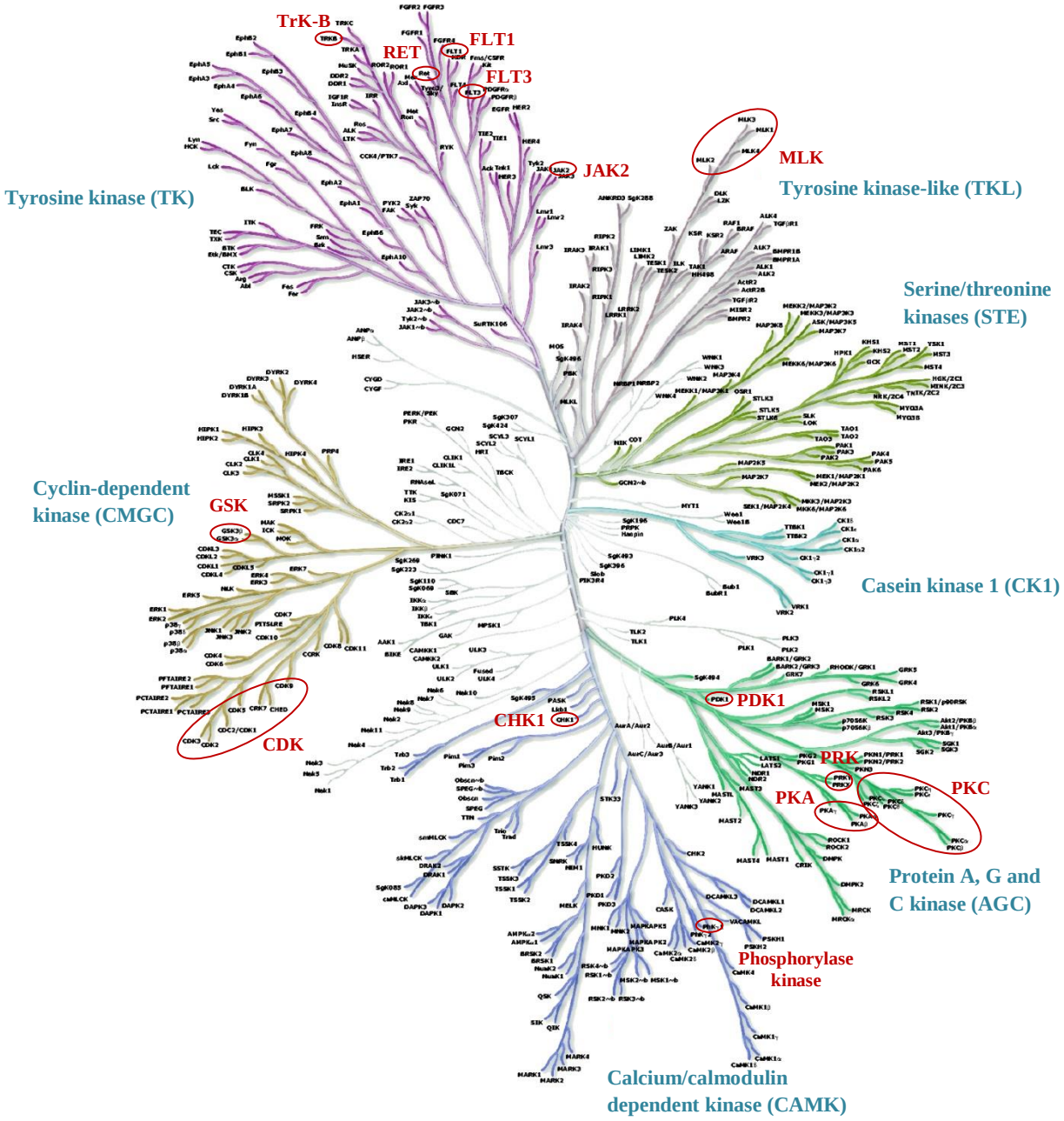



**Figure 1.3.2: The kinome, containing 518 kinases from seven different families**[32]

### *1.3.1.1 Kinases as drug targets*

Deregulated kinase activity has been implicated in a variety of human health conditions including disorders of the immune system, neurodegenerative disorders and diabetes, as well as cancer.[33] Since the discovery that staurosporine **1** was a nanomolar inhibitor of PKC in 1986, great interest has been generated into obtaining highly active and

selective protein kinase inhibitors either through chemical synthesis or screening of new natural products. The first kinase inhibitor to be approved by the FDA was imatinib **16** in 2001 (Figure 1.3.3), as a treatment for chronic myeloid leukemia. By April 2015, a total of 28 small-molecule kinase inhibitors have been approved for clinical use by the FDA, half of which were approved in the past 3 years.[34] These include erlotinib **17**, which is marketed as a treatment for non-small cell lung cancer, and sunitinib **18**, which is marketed as a treatment for renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumour.[35,36] A large number are also currently undergoing human clinical trials for cancer and other conditions.

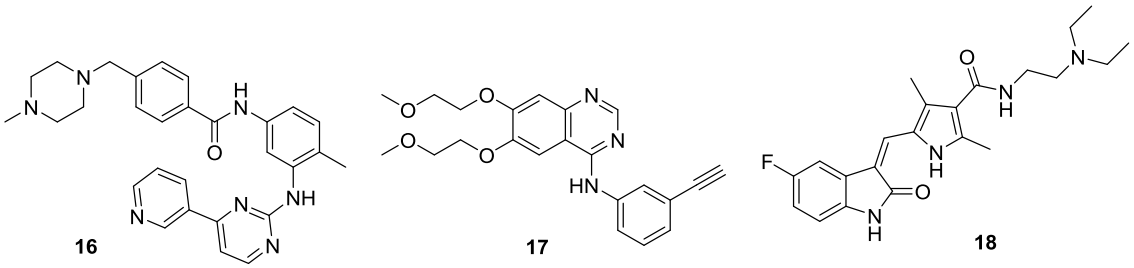



**Figure 1.3.3: Structure of imatinib** 16**, erlotinib** 17 **and sunitinib** 18

Many biologically relevant kinase inhibitors, including staurosporine **1**, have been shown to work by competing with ATP.[37] The interaction of staurosporine with the ATP-binding has been proven through the resolution of crystal structures of staurosporine bound to CDK2 (Figure 1.3.4) and PKA. This interaction has also been observed in crystal structures of various other indolocarbazoles with several kinases. These crystal structures show the indolocarbazole derivative being located in the ATP binding site, in the cleft between the two lobes of the protein kinase. The lactam group mimics the hydrogen bonding pattern of the adenine base of ATP by forming two hydrogen bonds to the backbone of the hinge between the N-terminal and C-terminal domains of the kinase. The sugar moiety then forms hydrogen bonds within the ribose binding site.

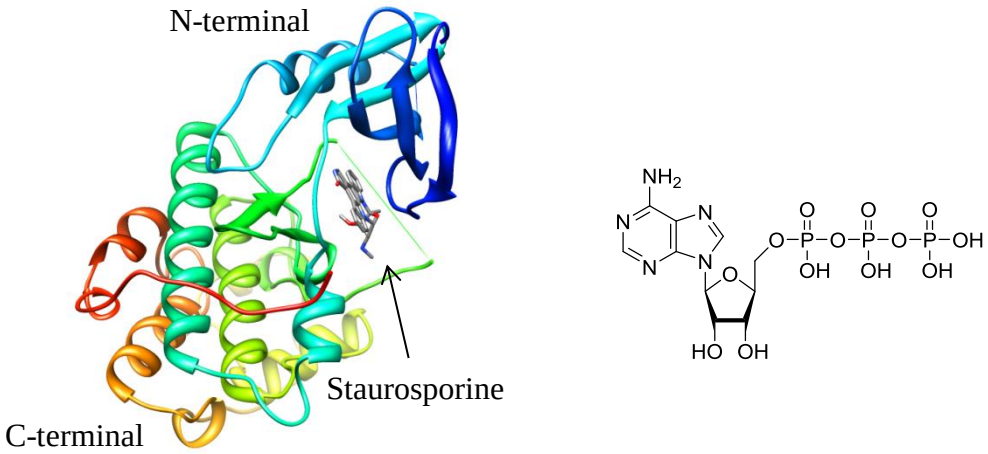



**Figure 1.3.4: Binding site of staurosporine 1 in CDK2[38,39]; structure of ATP**

Due to the similarity between ATP binding sites in different kinases, this approach can be limited in terms of selectivity. Staurosporine is a very close match for the ATP binding which makes it a highly potent but unspecific kinase inhibitor (Figure 1.3.5). However, it has been possible to obtain some selectivity by exploiting differences in the mode of ligand interaction in the ATP-binding pocket.[40]

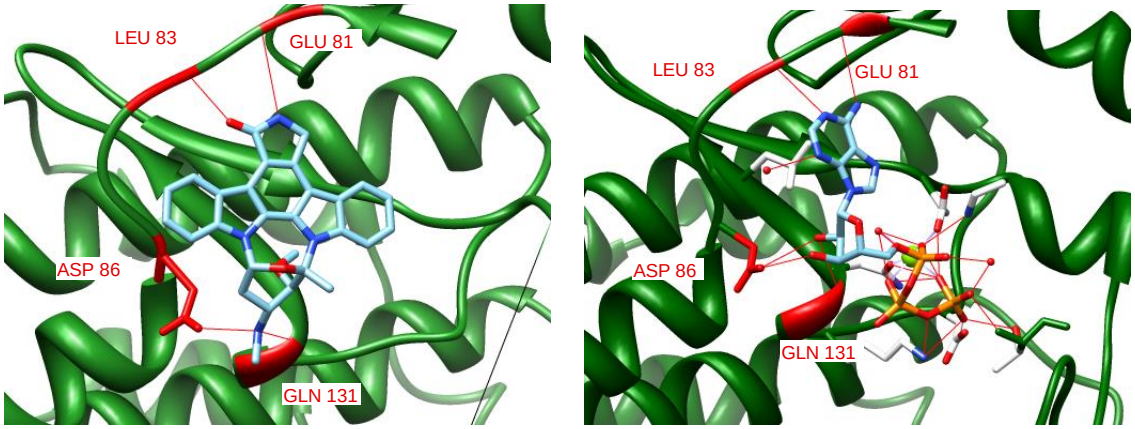



**Figure 1.3.5: Comparison between binding site of staurosporine 1[38] (left) and that of ATP[41] (right) in CDK2 (red lines indicate H-bonding interactions).[39]**

### *1.3.1.2 Staurosporine analogues as kinase inhibitors*

Although staurosporine **1** was unsuitable as a drug candidate itself, many groups have synthesised indolocarbazole analogues with a higher selectivity towards certain kinases

and these analogues will be discussed in this section. To date, structural modifications have included: (i) substitution or use of various functional groups on the F-ring, (ii) substitutions on the A and E rings, (iii) removal of the C(2)-C(2′) bond and (iv) modification of the sugar moiety (Figure 1.3.6).

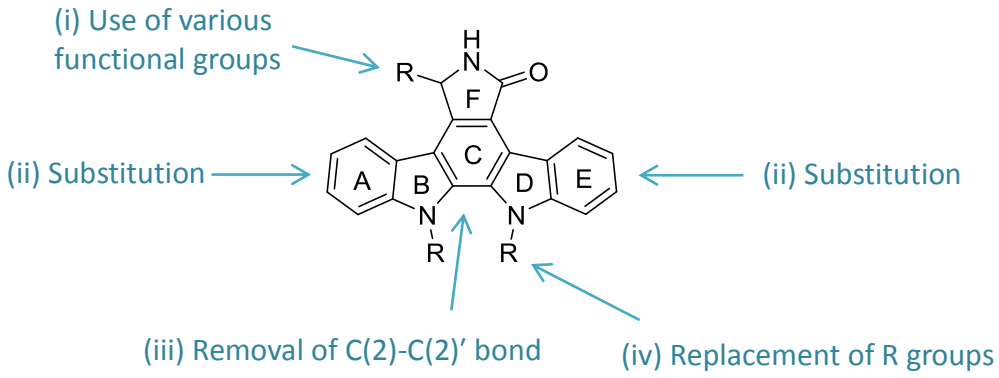


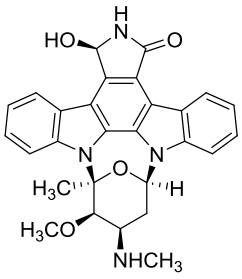

**Figure 1.3.6: Derivatisation of indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole**

These modifications led to the synthesis of kinase inhibitors with a wide variety of different structures with structural-activity relationships only being reliable for a particular kinase and a specific type of indolocarbazole. A number of general comparisons can be drawn, however.[13] Fully aromatic indolocarbazoles tend to be more active than their bisindolyl analogues.[42] The presence of a sugar moiety generally increased activity compared to the unsubstituted aglycone.[43] Also, the presence of a second carbonyl group in the F-ring is often better for non-glycosylated compounds, while the single carbonyl group seems to be better for glycosidic analogues. [23,42,44]

### *1.3.1.3 F-ring analogues*

The only naturally occurring indolocarbazole derivative that has undergone clinical trials to date is UCN-01 **5** (Figure 1.3.7). This close derivative of staurosporine only differs from the parent compound in that it contains an extra hydroxy group on the lactam moiety.[14] UCN-01 has anticancer properties itself and can also enhance the cytotoxicity of other anticancer drugs.[24] UCN-01 inhibits several protein kinases, including $Ca^{2+}$-dependant PKC isoforms ($IC_{50}$ ~30 nM), $Ca^{2+}$-independent isoforms

(~600 nM), cyclin-dependant kinases ($IC_{50}$ = 300-600 nM), and PDK1 ($IC_{50}$ = 5.0 nM).[45] This compound has entered several phase I/II clinical trials for treating various cancer types; however, its clinical development has been hindered by its high binding to human plasma proteins which causes undesirable pharmacokinetic effects.[46,47]

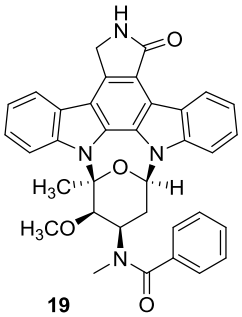



**5**

**Figure 1.3.7: Structure of UCN-01** 5

### *1.3.1.4 Analogues with modified sugar moiety*

A similar synthetic derivative, midostaurin **19** (Figure 1.3.8), which differs in structure from staurosporine only by the presence of a benzoyl group on the sugar moiety, is a potent inhibitor of a number of protein kinases including PKC, VEGF and FLT3.[48] Midostaurin has entered numerous clinical trials for treating various cancer types and its ability to suppress tumour growth is thought to be as a result of the inhibition of tumour angiogenesis in addition to directly inhibiting tumour cell proliferation.[46,49]




**19**

**Figure 1.3.8: Structure of midostaurin** 19

Gani *et al*. compared the binding strengths of staurosporine and midostaurin using kinase inhibition profile data from Karaman *et al*. to illustrate the effects of benzoylation on the selectivity of kinase inhibition across the kinome.[50,51] In general

binding strength is weakened by two orders of magnitude (green circles) on addition of the benzoyl group; however, this effect is not the same for every kinase. Kinases of the STE family are now inhibited to a much lesser extent (4-5 order of magnitude, red/orange circles), whereas MLK1, MLK3 and JAK3 are unaffected and their low nM binding is retained (blue circles). This demonstrates how even a small change in structure can lead to significant changes in the potency and selectivity of kinase inhibition of an indolocarbazole.

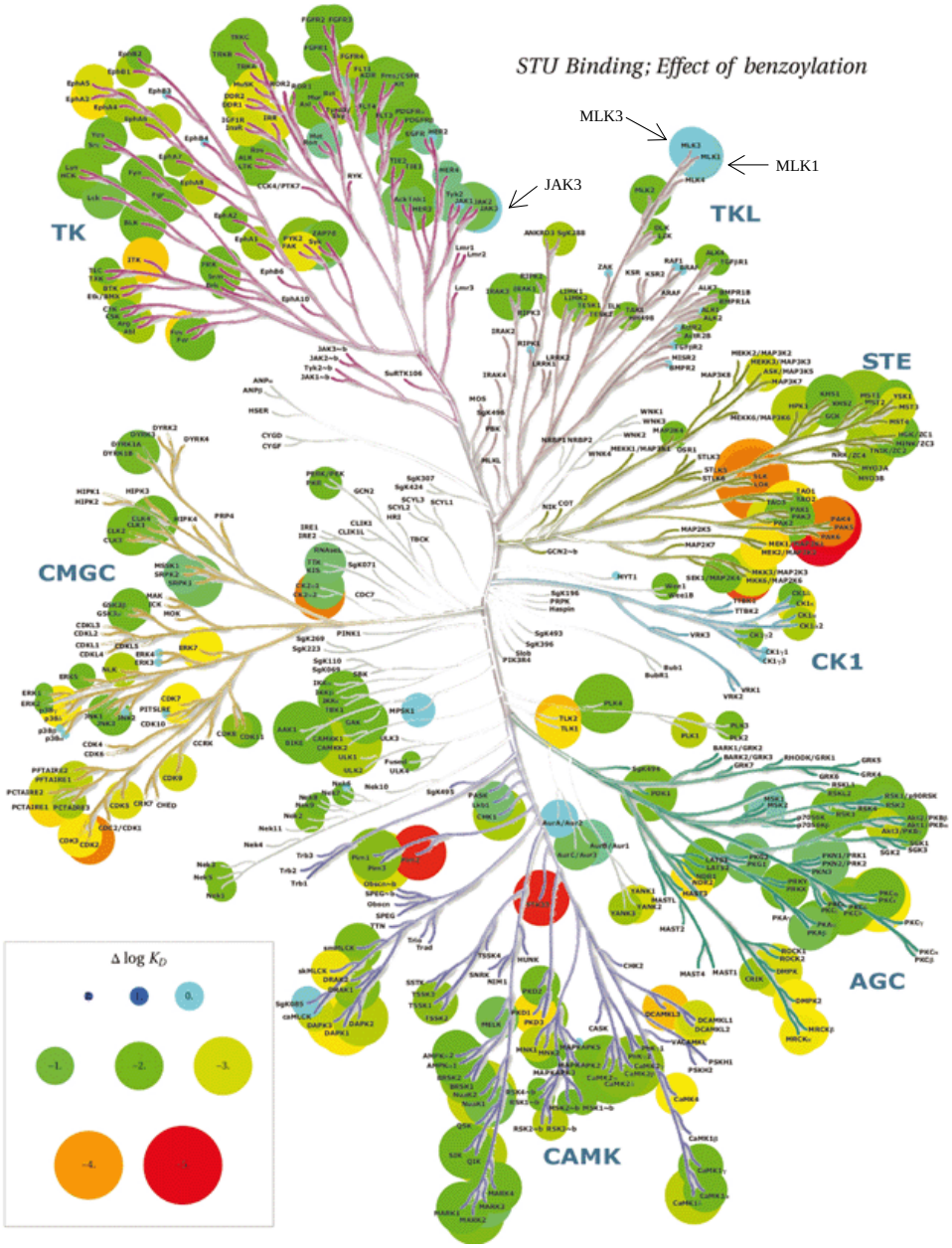
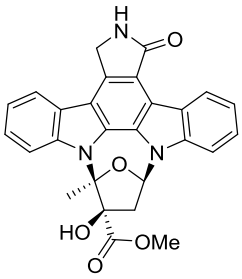
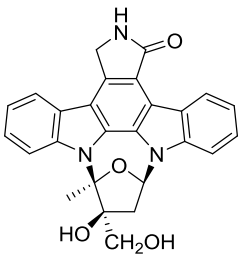



**Figure 1.3.9: Comparison of staurosporine and midostaurin binding.[51] The disk sizes represent the strength of staurosporine binding and the colours represent the effect of addition of the benzoyl group to staurosporine.**

Switching from a pyranose sugar to a furanose, as found in the naturally occurring K252a **20** (Figure 1.3.10), gave lestaurtinib **21**, a potent inhibitor of JAK2 kinase ($IC_{50}$ = 1 nM).[52] Lestaurtinib has displayed antitumour effects against pancreatic ductal adenocarcinoma, to inhibit growth of a trk-B overexpressing neuroblastoma, and to induce apoptosis in Hodgkin's lymphoma cells.[53,54,55] It has also been found to potently inhibit PRK1 ($IC_{50}$ = 8.6 nM), leading to anti-proliferative effects against prostate cancer cells, and also to interfere with FLT3 and RET tyrosine kinase.[56,57,58,59]



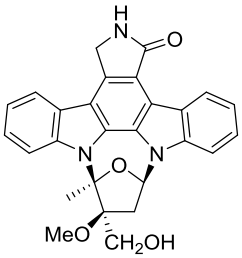

**20**



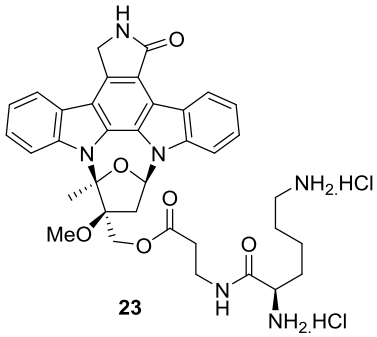

**21**

**Figure 1.3.10: Structure of K252a** 20 **and lestaurtinib** 21

Another pyranose analogue, CEP-751 **22** (Figure 1.3.11), has also been shown to have similar activity against trk-B, FLT3 and RET tyrosine kinase. A water-soluble prodrug of CEP-751, named CEP-2563 **23**, was developed and entered clinical trials for patients with advanced solid tumours, however these trials have been discontinued.




**22**




**23**

**Figure 1.3.11: Structure of CEP-751** 22 **and CEP-2563** 23

A 3,9-bis(ethylthiomethyl) derivative, CEP-1347 **24** (Figure 1.3.12), was reported to have neuroprotective properties and to be a potent MLK kinase inhibitor. CEP-1347 entered clinical trials for determining its success in the prevention of the progression of Parkinson's disease. However, 10 months into the trial it was announced that the data

were unlikely to provide evidence of any significant effect and the trial was discontinued.

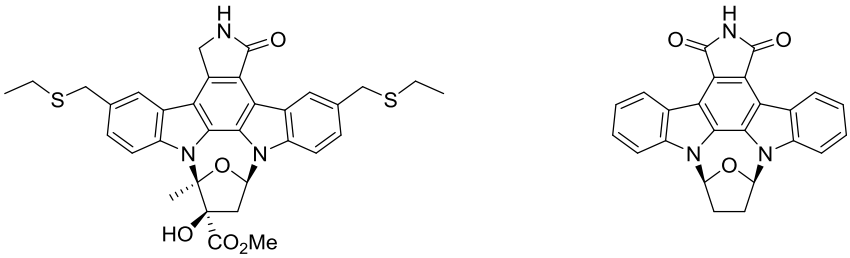



**Figure 1.3.12: Structure of CEP-1347** 24 **and SB-218078** 25

A simplified analogue with a maleimide and THF ring, SB-218078 **25** (Figure 1.3.12), showed a 15-fold selectivity for CHK1 ($IC_{50}$ = 15 nM) compared to CDK1 ($IC_{50}$ = 250 nM), and a 65-fold selectivity compared to PKC ($IC_{50}$ = 1000 nM).[60]

### *1.3.1.5 Bisindolylmaleimides*

Removal of the central C2-C2′ bond leads to the bisindolylmaleimides. Due to their increased conformational flexibility, the binding mode of bisindolylmaleimides may be more complex than that of planar indolocarbazoles.[61] Initially, GF 109203 X **26** and Ro 318220 **27** (Figure 1.3.13) were reported to be potent and selective inhibitors of protein kinase C ($IC_{50}$ = 10 nM and 20 nM, respectively).[62,63] However, they were later found to also potently inhibit several other kinases, such as MAPKAP kinase-1β ($IC_{50}$ = 50 nM and 3 nM, respectively) and GSK-3 ($IC_{50}$ = 360 nM and 6.8 nM, respectively).[64,65]

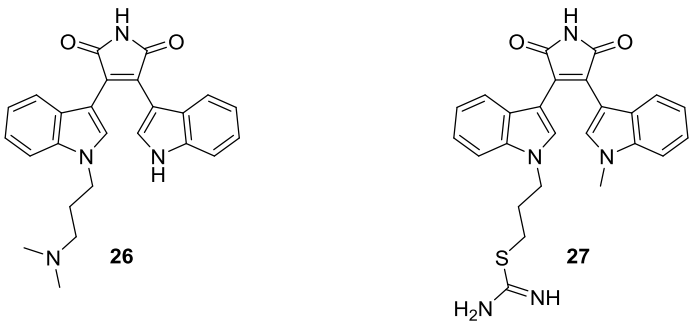



**Figure 1.3.13: Structure of GF 109203 X** 26 **and Ro 318220** 27

Another analogue, ruboxistaurin **28** (Figure 1.3.14), has been found to be a specific inhibitor of the PKC isoforms, PKCβ$_1$ ($IC_{50}$ = 4.7 nM) and PKCβ$_2$ ($IC_{50}$ = 5.9 nM).[66] It displays selectivity over other protein kinases and PKC isoforms such as PKCα ($IC_{50}$ = 360 nM), PKCγ ($IC_{50}$ = 300 nM), PKCε ($IC_{50}$ = 600 nM) and PDK1 ($IC_{50}$ = 750 nM).[67] Ruboxistaurin entered clinical trials for the treatment of diabetic peripheral retinopathy and was being investigated by Eli Lilly. In 2006, the company submitted a new drug application and received an approvable letter with requests for additional efficacy data. However, the application was withdrawn by the company in 2007.

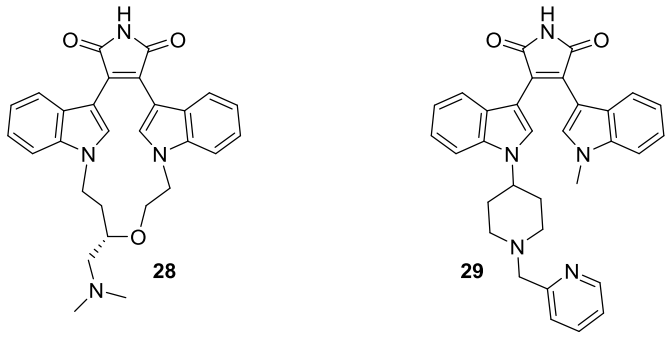



**Figure 1.3.14: Structure of ruboxistaurin** 28 **and enzastaurin** 29

Enzastaurin **29** (Figure 1.3.14) is a highly potent inhibitor of PKCβ ($IC_{50}$ = 6nM) and the AKT/PI3 pathway.[68] It shows moderate selectivity over other PKC isoforms including PKCα ($IC_{50}$ = 39nM), PKCγ ($IC_{50}$ = 83nM) and PKCε ($IC_{50}$ = 110 nM). Enzastaurin was also being investigated by Eli Lilly and underwent clinical trials for cancers such as glioblastoma, non-small cell lung cancer and colorectal cancer, but its development was halted in 2013.

In 2003, Kuo *et al*. synthesised a panel of novel macrocyclic bisindolylmaleimide derivatives which were found to be potent inhibitors of GSK-3*β* (Figure 1.3.15).[69] Although the bisindolyl analogue **30** was highly active against GSK-3*β*, it was not specific and was also active against several other kinases such as CDK1 and CDK2, VEGF-R and several PKC isoforms. Replacing one of the indole rings with 7-azaindole (compound **31**) had little effect on the potency or selectivity at GSK-3*β* but the replacement of both indoles with 7-azaindole (compound **32**) significantly increased the

selectivity. The bis-7-azaindolylmaleimide showed very low activity against a panel of 50 other kinases indicating a specific inhibitor of GSK-3*β*.

| Kinase assay | 30 | 31 | 32 |
|---|---|---|---|
| GSK-3*β* | 0.02 | 0.02 | 0.03 |
| CDK1 | 1.25 | 4.43 | >10 |
| CDK2 | 0.99 | 2.44 | >10 |
| VEGF-R | 1.45 | 3.56 | >10 |
| PKC*α* | 0.09 | 0.67 | >10 |
| PKC*β*II | 0.01 | 0.05 | 1.16 |
| PKC*θ* | 0.07 | 0.84 | >10 |
| PKA | >10 | >10 | >10 |

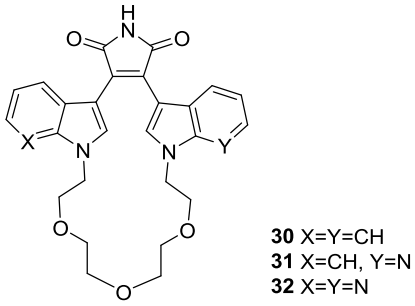



**Figure 1.3.15: $IC_{50}$ (μM) of kinase assays and structure of bisindolylmaleimide GSK-3*β* inhibitors 30, 31 and 32**

As inhibition of GSK-3*β* has been shown to reduce apoptosis signals it may be useful for the treatment of Alzheimer's disease and protection against cell death.[70,71] More recently, these bisindolylmaleimide GSK-3*β* inhibitors have been used to reveal the involvement of GSK-3*β* in the regulation of murine embryonic stem cell self-renewal.[72] These molecules were found to promote embryonic stem cell self-renewal which could enable the expansion of pluripotent embryonic stem cells for use in regenerative medicine and tissue engineering and as a source of differentiated cells for drug discovery and safety testing.

### 1.3.2 Topoisomerase I and other DNA interactions

DNA topoisomerases are found in all living organisms and are involved in the regulation of DNA supercoiling. As a result, they are enzymes which are involved in many cellular processes involving the separation of DNA strands such as replication, transcription, recombination and repair.[73] There are two types of DNA topoisomerases: type I which make a nick in a single strand of DNA and pass a single strand through the

nick and topoisomerase II which makes a nick in both strands of DNA and passes another double-stranded DNA segment through the nick.

**The mechanism of topoisomerase I (topo I) action begins with the enzyme binding to DNA to form DNA to form a macrocyclic complex (**

Figure 1.3.16). This is followed by the nicking step, where nucleophilic attack of the phenolic oxygen of an active site tyrosine residue on a DNA phosphodiester bond induces a single strand break in the DNA. Topo I then forms a covalent linkage to the 3′-end of the broken strand, while leaving a 5′-hydroxy group on the end free end. The DNA is then relaxed by controlled rotation of the 5′-hydroxy end of the break around the intact DNA strand, prior to religation.

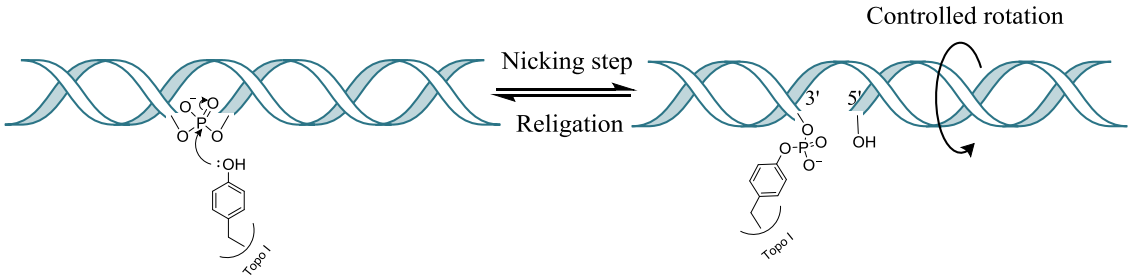



**Figure 1.3.16: Single strand DNA break by topo I**

### *1.3.2.1 Topoisomerase I as a chemotherapeutic target*

DNA topoisomerase I activity has been shown to be significantly raised in a number of different cancers, including colorectal, prostate and ovarian cancers, and is a target for different classes of drugs with antitumour or antibacterial applications.[74,75,76] In 1985, the DNA-topo I complex was found to be the target for the potent anticancer alkaloid, camptothecin **33** (Figure 1.3.17), which had been isolated from the Chinese tree *Camptotheca acuminate* by Wall *et al.* in 1966.[77,78] Although camptothecin did not progress beyond clinical trials due to harmful side-effects, derivatives such as topotecan **34** have since been developed.[79] In 1996, topotecan became the first topo I inhibitor to be approved for clinical use, initially in the treatment of ovarian cancer and later for use in the treatment of cervical and non-small cell lung cancer and has shown great promise as a drug to combat tumours.

These topo I poisons act as uncompetitive inhibitors by binding specifically to the DNA-enzyme complex resulting in a stabilised ternary complex, as shown in Figure 1.3.17. Topotecan intercalates at the site of DNA cleavage and mimics a DNA base pair. The topotecan molecule is stabilised by base-stacking interactions with both the upstream and downstream base pairs and displaces the reactive 5′-OH of the cleaved strand. This prevents religation of the DNA and so converts topo I into a DNA-damaging agent, causing cell damage which results in apoptosis.

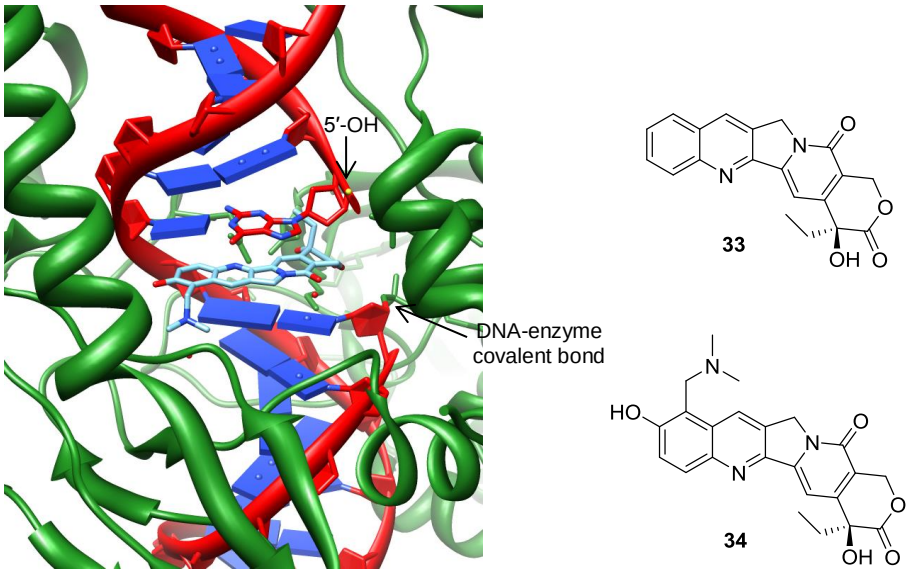



**Figure 1.3.17: Topotecan-DNA-enzyme ternary complex; structure of camptothecin** 33 **and topotecan** 34[79,39]

### *1.3.2.2 Indolocarbazoles with topo I activity*

On the discovery of rebeccamycin **7**, the exact mechanism for its antitumour activity was unknown, although it was reported that it was associated with extensive fragmentation of DNA in cancer cells.[17] Several years later, topo I was reported to be the cellular target for rebeccamycin by Yamashita *et al.*[80] The same study also found that although rebeccamycin itself was a moderately weak inducer of DNA cleavage, two related indolocarbazoles, KT6006 **35** and KT6528 **36** (Figure 1.3.18) also targeted topo I and were much more potent. Interestingly, in the absence of the topo I enzyme KT6528 **36** behaved as a DNA intercalator. This opened up new possibilities in that indolocarbazole compounds could not only selectively interact with ATP-binding sites of protein kinases but also other proteins with unique binding sites.

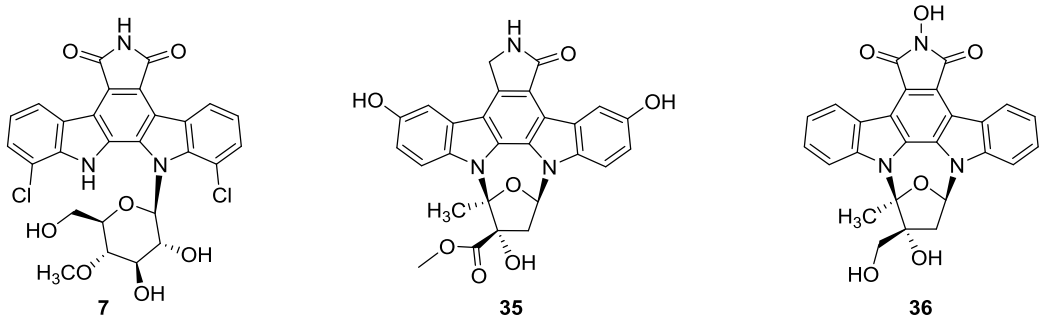



**Figure 1.3.18: Structure of rebeccamycin 7 and related indolocarbazoles KT6006 35 and KT6528 36**

Work by Bailly *et al.*, who investigated the DNA-binding properties of 21 indolocarbazoles by means of complementary spectroscopic techniques including electric linear dichroism, indicated that three necessary functional domains exist for anti-topo I activity (Figure 1.3.19).[81] Their results showed that the planar indolocarbazole core intercalates between two consecutive base pairs, aryl substitution with bulky groups being detrimental to activity. Bulky substituents were better tolerated on the maleimide nitrogen as long as they contained a hydrogen bond donor, suggesting interaction with the enzyme itself. The results also indicated the importance of the sugar moiety which they postulated to reside in one of the helical grooves. For analogues where the sugar links both indole nitrogens or where the non-glycosylated nitrogen is methylated, significant topo I inhibition is unlikely to be displayed.

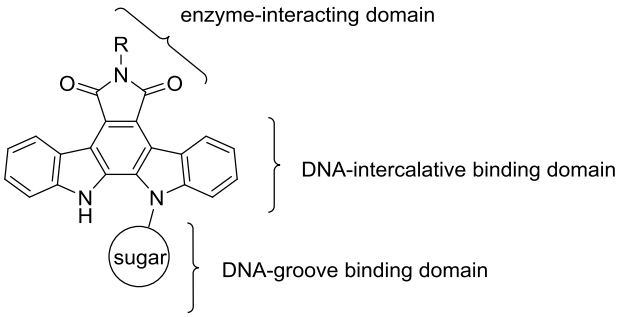
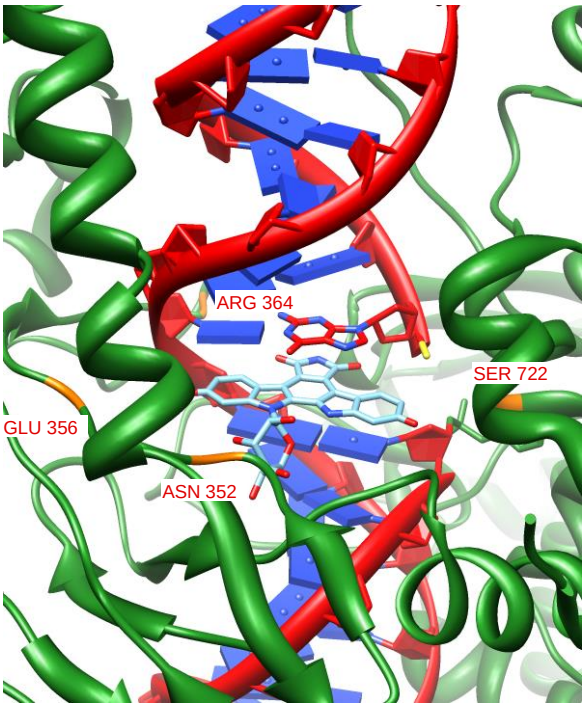
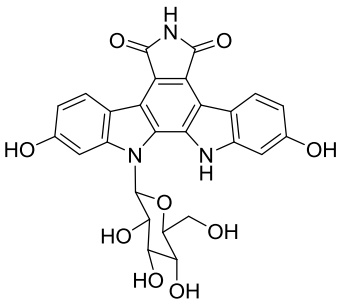



**Figure 1.3.19: SAR of topo I active indolocarbazoles[82]**

The crystal structure of the indolocarbazole SA315F **37** bound to the topo I-DNA complex, reported by Staker *et al.* in 2005, supported these conclusions and revealed that the indolocarbazoles do intercalate DNA at the site of topo I cleavage, in a similar

fashion to topotecan (Figure 1.3.20).[79] The glycosylated indole ring stacks with bases on the intact DNA strand and the non-glycosylated indole ring stacks with bases on the cleaved strand. The maleimide ring projects into the minor groove side of the intercalation binding pocket, with one of its two carbonyls being 2.7 Å from Arg364, while the glycoside portion of the molecule is in the major groove close to Asn352. The indolyl hydroxy groups are also likely to interact with the topo I enzyme, one being located 4.0 Å from Glu 356 and the other being 3.3 Å from Ser 722.



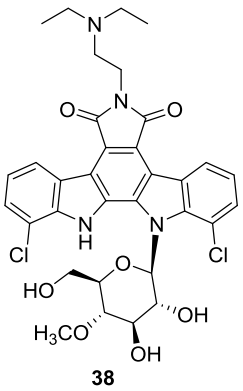




**37**

**Figure 1.3.20: Indolocarbazole SA315F 37 bound to Topoisomerase I-DNA complex.[79,39] The topo I enzyme is green with specific amino acids highlighted in orange, the DNA strand is blue/red and the indolocarbazole is pale blue.**

Indolocarbazoles are the most studied family of topoisomerase I inhibitors after the camptothecins. Despite the potent antitumour activity of rebeccamycin **7**, its limited aqueous solubility posed problems for further evaluation and so the design of analogues with improved solubility was initiated. Substitution with an *N*-diethylaminoethyl group on the maleimide moiety gave becatecarin **38** (Figure 1.3.21), which possessed the desired solubility and also an increase in antitumour activity.[83] Unlike rebeccamycin which was only a weak DNA intercalator, becatecarin exhibited strong DNA intercalation resulting in effective topoisomerase I and II inhibition.[84] Becatecarin has undergone clinical trials for the treatment of several different cancers, including

leukemias and cancers of the colon, breast, ovary and lung among others.[49] Although initial results from phase I clinical studies were promising, phase II trials revealed only modest activity with considerable myelosupression.[85,86,87]




**38**

**Figure 1.3.21: Structure of becatecarin** 38

During precursor feeding fermentation experiments of *L. aerocolonigenes* with fluorine-substituted tryptophans a series of novel fluoroindolocarbazoles (**39**, **40**, **41** and **42**, Figure 1.3.22) were produced and found to be more active against leukaemia than rebeccamycin.[88] Compound **41**, in particular, was found to be a potent inducer of topo I mediated DNA cleavage ($IC_{50}$ = 18 nM), more potent than camptothecin **33** in the same experiment ($IC_{50}$ = 38 nM).[84,89] A derivative of this, BMS-250749 (**42**), was later synthesised with improved antitumour effects and topo I activity ($IC_{50}$ = 2 nM) and this compound entered clinical trials in 2005 with no reported data since.[89]

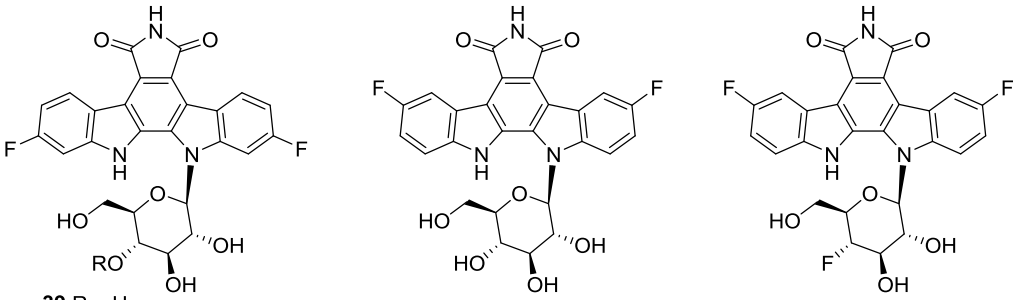



**Figure 1.3.22: Structure of fluoroindolocarbazoles (**39**,** 40**,** 41 **and** 42**)**

In 1991, a new compound, BE-13793C **43** (Figure 1.3.23), was isolated during a screening program for new antitumour substances.[90] Like rebeccamycin **7**, this

compound was found to be active against topoisomerases and inhibited the growth of doxorubicin-resistant or vincristine-resistant P388 murine leukaemia cell lines as well as the parent P388 cell line. Again, the low water solubility of this compound hampered its biological evaluation; however, it did become the basis of another series of clinical candidates. The main aim of the derivatisation studies was to improve aqueous solubility and this was achieved by adding a carbohydrate unit and by modifying the maleimide moiety. A 6-*N*-formylamino derivative, NB-506 **44**, had increased water solubility and significant anticancer activity.[91,93] NB-506 entered phase I clinical trials for the treatment of solid tumours in ovarian and breast cancer patients.[49]

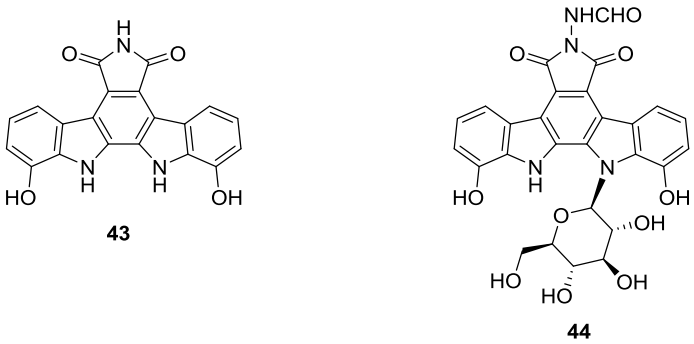
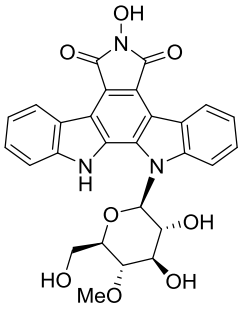



**Figure 1.3.23: Structure of BE-13793C** 43 **and NB-506** 44

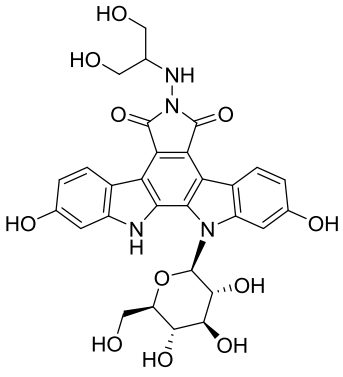
It is interesting to note that topo I is a dual enzyme which, in addition to its DNA-relaxing activity, also has an independent protein kinase action.[94] The enzyme specifically phosphorylates arginine-serine-rich splicing factors which control the expression of particular genes. Several indolocarbazole antitumour agents displayed inhibitory action against the kinase activity of topo I in addition to inhibiting its DNA-relaxing activity, including NB-506 **44** and a hydroxymaleimide R-3 **45** (Figure 1.3.24).[92,95] These effects were studied in detail using hydroxymaleimide R-3 **45** which is a highly active topo I inhibitor and cytotoxic against murine leukaemia cell line P388 ($IC_{50}$ = 0.28 μM) but also shows discrete anti-topo I kinase activity in the absence of DNA ($IC_{50}$ = 1-2 μM). A mutant topo I enzyme that lacked DNA cleavage/religation activity but still retained its phosphotransferase activity, was used without loss of kinase action indicating that the two effects are separate.[95] This action is specific as compounds active against the kinase activity of topo I are not necessarily active against other kinases.[96]




**45**

**Figure 1.3.24: Structure of hydroxymaleimide R-3** 45

Further modifications to NB-506 **44** by replacing the formylamino group with a more polar group and moving the hydroxy groups on the indolocarbazole portion from positions 1 and 11 to positions 2 and 10 gave edotecarin **46** (Figure 1.3.25).[97] Edotecarin had greater water solubility and plasma stability while possessing the same topo I inhibitory activity and cytotoxicity as NB-506.[98,99] The compound was developed by Pfizer and has undergone phase III clinical trials for treatment of glioblastoma multiforme and several phase I and II clinical trials against breast and gastric cancers; however, trials have since been discontinued.[49,100]




**46**

**Figure 1.3.25: Structure of edotecarin** 46

## 1.4 Conclusion

The biological activity of a particular indolocarbazole was thought to be effected through a single mode of action; however, it is now clear that many analogues act through more than one mechanism. Although rebeccamycin **7** is a topo I inhibitor, the antitumour activities of different rebeccamycin derivatives could often not be explained by their anti-topo I or DNA-binding activities.[23,101] Similarly, although K252a is active through kinase inhibition, some K252a derivatives were found to be active against topo I, as well as being kinase inhibitors.[102,103] This demonstrates that even small modifications to the indolocarbazole structure can cause a change in the preferred mode of its biological action.

The prediction of the biological targets for these molecules is challenging at present due to the literature not being complete in respect of bioactivity. This is because in the past large scale protein kinase profiling was not possible and most inhibitors could only be screened against a limited number of kinases. Inhibitors can now be tested against panels of hundreds of kinases and this information will greatly assist the design and discovery of new, highly potent and specific, drug candidates.[51]

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# 2 Chemical Introduction

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## 2.1 Chemical routes to indolo[2,3-*a*]carbazole derivatives

Indolocarbazoles are a class of pentacyclic heteroaromatic compounds consisting of a carbazole moiety fused to an indole unit *via* its five-membered ring. There are five different isomeric ring systems available for indolocarbazoles, however as almost all indolocarbazoles isolated from nature are indolo[2,3-*a*]carbazoles **3** (Figure 2.1.1), synthetic interest has mostly focused on this isomer.

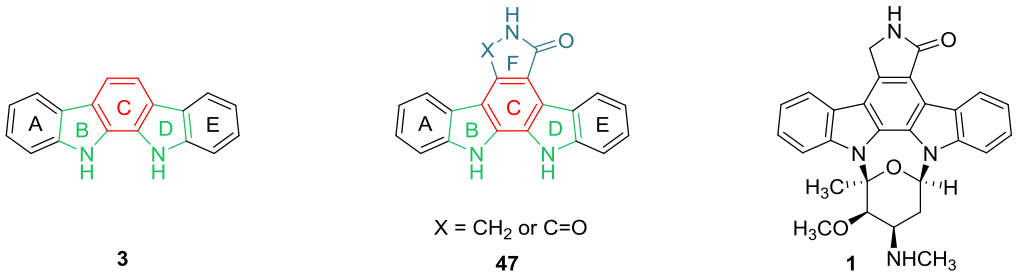



**Figure 2.1.1: Structure of indolo[2,3-*a*]carbazoles**

Prior to the isolation of the first natural indolocarbazole, staurosporine **1**, syntheses of indolo[2,3-*a*]carbazoles were scarce and focussed on the synthesis of the parent indolocarbazole **3**. The discovery of the biological potential of these compounds, which generally contain an extra F-ring (**47**, Figure 2.1.1), attracted a much greater interest and a variety of different approaches and methods were developed.

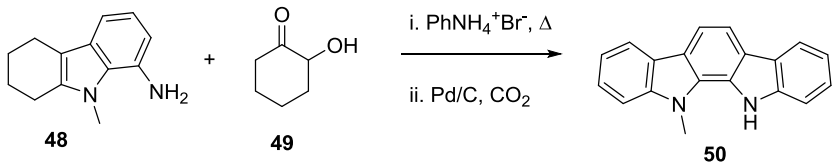
For review purposes, the methods for the synthesis of indolo[2,3-*a*]carbazole platform **3** can be broadly classified into 3 types depending on the final ring closing step: B/D type (Section 2.2), C type (Section 2.3) and multiple ring closing (Section 2.4). B/D type routes target indolocarbazoles by indole ring synthesis to close the 2 five-membered rings. C type routes involve the initial synthesis of a bisindolyl intermediate, some of which have been found to be very interesting compounds in their own right, followed by cyclisation to form the central C ring. The multiple ring closing strategies involve the closing of more than one ring simultaneously. In general, each of these strategies is useful for the synthesis of particular classes of derivatives and the choice of method depends strongly on the final target.

## 2.2 B/D type

B/D type routes are characterised by the key formation of one or both of the five-membered B and D rings. This has been accomplished using a Bischler-Möhlau indole synthesis, Fischer cyclisation or Cadogan nitrene insertion to close the rings.

### 2.2.1 Bischler-Möhlau indole synthesis

The first synthesis of an indolo[2,3-*a*]carbazole was achieved *via* a Bischler-Möhlau indole synthesis.[1,2] In 1956, Tomlinson and co-workers reported that the condensation of 8-amino-1,2,3,4-tetrahydro-9-methyl-9*H*-carbazole **48** with 2-hydroxycyclohexanone **49**, followed by dehydrogenation produced the *N*-methyl derivative **50** (Scheme 2.2.1).[3] However, attempts to synthesise the parent indolo[2,3-*a*]carbazole **3** via an analogous approach failed.

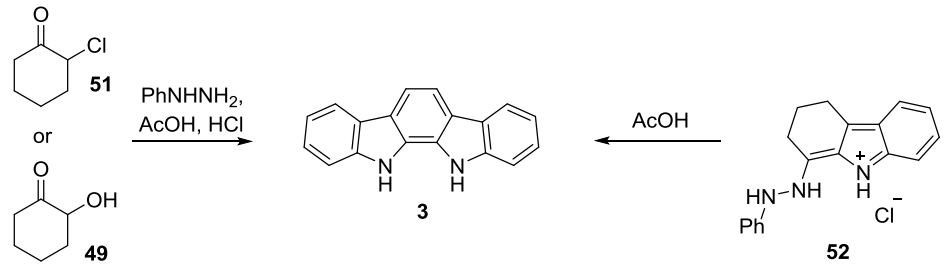



Scheme 2.2.1

### 2.2.2 Fischer indole synthesis

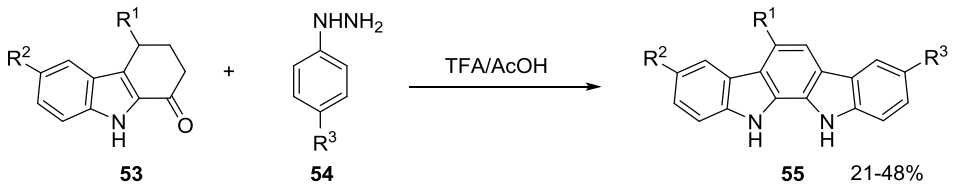
These routes are characterised by the reaction of phenylhydrazine with cyclohexanone derivatives in a classical Fischer indole synthesis.[4,5]

In 1957, Bhide *et al.* developed a double Fischer indole synthesis of 2-chlorocyclohexanone **51** or 2-hydroxycyclohexanone **49** with phenylhydrazine which, upon dehydrogenation, provided indolo[2,3-*a*]carbazole **3** (Scheme 2.2.2).[6] Mann and Willcox used a similar method to synthesise indolo[2,3-*a*]carbazole **3** by Fischer indole cyclisation of 1,2,3,4-tetrahydro-9*H*-carbazol-1-one phenylhydrazone hydrochloride **52**.[7]



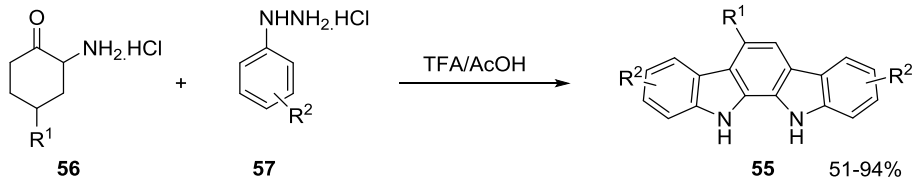

**Scheme 2.2.2**

Several years later, another application of this methodology was reported by Royer *et al*. who described the synthesis of various pentacyclic molecules including some indolocarbazoles from tricyclic ketones **53**.[8] The Fischer indole synthesis was performed in a mixture of acetic acid and trifluoroacetic acid, reacting the ketones with substituted arylhydrazines **54** to form the tjipanazoles analogues **55** (Scheme 2.2.3).




**Scheme 2.2.3**

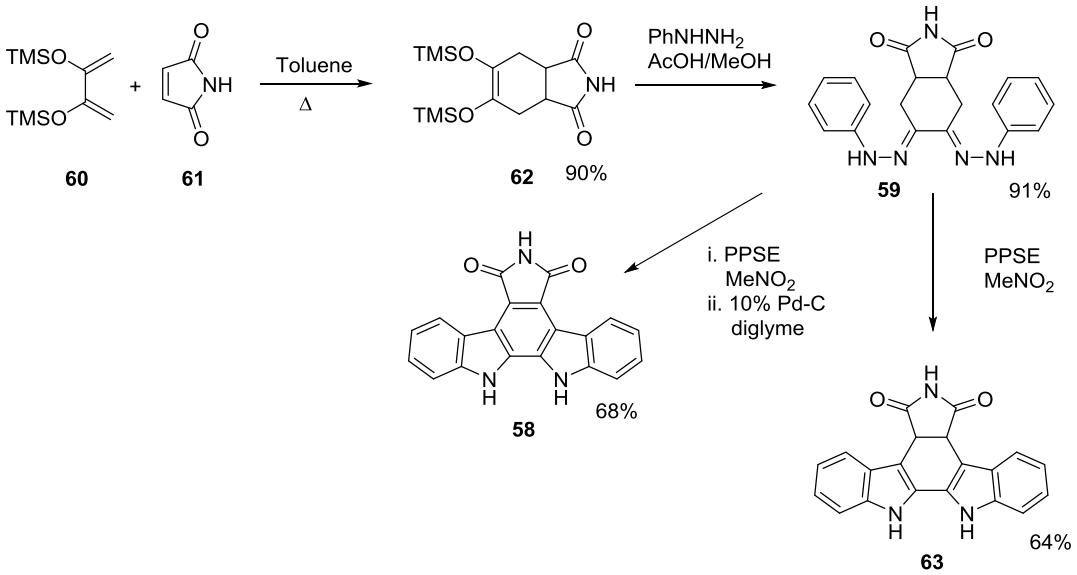
In 2005, Hu and Chen utilised a one-pot double Fischer indole synthesis in the synthesis of a number of novel biologically active tjipanazoles **55**.[9] In this route, the reaction of 2-amino-cyclohexanone hydrochloride **56** with a substituted arylhydrazine hydrochloride **57** in acidic media (usually 95% AcOH/TFA) to form the indolocarbazole (Scheme 2.2.4).




**Scheme 2.2.4**

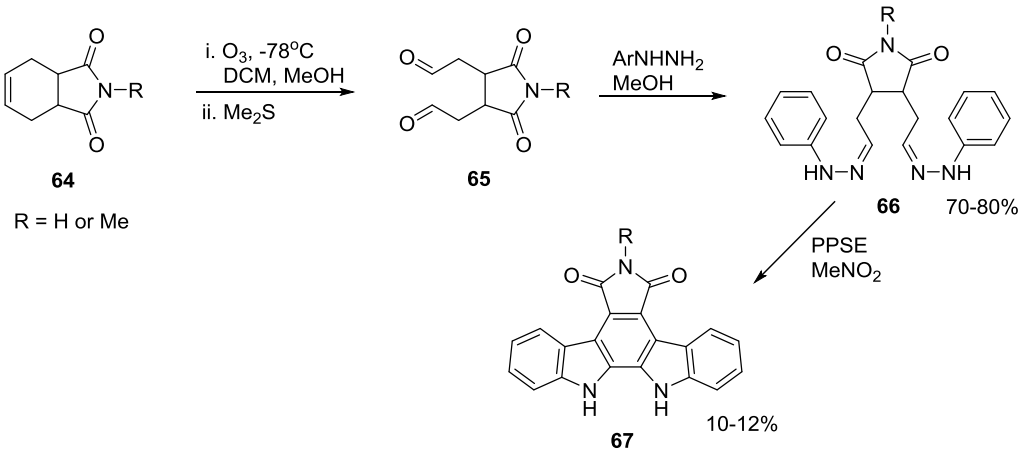
In 1988, Bergman and Pelcman described the synthesis of a number of symmetrical indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazoles, including arcyriaflavin A **58** (Scheme 2.2.5),

using a similar double Fischer indole synthesis.[10] The bis(phenylhydrazone) **59** was synthesised by reacting 2,3-bis[(trimethylsilyl)oxy]butadiene **60** with maleimide **61** in a Diels-Alder reaction and then treating the resulting cycloadduct **62** with 3 equivalents of phenylhydrazine. Polyphosphoric acid trimethylsilyl ester (PPSE) induced cyclisation was then achieved to give the dihydro compound **63** which partially converted to fully aromatic **58** on recrystallisation. Indolocarbazole **58** was prepared more successfully by subjecting the crude product **63** to dehydrogenation using Pd-C in diglyme.




**Scheme 2.2.5**

Gribble and Berthel concurrently developed a similar route with an alternative method for achieving the bis(phenylhydrazone) intermediate.[11] Tetrahydrophthalimides **64** were subjected to ozonolysis at -78 °C, followed by treatment with dimethyl sulfide to give the dialdehyde intermediates **65**, which were not isolated due to problematic polymerisation. The crude dialdehydes were then reacted with an arylhydrazine to give the desired bis(phenylhydrazone)s **66** in 70-80% yield. Fischer indole cyclisation with PPSE and nitromethane then gave the indolocarbazoles **67** in 10-12% yield (Scheme 2.2.6).

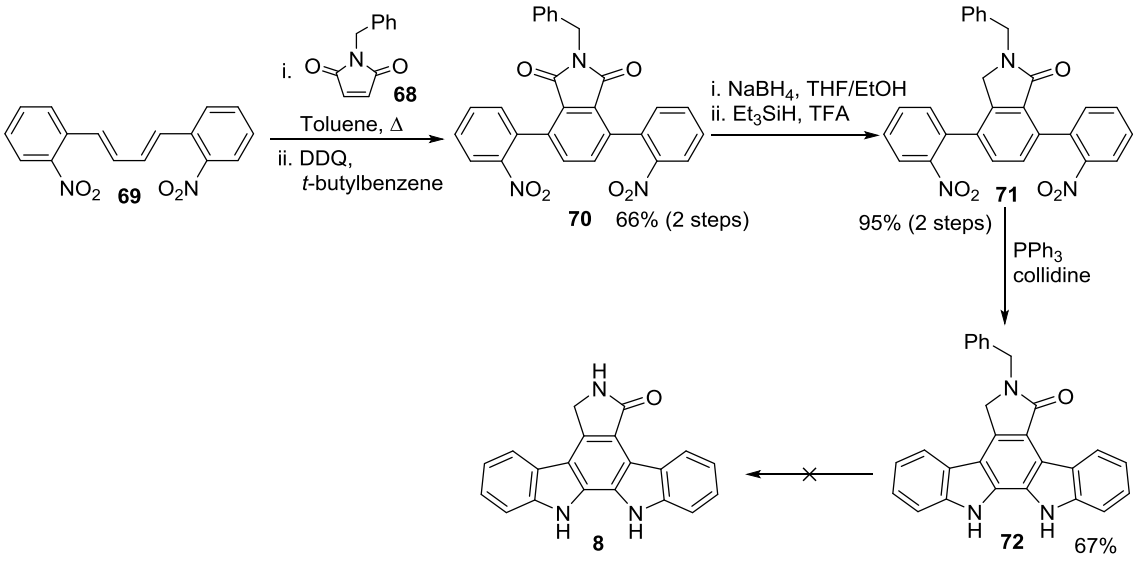



**Scheme 2.2.6**

### 2.2.3 Nitrene insertion

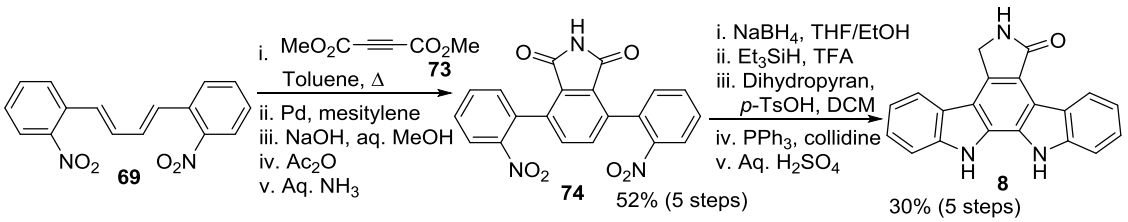
In 1965 Cadogan *et al*. reported a new route to carbazoles *via* reduction of nitro compounds by triethyl phosphite or triphenylphosphine.[12] This strategy has since been utilised in a number of routes to indolo[2,3-*a*]carbazoles and has been found to proceed through nitrene insertion.

The first of these routes was reported by Raphael and co-workers, in 1990, with the aim of synthesising a lactam *N*-protected staurosporinone whose protecting group could be either removed to form staurosporinone **8** or retained for an *N*-glycosylation step to form staurosporine **1**.[13] Initially, a benzyl group was employed and the route began with a Diels-Alder reaction between *N*-benzyl maleimide **68** and dinitro compound **69** to give the adduct which was oxidised to give compound **70** using DDQ. The maleimide was then reduced with sodium borohydride to give the hydroxyl lactam, which was further reduced with triethylsilyl hydride-trifluoroacetic acid to give the lactam **71**. Attempted deoxygenation with triethyl phosphite resulted in the formation of a mixture of the mono- and di-*N*-ethylated products; however, deoxygenation with triphenylphosphine achieved the required double nitrene insertion to give *N*-benzyl staurosporinone **72**. Unfortunately **72** could not be deprotected to form staurosporinone **8** (Scheme 2.2.7). The route was also attempted with variety of other protecting groups; however, they all failed at some point in the sequence.




**Scheme 2.2.7**

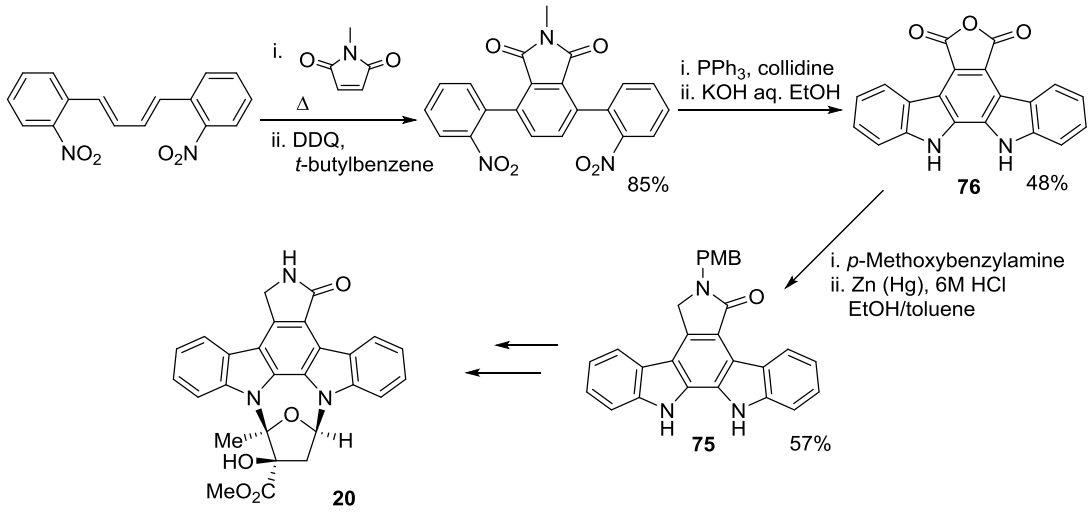
The route was then attempted without a protecting group starting from a Diels-Alder reaction of dimethyl acetylenedicarboxylate **73** and compound **69**, followed by palladium-mediated aromatisation and conversion to the anhydride (Scheme 2.2.8). The anhydride was then treated with aqueous ammonia to form the maleimide **74**. The lactam **8** was then formed through treatment with sodium borohydride, followed by triethylsilyl hydride-trifluoroacetic acid. Although double nitrene insertion was successfully achieved with triphenylphosphine, the resulting staurosporinone **8** could not be separated from the triphenylphosphine oxide side product. The lactam nitrogen was instead protected using dihydropyran with *p*-toluenesulfonic acid which was then smoothly converted to the protected staurosporinone using triphenylphosphine and subsequently deprotected using aqueous sulfuric acid to give staurosporinone.




**Scheme 2.2.8**

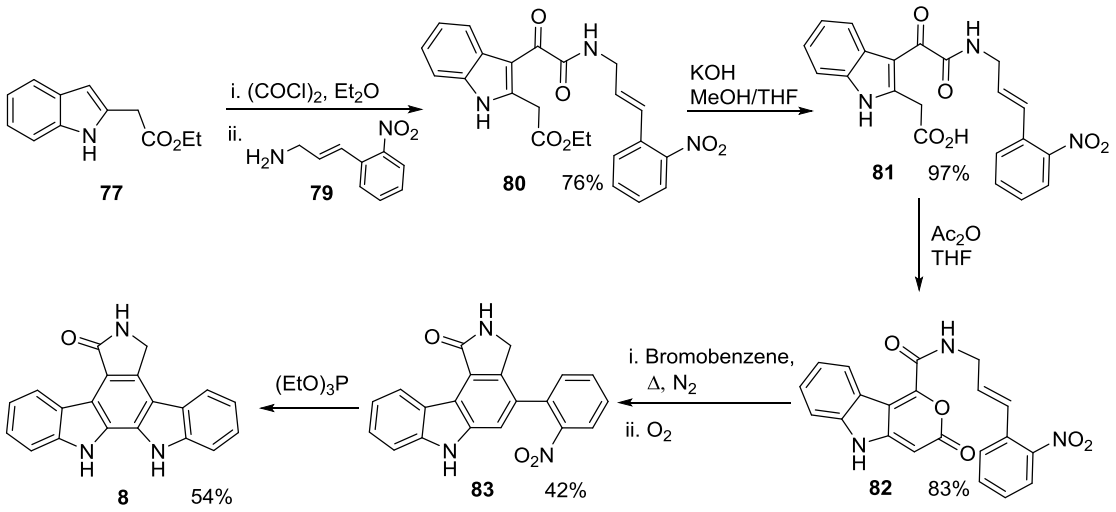
Several years later, Lowinger *et al*. followed a similar route to synthesise a *p*-methoxybenzyl protected staurosporinone **75** *via* a maleic anhydride intermediate **76**,

which was then successfully glycosylated and deprotected to form K252a **20** (Scheme 2.2.9).[14]



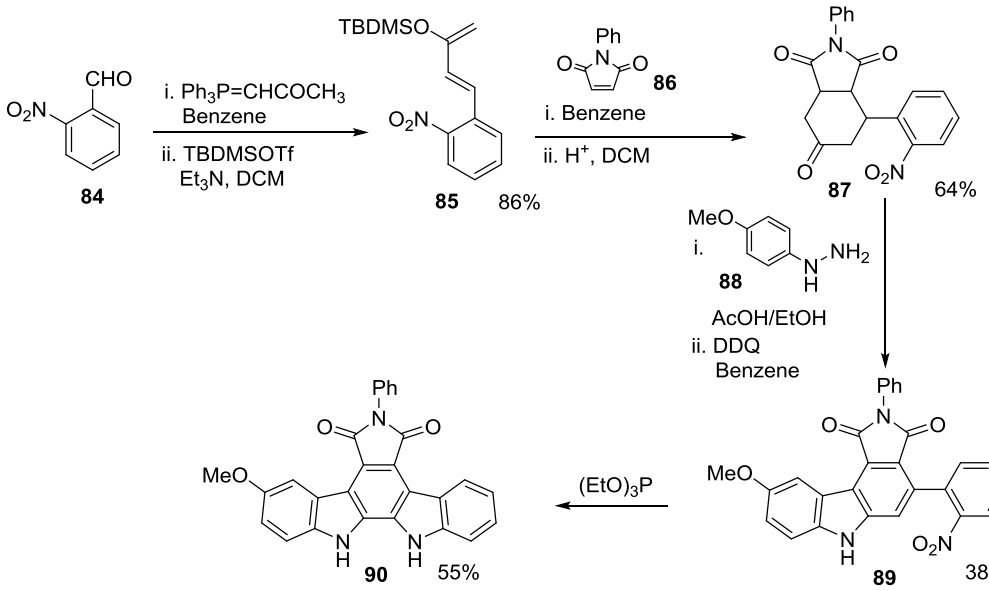

**Scheme 2.2.9**

In 1992, Moody *et al*. developed a route to staurosporinone **8** with an intramolecular Diels-Alder reaction followed by a nitrene insertion as the key steps (Scheme 2.2.10).[15] This was achieved without the need for protecting groups on either the lactam or indole nitrogens. Starting from the acylation of ethyl indole-2-acetate **77** with oxalyl chloride, the resulting indole-3-glyoxalyl chloride **78** was quenched with 2-nitrocinnamyl amine **79** to form the amide **80**. Hydrolysis to the acid **81** was effected using potassium hydroxide prior to cyclodehydration using acetic anhydride to give the Diels-Alder substrate **82**. The Diels-Alder reaction was initially attempted by heating **82** in refluxing bromobenzene open to air but although the indolocarbazole was formed, the lactam had also been oxidised to the maleimide. When heated under nitrogen, a reduced dihydrocarbazole was formed after 4 hours. However, it was found that if the nitrogen inlet on the reaction vessel was removed at this point and heating was continued for a further 4 hours, air oxidation led to the formation of the desired carbazole **83**. Triethyl phosphite induced nitrene insertion then yielded the indolo[2,3-*a*]carbazole **8**. A small amount of *N*-ethylation was observed, however this impurity was easily removed using column chromatography.



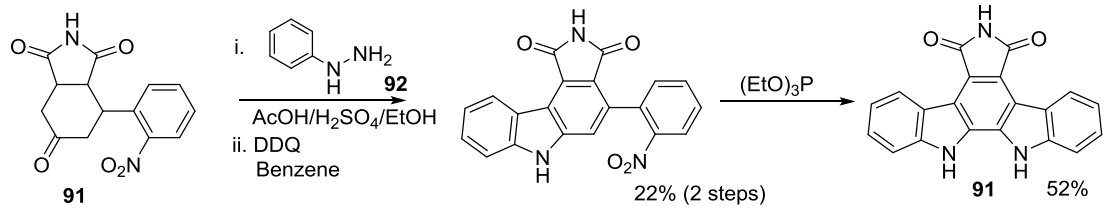

**Scheme 2.2.10**

Tomé and co-workers developed a different route with a nitrene insertion as the final step (Scheme 2.2.11).[16] Starting from commercially available 2-nitrobenzaldehyde **84**, the first step was a Wittig reaction to form an enone which was treated with *t*-butyldimethylsilyl triflate and triethylamine to form diene **85**. The diene then reacted with *N*-phenylmaleimide **86** in a Diels-Alder step, similar to some reported previously by the group, and the silyl ether was hydrolysed forming **87**.[17] A Fischer indole synthesis with *p*-methoxyphenylhydrazine **88** followed by treatment with DDQ formed the carbazole **89**, which was then heated with triethyl phosphite to form the final indole ring **90**. This route provided a more versatile route to the synthesis of unsymmetrical staurosporine analogues as a variety of substituted aryl hydrazones could be used in the Fischer reaction step to introduce diversity to the A-ring.



Scheme 2.2.11

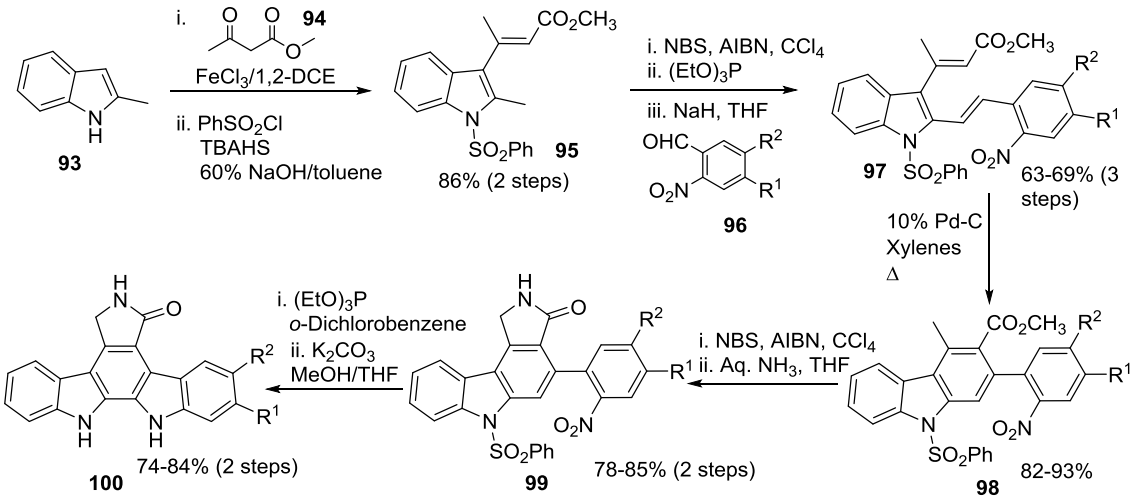
The same methodology was also successfully applied to the synthesis of arcyriaflavin A **58**, using an unprotected succinimide **91** and phenylhydrazine **92**, with the steps proceeding in similar yields (Scheme 2.2.12).



Scheme 2.2.12

Rajeshwaren and Mohanakrishnan have recently reported a route to a series of unsymmetrical indolocarbazoles starting from 2-methylindole **93**, involving electrocyclisation and nitrene insertion reactions (Scheme 2.2.13).[18] Treatment of 2-methylindole with methylacetoacetate **94** in the presence of iron(III) chloride (20 mol%), followed by phenylsulfonylation gave protected **95**. Selective bromination at the 2-methyl position was achieved using NBS in the presence of a catalytic amount of AIBN and the bromide was reacted with triethylphosphite to form the phosphonate ester. A Wittig-Horner reaction with a series of functionalised 2-nitroarylaldehydes, containing chloro, fluoro or methoxy substituents **96**, was accomplished using sodium hydride in dry THF to form **97** which were converted to the 2-nitroarylcarbazoles **98** in

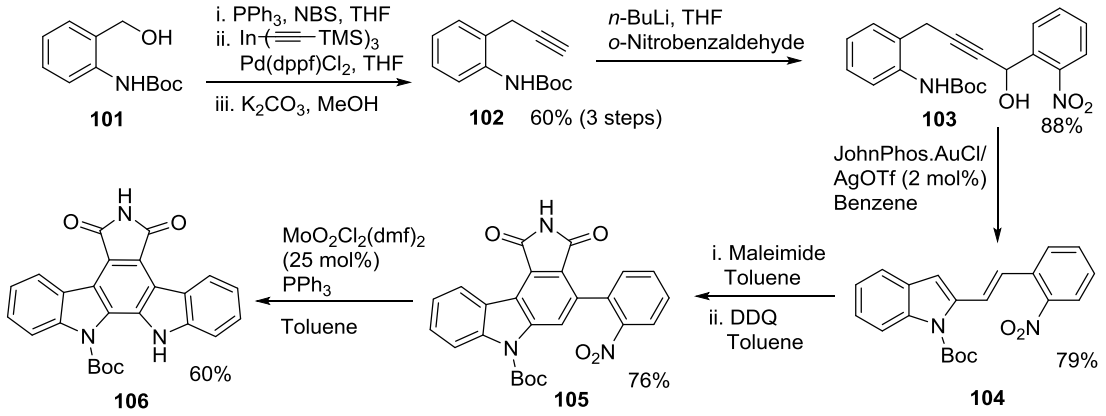
an electrocyclisation step. The lactam moiety **99** was formed by initial treatment with NBS in the presence of AIBN, followed by treatment with aqueous ammonia in THF. The final ring-closing step was effected using triethylphosphite in 1,2-dichlorobenzene which, following deprotection, gave the desired substituted indolo[2,3-*a*]carbazoles **100**. The author noted that *N*-ethylation was not observed during the nitrene insertion step and postulated that the higher yield could be attributed to the nitrene insertion taking place *meta*- to the amide carbonyl unlike Moody's route where the insertion occurred *para*- to the amide and only proceeded in 54% yield (see Scheme 2.2.10).




**Scheme 2.2.13**

Recently, Borrero *et al*. reported a new strategy to mono-substituted indolocarbazoles involving a gold-catalysed tandem dehydrative cyclisation/Diels-Alder reaction of a propargyl alcohol (Scheme 2.2.14).[19] The route begins with the known Boc-protected 2-aminobenzyl alcohol **101** which was converted to the alkyne **102** by initial bromide formation followed by palladium-catalysed cross-coupling with tris(trimethylsilylacetylene)-indium before deprotection of the silyl group with potassium carbonate. Deprotonation followed by the addition of 2-nitrobenzaldehyde formed a substrate **103** for the Au-mediated cyclisation which was achieved using the optimised conditions of JohnPhos.AuCl with silver triflate and benzene as solvent. The resulting 2-substituted indole **104** was then heated with maleimide in toluene and following DDQ oxidation **105** was formed. The final nitrene insertion proved difficult with attempted conditions involving trivalent organophosphorus reagents, Grignard reagents and a variety of catalytic and oxidative amination methods resulting in

decomposition or partial removal of the Boc group. The transformation was finally achieved using the $MoO_2Cl(dmf)_2$ catalyst to form the fully cyclised indolocarbazole **106**.




**Scheme 2.2.14**

## 2.3 C Type

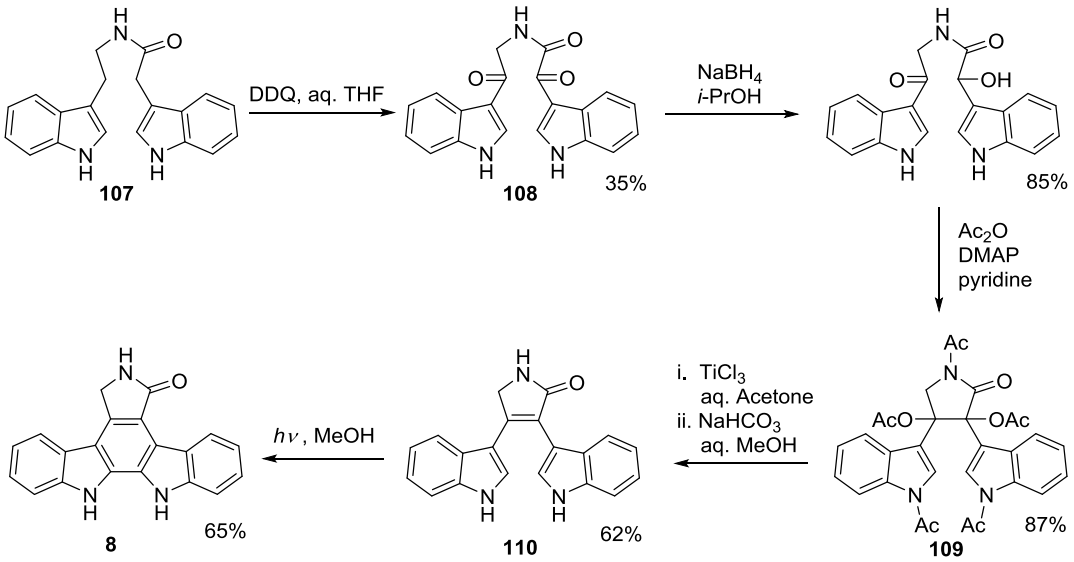
C type routes are some of the most highly versatile and heavily utilised routes to indolocarbazoles and their analogues. They are characterised by initial formation of bisindole precursor prior to cyclisation to form the final C ring. The two indoles of the bisindole precursors can either be connected at the C-3 position, as in the bisindolylmaleimides, or the C-2 position.

### 2.3.1 Cyclisation from bisindolylmaleimide intermediates

As well as being useful intermediates in the synthesis of indolo[2,3-*a*]carbazoles, many bisindolylmaleimides have been shown to be potent inhibitors of various protein kinases in their own right. Therefore, in this section the syntheses of bisindolylmaleimides not synthesised as precursors to indolo[2,3-*a*]carbazoles will also be included. The final step to the fully aromatic indolocarbazole involves the formation of the 2,2′ carbon-carbon bond.

### *2.3.1.1 Early routes*

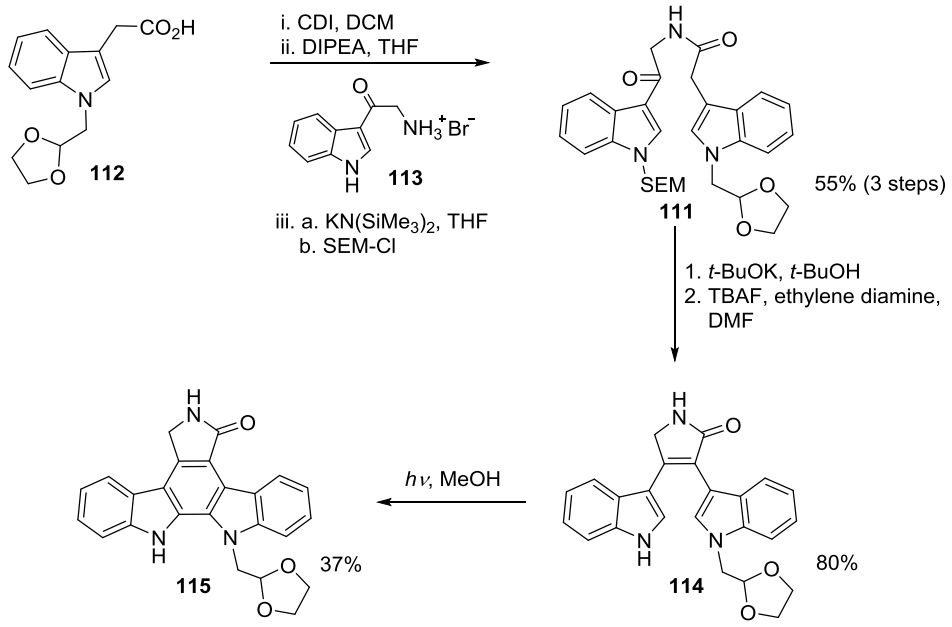
The first synthesis of staurosporinone **8** was reported by Winterfeldt and co-workers in 1983 and involved a final C-ring closing step (Scheme 2.3.1).[20] Initially, bisindole amide **107** was oxidised using DDQ to form trione **108**. The trione was then partially reduced using sodium borohydride and polyacetylation was performed forming **109**. Deacylation using titanium trichloride in aqueous acetone followed by sodium bicarbonate in aqueous methanol gave the bisindole compound **110**, which was converted to the fully aromatic indolocarbazole **8** *via* a photocyclisation step.




**Scheme 2.3.1**

The group later reported a variation which allowed the synthesis of unsymmetrically *N*-substituted indolocarbazoles and also changed the reductive cyclisation step to a more simple base hydrolysis (Scheme 2.3.2).[21] The bisindole amide intermediate **111** was prepared by reacting the *N*-alkylated acid **112** with amino ketone **113** in the presence of *N*-*N*′ carbonyl diimidazole (CDI). Preliminary experiments indicated the necessity of the protection of both indole nitrogens for the base hydrolysis and so a SEM group was introduced prior to lactam formation. This was achieved through treatment with potassium *tert*-butoxide followed by deprotection to form the unsaturated lactam **114**. A final photocyclisation step then formed the indolo[2,3-*a*]carbazole **115**. It is interesting to note that the cyclisation reaction now proceeds in a lower yield of 37% when an

indole *N*-substituent is present in **115**, compared to the 65% yield achieved to form cyclised **8** in which both indoles are unsubstituted.

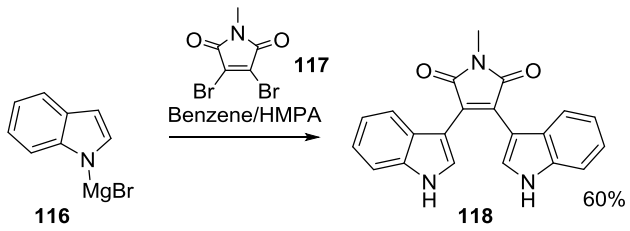



**Scheme 2.3.2**

### *2.3.1.2 Grignard and related routes*

These routes involve a type of indole electrophilic substitution where the indoles are initially treated with a Grignard reagent or non-nucleophilic base to activate the C-3 position, prior to reaction with 3,4-dihalomaleimides.

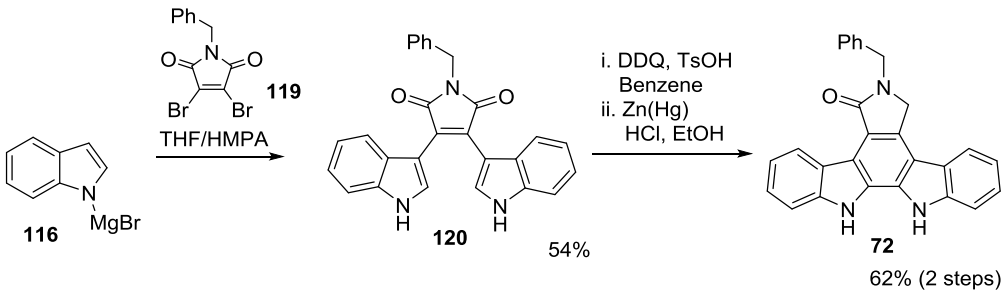
The first reported synthesis of a bisindolylmaleimide was reported by Steglich *et al.* in 1980.[22] This was accomplished by reaction of indolyl magnesium bromide **116** with 2,3-dibromo-*N*-methylmaleimide **117** to give the methyl protected bisindolylmaleimide **118** in 60% yield (Scheme 2.3.3).




**Scheme 2.3.3**

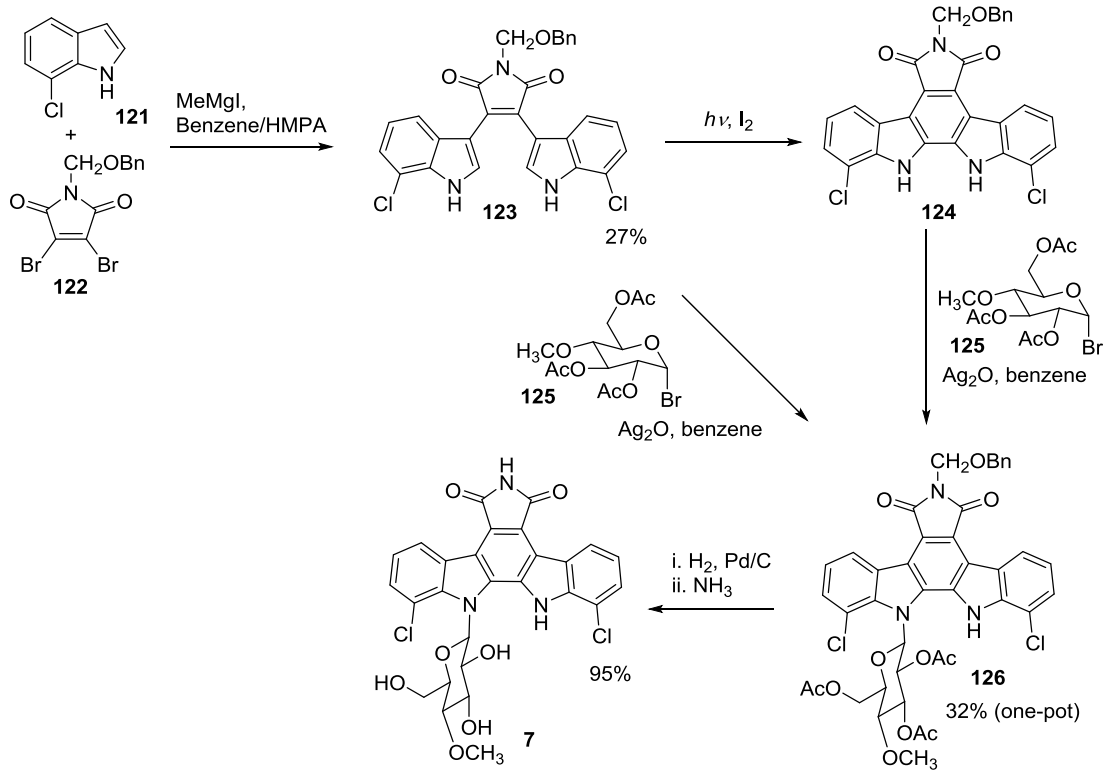
This method was later applied by Weinreb and Kaneko for their syntheses of benzyl protected staurosporinone **72** and rebeccamycin **7** respectively.[23,24]

Weinreb *et al*. coupled indole magnesium bromide **116** with *N*-benzyl-3,4-dibromomaleimide **119** to form the bisindolylmaleimide **120** which was then cyclised to the indolocarbazole *via* DDQ oxidation (Scheme 2.3.4). A Clemmenson reduction was then used to convert the maleimide to the required lactam **72**, the first reported use of this method for the transformation. The group did not report conversion to unprotected staurosporinone **8**.



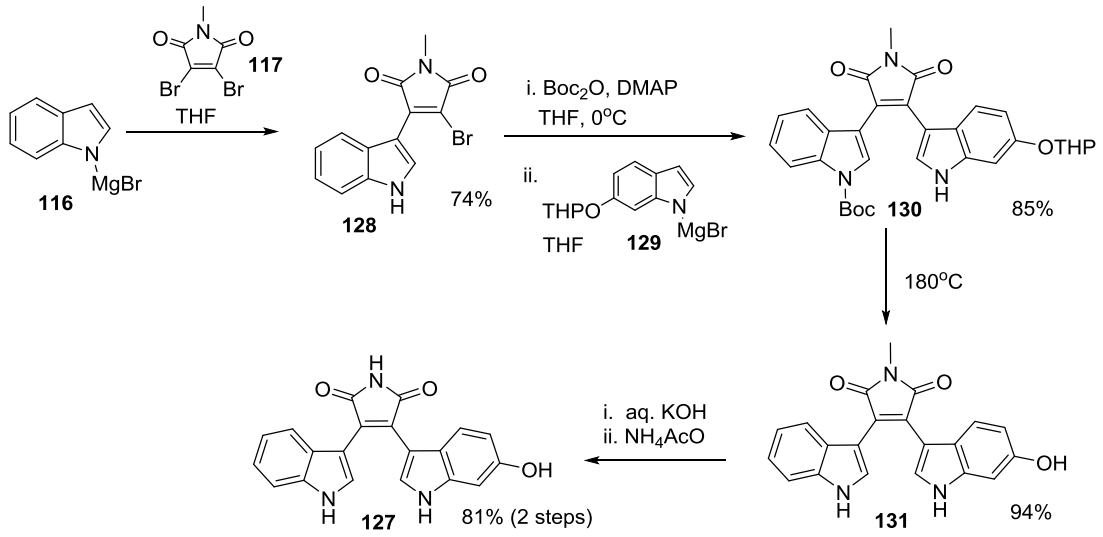

**Scheme 2.3.4**

For rebeccamycin **7**, Kaneko *et al.* coupled 7-chloroindole **121** with *N*-benzyloxymethyl-2,3-dibromomaleimide **122** under Grignard conditions in benzene containing a catalytic amount of HMPA and the desired bisindolylmaleimide product **123** was formed in 27% yield (Scheme 2.3.5). Cyclisation to indolocarbazole **124** could be effected by irradiating with mercury lamps in the presence of iodine and air but a one-pot method was also developed where cyclisation and glycosylation could both be effected in refluxing benzene in the presence of silver oxide and the bromo glycoside **125** with the *N*-glycoside **126** being obtained in 32% yield. Deprotection of the benzyloxymethyl group by hydrogenation and the acetyl groups by ammonolysis gave rebeccamycin **7** in 95% yield.



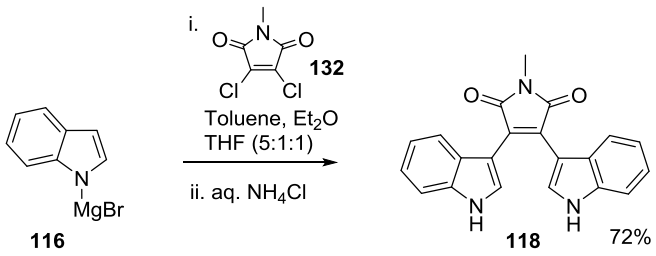

**Scheme 2.3.5**

Several years later Brenner *et al.* used a similar Grignard reaction from a protected dibromomaleimide **117** to achieve the synthesis of unsymmetrically substituted arcyriarubin B **127** (Scheme 2.3.6).[25] The condensation of indole Grignard **116** with 2,3-dibromo-*N*-methylmaleimide **117** previously carried out in ether/benzene mixtures gave a mixture of the mono- and bisindolylmaleimides. They found that the outcome of this reaction is strongly dependent on the solvent with toluene giving the bisindolyl compound **118** in 70% yield and THF giving the monoindolyl compound **128** in 74% yield. The monoindolyl compound could then be used for the preparation of the unsymmetrically substituted bisindolylmaleimide through initial conversion to its *N*-*tert*-butyloxycarbonyl derivative to prevent salt formation followed by reaction with 2 equivalents of substituted indole Grignard **129** in THF to give the Boc-protected **130** in 85% yield. Deprotection was carried out by heating the compound to 180°C to give **131**. Conversion of the methylmaleimide to the maleimide was then carried out in the usual method to give arcyriarubin B **127**.



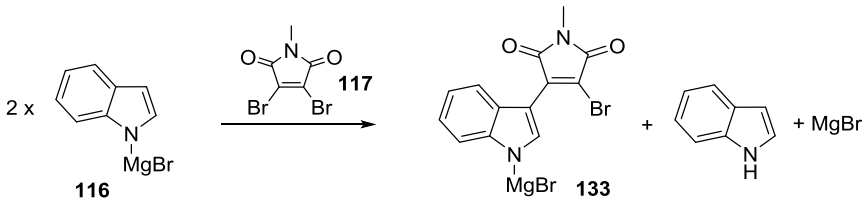

**Scheme 2.3.6**

Faul *et al.* investigated the Grignard route in greater detail.[26] They found the dichloromaleimides **132** to have comparable reactivity to the dibromo analogues as well as being cheaper and more readily available. They also found the synthesis to be solvent dependent and found the optimal solvent system toluene/ether/THF (5:1:1) for the reaction. Addition of aqueous ammonium chloride to the reaction mixture eliminated the need for chromatography and, on incorporation of all these improvements, the synthesis of **118** was scaled up to 770 g with a yield of 72% (Scheme 2.3.7).



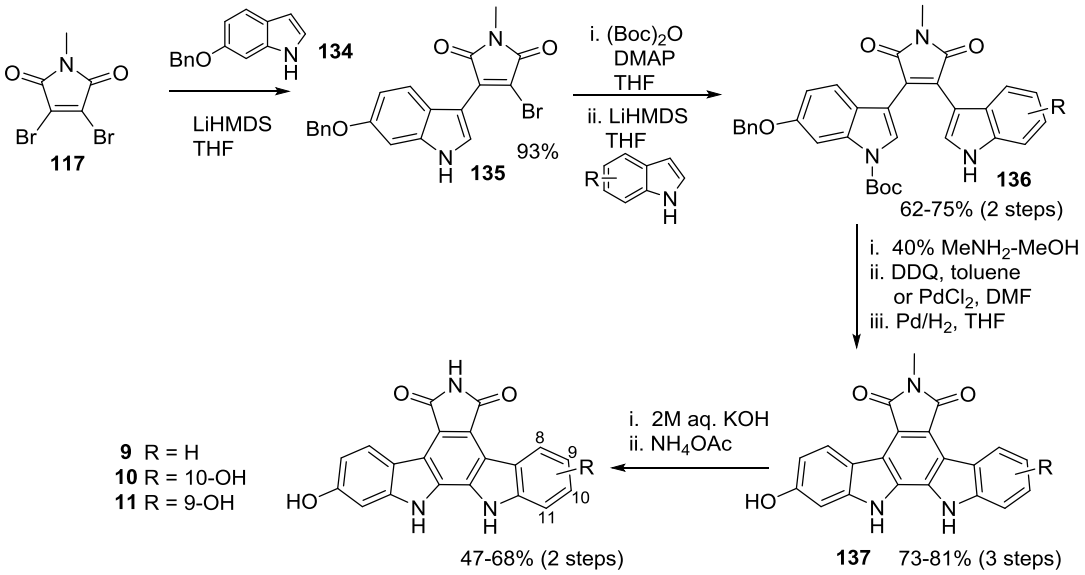

**Scheme 2.3.7**

One disadvantage of the Grignard route is that it requires a large excess of the indole Grignard reagent **116** to obtain a high yield. This is due to the monoindolylmaleimide reacting with the other indolylmagnesium bromide **116** to form **133** and indole when just two equivalents are used, as shown in Scheme 2.3.8.



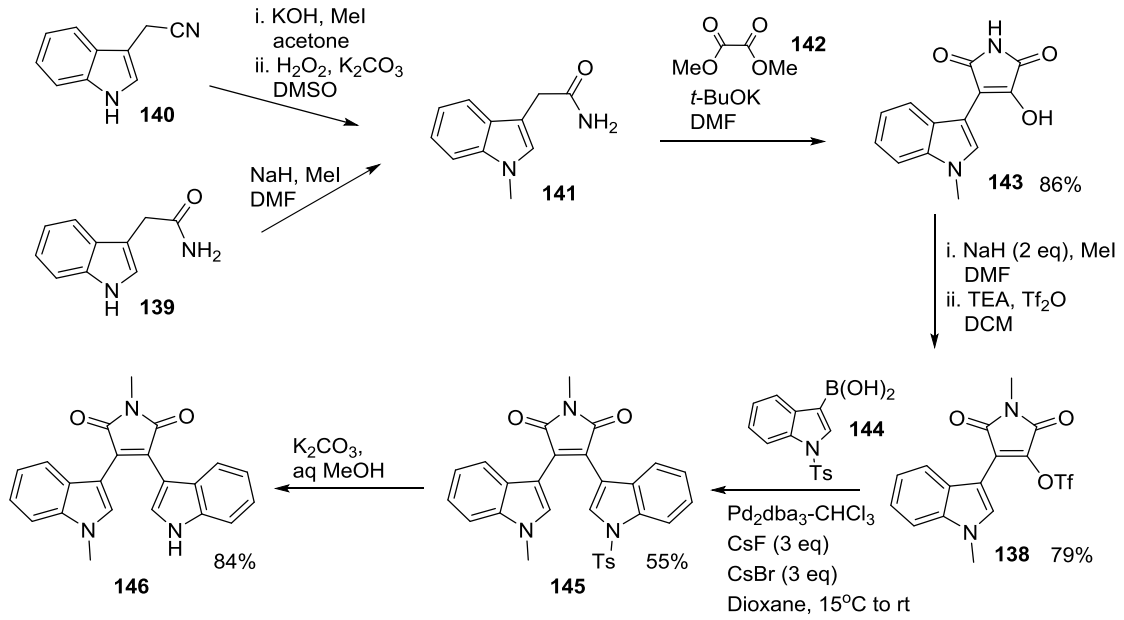

**Scheme 2.3.8**

Ohkubo *et al.* examined several bases including *n*-BuLi, LDA, LiHMDS, NaHMDS and KHMDS as an alternative to the Grignard reagent, finding LiHMDS to be the most effective.[27] They found that just one equivalent of indole is sufficient for indolylation when using a non-nucleophilic base and used this method to synthesise arcyriaflavin A **9**, arcyriaflavin B **10** and arcyriaflavin C **11** (Scheme 2.3.9). One equivalent of 6-benzyloxyindole **134** and 2,3-dibromo-*N*-methyl-maleimide **117** were coupled using a two equivalents of LiHMDS in THF to give the monoindolyl **135** compound in 93% yield. With 1 equivalent of indole, use of LDA also gave a relatively high yield of 76%, but NaHMDS and KHMDS gave lower yields with *n*BuLi only giving a trace of the desired product. The indole product was then protected with a Boc group and then reacted with several different indoles in the presence of two equivalents of LiHMDS to form the corresponding bisindole compounds **136** in good yields. Following deprotection, cyclisation and conversion from the methylmaleimide **137** to the maleimide gave the arcyriaflavins **9**, **10** and **11**.




**Scheme 2.3.9**

Neel *et al.* employed a different strategy utilising a Suzuki cross coupling reaction as a key step from an indolylmaleimide triflate intermediate **138** (Scheme 2.3.10).[28] This intermediate was obtained *via* initial methylation of indole-3-acetamide **139** or by methylation of indole acetonitrile **140** followed by conversion to the amide **141**. The amide **141** was then cyclised with dimethyl oxalate **142** and potassium *tert*-butoxide in DMF to form the maleimide **143** which was subsequently methylated and converted to the triflate **138**. The triflate was then coupled with protected 3-indole boronic acid **144** in a Suzuki reaction with the $Pd_2(dba)_3$ chloroform complex in the presence of cesium fluoride and caesium bromide to give the desired bisindolylmaleimide **145** in 55% yield. The tosyl group was then removed using potassium carbonate in aqueous methanol yielding bisindolylmaleimide **146**.



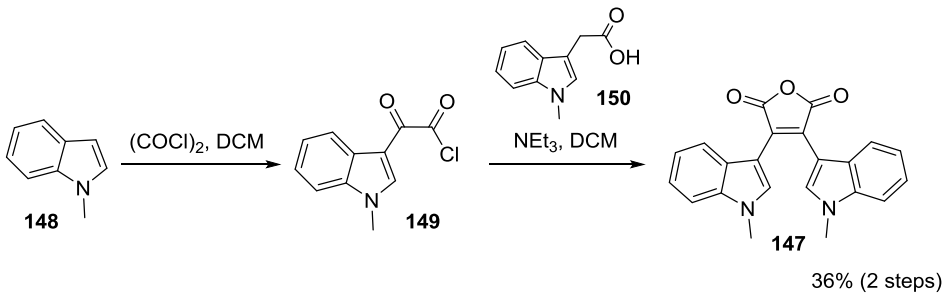

**Scheme 2.3.10**

### *2.3.1.3 F-ring routes*

This section discusses routes where the F-ring is formed directly from two functionalised indole precursors.

In 1990, Davis *et al.* reported a new route to indolylaryl maleic anhydrides using a Perkin condensation between indolyl-3-glyoxylyl chlorides with aryl acetic acids (Scheme 2.3.11).[29] For the synthesis of bisindolyl compound **147**, methyl indole **148**

was treated with oxalyl chloride to form 1-methylindol-3-glyoxylyl chloride **149** which was then used directly in the reaction with 1-methyl-3-indolylacetic acid **150** in the presence of triethylamine to give the maleic anhydride in 36% isolated yield. One disadvantage of this route is that the reaction requires the protection of the indole nitrogens.




**Scheme 2.3.11**

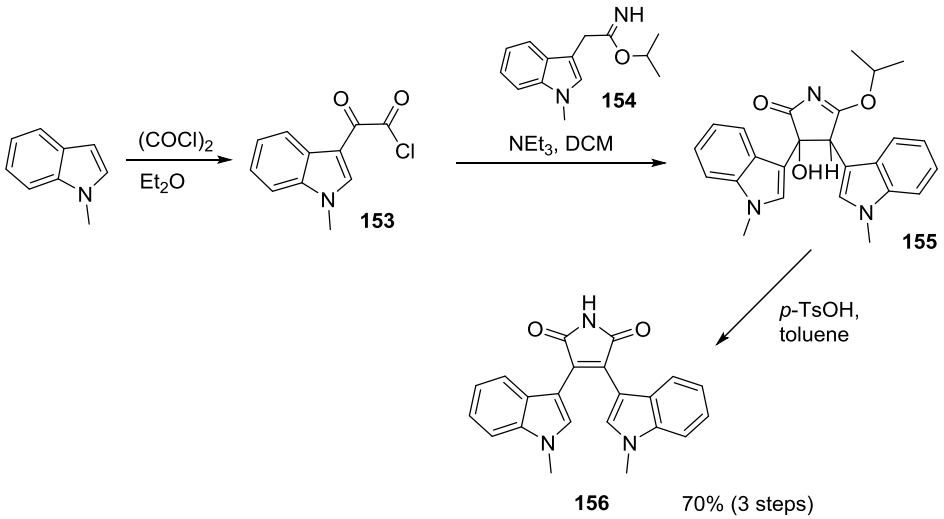
Later that year the group reported a mild conversion of maleic anhydrides **151** into maleimides **152** using HMDS/MeOH (Scheme 2.3.12).[30] This procedure gave excellent yields for a variety of maleic anhydrides and did not affect more sensitive functional groups such as nitriles and esters.




**Scheme 2.3.12**

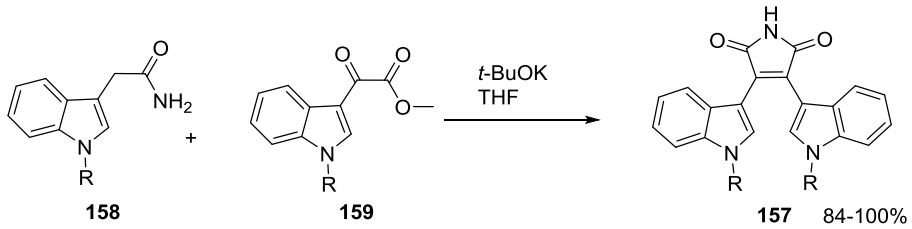
In 1993, Bit *et al*. developed a new coupling reaction which allowed direct access to the bisindolylmaleimide system involving the reaction of glyoxylyl chlorides with acetimidates (Scheme 2.3.13).[31] Reaction of an *N*-substituted indole-3-glyoxylyl chloride **153** with an indole-3-carboximidate **154** in the presence of excess triethylamine gave the hydroxypyrrolone **155**, which underwent dehydration and hydrolysis to the maleimide **156** upon treatment with *p*-toluenesulfonic acid. An alternative method for the dehydration and hydrolysis of acid sensitive groups was also developed using trifluoroacetic acid in pyridine and a panel of *N*-substituted bisindolylmaleimides were synthesised. The advantage of this route was that it tolerates an unprotected indole

nitrogen on the imidate and is higher yielding than the Perkin route based on the indole starting material. However, the acetimidate starting materials are less readily available.




Scheme 2.3.13

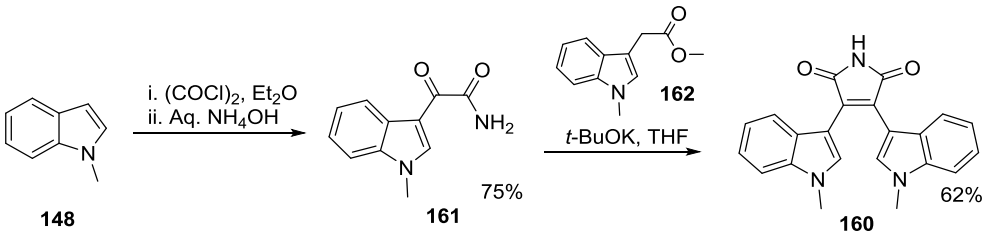
In 1998, Faul *et al.* described a synthesis of *N*-substituted bisindolylmaleimides **157** involving the reaction of indole-3-acetamides **158** with methyl indolyl-3-glyoxylates **159** using a 1.0 M solution of potassium *tert*-butoxide in THF (Scheme 2.3.14).[32] As indolyl-3-glyoxylyl chlorides were successful in Davis's Perkin condensation, the reaction was also attempted with indolyl-3-glyoxylyl chloride instead of indolyl-3-glyoxylyl ester however the yield dropped from a quantitative yield to 68%. This reaction was found to tolerate an unprotected indole nitrogen on either starting material and indole-3-acetamides with alkyl *N*-substituents containing a variety of functional groups (OH, OTr, and $NMe_2$) with yields ranging from 84% to 100%. The main disadvantage of the method is that indole-3-acetamides are relatively expensive.




Scheme 2.3.14

In 2006, Roy *et al.* developed a method for the synthesis of bisindolylmaleimides **160** along with other indolylaryl and indolylheteroaryl maleimides by reacting *N*-

methylindole-3-glyoxylamide **161** with methyl aryl acetates **162** in the presence of potassium *tert*-butoxide in THF (Scheme 2.3.15).[33] *N*-Methylindole-3-glyoxylamide **161** was prepared from *N*-methylindole **148** through treatment with oxalyl chloride followed by aqueous ammonium hydroxide. This method was found to be successful for a range of aryl and heteroaryl derivatives, although it was found to be unsuitable for an unprotected indole.

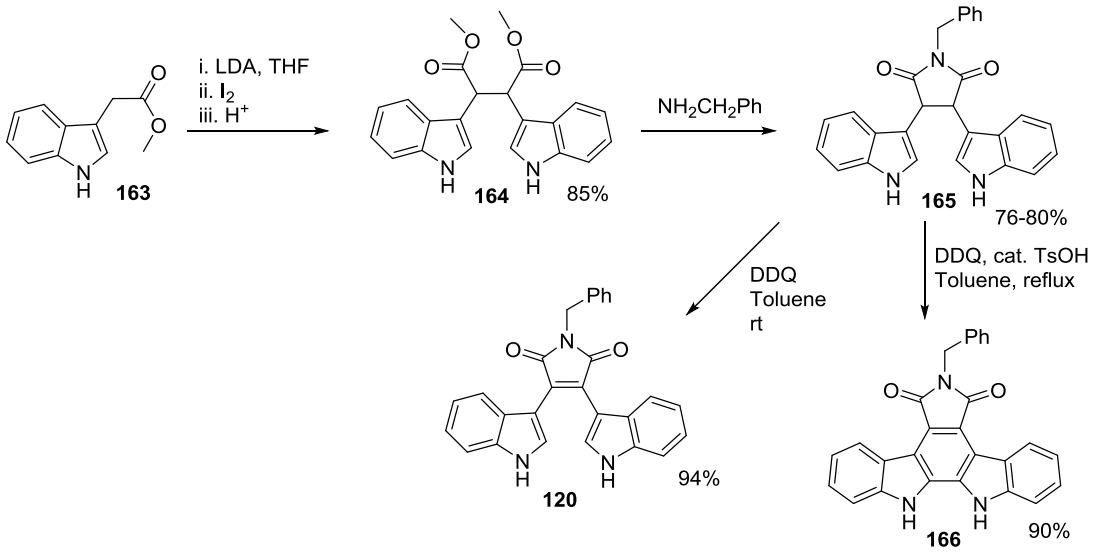



**Scheme 2.3.15**

### *2.3.1.4 Via other 3,3'-bisindolyl intermediates*

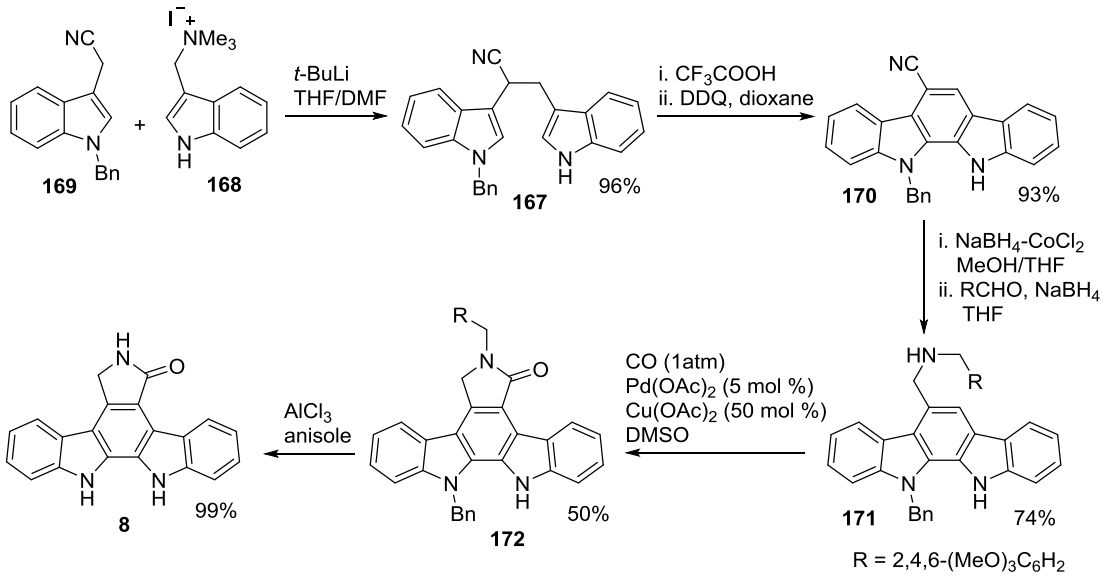
These routes initially form a functionalised bisindolyl compound which can subsequently be reacted to form the F-ring.

In 1987, Bergman and Pelcman developed a biomimetic synthesis *via* oxidative coupling of the dianion derived from indolylmethylacetate **163** (Scheme 2.3.16).[34] Diester **164** was formed by the addition of 2 equivalents of LDA to indolylmethylacetate **163** in THF, followed by coupling with 0.5 equivalents of iodine and an acidic workup. Heating with benzylamine gave the succinimide **165**, which upon oxidation with DDQ afforded the bisindolylmaleimide **120**. Heating the succinimide **165** to reflux with DDQ and a catalytic amount of *p*-toluenesulfonic acid afforded the indolocarbazole **166**.



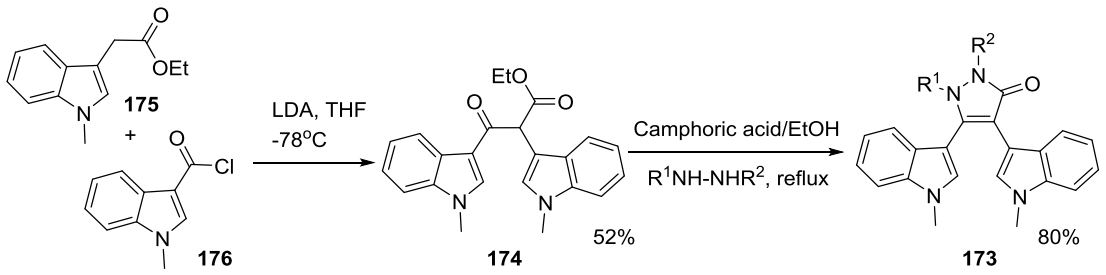

**Scheme 2.3.16**

In 2006, Orito and co-workers reported a method for the synthesis of staurosporinones *via* a bisindolyl intermediate functionalised with a nitrile **167** (Scheme 2.3.17).[35] The route began with the reaction of gramine methyl iodide **168** with 3-(*N*-benzylindolyl)acetonitrile **169** in the presence of *t*-butyllithium to form bisindole **167**. Oxidative cyclisation to indolocarbazole **170** was successfully achieved through treatment with trifluoroacetic acid followed by DDQ. Reduction of the cyano group to form a primary amine was achieved using sodium borohydride and cobalt chloride, and the amine was condensed with (2,4,6-trimethoxy)benzylaldehyde to form an imine which was subsequently reduced using sodium borohydride to the secondary amine **171**. Carbonylation was then achieved with palladium acetate and copper acetate in DMSO under a carbon monoxide atmosphere to form the protected lactam **172** before treatment with aluminium chloride in anisole resulted in deprotected staurosporinone **8**.




**Scheme 2.3.17**

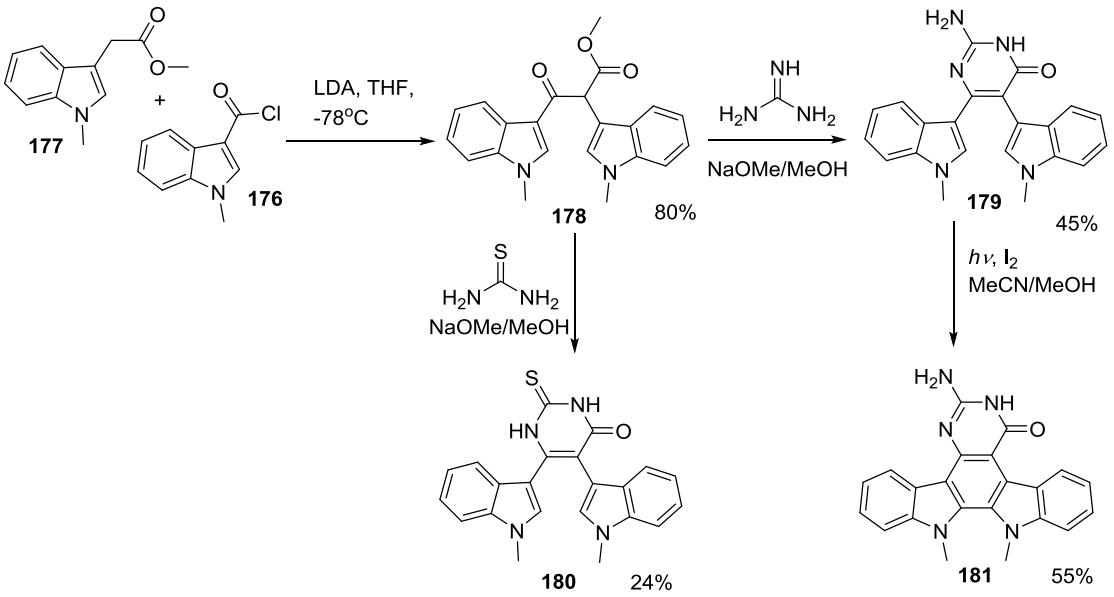
In 2006, Braña *et al*. reported a route to bisindolylpyrazolones **173** *via* a β-ketoester intermediate **174** (Scheme 2.3.18).[36] The β-ketoester was formed by initial deprotonation of ethyl-(1-methyl-1*H*-indol-3-yl)acetate **175** using LDA, followed by the addition of acid chloride **176** previously synthesised by the treatment of 1-methyl-1*H*-indole-3-carboxylic acid with thionyl chloride and DMF. The pyrazolones **173** were then formed by exposing the β-ketoester **174** to the appropriate hydrazine in refluxing ethanol.




**Scheme 2.3.18**

Pierce *et al*. developed a similar method for synthesising a β-ketoester intermediate in their route to novel 5,6-bisindolylpyrimidin-4-ones (Scheme 2.3.19).[37] This route began from methyl 2-(1-methyl-1*H*-indol-3-yl)acetate **177** which was deprotonated using

LDA and treated with 1-methyl-1*H*-indole-3-carbonyl chloride **176** to form the β-ketoester **178**. In this case the acid chloride was obtained by treating the corresponding carboxylic acid with oxalyl chloride resulting in a much cleaner reaction compared to the previously reported thionyl chloride reaction, which was limited by formation of a considerable amount of the 2-chlorinated acid chloride side product. Coupling of the β-ketoester intermediate with guanidine and with thiourea was then successfully achieved to form the 5,6-bisindolylpyrimidin-4-ones **179** and **180** under base-mediated conditions.



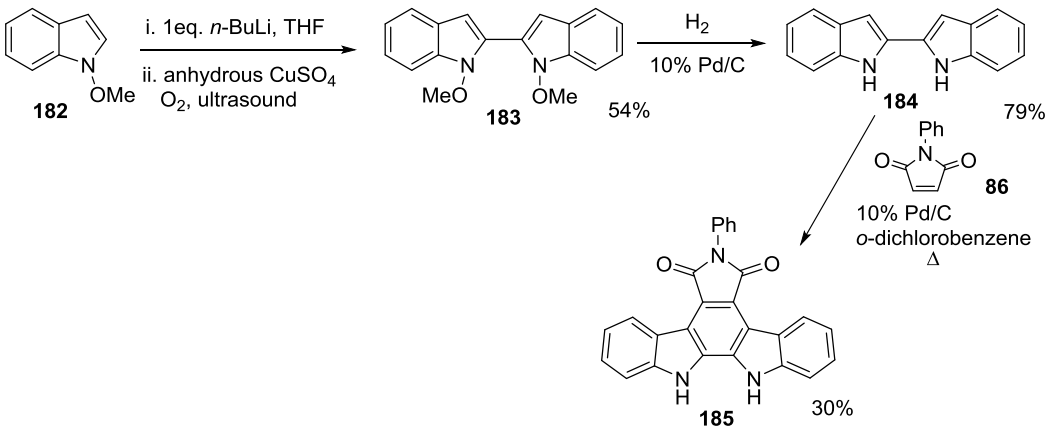

**Scheme 2.3.19**

Isocytocine derivative **179** was then cyclised to the corresponding indolocarbazole **181** in a photocyclisation step.[38]

### 2.3.2 Cyclisation from 2,2′-bisindolyl derivatives

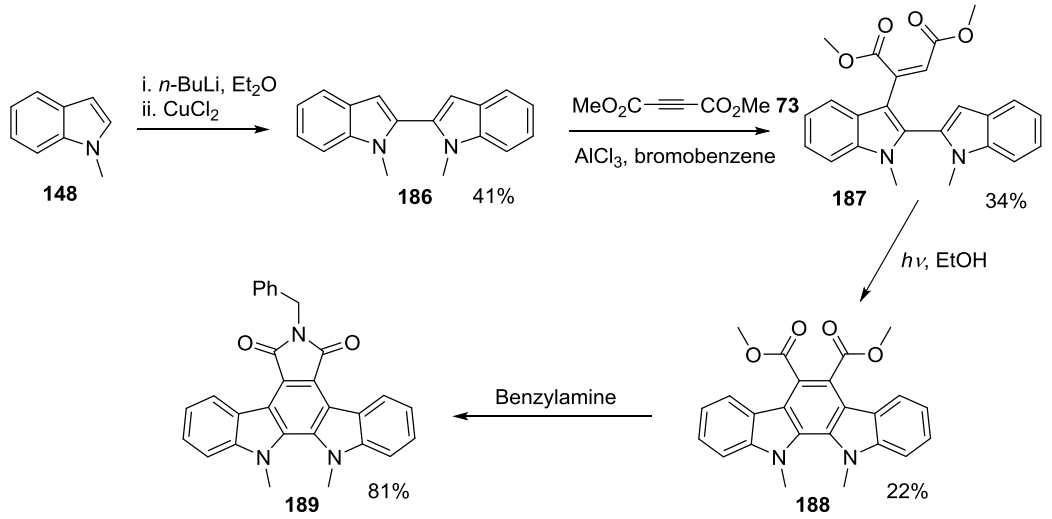
C-ring type routes have also been achieved from a 2,2′-bisindolyl starting material using a variety of different approaches. The first of these routes was reported by Somei and Kodama in 1992 and used a Diels-Alder reaction as the key step (Scheme 2.3.20).[39] 1-Methoxyindole **182** was lithiated using *n*-butyllithium to form 2-lithio-1-methoxyindole which was then treated with anhydrous copper sulfate under an oxygen atmosphere with ultrasound stirring to form 2,2′-bis(1-methoxyindole) **183**. Removal of the methoxy

groups was achieved by hydrogenation with 10% Pd/C affording 2,2′-bisindole **184**. Diels-Alder cycloaddition with *N*-phenylmaleimide **86** containing a catalytic amount of 10% Pd/C for dehydrogenation then formed the indolo[2,3-*a*]carbazole derivative **185**.



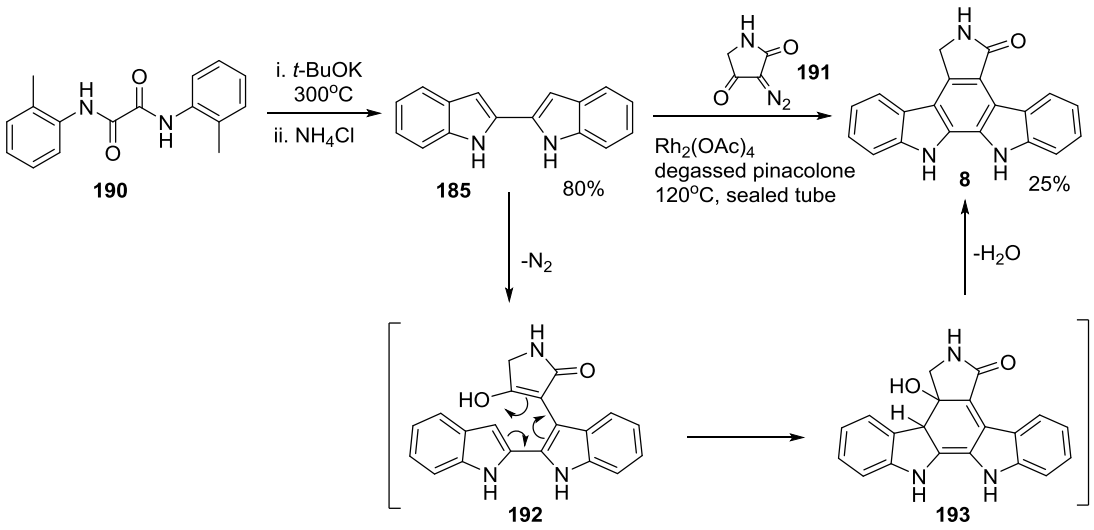

**Scheme 2.3.20**

In 1995, Pindur *et al*. reported a different strategy utilising a photochemically induced cyclisation (Scheme 2.3.21).[40] 2,2′-Bis(*N*-methylindole) **186** was formed by treating *N*-methyl indole **148** with *n*-butyllithium, followed by anhydrous copper (II) chloride. The bisindole intermediate **186** was then reacted with dimethyl acetylenedicarboxylate **73** to form **187**. Photochemically induced 1,6-electrocyclisation of **187** gave indolo[2,3-*a*]carbazole **188** which could then be treated with benzylamine to give the maleimide derivative **189**.




**Scheme 2.3.21**

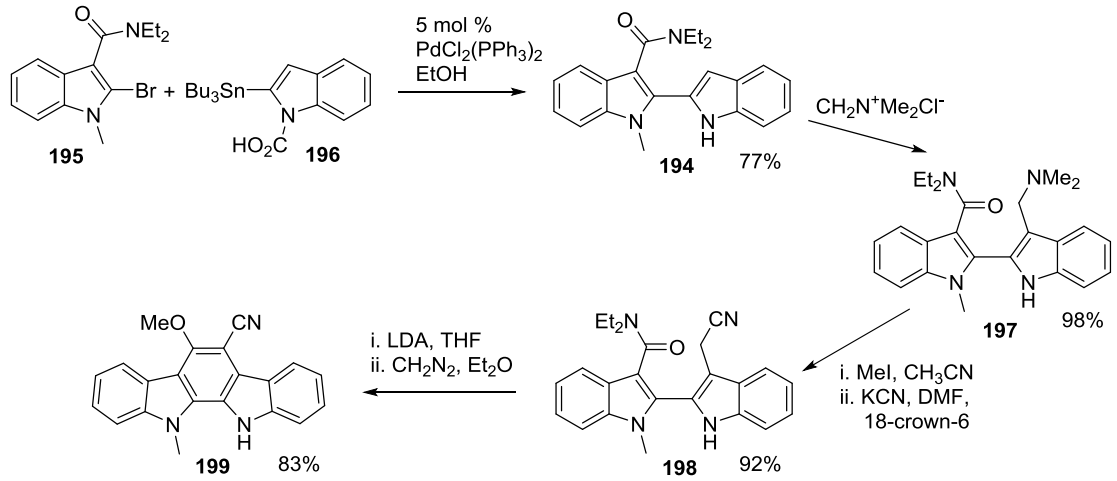
In 1997, Wood *et al*. employed novel rhodium carbenoid chemistry to synthesise a range of different indolo[2,3-*a*]carbazoles including staurosporinone **8**.[41] This was achieved by treating 2,2′-bisindole **184** with a number of diazo lactams in the presence of a rhodium catalyst (Scheme 2.3.22). 2,2′-Bisindole **184** was synthesised using a double Madelung cyclisation procedure from **190** that had been reported a couple of years earlier by Bergman *et al*.[42] Reaction with diazo lactam **191** and rhodium (II) acetate followed by electrocyclisation and dehydration *via* the proposed intermediates shown (**192** and **193**), yielded the indolo[2,3-*a*]carbazole **8**. The identification of a suitable solvent for this step proved challenging with the substrate only being sparingly soluble in most solvents typically employed in rhodium carbenoid reactions resulting in very low yields. The use of sterically bulky pinacolone and also more rigorous deoxygenation techniques increased the yield to 25%, with the introduction of a lactam protecting group increasing the yield further still.




**Scheme 2.3.22**

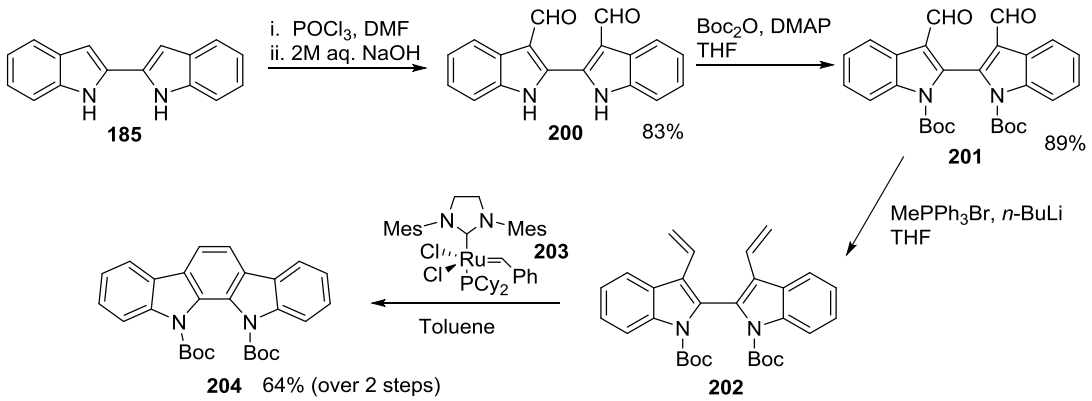
In 2004, Cai and Snieckus developed a route starting from a 2,2′-bisindole intermediate synthesised using a Stille cross-coupling reaction (Scheme 2.3.23).[43] While initial attempts at forming the bisindole **194** using Suzuki-Miyaura conditions had proved unsuccessful, the reaction of 2-bromoindole carboxamide **195** with the 2-stannylated *N*-carboxyindole **196** gave the desired decarboxylated bisindolyl intermediate **194** in good yield. The intermediate was treated with Eschenmoser's salt to give the gramine **197**, which was then treated with iodomethane followed by potassium cyanide with 18-

crown-6 yielding the nitrile **198**. Cyclisation was then achieved using LDA and the unstable resulting phenol was immediately methylated using diazomethane to form **199**.




**Scheme 2.3.23**

Another strategy involved the use of a metathesis reaction as the key step and was reported by Pelly *et al*. in 2005 (Scheme 2.3.24).[44] 2,2′-Bisindole **184** was synthesised as before, using the route reported by Bergman *et al*. (see Scheme 2.3.22), before being subjected to standard Vilsmeier-Haack reaction conditions to give the dialdehyde **200**. Protection as the bis-carbamate **201** was followed by use of the Wittig reaction to form dialkene **202**, which was then treated with Grubbs second generation catalyst **203** to form the indolo[2,3-*a*]carbazole **204**.

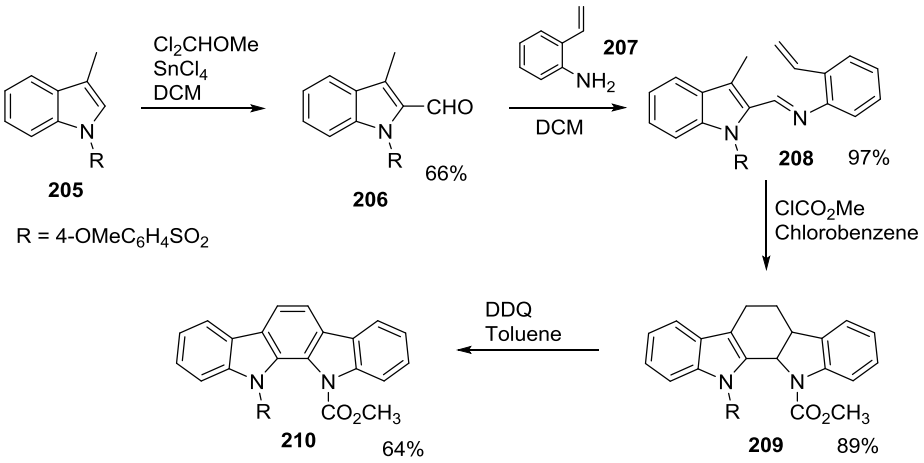



**Scheme 2.3.24**

## 2.4 Multiple ring

A few routes employ strategies which involve the formation of the B and/or D rings and the C ring simultaneously. This has been achieved using [4 + 2] cycloaddition and palladium(0)-catalysed polyannulation as the key step.

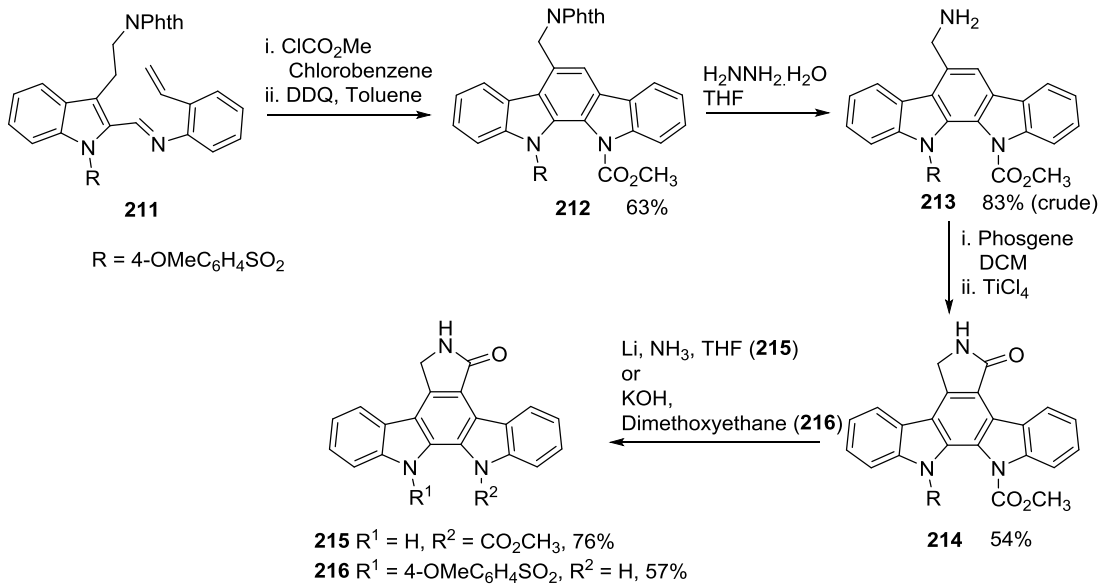
In 1983, Magnus *et al*. reported a route involving an indole-2,3-quinodimethane intermediate to synthesise an indolo[2,3-*a*]carbazole (Scheme 2.4.1).[45] Formylation of *N*-4-methoxybenzenesulfonyl-3-methylindole **205** was achieved through treatment with stannic chloride in DCM and 1,1-dichlorodimethyl ether. Condensation of the resulting aldehyde **206** with 2-amino styrene **207** gave the imine **208** which formed the pentacyclic carbamate **209** when treated with methylchloroformate and DDQ-mediated dehydrogenation gave the parent indolocarbazole **210**.




**Scheme 2.4.1**

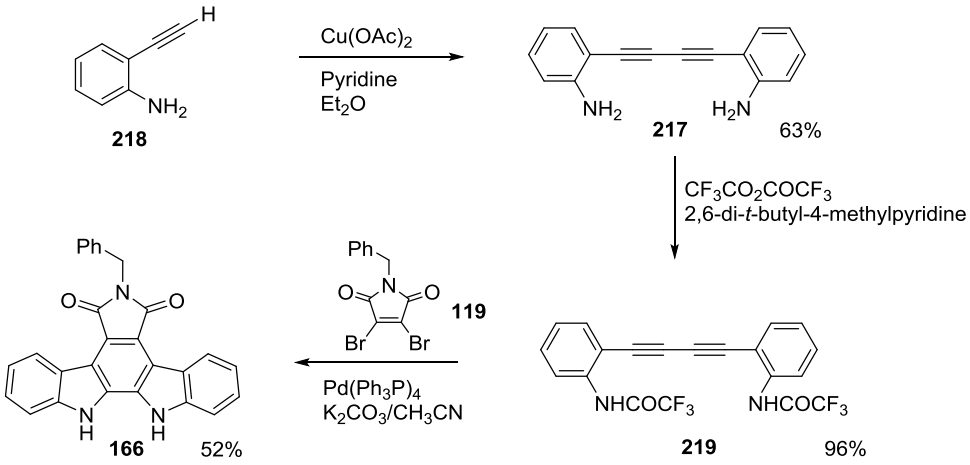
Several months later, the group reported an adaptation of the route to synthesise a tryptamine derivative which could then be used to access indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazoles (Scheme 2.4.2).[46] The phthalimide protected tryptamine intermediate **211** was synthesised and converted to the indolo[2,3-*a*]carbazole **212** using the same route as originally published. The phthalimide protecting group was then removed using hydrazine hydrate and treatment of the resulting amine **213** with phosgene followed by titanium tetrachloride formed the pyrrole ring **214**. It was found that each protecting group could be selectively removed, with lithium and ammonia in THF removing just the sulfonyl group to form **215** and potassium hydroxide in dimethoxyethane selectively

removing the carbamate forming **216**, which would allow for the regioselective attachment of carbohydrate moieties if desired.




**Scheme 2.4.2**

In 1995, Saulnier *et al*. reported the synthesis of a rebeccamycin analogue by a novel palladium(0)-catalysed polyannulation reaction, which formed 4 bonds and 3 rings in a single step from a 1,3-diacetylene intermediate (Scheme 2.4.3).[47] Diacetylene **217** was formed from 2-ethynylaniline **218** using copper acetate in a mixture of pyridine and diethyl ether and was then treated with trifluoroacetic anhydride to form the precursor for the polyannulation step **219**. The key reaction was carried out by combining **219** with *N*-benzyl-3,4-dibromomaleimide **119** in the presence of anhydrous potassium carbonate and tetrakis(triphenylphosphine)-palladium (0) (7 mol%) in dry acetonitrile under argon, simultaneously forming the indole B and D rings and the central C ring of indolocarbazole **166**.




Scheme 2.4.3

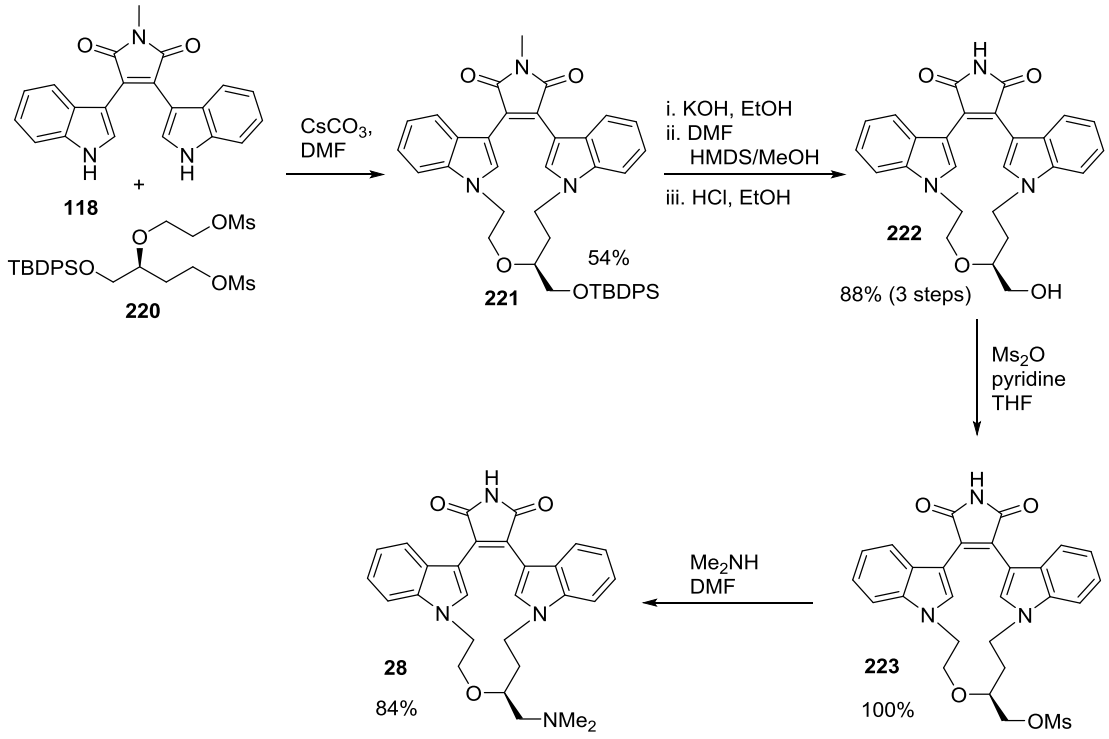
## 2.5 Synthesis of substituted bisindolylmaleimides

Much of the earlier work on the synthesis of substituted staurosporine analogues focussed on the use of carbohydrate *N*-substituents similar to that of the original natural product. However, several of the analogues evaluated in clinical trials contain more simplified alkyl or macrocyclic *N*-substituents. Addition of the substituents can be easily achieved using sodium hydride and the appropriate alkyl halide; however, the difficulties arise when differentiation between the two indole nitrogens is required for the synthesis of unsymmetrically substituted indolocarbazole/bisindolylmaleimide targets. The formation of a macrocycle by linking the indole nitrogens with a carbon chain also presents its own complications. The various strategies towards overcoming these challenges are discussed in the following section.

### 2.5.1 Synthesis of macrocycles

The first work towards synthesis of macrocyclic bisindolylmaleimide derivatives was reported by Jirousek *et al*. who in 1995 reported the synthesis of a panel of *N*,*N*′ bridged derivatives.[48] One of these compounds, later named ruboxistaurin **28**, was subsequently found to be a highly potent and selective protein kinase inhibitor and was developed for clinical trials for use in the treatment of diabetic peripheral retinopathy.

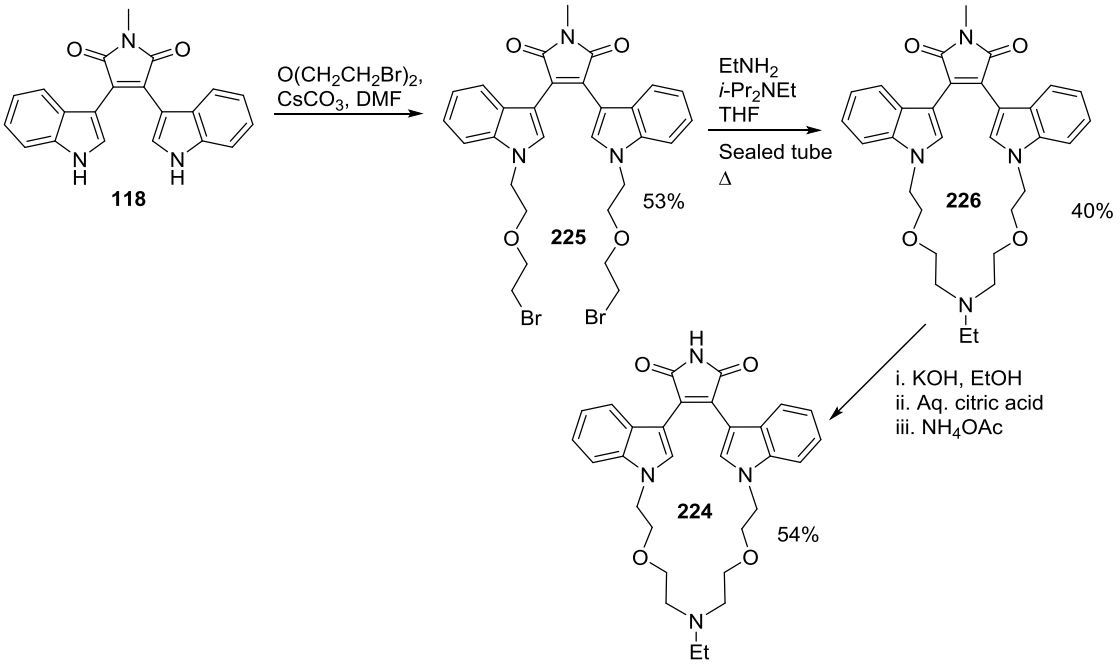
The initial route involved the stepwise alkylation of 2,3-(bis)indolyl-*N*-methyl-maleimide **118** by a previously formed *N*-*N*′ bridging component before being further developed to combine both alkylation steps.[49] This was achieved by slowly adding a mixture of the bisindolylmaleimide **118** and bismesylate **220** to a slurry of caesium carbonate in DMF to form the macrocycle **221** (Scheme 2.5.1). The maleimide methyl group was then removed through treatment with potassium hydroxide followed by HMDS and methanol in DMF and the alcohol was deprotected under acidic conditions forming **222**. The alcohol was activated *via* the mesylate **223** which was then displaced using dimethylamine to form ruboxistaurin **28**.




**Scheme 2.5.1**

In 2003, Zhang *et al*. reported a route to a series of novel macrocyclic bisindolylmaleimides containing linkers with multiple heteroatoms (Scheme 2.5.2). Lead compound **224** was found to be highly active against PKC-β and GSK-3β and showed high selectivity in a screening panel of ten other kinases. These workers took a different approach to Jirousek *et al*. and initially attached two separate but identical *N*-substituents before linking those to form the macrocycle. Starting from *N*-methyl maleimide **118**, bis-*N*-alkylation of the indole nitrogens was achieved using an excess of 2-bromoethyl ether in the presence of caesium carbonate. The dibromide **225** was then

heated with 13 equivalents of ethylamine in the presence of Hünig's base and THF in a pressure tube to form a mixture of the desired cyclised amine **226** and acyclic mono- and di-amines. To ensure the monoamine was converted to the cyclised product **226** and not the unwanted diamine, all volatiles were evaporated before the residue was heated in refluxing dioxane for 6 hours. Removal of the *N*-methyl group from the maleimide was then achieved by initial treatment with potassium hydroxide followed by citric acid and then reacting with neat ammonium acetate.



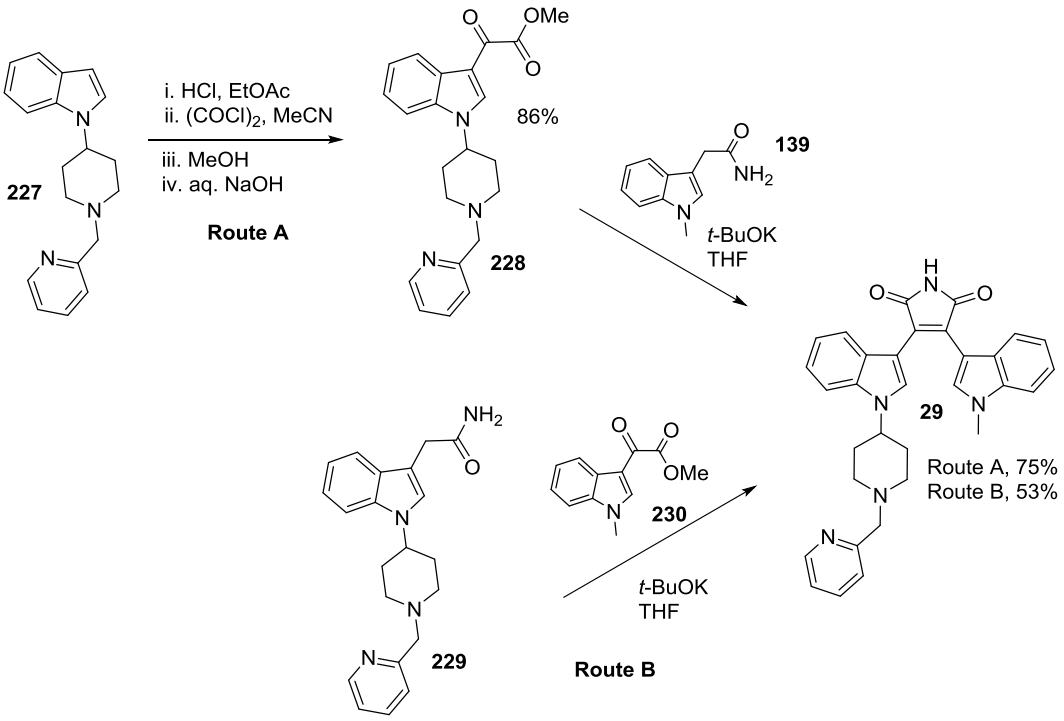

**Scheme 2.5.2**

## 2.5.2 Synthesis of mono-alkylated bisindolylmaleimides

There are two main strategies for the synthesis of mono-alkylated or unsymmetrically alkylated bisindolylmaleimides: one involves the addition of the substituent to one of the indole precursors prior to coupling to forming the bisindolyl structure and the other involves synthesising a suitable bisindolyl intermediate and achieving selective mono-alkylation at this point.

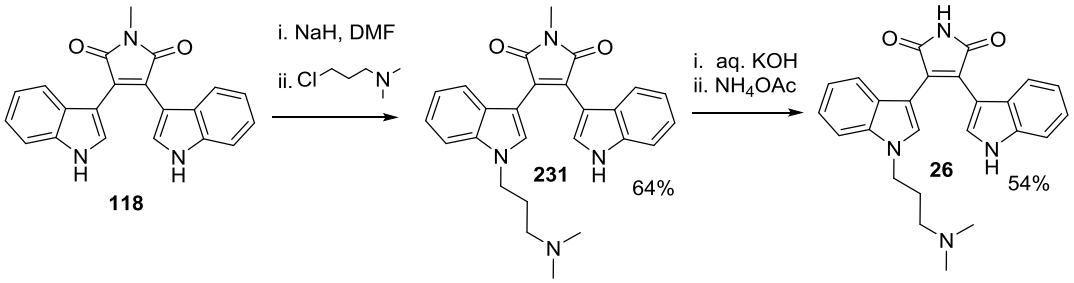
In 2003, Faul *et al*. reported the synthesis of a series of *N*-(azacycloalkyl) bisindolylmaleimides, one of which was selected for clinical evaluation for glioblastoma and colorectal cancer and later named enzastaurin **29**.[50] The

bisindolylmaleimide portion was synthesised using methodology previously reported by the group involving the reaction of an indole-3-glyoxylate ester with an indole-3-acetamide. Two comparable routes were then developed to introduce the *N*-(azacycloalkyl) group (Scheme 2.5.3). The first involved preparation and conversion of the HCl salt of *N*-(azacycloalkyl) indole **227** to the corresponding glyoxylate ester **228** and reacting this with 2-(1-methyl-1*H*-indol-3-yl)acetamide **139** in the presence of potassium *tert*-butoxide to form the bisindolylmaleimide **29**. The second involved the initial synthesis of the substituted indole-3-acetamide **229** which could then be reacted with methyl 2-(1-methyl-1*H*-indol-3-yl)-2-oxoacetate **230** under the same base-mediated conditions to form **29**.




**Scheme 2.5.3**

Toullec *et al*. used a different approach to the synthesis of a mono-alkylated bisindolylmaleimide in their synthesis of GF 109203 X **26** (Scheme 2.5.4).[51] Instead of introducing the indole substituent from the beginning of the synthesis, their route began with the synthesis of an unsubstituted bisindolylmaleimide **118** using Steglich's procedure which was then mono-alkylated using sodium hydride and the appropriate acid chloride to form **231**. The free maleimide **26** was then formed through treatment with potassium hydroxide followed by ammonium acetate.




**Scheme 2.5.4**

The yields for the late-stage selective mono-alkylation of a bisindolyl intermediate are generally observed to be lower than the yields for the routes where the substituent is incorporated onto one of the indole precursors. Also, *N*-protection of the maleimide is required for the routes involving alkylation at a later stage and usually involves the use of a methyl or benzyl group which can be difficult to remove, further reducing the yield; and so, introducing the substituent at the beginning is a better choice when a single target is desired. However, the mono-alkylation of a common intermediate is still beneficial when the target is a panel of similar compounds.

## 2.6 Conclusion

Due to the diverse and extensive biological profiles of compounds of the indolocarbazole family, much progress has been achieved on the development of synthetic strategies of indolocarbazoles since the first reported synthesis in 1956. While early syntheses were generally based on methods used for general indole synthesis, with the growing interest in this field a much wider range of diverse routes have been reported.

The indole forming B/D routes were successful in forming certain types of indolocarbazoles; however, they were not without limitations. Although routes based on Fischer indole synthesis can successfully achieve symmetrical and unsubstituted indolocarbazoles in high yields and are formed from easily accessible starting materials, derivatisation is difficult and they are limited to employing only 2 or 4-substituted phenylhydrazine substrates as precursors.[52] Cadogen insertions can suffer from low yields and unwanted *N*-ethylated side-products; although some groups reported high yields and postulated that substitution patterns influenced the success. High yields could also be obtained from 2,2′ bisindole precursors but these routes often required harsh conditions and it was more difficult to obtain an unsymmetrical indolocarbazole. The cyclisation of bisindolylmaleimides is by far the most popular route due to the short routes to the bisindolylmaleimide intermediates from readily available starting material, but the cyclisation itself can be difficult and the success is strongly influenced by changes to the F-ring and the presence of *N*-substituents. Many routes also suffer from poor solubility which can be a particular problem in routes with intermediates containing unmasked polar heterocyclic functionality.

Synthesis of unsymmetrically *N*-substituted analogues has been achieved both by initial alkylation of the indole precursors and by selective alkylation of bisindolylmaleimide intermediate and the choice of route depends on the desired target. The synthesis of macrocyclic bisindolylmaleimides with interesting and potent biological activity has also been described.

It can be noted that the vast majority of reported compounds contain either a lactam or maleimide F-ring and there have been few reports of F-ring derivatisation in the literature to date. Thus, the development of novel synthetic strategies to these novel F-ring analogues is of paramount interest to explore their potential effects on biological activity *via* diversification of existing H-bonding networks or increased solubility.

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# 3 Aims and Objectives

## 3.1 Background

As outlined in the introductory chapters, the discovery of the excellent anticancer properties of the natural product staurosporine **1**, has provoked great interest from medicinal chemists, biologists and pharmaceutical companies alike.[1,2] Staurosporine was also found to be a nanomolar inhibitor of protein kinase C and so much of this interest has focused on the development of selective and potent protein kinase inhibitors with potential therapeutic applications.[3] Staurosporine and other indolocarbazoles have been shown to inhibit kinases by competing with ATP, but due to the similarity between ATP binding sites in different kinases this approach can be limited in terms of selectivity.[4] Critically there are differences to exploit, however, and therefore it is imperative to discover new chemical entities that are both potent and selective.

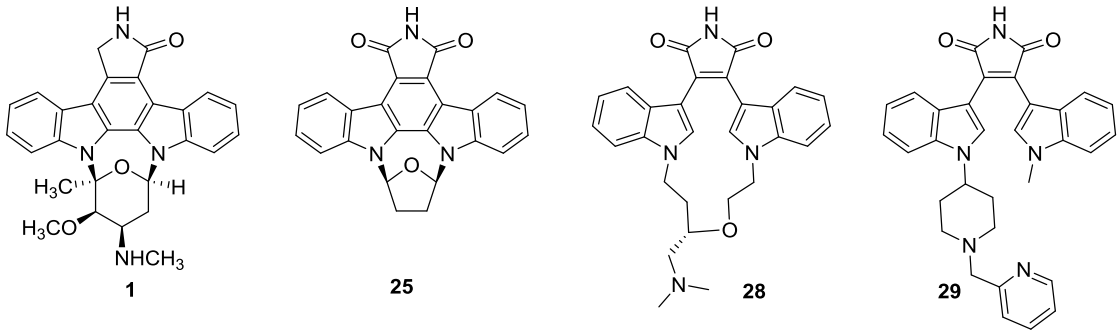
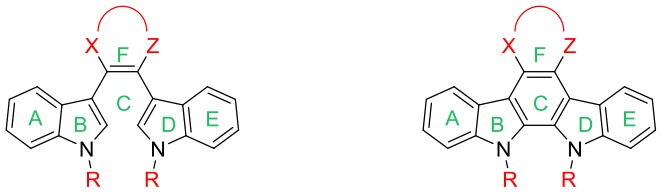



**Figure 3.1.1: Structures of staurosporine 1, SB-218078 25, ruboxistaurin 28 and enzastaurin 29.**

Some success has been achieved in the synthesis of analogues with a higher selectivity towards individual kinases. One such compound, SB-218078 **25**, showed a 15-fold selectivity for CHK1 ($IC_{50}$ = 15 nM) compared to CDK1 ($IC_{50}$ = 250 nM), and a 65-fold selectivity compared to PKC ($IC_{50}$ = 1000 nM).[5] A simple modification has led to the bisindolylmaleimides many of which have been shown to be highly selective inhibitors of individual protein kinases. Examples are ruboxistaurin **28** and enzastaurin **29**, which are highly active and selective inhibitors of protein kinase C-β.[6,7] However, although a small number of this class of compounds have undergone clinical trials, to date none have progressed to be used in anticancer therapy.

## 3.2 Overall Aims

The overall objective of this project is to synthesise novel anticancer agents by modification of the bisindolemaleimide structure **232** and indolocarbazole structure **233** with the aim of improving the key enzyme binding characteristics to increase selectivity and potency.




**232** **233**

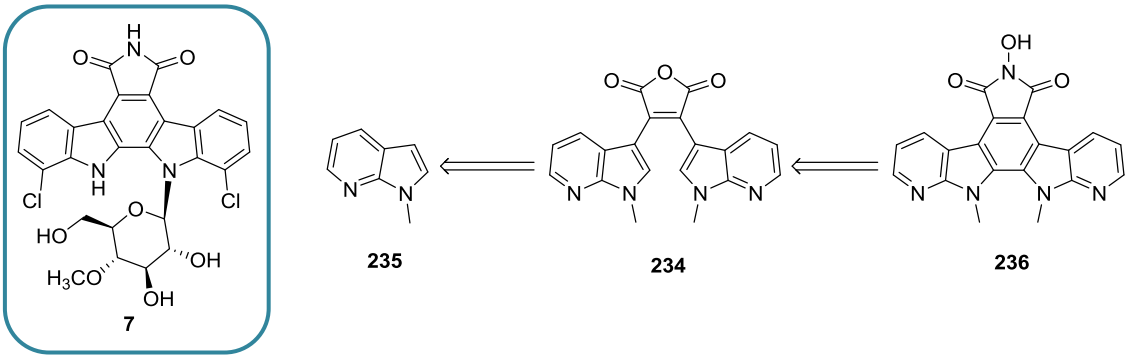
**Figure 3.2.1**

This will be achieved by firstly utilising novel heterocycles on the connecting F-ring. A common motif in these biologically active indolocarbazoles is the lactam/maleimide and it was proposed that changes to this ring will result in promising diversification of existing H-bonding networks necessary for biological activity. Further diversification will be achieved through substitution on the indole nitrogens, adding further possibilities for protein-ligand interactions and also giving additional properties such as increased solubility. Work can then be carried out on the final ring closing stage to achieve the indolocarbazole analogues of these compounds. Once synthesised, the biological activity of all target compounds will then be assessed.

## 3.3 Development of Synthetic Targets

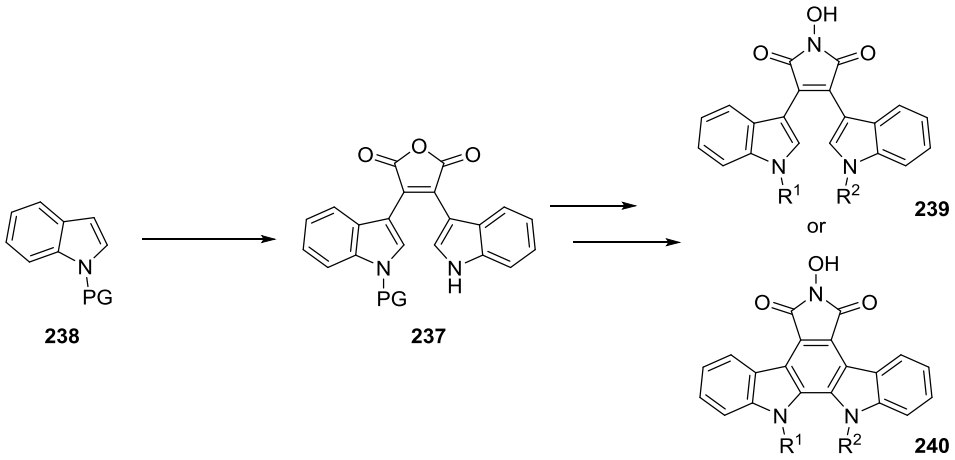
Previous work within the group developed a synthetic strategy to *N*-methyl azaindolylmaleimides and their hydroxymaleimide analogues synthesised *via* a maleic anhydride intermediate **234** and starting from *N*-methyl azaindole precursors **235**. It was anticipated that the incorporation of a hydroxy group would modify the F-ring protein-interacting pharmacophore by altering the position of the H-bond donor, which could lead to novel derivatives with interesting biological profiles. This identified a lead hydroxymaleimide **236** which was highly active in NCI screening and also possessed a

very high correlation with a series of camptothecin derivatives using the COMPARE algorithm, indicating action through topo I inhibition.[8] This topo I activity is unsurprising as indolocarbazoles such as rebeccamycin **7** and its analogues are known inhibitors of topo I.[9]




**Scheme 3.3.1**

The original approach involves the use of *N*-methylated indole starting materials which limits modification at this position as these cannot be removed later in the synthesis. As *N*-substituents can greatly influence both the mode and level of biological activity, one of the main aims in this thesis is to develop a more flexible route which would allow for a variety of different *N*-substituents to be incorporated from a key maleic anhydride intermediate **237**. The design of a route capable of yielding derivatives with unsubstituted indole nitrogens was also essential, as the presence or absence of one free indolic nitrogen has been shown to influence the mode of action of indolocarbazoles, and also as the final ring closing step to form the planar indolocarbazole core tends to be more successful if at least one indole nitrogen is unprotected. This would be achieved starting from indole building blocks that were either unprotected or possessed a protecting group **238** that could be removed later in the synthesis, and used to synthesise a panel of novel hydroxymaleimide derivatives of structures **239** and **240** **239,240239,240**(Scheme 3.3.2).




Scheme 3.3.2

Once a suitable route had been identified, the next goal would be to synthesise a series of novel hydroxymaleimides with varied alkyl *N*-substituents to incorporate lipophilicity in both the bisindole **241** and the indolocarbazole **242** series (Figure 3.3.1). The addition of functionalised chains incorporating hydrophilicity would also be explored yielding derivatives such as **243** and **244**. The functionalised carbon chains are similar to that found on ruboxistaurin **28** and enzastaurin **29**.

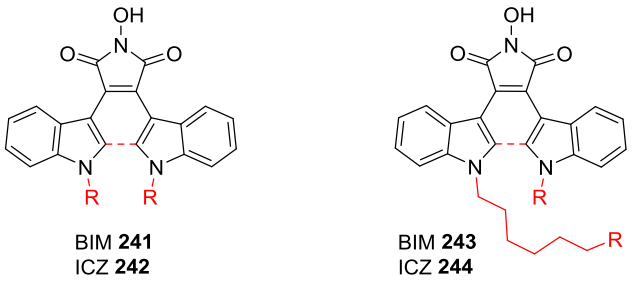



Figure 3.3.1: Structures of target compounds

Once the synthesis of a panel of novel hydroxymaleimides had been completed, the emphasis would then turn to replacement of the hydroxy group to form compounds of general structure **245** and **246**. Very little work has been described in the area of F-ring modification and in particular very few 6-membered rings have been reported.[10] Six-membered uracil rings were of particular interest due to the possibility for more H-bonding interactions leading to greater specificity. Also, the uracil moiety plays a significant role in biological systems as it is a fundamental component of DNA, being

present in the pyrimidine bases thymine and cytosine, and so derivatives containing this structure may be more likely to interact with DNA and act as topoisomerase inhibitors.

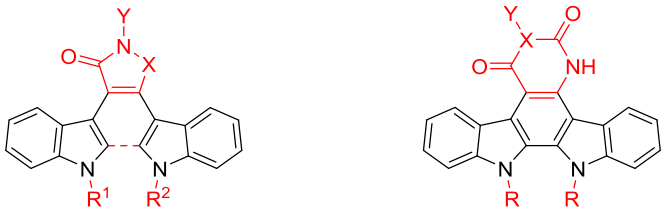



**Figure 3.3.2: Structures of target compounds**

## 3.4 Biological Evaluation

The biological effects of novel final compounds synthesised would then be assessed *via* several different methods. Submission to the NCI 60-cell line screen gives an overall picture of the anticancer activity of the compounds as well as any specificity of a compound towards individual cancer cell lines. Each compound is screened for activity against 60 human cancer cell lines including lines from cancers such as leukaemia, non-small cell lung cancer, melanoma, and cancers of the colon, CNS, ovary, kidney, prostate and breast. This screening program is run by the Developmental Therapeutics Program (DTP) of the NCI.

For further insight into the biological mechanism of these derivatives, final compounds would also be screened against a panel of individual kinases. This assessment is carried out in collaboration with the Kinase Inhibitor Specialised Screening Facility (KISSf) in Station Biologique de Roscoff, France. Compounds will also be suitable for evaluation using a topo I-mediated DNA cleavage assay to assess their ability to act as topo I inhibitors.

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# 4 Chemical Results and Discussion

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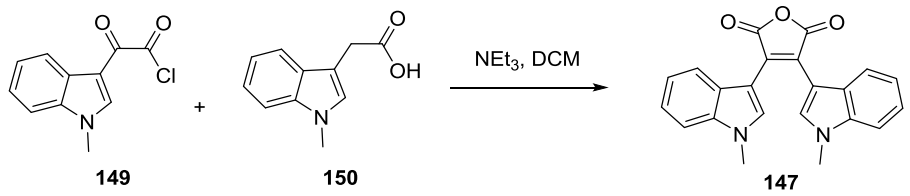
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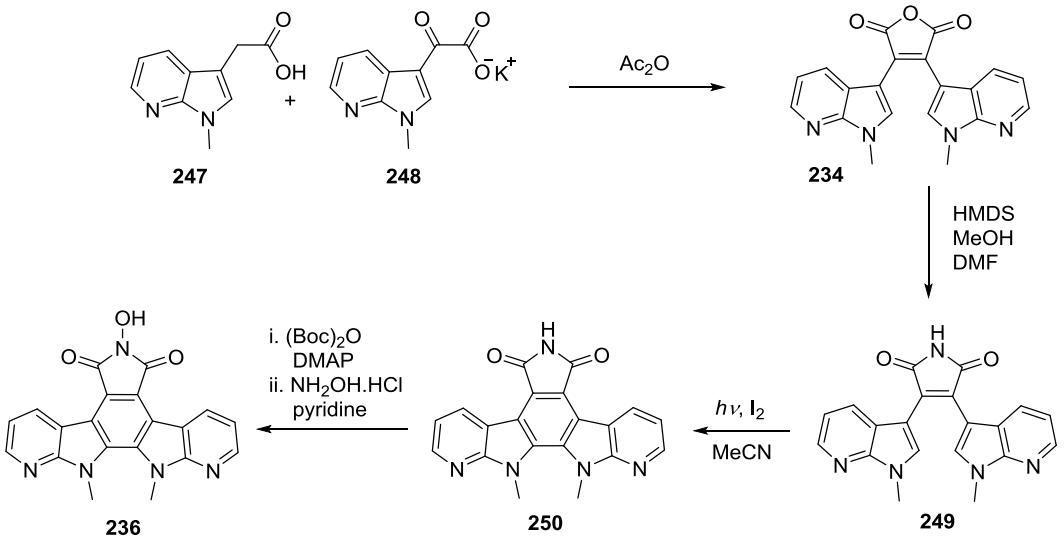
## 4.1 Initial synthetic strategy

Indolylaryl maleic anhydrides were first prepared by a Perkin-type condensation by Davis *et al*.[1] For the synthesis of the bisindolyl compound they reacted 1-methylindol-3-glyoxylyl chloride **149** with 1-methyl-3-indolylacetic acid **150** in the presence of triethylamine to give the maleic anhydride **147** in 36% isolated yield, (Scheme 4.1.1). The reaction was found to fail with unprotected indoles.




Scheme 4.1.1

Previous work within the group used a variation of this reaction involving the condensation of the acetic acid **247** with the potassium glyoxylate salt **248** in acetic anhydride to synthesise a bis(7-azaindol-3-yl) maleic anhydride **234**.[2] Photocyclisation of the maleic anhydride was unsuccessful, however on conversion to the maleimide **249**, cyclisation to the indolocarbazole **250** proceeded smoothly. The hydroxymaleimide **236** was obtained *via* initial Boc-protection followed by treatment with hydroxylamine hydrochloride in pyridine, (Scheme 4.1.2).



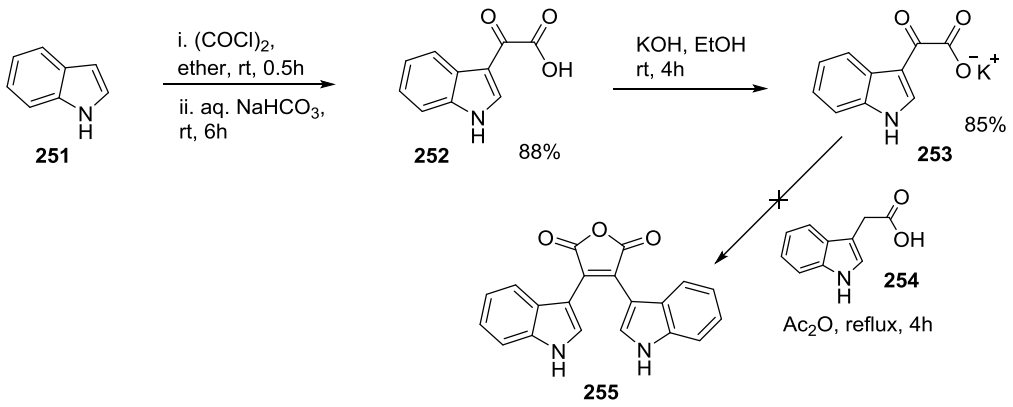

Scheme 4.1.2

These routes involve the use of indoles with methyl substituents on the indole nitrogen which cannot be replaced later in the synthesis. As a result, the goal was to adapt the synthesis to include cleavable protecting groups to form a versatile intermediate which could then be used to synthesise a panel of compounds with varying substituents on one or both nitrogens.

## 4.2 Synthesis and derivatisation of 1-hydroxy-3,4-di(1*H*-indol-3-yl)-1*H*-pyrrole-2,5-diones

### 4.2.1 Synthesis of maleic anhydride intermediates

A solution of indole **251** in diethyl ether was treated with oxalyl chloride to form the glyoxalyl chloride intermediate as a yellow suspension. After stirring for 30 minutes at room temperature, a solution of sodium bicarbonate was added cautiously to quench any remaining oxalyl chloride and to hydrolyse the acid chloride. After 16 hours of stirring the yellow solid was collected by vacuum filtration and then dried overnight at 40 °C to give 2-(1*H*-indol-3-yl)-2-oxoacetic acid **252** in 88% yield. Treatment with 1 equivalent of potassium hydroxide in ethanol gave the potassium salt **253** as a white solid which was collected by vacuum filtration and dried overnight at 40 °C in 85% yield. Initial attempts to couple the potassium 2-(1*H*-indol-3-yl)-2-oxoacetate **253** with unprotected indole-3-acetic acid **254** using acetic anhydride to form the unsubstituted maleic anhydride **255** were unsuccessful (Scheme 4.2.1) as for the similar procedure reported by Davis *et al*. (see Scheme 4.1.1).



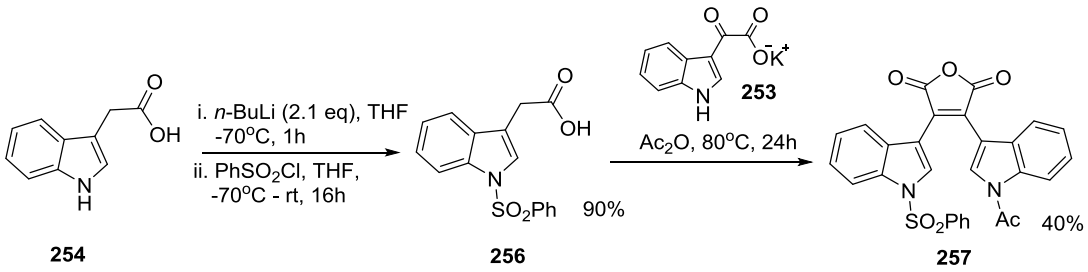

**Scheme 4.2.1**

The next attempt involved the use of a benzenesulfonyl protected indole-3-acetic acid. This was achieved using a method outlined by Roy *et al*. which involved the addition of *n*-BuLi to a solution of indole-3-acetic acid **254** in THF while maintaining the temperature at -70 °C followed by stirring for 1 hour at this temperature.[3] A solution of benzenesulfonyl chloride in THF was then added and the reaction was stirred for 16 hours while slowly warming to room temperature. Following an acidic work-up and recrystallisation from ethyl acetate this method gave 2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]acetic acid **256** as an off-white solid in 70% yield. It was found that allowing the reaction mixture to warm to room temperature more rapidly significantly increased the reaction yield. When the reaction vessel was removed from the low temperature bath immediately after the addition of benzenesulfonyl chloride, the internal temperature rose to room temperature after ~1 hour and the yield increased to 90% (Scheme 4.2.2), slightly higher than the reported yield of 84%.

Protected acid **256** was then reacted with potassium 2-(1*H*-indol-3-yl)-2-oxoacetate **253** in acetic anhydride at reflux for 4 hours. TLC analysis of the dark brown solution showed a bright yellow spot characteristic of bisindolyl maleic anhydrides and significantly less polar than either starting material, but also indicated a mixture of other side products. Following removal of solvent under reduced pressure and washes with saturated sodium bicarbonate, the crude residue was purified using careful column chromatography to give a bright yellow solid. Analysis using proton NMR confirmed the presence of a bisindolyl structure but showed an unexpected 3H singlet at 2.69 ppm and the absence of an NH signal. Further analysis using $^{13}C$ NMR and mass spectrometry confirmed the product to be novel 3-(1-acetyl-1*H*-indol-3-yl)-4-[1-

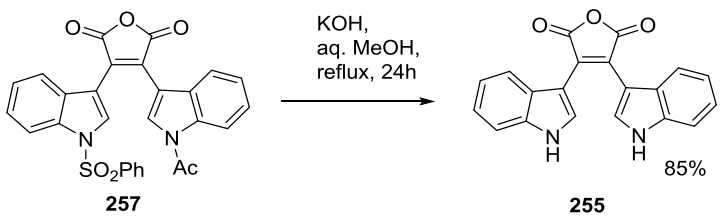
(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **257** which had been isolated in 24% yield.

In an attempt to reduce the number of side products being formed the reaction was repeated at a lower temperature of 80 °C and for a longer reaction time of 24 hours. This resulted in the formation of a yellow suspension which, after cooling, was collected by vacuum filtration. Suspension in boiling ethyl acetate followed by hot filtration and removal of solvent under reduced pressure gave the desired product **257** in 33% yield. Work-up of the original acetic anhydride filtrate followed by recrystallisation from ethyl acetate gave additional product increasing the yield to 40% (Scheme 4.2.2). Although only moderate, this yield was considered quite satisfactory due to the bulk of the substituents at the *N*-position. A limited probe of equivalent ratios was undertaken but no increase in percentage yield was observed.



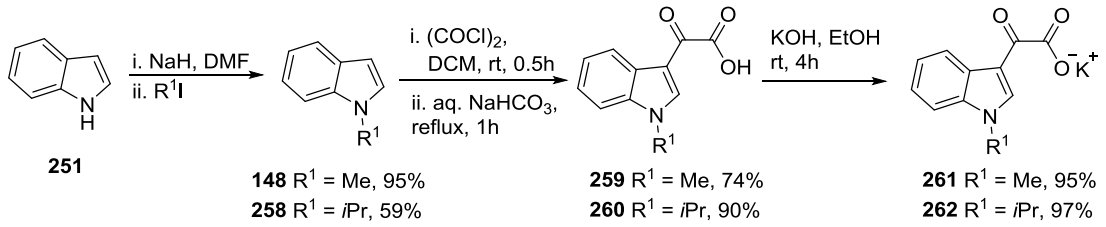

**Scheme 4.2.2**

Removal of both protecting groups was achieved by heating to reflux with potassium hydroxide in 4:1 methanol/water for 24 hours (Scheme 4.2.3). As the starting material and product spots overlapped on TLC analysis, the reaction had to be monitored by proton NMR. Upon completion, the solvent was removed and an acidic work-up gave 3,4-di(1*H*-indol-3-yl)furan-2,5-dione **255** as a red foamy solid which was recrystallised from chloroform to give a red powder in 85% yield.




**Scheme 4.2.3**

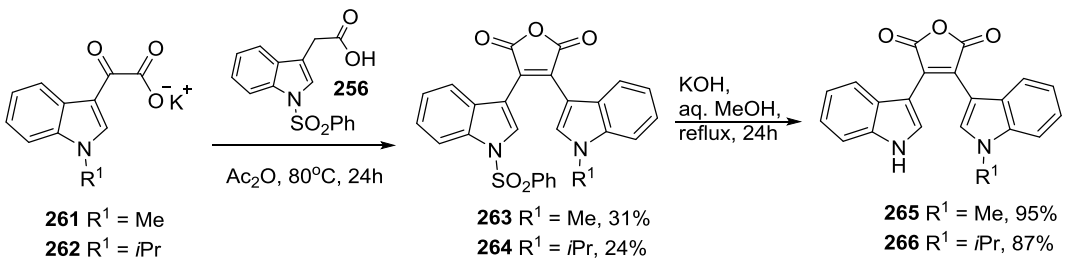
Work then began on the synthesis of substituted maleic anhydrides. It was predicted that coupling an alkylated potassium 2-(1*H*-indol-3-yl)-2-oxoacetate and 2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]acetic acid **256** would increase the yield of the Perkin-type step compared to using unprotected potassium 2-(1*H*-indol-3-yl)-2-oxoacetate. This would incorporate one *N*-substituent from the start while still having a removable protecting group on the second indole nitrogen which could be replaced later on. Indole **251** was methylated by initial deprotonation using sodium hydride in DMF followed by the addition of iodomethane. After work-up, 1-methyl-1*H*-indole **148** was isolated as a pale yellow oil in 95% yield. 1-Isopropyl-1*H*-indole **258** was synthesised in a similar manner although with a longer reaction time due to the less reactive nature of 2-iodopropane compared to iodomethane and following purification using hexane/ethyl acetate column chromatography gave the product as a pale yellow oil in 59% yield. The two substituted indoles were then converted to the oxoacetic acids **259** and **260** and subsequently to their corresponding glyoxalate salts to give potassium 2-(1-methyl-1*H*-indol-3-yl)-2-oxoacetate **261** and potassium 2-(1-isopropyl-1*H*-indol-3-yl)-2-oxoacetate **262** in 70% and 87% yields respectively (over two steps), (Scheme 4.2.4).




**Scheme 4.2.4**

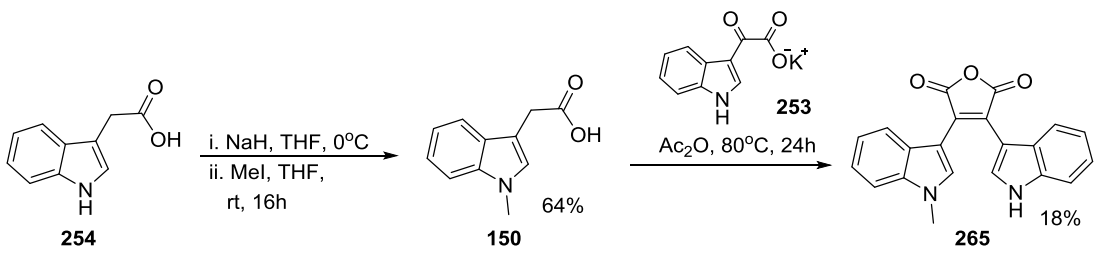
Potassium 2-(1-methyl-1*H*-indol-3-yl)-2-oxoacetate **261** was then reacted with 2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]acetic acid **256** in acetic acid at 80 °C for 24 hours, resulting in an orange solution. The solvent was removed and following work-up an orange gummy residue was obtained. In this case, recrystallisation of the maleic anhydride product **263** was unsuccessful and so purification was achieved using hexane/ethyl acetate column chromatography to give pure product **263**, isolated in 31% yield. The reaction was also carried out using potassium 2-(1-isopropyl-1*H*-indol-3-yl)-2-oxoacetate **262** with similar results and a yield of **264** of 24%. It is likely that the lower yields in these two cases is caused by the fact that a considerable amount is lost on purification due to very close spots on TLC analysis, whereas the initial maleic

anhydride **257** (Scheme 4.2.2) could be purified by recrystallisation, avoiding this issue. In both cases, deprotection was achieved in high yields of 95% and 87% to give 3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **265** and 3-(1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione **266**, respectively, (Scheme 4.2.5).




**Scheme 4.2.5**

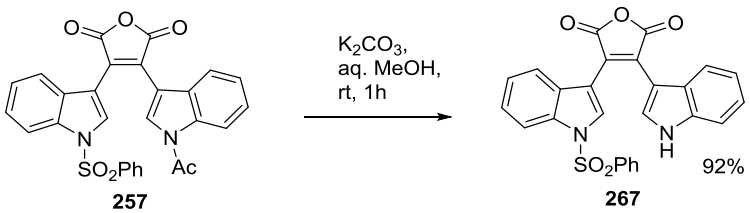
As only protection of the indole-3-acetic acid nitrogen was needed for the reaction to be successful, synthesis of 3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **265** was also attempted using 2-(1-methyl-1*H*-indol-3-yl)acetic acid **150** and unprotected potassium 2-(1*H*-indol-3-yl)-2-oxoacetate **253**, eliminating two steps for the protection and deprotection. 2-(1-Methyl-1*H*-indol-3-yl)acetic acid **150** was formed by treatment of a solution of indole-3-acetic acid **254** in THF with sodium hydride, followed by iodomethane to give the product in 64% yield (Scheme 4.2.6). Again, the product of the Perkin-type reaction **265** had to be purified by column chromatography isolating the maleic anhydride in 18% yield.




**Scheme 4.2.6**

As 3-(1-acetyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **257** was the maleic anhydride formed with the highest yield and took the shortest amount of time to purify, it was decided to use this as the main intermediate. Although the fully deprotected maleic anhydride derivative **255** would be useful for forming compounds

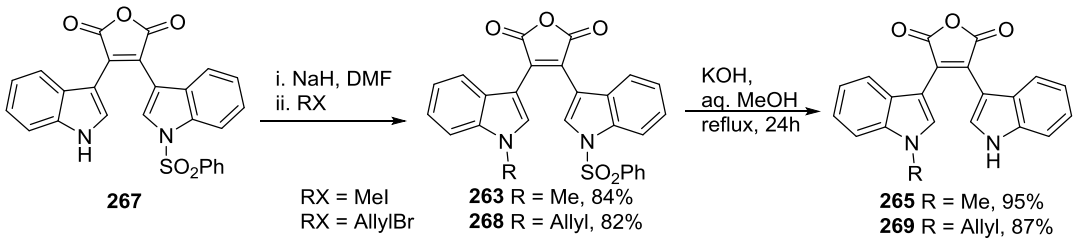
with two identical nitrogen substituents, a maleic anhydride intermediate with just one indolic nitrogen protected and the other free was also desired as a starting point to final compounds with differing nitrogen substituents. 3-(1-Acetyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **257** was treated with the milder base, potassium carbonate, in 4:1 methanol/water for 1 hour at room temperature. Following a similar work-up to the previous deprotection, 3-(1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **267** was formed as an orange solid in an excellent yield of 92% (Scheme 4.2.7).




**Scheme 4.2.7**

In another route to 3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **265**, benzenesulfonyl protected maleic anhydride intermediate **267** was treated with sodium hydride in DMF, allowed to stir for 30 minutes and then treated with iodomethane. Following work-up, 3-(1-methyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **263** was isolated in 84% yield as an orange foamy solid. Deprotection could again be achieved by heating to reflux with potassium hydroxide in 4:1 methanol/water for 24 hours to give 3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **265** as a red solid in 95% yield (Scheme 4.2.8). The major advantage to this route was that as it avoids the need for column chromatography of complex mixtures, it could be successfully scaled up to be carried out on a multigram scale.

A similar route was used to synthesise an analogous allyl derivative. For this, 3-(1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **267** was treated with sodium hydride in DMF, followed by allyl bromide to give 3-(1-allyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **268** as an orange foamy solid in 82% yield which was then treated with potassium hydroxide in aqueous methanol to give 3-(1-allyl-1*H*-indol-3-yl)-4-(1*H*-indol-3-yl)furan-2,5-dione **269** as a red solid in 87% yield (Scheme 4.2.8).




**Scheme 4.2.8**

### *4.2.1.1 Spectroscopic properties*

HMBC experiments were used to identify which set of indole protons corresponded to which *N*-substituted indole. In the case of **257** (Figure 4.2.1), a correlation is seen between the carbonyl group carbon of the acyl group and one of the C(2)-H protons (blue arrows) confirming those to be on the same indole moiety. The C(2)-H proton also shows a correlation to a quaternary carbon (green arrow), which also shows a correlation to a C(5)-H and a C(7)-H proton (both green arrows). The corresponding C(4)-H and C(6)-H protons are then easily identified using a COSY experiment.

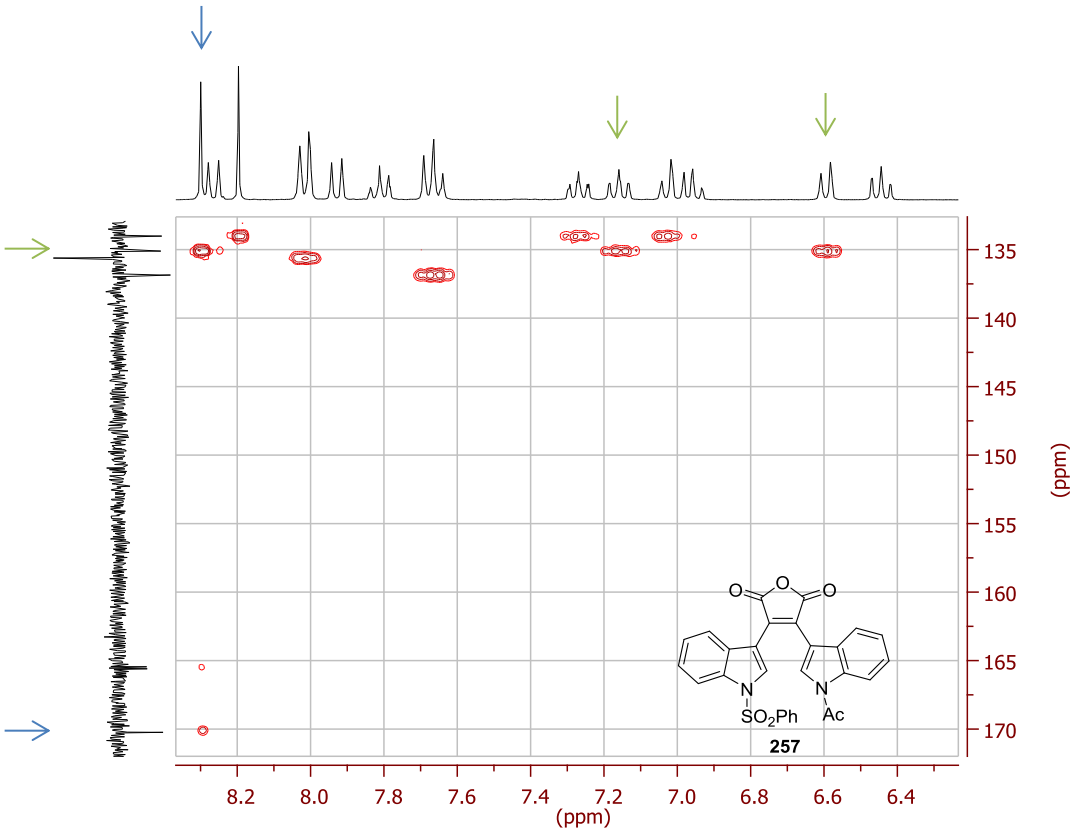



**Figure 4.2.1: HMBC (Heteronuclear Multiple Bond Correlation) 2D spectrum for 257 in DMSO-$d_6$ at 300 MHz**

All other benzenesulfonyl protected maleic anhydride intermediates were assigned in a similar fashion as the alkyl carbon attached to the indolic nitrogen showed a correlation to the nearby C(2)-H proton.

The proton NMR spectra of the bisindolyl compounds containing a benzenesulfonyl protecting group show some interesting characteristics (Figure 4.2.2). The mono-methylated maleic anhydride **265** shows only a slight separation of peaks from symmetrical precursor **255**. A much bigger change is observed on addition of a benzene sulfonyl group (**263**), however. All protons on the benzenesulfonyl protected indole are significantly deshielded, due to the addition of the electron withdrawing group. The C(2)-H proton of the methylated indole is also deshielded. Interestingly, significant shielding is observed of the C(6 and 7)-H protons of the methylated indole. On changing the methyl group to an acyl group (**257**), the most noticeable difference is the deshielding of the C(4)-H proton on that ring, which undergoes a change in chemical shift of over 0.8 ppm (green arrows).

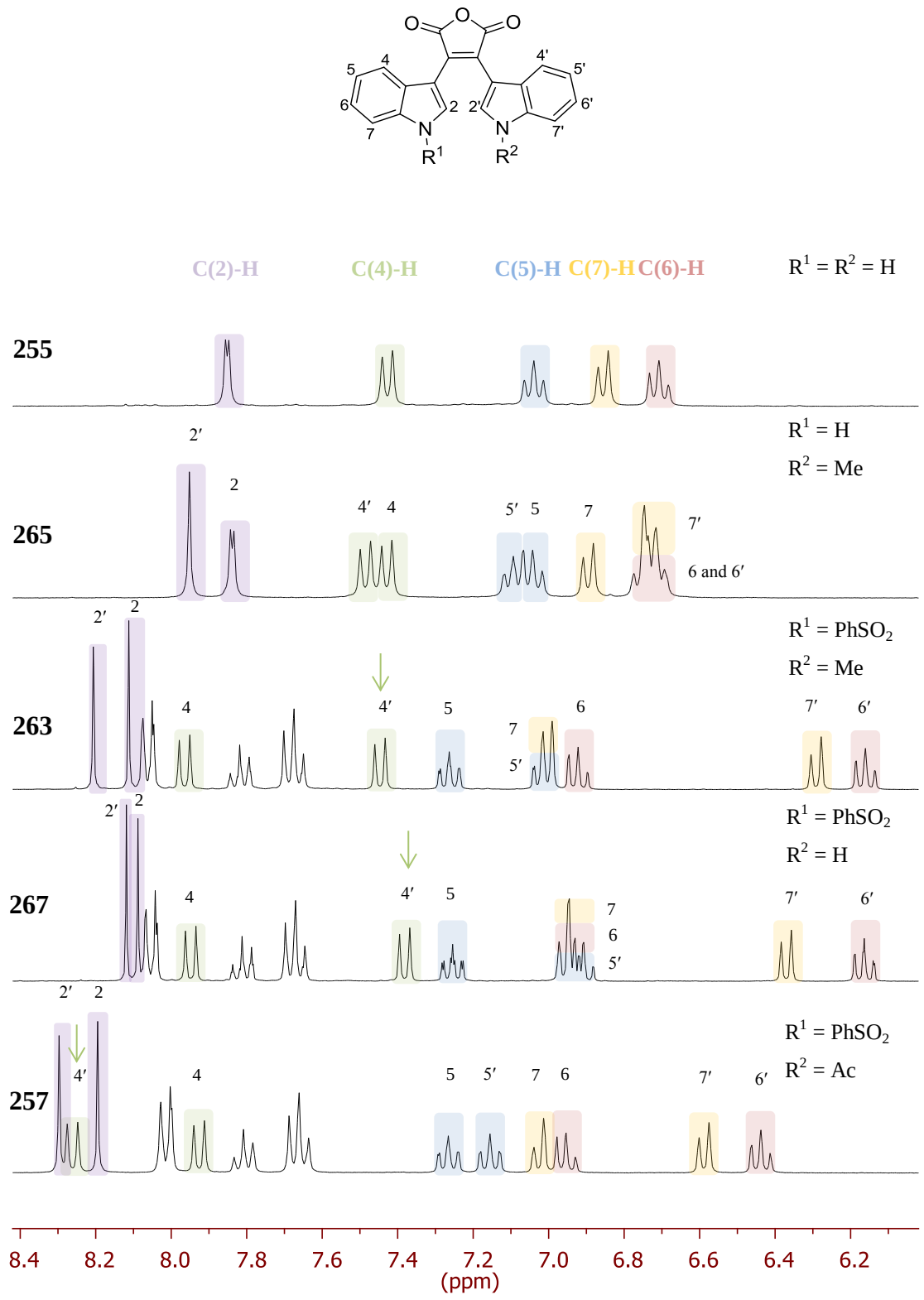
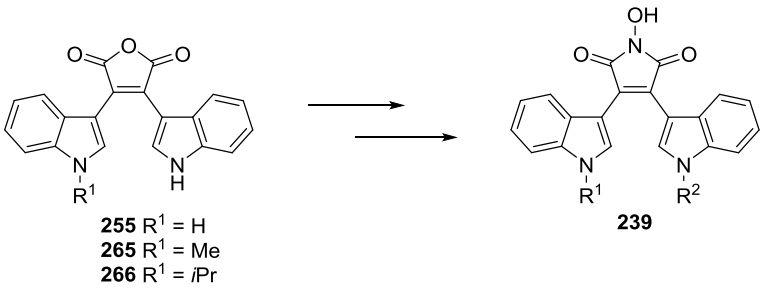



**Figure 4.2.2: Stacked proton NMR plot of maleic anhydride derivatives** 255**,** 265**,** 263**,** 267 **and** 257 **in DMSO-$d_6$ at 300 MHz**

It is also useful to note that the C(2)-H proton of indoles with an unsubstituted indole nitrogen are often split due to coupling with the NH proton (see spectra for **255** and **265** Figure 4.2.2). Although not always observed, this splitting can mean that the C(2)-H protons of an *N*-substituted and unsubstituted indole can be distinguished easily.

### 4.2.2 Derivatisation of maleic anhydride intermediates

With some key intermediates now synthesised, derivatisation of three of these (**255**, **265** and **266**) was undertaken to form a panel of maleic anhydride derivatives which could then be converted to their hydroxymaleimide analogues **239** (Scheme 4.2.9). A selection of *N*-substituents was chosen to incorporate (i) lipophilicity with both small and bulkier groups, (ii) hydrophilicity in the form of functionalised 6-carbon chains and (iii) conformational restriction where the nitrogens are linked by a tether.




**255** $R^1$ = H
**265** $R^1$ = Me
**266** $R^1$ = *i*Pr

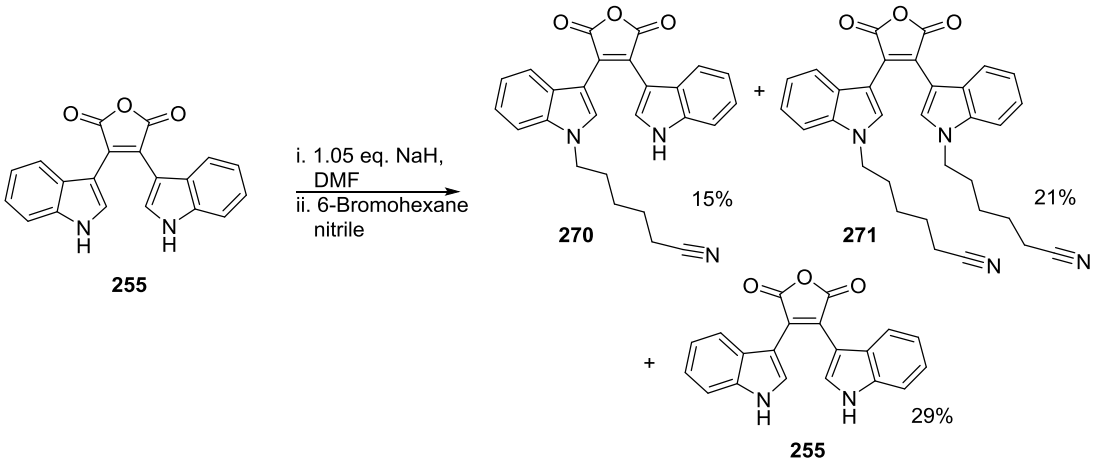
**239**

**Scheme 4.2.9**

#### *4.2.2.1 Derivatisation of unsubstituted intermediate*

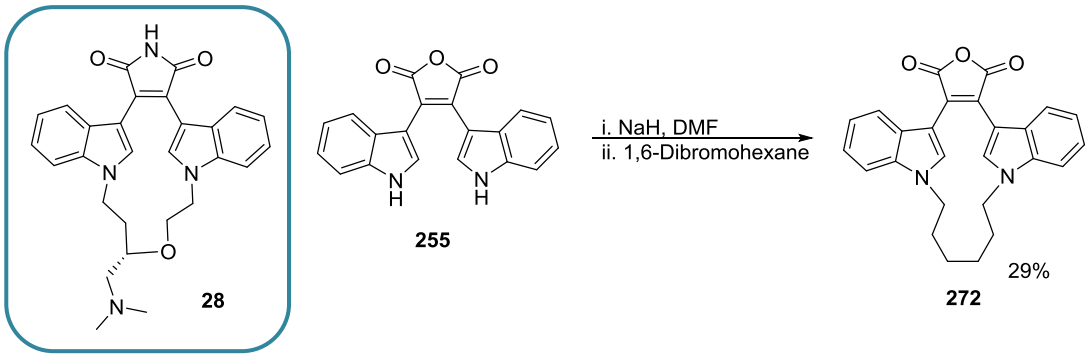
The possibility that mono-alkylation of unsubstituted **255** could be achieved was next explored. 3,4-Di(1*H*-indol-3-yl)furan-2,5-dione **255** was treated with 1.05 equivalents of sodium hydride and allowed to stir for 30 minutes changing from a red to a deep purple solution. 6-Bromohexane nitrile was then added and the reaction was stirred for 16 hours, slowly returning to a red solution. TLC analysis showed three spots close together which, following work-up, were separated using careful column chromatography. Mono-alkylated product **270** was isolated in 15% yield, di-alkylated product **271** was isolated in 21% yield and 29% unreacted starting material was also recovered (Scheme 4.2.10). Although selective mono-alkylation was not achieved, this

strategy did incorporate more diversity which could subsequently be used in the synthesis of further novel compounds.




**Scheme 4.2.10**

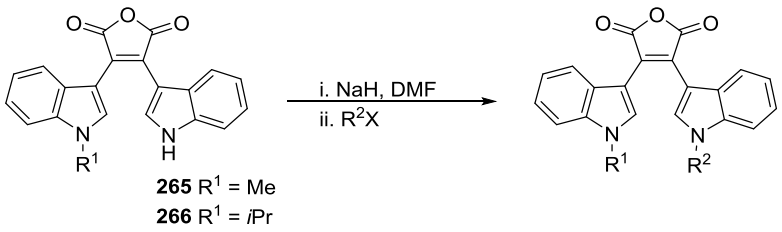
Conformationally restricted macrocyclic bisindolylmaleimides have been shown to be highly potent and selective inhibitors of individual kinases and even of individual isoforms. For example, the bisindolylmaleimide ruboxistaurin **28** selectively inhibits PKC*β* and PKC*β*2 over PKC*α*.[4] Initially, a bisindolyl hydroxymaleimide with a 6 carbon chain was synthesised, the same length as seen in ruboxistaurin (Scheme 4.2.11). The maleic anhydride precursor was formed by first treating 3,4-di(1*H*-indol-3-yl)furan-2,5-dione **255** with 2.2 equivalents of sodium hydride and stirring for 40 minutes resulting in a dark blue solution. 1,6-Dibromohexane was then added and the mixture was allowed to warm to room temperature slowly while stirring for 16 hours. The reaction was carried out at 0 °C and with 1.05 equivalents of dibromohexane to limit di-alkylation and the reaction mixture was at a much higher dilution than usual to limit dimer formation. Despite initial solvation problems with column chromatography while using hexane/ethyl acetate as eluent, employing a hexane/DCM solvent system gave a reddish/purple solid found to be the desired macrocyclic maleic anhydride **272** in 29% yield. The remainder of the crude material contained a complex mixture which was not successfully separated.




**Scheme 4.2.11**

### *4.2.2.2 Derivatisation of* N*-methyl and* N*-isopropyl intermediate*

Derivatisation of the methyl **265** and isopropyl intermediates **266** was then undertaken. For each, the indole nitrogen was deprotonated using sodium hydride in DMF and then treated with iodomethane, iodoethane or 6-bromo hexanenitrile to give a panel of unsymmetrically di-alkylated bisindolyl maleic anhydrides (Table 4.2.1). All derivatives in this panel are novel with the exception of bis-methyl **147**.[1]




| Compound | $R^1$ | $R^2$ | X | Yield |
|---|---|---|---|---|
| **147** | Me | Me | I | 80% |
| **273** | Me | Et | I | 72% |
| **274** | Me | $-CH_2(CH_2)_4CN$ | Br | 40% |
| **275** | *i*Pr | Me | I | 46% |
| **276** | *i*Pr | Et | I | 51% |
| **277** | *i*Pr | $-CH_2(CH_2)_4CN$ | Br | 33% |

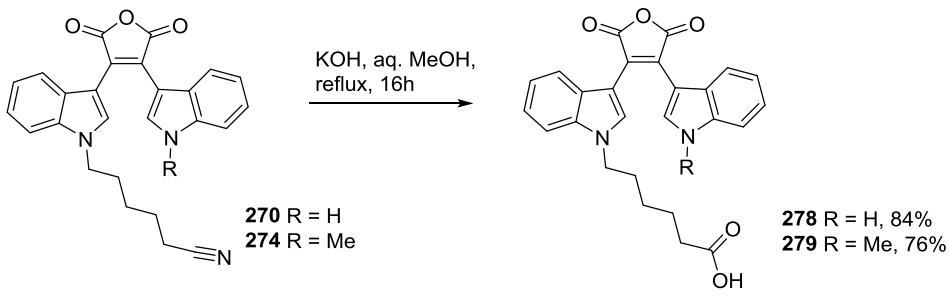
**Table 4.2.1: Isolated yields of disubstituted maleic anhydrides**

The hexane nitrile derivatives were formed in lower yields than the other derivatives, probably due to the fact that the bromo alkane was used which would be less reactive

than the iodo alkanes. The yields for alkylation of the isopropyl intermediate were also found to be lower, possibly due to the presence of a more bulky substituent.

#### *4.2.2.3 Synthesis of acids*

To incorporate a more polar side chain, the two compounds with hexanenitrile side chains **270** and **274** were converted to their corresponding carboxylic acids. This was achieved by treating the nitriles with potassium hydroxide in aqueous methanol and heating to reflux for 16 hours until TLC showed complete consumption of starting material and the presence of a new, highly polar component. After acidic work-up, the two carboxylic acids 6-{3-[4-(1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanoic acid **278** and 6-{3-[4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanoic acid **279** were obtained as red solids in yields of 84% and 76% respectively (Scheme 4.2.12). In each case, IR analysis showed the expected disappearance of the characteristic nitrile signal at ~2245 $cm^{-1}$ and the appearance of a new signal at ~1700 $cm^{-1}$ characteristic of the carboxylic acid C=O stretch.

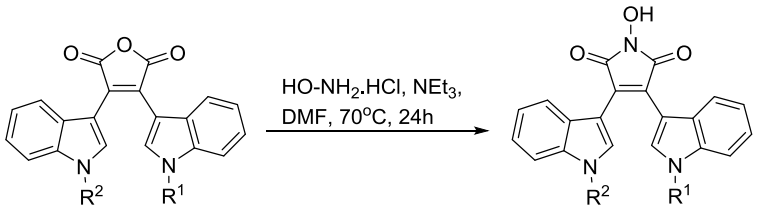



**Scheme 4.2.12**

### 4.2.3 Synthesis of hydroxymaleimide analogues

All maleic anhydrides were then converted to their corresponding hydroxymaleimide derivatives to form a panel of bisindolylmaleimide analogues with potentially interesting and potent biological activity. The transformation was achieved using 5 equivalents of hydroxylamine hydrochloride and 5 equivalents of triethylamine in DMF at 70 °C for 24 hours and the hydroxymaleimides were isolated in high yields (Table 4.2.2). All hydroxymaleimides in this panel are novel with the exception of

unsubstituted **280**.[5] The majority of compounds in this table were selected for assessment using the NCI 60 cell line screen and certain compounds were also selected for assessment of activity against a panel of individual kinases. The results of this analysis will be discussed in Chapter 5.




| Starting Material | Product | $R^1$ | $R^2$ | Yield |
|---|---|---|---|---|
| **255** | **280** | H | H | 87% |
| **270** | **281** | $-CH_2(CH_2)_4CN$ | H | 86% |
| **271** | **282** | $-CH_2(CH_2)_4CN$ | $-CH_2(CH_2)_4CN$ | 87% |
| **278** | **283** | $-CH_2(CH_2)_4CO_2H$ | H | 86% |
| **272** | **284** | $-CH_2(CH_2)_4CH_2-$ | | 82% |
| **265** | **285** | Me | H | 83% |
| **147** | **286** | Me | Me | 84% |
| **273** | **287** | Me | Et | 69% |
| **274** | **288** | Me | $-CH_2(CH_2)_4CN$ | 82% |
| **279** | **289** | Me | $-CH_2(CH_2)_4CO_2H$ | 79% |
| **266** | **290** | *i*Pr | H | 77% |
| **275** | **291** | *i*Pr | Me | 93% |
| **276** | **292** | *i*Pr | Et | 80% |
| **277** | **293** | *i*Pr | $-CH_2(CH_2)_4CN$ | 77% |

**Table 4.2.2: Isolated yields of hydroxymaleimides**

### *4.2.3.1 Spectroscopic properties*

Again, 2D NMR experiments were used to aid the assignments of these unsymmetrical bisindolyl compounds. The assignment of hydroxymaleimide **281** is shown as an example (Figure 4.2.3). A correlation is seen between the $CH_2$ protons adjacent to the indole nitrogen and a quaternary carbon on the same indole moiety (blue arrows). This quaternary carbon also shows a correlation to the C(2)-H proton, identified as the only singlet (at 7.7 ppm), and to two other protons on the same ring (green arrows).

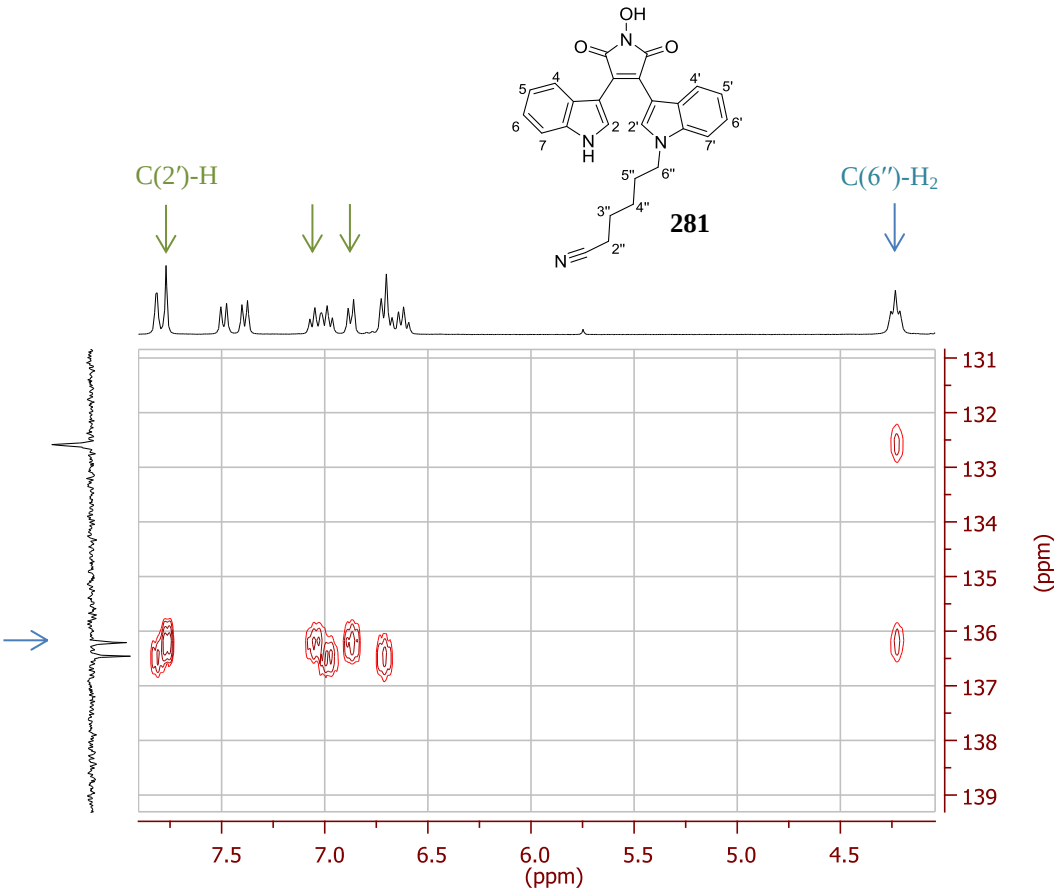



**Figure 4.2.3: HMBC 2D spectrum for 281 in DMSO-$d_6$ at 300 MHz**

A COSY experiment is then used to assign those protons and the remaining protons (Figure 4.2.4). The doublet at 7.49 ppm shows a correlation to the triplet at 7.05 ppm (identified from the HMBC to be on the hexanenitrile substituted indole), confirming these to be C(4′)-H and C(5′)-H. Both the C(5′)-H proton and the C(7′)-H proton at 6.87 ppm (identified from the HMBC to be on the hexanenitrile substituted indole) show a correlation to the 2H mutiplet at 6.67-6.74 ppm, indicating that the multiplet contains the C(6′)-H proton. The remaining signals can be attributed to the other indole ring and can be assigned in a similar fashion.

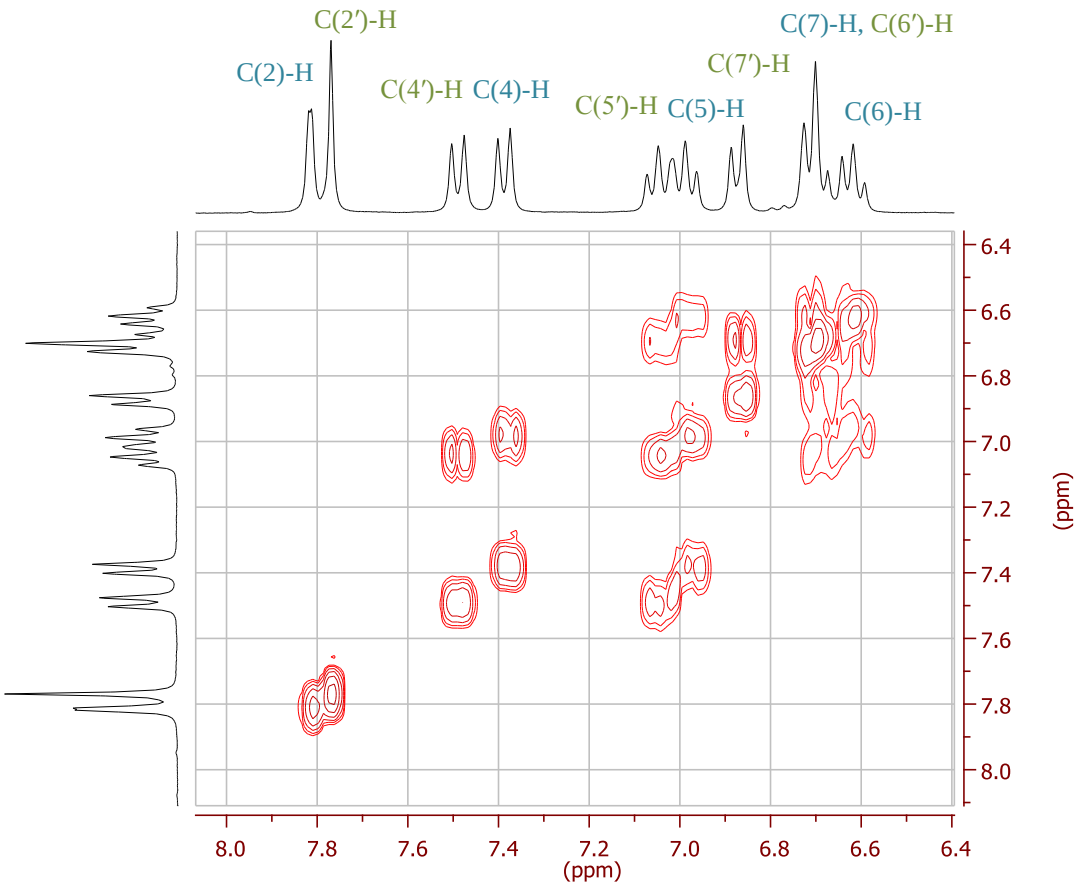



**Figure 4.2.4: COSY (Correlation Spectroscopy) 2D spectrum for 281 in DMSO-$d_6$ at 300 MHz (protons on hexanenitrile substituted indole are assigned in green and unsubstituted indole in blue)**

The peaks in the aliphatic region were also assigned using a COSY experiment (Figure 4.2.5). The most deshielded triplet at 4.23 ppm is identified as C(6″)-$H_2$ due to the correlations seen to atoms in the indole ring in the HMBC experiment. The C(6″)-$H_2$ protons couple to a quintet at 1.73 ppm corresponding to the C(5″)-$H_2$ protons, which also couple to the multiplet at 1.25-1.36 ppm corresponding to the C(4″)-$H_2$ protons. These in turn couple to the C(3″)-$H_2$ protons at 1.56 ppm, which also couple to the triplet at 2.44 ppm corresponding to the C(2″)-$H_2$ protons.

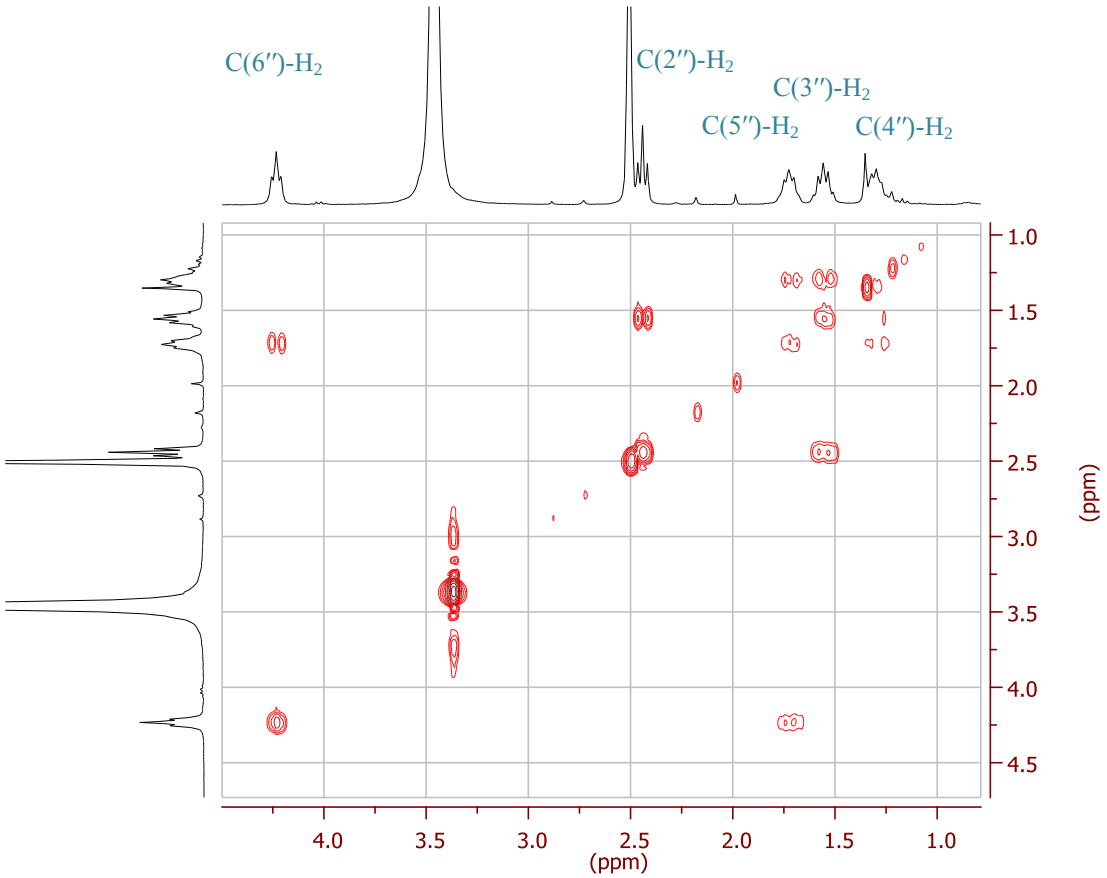



**Figure 4.2.5: COSY 2D spectrum of alkyl region of** 281

The NMR spectra of the hydroxymaleimides are very similar to those of the maleic anhydrides with some slight shielding and the addition of a broad singlet at about 10.5 ppm corresponding to the hydroxyl proton (Figure 4.2.6). The aromatic regions of hydroxymaleimide **281** and the corresponding maleic anhydride **270** are shown below for comparison. Due to the similarity between the two, 2D experiments were routinely run for the hydroxymaleimides to aid their assignments and the maleic anhydrides were then assigned from their corresponding hydroxymaleimide.

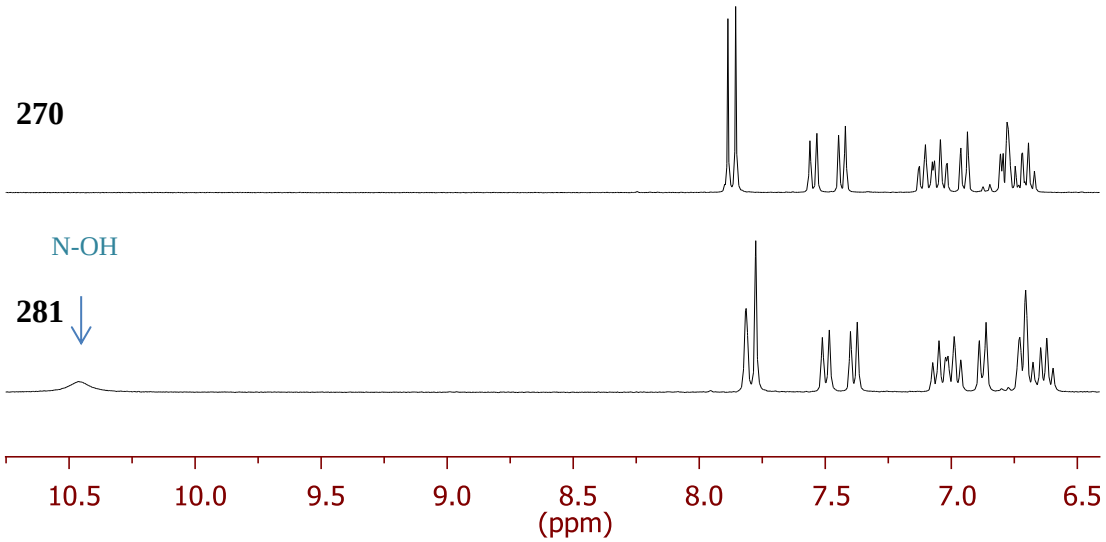



**Figure 4.2.6: Stacked proton NMR spectra of** 270 **and** 281 **in DMSO-$d_6$ at 300 MHz**

## 4.3 Synthesis of indolo[2,3-*a*]carbazoles

It has been well documented in the literature that indolo[2,3-*a*]carbazoles possess unique and more potent activity compared to their bisindole analogues. For example, Sanchez-Martinez *et al*. revealed how a series of indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazoles consistently displayed greater inhibitory activity towards CDK2 and CDK4 compared to their corresponding bisindolyl analogues.[6] The group reported that a series of ten substituted indolocarbazoles of structure **294** were 8-42 fold more active in head-to-head comparison with their corresponding bisindolylmaleimides **295**. Thus, attention then turned to forming these fully aromatic analogues *via* oxidative cyclisation to form the necessary C-C bond.

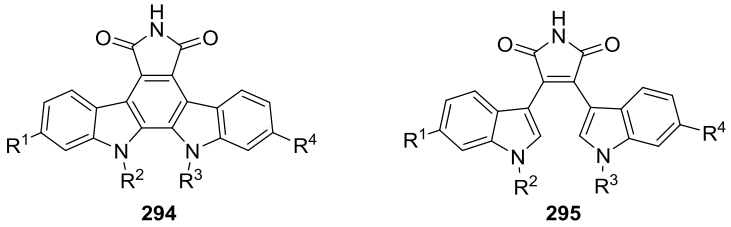



**Figure 4.3.1: General structure of series of indolocarbazoles and bisindolylmaleimides**

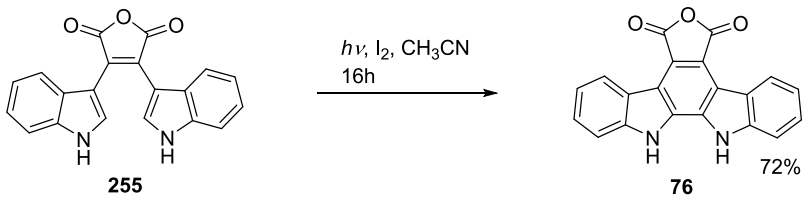
### 4.3.1 Aromatisation of unsubstituted bisindoles

As the success of these reactions can vary greatly depending on the substrate, with both F ring type and *N*-substitution having a considerable effect, it was decided that the optimum strategy would be to identify a substrate that cyclises cleanly with a high yield and use this as the starting material for further derivatisation. The three most commonly used oxidants for this type of transformation documented in the literature are (i) DDQ,[7,8] (ii) Pd(II)[9,10] and (iii) oxidative photocyclisation.[11,12] A photocyclisation step was the most desirable as it would be considerably cheaper than the other two options, especially palladium based reagents which often have to use used in excess. A light-mediated reaction would also potentially be much cleaner, especially compared to DDQ where the reduced by products can be difficult to remove. These two points would be of particular importance if the step was scaled up to a multi-gram scale as planned.

#### *4.3.1.1 Synthesis of furo[3,4-c]indolo[2,3-a]carbazole-5,7(12H,13H)-dione*

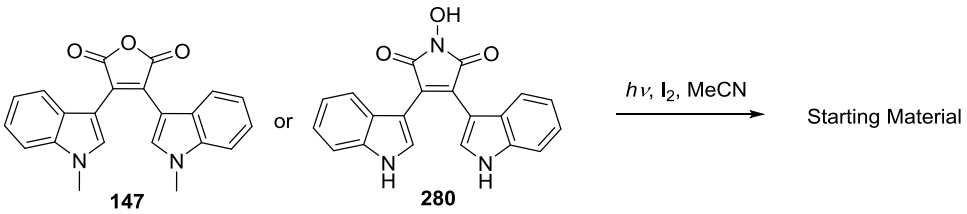
Maleic anhydride derivative **255** was initially selected for cyclisation as the cyclised maleic anhydride indolocarbazole would serve as a highly versatile intermediate with numerous options for *N*-alkylation and further F-ring derivatisation. The bisindolyl compound was dissolved in acetonitrile and a catalytic amount of iodine was added. Light irradiation was achieved using a standard high pressure mercury lamp. Over the course of the reaction the original bright red solution slowly changed to a yellow suspension which, when TLC analysis showed complete consumption of starting material, was collected by vacuum filtration and washed with saturated aqueous sodium thiosulfate to remove traces of iodine. Initially, this reaction was carried out on a 100 mg scale with a concentration of 1 mg/mL forming **76** with a moderate yield of 54%. Fortunately, as the scale of the reaction was increased an increase in yield was also

observed, with 1 g of starting material being converted to aromatised product in 72% yield with the same reaction concentration (Scheme 4.3.1). The difference in yield is most likely caused by a difference in reaction temperature. The small volume of the 100 mL reaction is efficiently heated by the bulb and is observed to reflux for the duration of the reaction while the heat from the bulb is not sufficient to heat the larger 1 L reaction to its boiling point. Due to the yield differential, it can be postulated that fewer impurities are formed at the lower temperature in the larger flask resulting in a higher yield of the desired product.




**Scheme 4.3.1**

The cyclisations of unsubstituted hydroxymaleimide **280** and bis-methyl maleic anhydride **147** were also attempted (Scheme 4.3.2). However, in both cases only starting material was recovered. This was unsurprising as there are no reports in the literature of the cyclisation of a bisindolyl compound with a hydroxymaleimide F-ring and it has been reported that cyclisations of bis-*N*-alkylated analogues are generally challenging.[13,14]




**Scheme 4.3.2**

### *4.3.1.2 Spectral comparison with bisindoles*

The aromatic regions of proton NMR spectra for three related maleic anhydride derivatives are stacked for comparison (Figure 4.3.2): one is the unsubstituted bisindolyl maleic anhydride **255** where the indoles are relatively free to rotate, the second has

restricted movement due to the nitrogens being linked by a tether **272** and the third is fully aromatic **76**. The most interesting feature is the change in chemical shift of the C(4)-H proton from 7.43 ppm in bisindolyl **255** to 8.69 ppm in indolocarbazole **76**, with the partially conformationally restricted **272** being about midway between the two. This is due to a magnetic anisotropic effect caused by the π-electrons in the carbonyl group. As the indoles become more fixed in position the C(4)-H protons are also pushed closer to the magnetic anisotropy deshielding zone of the carbonyl group causing them to be shifted downfield.

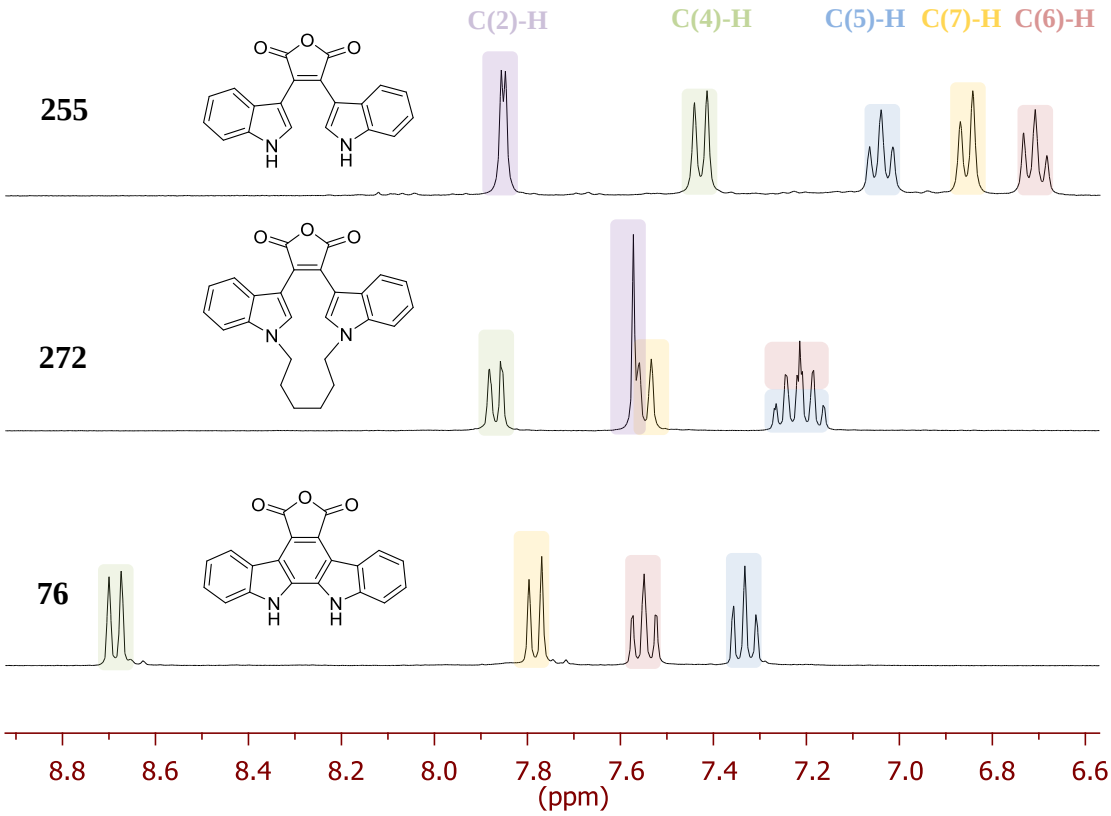
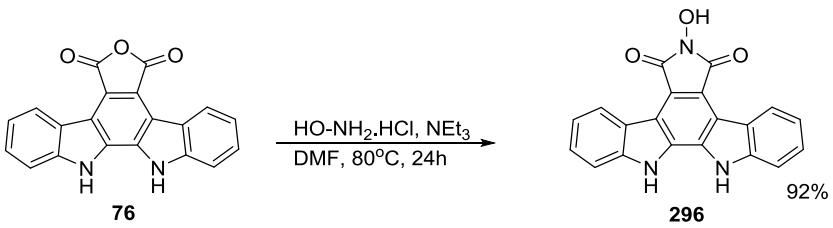



**Figure 4.3.2: Stacked proton NMR spectra of 255, 272 and 76 in DMSO-$d_6$ at 300 MHz**

It is also interesting to note that C(6)-H and C(7)-H are also shifted downfield going from **255** to **272** to **76**, however C(5)-H is much less affected. The disappearance of the C(2)-H signal in **76** confirms the formation of the final carbon-carbon bond in the fully aromatic system.

The maleic anhydride **76** was then converted to the corresponding hydroxymaleimide using 5 equivalents of hydroxylamine hydrochloride and 5 equivalents of triethylamine

in DMF at 80 °C for 24 hours. After cooling to room temperature, the reaction mixture was poured into 1 M aqueous HCl to precipitate the product and remove any excess triethylamine. The red solid was then collected using vacuum filtration to give **296** in 92% yield (Scheme 4.3.3).



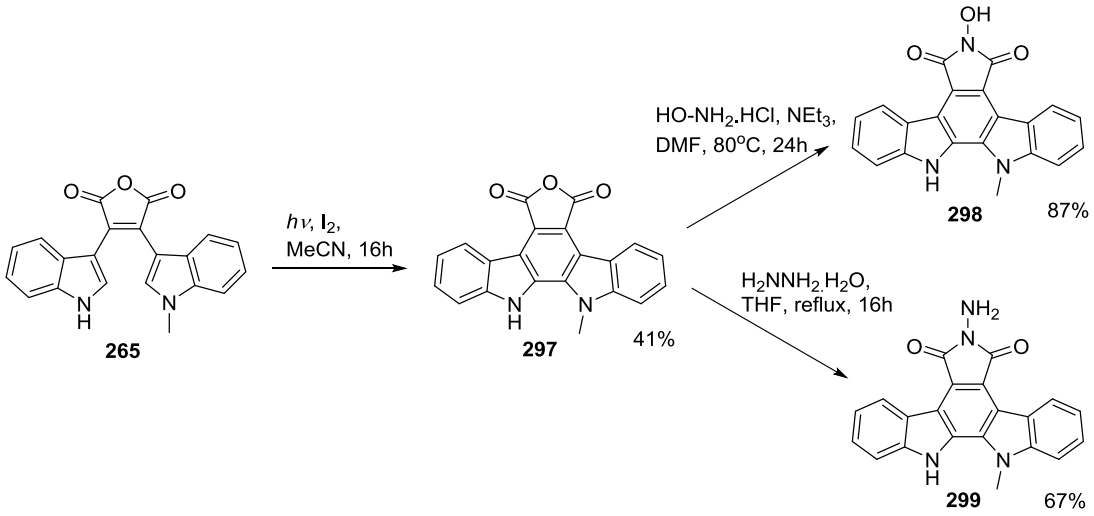

**76** **296**

**Scheme 4.3.3**

## 4.3.2 Incorporation of methyl and allyl substituents

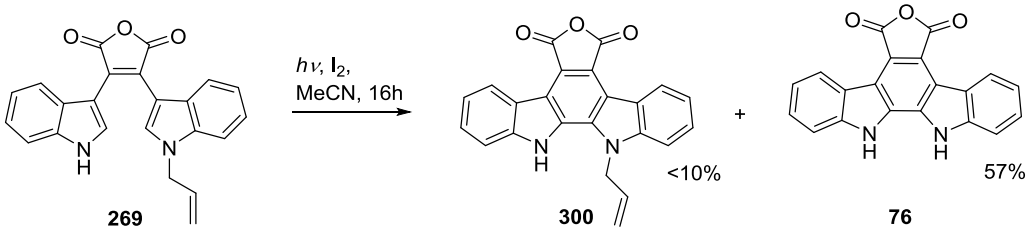
### *4.3.2.1 Synthesis of mono-alkylated derivatives*

A similar method was used to prepare a mono-methylated derivative. Photocyclisation of the appropriate bisindolyl maleic anhydride **265** under the same conditions outlined in the previous section gave indolocarbazole **297** as a yellow solid in 41% yield. The fully aromatic maleic anhydride was then converted to the corresponding hydroxymaleimide **298** with a yield of 87%. Transformation of maleic anhydride derivative **297** to an aminomaleimide **299** was also achieved using a similar method by heating to reflux in THF with 5 equivalents of hydrazine hydrate for 16 hours forming the product as an orange solid in 67% yield (Scheme 4.3.4).




**Scheme 4.3.4**

Expanding the alkyl chain to incorporate an alkene which could subsequently be converted to a number of different functional groups was next attempted. Photocyclisation of the allylated bisindolyl compound **269** was initially undertaken but the yellow precipitate formed was shown to be deallylated indolocarbazole **76** on analysis which was formed in 57% yield. Concentration of the filtrate resulted in the precipitation of more yellow solid which was found upon analysis to be the desired product **300** but with a less than 10% yield (Scheme 4.3.5). Due to the low yield it was decided not to continue with this route; however, due to the high yield of deallylated **76**, the use of an allyl substituent as a cleavable protecting group will be investigated in the future.




**Scheme 4.3.5**

### *4.3.2.2 Spectroscopic properties*

Complete assignment of the $^{1}$H NMR spectra of the unsymmetrically substituted indolocarbazoles proved more challenging than for the analogous bisindolyl compounds

due to more overlapping of the related signals from each indole ring. As can be seen from the stacked spectra of mono methyl bisindolyl compound **285** and indolocarbazole **298** (Figure 4.3.3) the C(7)-H protons in **285** are clearly distinguishable and can be assigned using 2D experiments, whereas in **298** they overlap almost completely and cannot be separately assigned. The same can be seen for the C(4)-H and C(5)-H protons which can be distinguished in **285** but completely overlap in **298**. The C(2)-H protons again disappear in **298** due to the formation of the final C-C bond and an overall deshielding effect is observed.

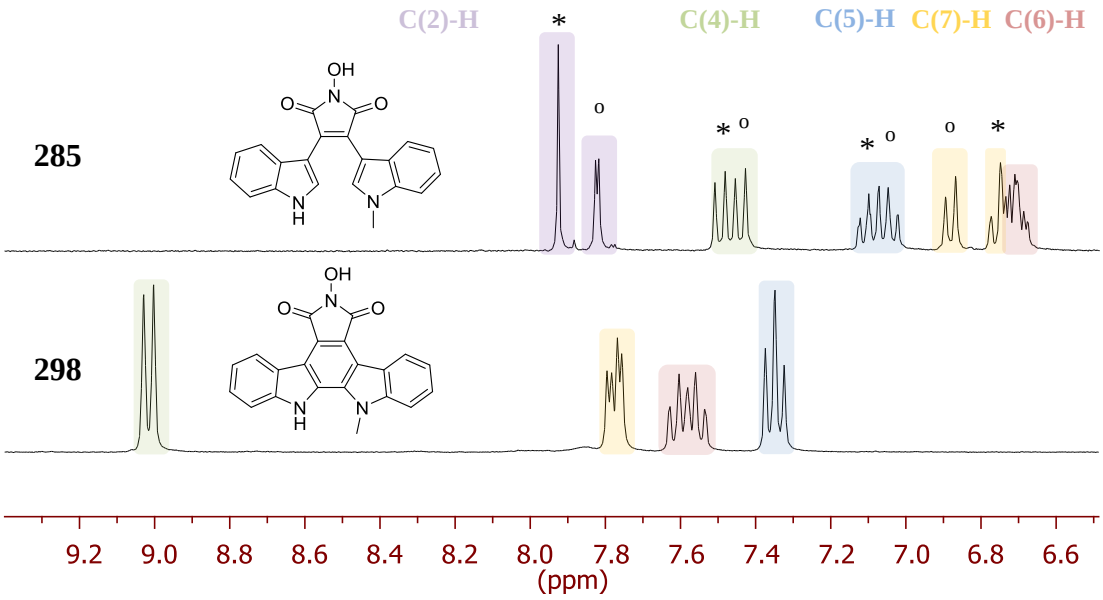
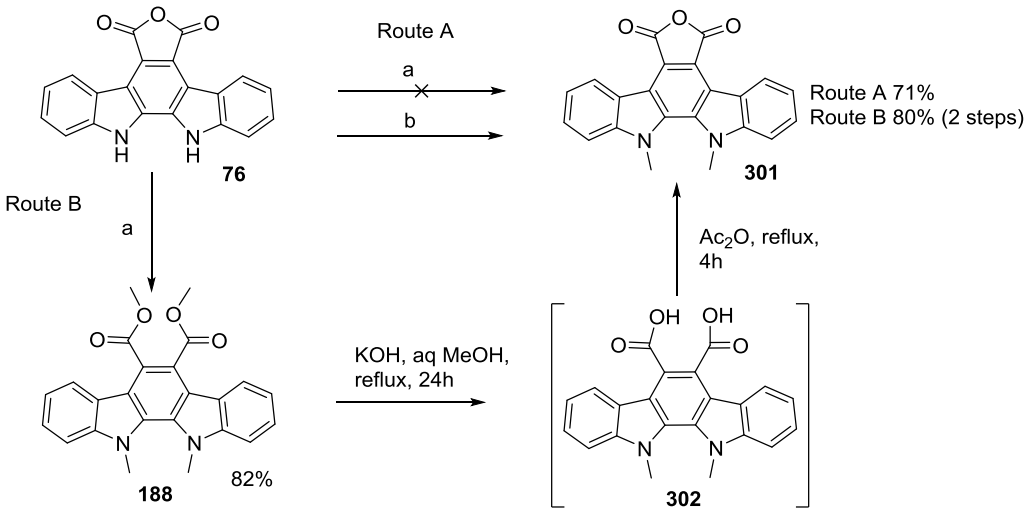



**Figure 4.3.3: Stacked spectra of bisindolyl** 285 **and indolocarbazole** 298 **in DMSO-$d_6$ at 300 MHz (*assigned proton on methylindole ring, °assigned proton on unsubstituted indole).**

### *4.3.2.3 Synthesis of di-alkylated derivatives*

With the cyclisation of unsubstituted **255** to **76** occurring cleanly, in high yield and on a reasonable scale it was decided to keep this as the main cyclisation step and to form all further derivatives from indolocarbazole **76**. Synthesis of a dimethyl maleic anhydride derivative **301** was then undertaken by initial deprotonation using 3 equivalents of sodium hydride, resulting in a dark blue solution, followed by the addition of iodomethane. TLC analysis of the resulting pale yellow solution after 16 hours indicated the presence of remaining starting material and so a further portion of sodium hydride was added followed by iodomethane and the reaction was stirred for a further 6 hours. The reaction mixture was poured into water and the precipitate collected by vacuum

filtration as an off-white solid. Analysis using proton NMR showed a symmetrical molecule with an extra 6H singlet at 4.03 ppm as well as the expected 6H singlet at 4.24 ppm for the *N*-methyl protons indicating two extra methyl groups. HRMS analysis confirmed the presence of an extra four methyl groups and an oxygen from the starting material and it was proposed that this compound formed due to ring opening of the maleic anhydride to form a diester **188** in 82% yield (Scheme 4.3.6).




Conditions: (a) i. NaH, DMF, rt; ii. $CH_3I$, rt; (b) i. NaH, DMF, -12 $^oC$; ii. $CH_3I$, rt, -12 $^oC$ to rt

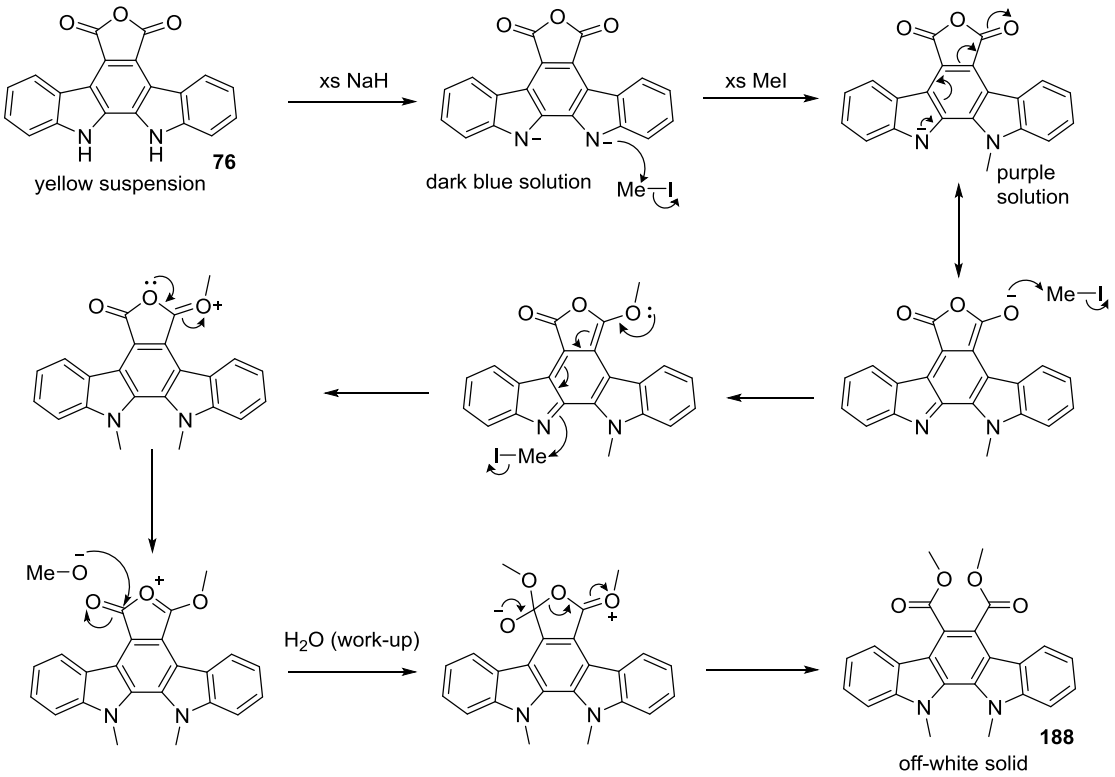
**Scheme 4.3.6**

Conversion of the diester **188** to the desired maleic anhydride **301** was achieved by initial treatment with potassium hydroxide in aqueous methanol and heating to reflux for 24 hours before the solvent was evaporated under reduced pressure and the resulting residue dissolved in water and acidified to pH 2 to form the diacid **302** which due to solubility issues was not analysed and was used directly in the next step. The off-white precipitate was collected using vacuum filtration and allowed to dry fully overnight at 40 °C. The di-acid intermediate was then heated to reflux for 4 hours in acetic anhydride to form the maleic anhydride in 80% yield (Scheme 4.3.6).

In an attempt to obtain dimethyl **301** without unwanted ring opening of the maleic anhydride, the reaction was repeated at a lower temperature. A solution of the unsubstituted maleic anhydride **76** in DMF was stirred in an ice/salt bath until the internal temperature dropped to -12 °C before the addition of sodium hydride. The reaction was stirred for 45 minutes resulting in a dark blue solution to ensure complete

deprotonation before iodomethane (2.1 eq) was added dropwise. The reaction mixture was allowed to warm slowly to room temperature in the bath over 16 hours. TLC analysis at this point showed the complete consumption of starting material and so the now yellow reaction mixture was poured into 1 M aqueous HCl and the precipitate collected using vacuum filtration. Recrystallisation from DMSO gave maleic anhydride derivative **301** in 71% yield.

The proposed mechanism for the formation of the diester in outlined in Scheme 4.3.7. Excess sodium hydride results in the deprotonation of both indole nitrogens of the yellow suspension of **76** resulting in the dianion. Formation of the dianion is characterised by a dark blue coloured solution, in contrast to the monoanion which is purple regardless of whether the second indole unit is alkylated or not. On addition of the methyl iodide there is a colour change from blue to purple over a period of five minutes and this purple solution then eventually changes to yellow. The first step after addition of methyl iodide is likely to be addition of one methyl group to one indole nitrogen; however addition of a methyl group to the second indole nitrogen is more difficult due to steric hindrance from the first group and, as a result, is slower. The electrons delocalised onto the carbonyl group of the maleic anhydride can then attack the iodomethane resulting in methylation at this position. As iodomethane is a very reactive methylating agent, it is possible for methylation to occur through the attack of a lone pair, without the presence of an anion. It is likely that the lone pair of the unsubstituted indole nitrogen aided by delocalisation of electrons on the now-methylated oxygen can attack a third molecule of iodomethane resulting in methylation at this position. Water added to quench the reaction reacts with excess sodium hydride and iodomethane resulting in the formation of sodium methoxide which can then attack the second carbonyl resulting in ring opening to form the diester **188**.




**Scheme 4.3.7**

The 82% yield suggests that this is an efficient process under these conditions; however, in reactions where an excess of iodomethane but not sodium hydride was present, ring opening did not occur (see Section 4.3.4.1). This suggests that in the absence of excess base to form the methoxide necessary for ring opening, addition of water results in the loss of the methyl group from initial *O*-methylation as methanol.

The anisotropic effect from the π-electrons in the carbonyl group on the C(4)-H protons in the maleic anhydrides is again evident when the proton NMR spectrum of the maleic anhydride **301** is compared to the diester **188** (Figure 4.3.4). A difference in chemical shift of 0.89 ppm upfield is observed going from the maleic anhydride where the carbonyl groups are fixed in position to the di-ester where they can move away from the top of the indole ring.

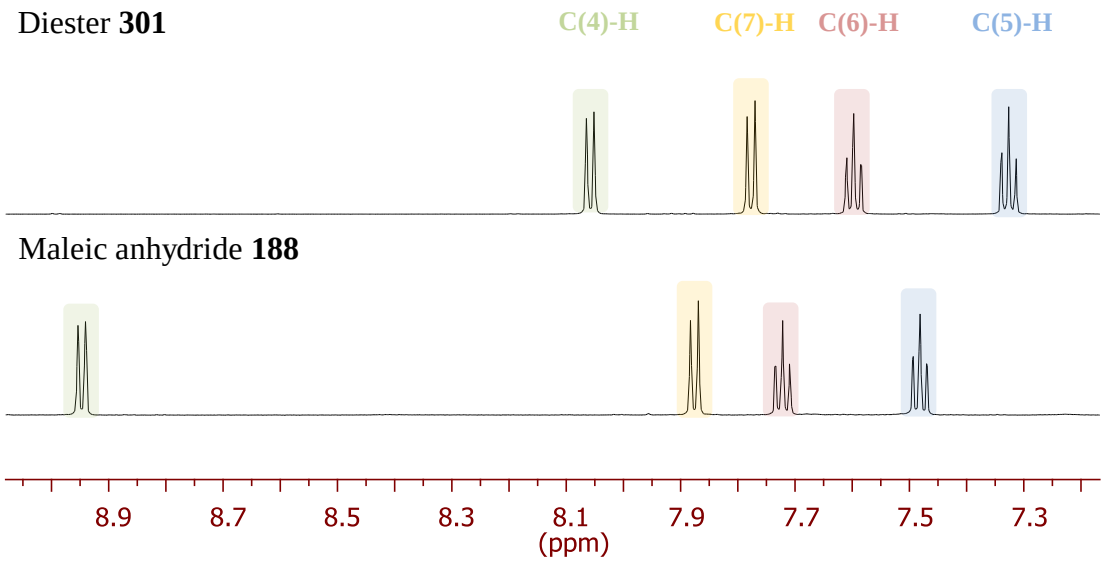
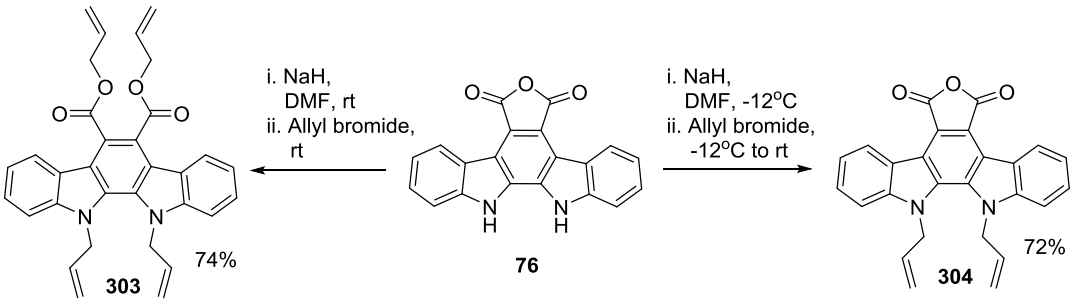


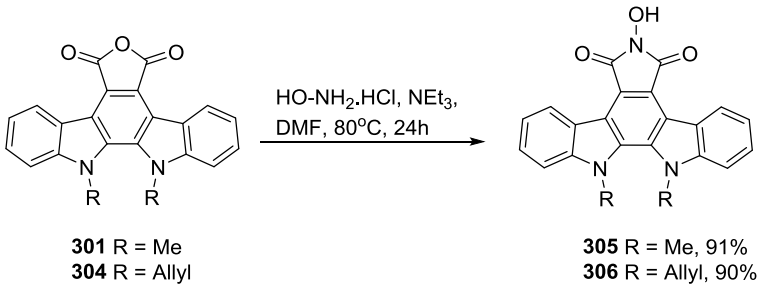

**Figure 4.3.4: Stacked spectra of** 301 **and** 188 **in DMSO-$d_6$ at 300 MHz**

Treatment of unsubstituted **76** with sodium hydride followed by allyl bromide at room temperature again resulted in ring opening of the maleic anhydride with the addition of four allyl groups **303**. Repetition of the reaction at -12 °C formed the desired diallyl maleic anhydride **304** as a bright yellow solid in 72% yield (Scheme 4.3.8).




**Scheme 4.3.8**

Both the dimethyl **301** and diallyl **304** maleic anhydrides were successfully transformed to their hydroxymaleimide analogues, **305** and **306** respectively, in high yields (Scheme 4.3.9).



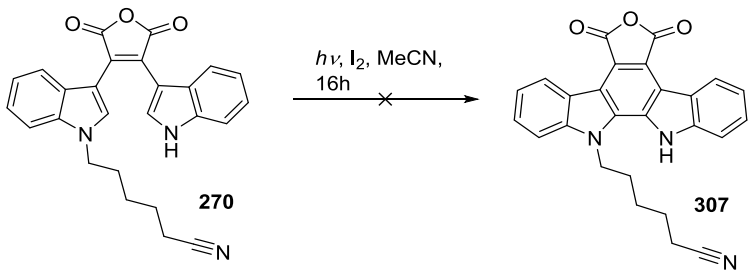

**301** R = Me
**304** R = Allyl

**305** R = Me, 91%
**306** R = Allyl, 90%

**Scheme 4.3.9**

### 4.3.3 Synthesis and derivatisation of 6-[5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl]hexanenitrile

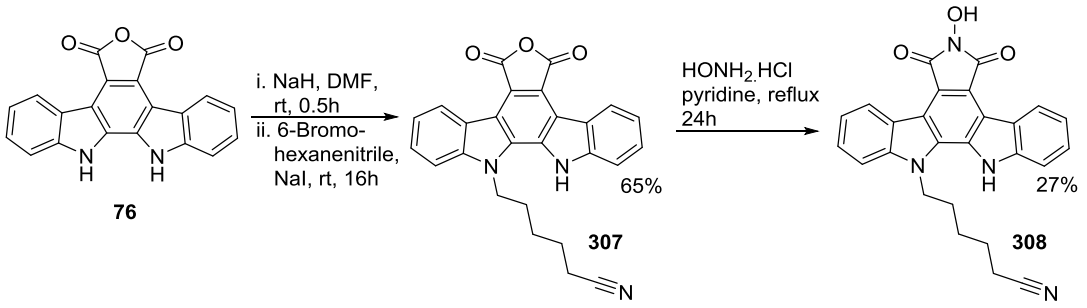
Work then began on the synthesis of an indolocarbazole maleic anhydride with a longer functionalised chain *N*-substituent. Initially, attempts focussed on the conversion of bisindole compound **270** to indolocarbazole **307** in a photocyclisation step, however irradiation of **270** for 24 hours as for previous reactions only returned starting material (Scheme 4.3.10). This is probably due to the bulkier *N*-substituent making it more difficult for the two indoles to get into the correct position for the reaction to occur.




**270** **307**

**Scheme 4.3.10**

Fortunately, treatment of the unsubstituted cyclised maleic anhydride **76** with sodium hydride followed by 6-bromohexane nitrile and sodium iodide resulted in mono-alkylation again probably due to steric hindrance of the more bulky chain combined with the less reactive alkyl halide compared to iodomethane and allyl bromide. The mono-alkylated product **307** was isolated as a yellow solid in 65% yield (Scheme 4.3.11).

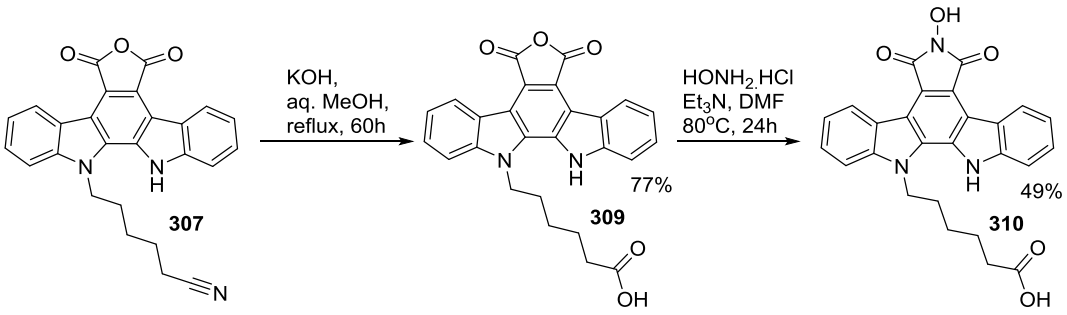



**Scheme 4.3.11**

Treatment of nitrile **307** with hydroxylamine hydrochloride and triethylamine in DMF only returned starting material. The reaction was repeated with 10 equivalents of both hydroxylamine hydrochloride and triethylamine with a higher reaction temperature of 120 °C but even after 48 hours none of desired hydroxymaleimide **308** had formed. The reaction was then repeated with hydroxylamine hydrochloride and pyridine as the amine base and solvent, at reflux, for 24 hours before TLC analysis showed complete consumption of starting material. This reaction was not as clean as the usual method with several unidentified side products forming, but, after careful column chromatography hydroxymaleimide **308** was isolated as an orange solid in 27% yield.

### *4.3.3.1 Derivatisation of nitrile*

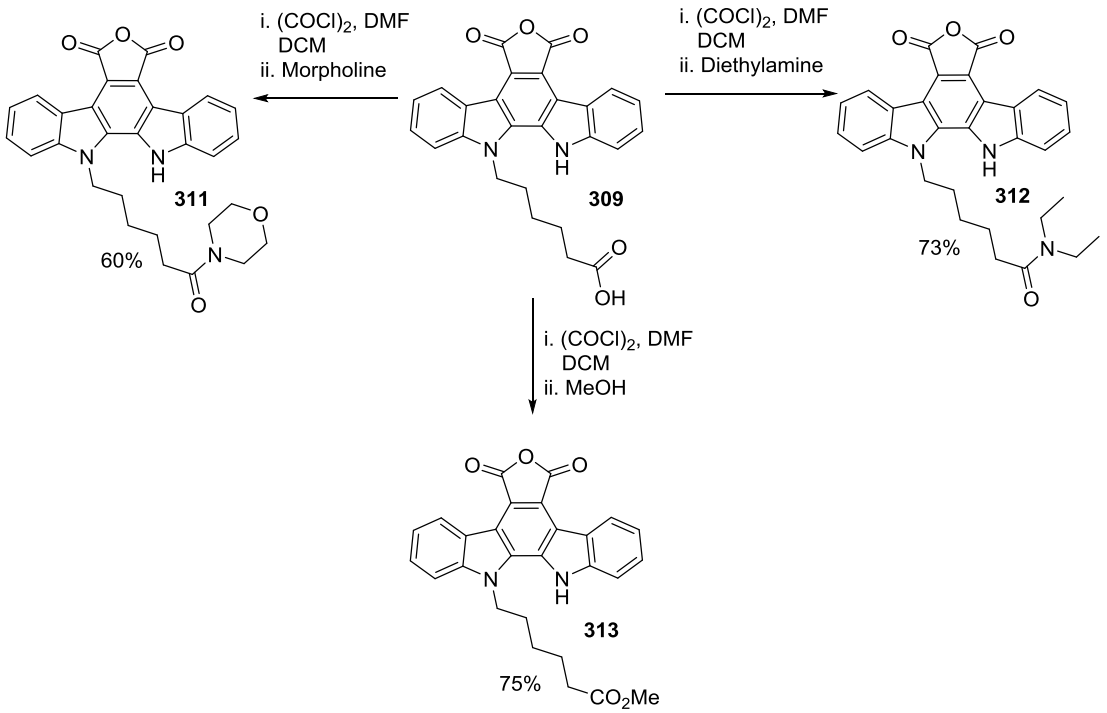
In order to access a series of functionalised chains it was decided to first convert the nitrile to the more versatile carboxylic acid. The was achieved through treatment of nitrile **307** with potassium hydroxide in aqueous methanol and heating to reflux for 60 hours until TLC analysis confirmed complete consumption of starting material. After an acidic work-up, the yellow solid was recrystallised from acetone to give carboxylic acid **309** in 77% yield (Scheme 4.3.12).




**Scheme 4.3.12**

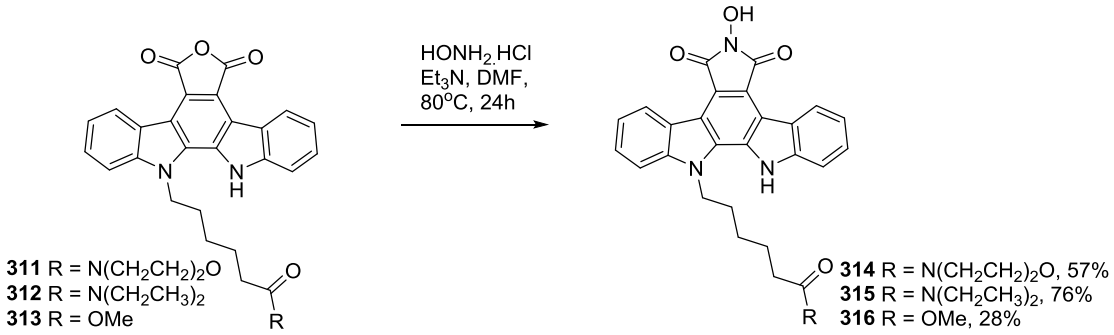
Transformation of acid **309** to the corresponding hydroxymaleimide was achieved using the usual method to give **310** as a red solid in 49% yield (Scheme 4.3.12).

The preparations of two amides and a methyl ester were then carried out *via* initial activation of carboxylic acid **309** to the acid chloride (Scheme 4.3.13). This was achieved through addition of oxalyl chloride and a catalytic amount of DMF to the yellow suspension in DCM resulting in a slight fizzing and gradual dissolution to form a pale yellow solution. When gas evolution had ceased, the solvent was evaporated under reduced pressure and the residue was treated with an amine or alcohol. When morpholine was added, amide **311** was isolated as a yellow solid in 60% yield after trituration with hot acetone. Addition of diethylamine resulted in a gummy material which stuck to the sides of the flask and so a small amount of DCM was added after 2 hours to increase solubility. The resulting solution was stirred for a further 1 hour before work-up and purification by trituration with hot acetone gave amide **312** in 73% yield. Addition of methanol to the acid chloride resulted in the formation of a bright yellow precipitate which, after 2 hours, was collected by vacuum filtration and triturated with hot acetone to give ester **313** in 75% yield.



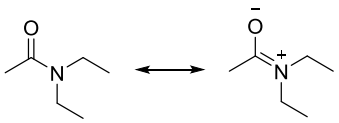

**Scheme 4.3.13**

The three maleic anhydrides **311**, **312** and **313** were then transformed to their corresponding hydroxymaleimides through treatment with hydroxylamine hydrochloride and triethylamine in DMF (Scheme 4.3.14). Morpholine derivative **314** was isolated in 57% yield after column chromatography as a red solid, diethyl derivative **315** was isolated in 76% yield as a yellow/orange solid without the need for column chromatography and methyl ester **316** was isolated in 28% yield after column chromatography.




**Scheme 4.3.14**

Due to the partial double-bond character of amides leading to restricted rotation around the N-C bond, the ethyl groups of diethyl amides **312** and **315** show two separate sets of peaks.



This can be seen in the proton NMR spectrum of **315** (Figure 4.3.5) where the $CH_3$ signals appear as two triplets at 0.83 and 0.85 ppm (red arrows) and the $CH_2$ signals appear as two quartets at 3.04 and 3.11 ppm (blue arrows). Separate signals are also seen in the $^{13}C$ spectrum where the $CH_3$ peaks appear at 13.4 and 14.6 ppm (red arrows) and the $CH_2$ peaks appear at 39.7 and 41.6 ppm (blue arrows). This effect is also seen for morpholine derivatives **311** and **314**.

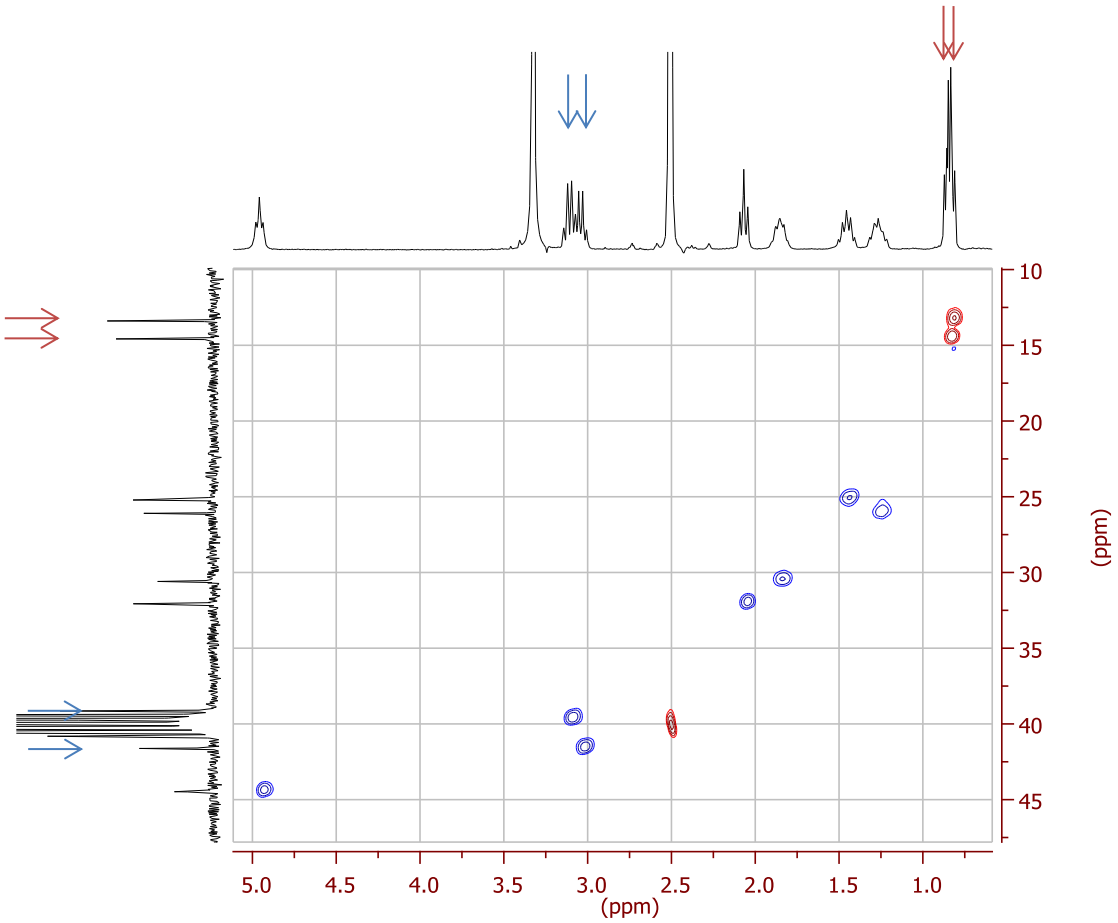


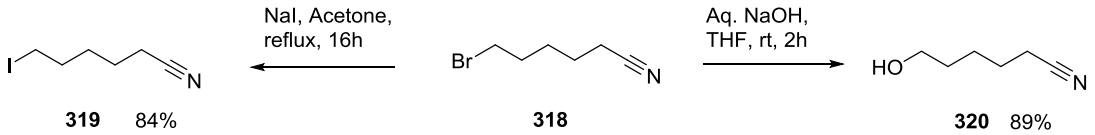

**Figure 4.3.5: HSQC 2D spectrum of diethyl amide** 315

### *4.3.3.2 Synthesis of 6-[6-hydroxy-13-methyl-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12(13*H*)-yl]hexanenitrile*

The presence or absence of one free indolic nitrogen has been shown to influence the mode of action of indolocarbazoles and so the effect of the addition of a second *N*-substituent was investigated. Initially, compounds where one of the substituents was a methyl group were targeted. The initial strategy involved the alkylation of 12-methylfuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **297** with a second functionalised substituent, starting with a hexanenitrile chain to form di-substituted **317**. Although treatment of **297** with sodium hydride resulted in a dark purple solution indicating deprotonation had occurred, subsequent addition of 6-bromohexanenitrile followed by stirring for 24 hours returned only starting material.

Conversion of the 6-bromohexane nitrile **318** to more reactive 6-iodohexanenitrile **319** in a Finkelstein reaction with sodium iodide in acetone was achieved in 84% yield (Scheme 4.3.15). However, alkylation of **297** with 6-iodohexanenitrile **319** also failed, again with only starting material recovered.

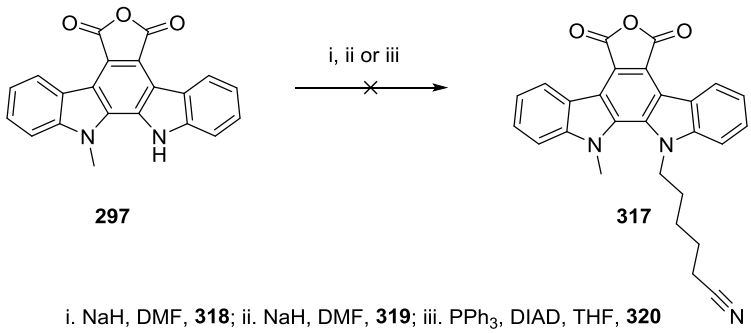
Another method for *N*-alkylation of indolocarbazole-type compounds reported in the literature is *via* a Mitsonobu reaction.[15] 6-Hydroxyhexanenitrile **320** was synthesised from 6-bromohexanenitrile **318** through treatment with 10% aqueous sodium hydroxide in THF followed by an acidic work-up to give a colourless oil in 89%.




**319** 84% **318** **320** 89%

**Scheme 4.3.15**

For the Mitsonobu reaction, mono-methylated **297** along with triphenylphosphine, 6-hydroxyhexanenitrile **320** and THF were cooled to -78 °C under a nitrogen atmosphere. Diisopropyl azodicarboxylate was then added and the mixture was allowed to warm to room temperature over 16 hours. After work-up, proton NMR analysis showed only starting material to be present (Scheme 4.3.16).



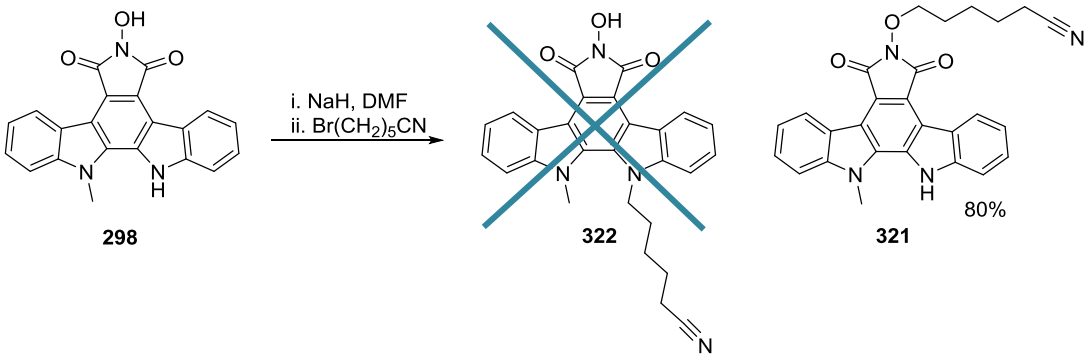

**297** **317**

i. NaH, DMF, **318**; ii. NaH, DMF, **319**; iii. $PPh_3$, DIAD, THF, **320**

**Scheme 4.3.16**

Alkylation of the hydroxymaleimide analogue was then attempted with the prediction that sodium hydride would be a strong enough base to deprotonate both the hydroxy group and indolic nitrogen and that alkylation would occur on the more nucleophilic nitrogen. Treatment of a solution of 6-hydroxy-12-methyl-12,13-dihydro-5*H*-

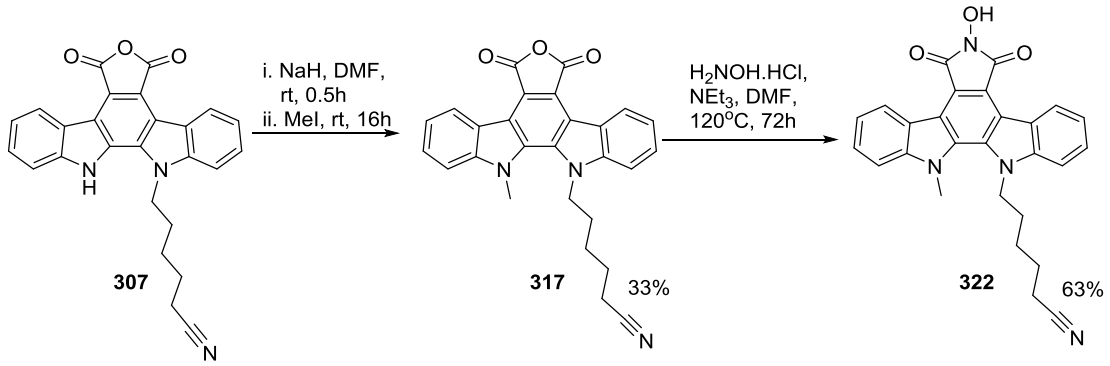
indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **298** in DMF with sodium hydride followed by 6-bromohexanenitrile gave a yellow/orange solid which proton NMR analysis showed to have an extra 5 $CH_2$ signals indicating alkylation had occurred. However, the absence of a singlet between 10 and 11 ppm characteristic of the hydroxymaleimides and the presence of a singlet at 12.02 ppm, which is characteristic of the proton attached to the indole nitrogen suggested alkylation had occurred on the oxygen to give product **321** in 80% yield and not **322** as expected (Scheme 4.3.17).




**Scheme 4.3.17**

The order of substitution was then reversed with an attempt to alkylate 6-[5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl]hexanenitrile **307** with the more reactive and less sterically hindered iodomethane. Treatment of a solution of **307** in DMF with sodium hydride followed by iodomethane gave desired di-substituted product **317** as a yellow solid in 33% yield (Scheme 4.3.18).

In this case, the maleic anhydride was successfully converted to the hydroxymaleimide using hydroxylamine hydrochloride and triethylamine in DMF although the reaction time had to be increased to 72 hours with the temperature increased to 120 °C after the first 24 hours. After work-up and purification using column chromatography, hydroxymaleimide **322** was isolated in 63% yield as an orange solid.

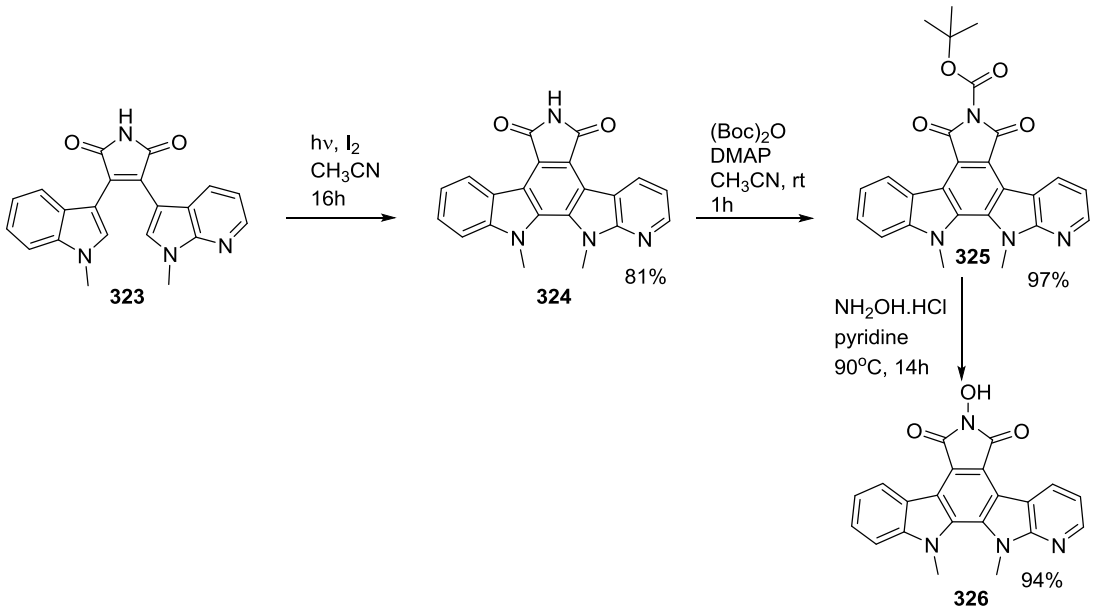



**Scheme 4.3.18**

### *4.3.3.3 Synthesis of 6,6'-(6-hydroxy-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-12,13-diyl)dihexanenitrile*

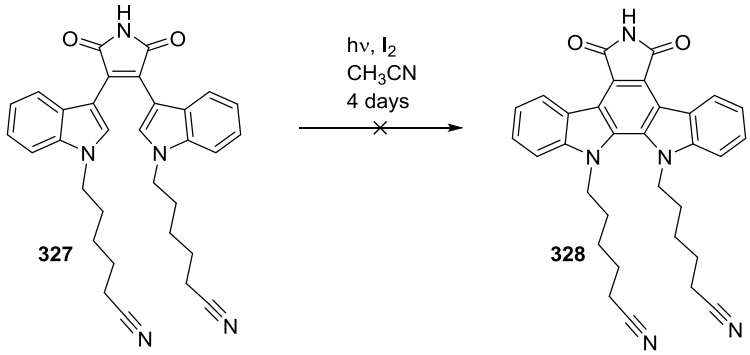
Due to the promising results from 6,6'-[3,3'-(1-hydroxy-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile **282** in NCI screening, an analogous indolocarbazole was a key target. The alkylation of unsubstituted indolocarbazole **76** with bromohexanenitrile and sodium iodide had been shown to give exclusively mono-substituted product **307** as did a repeat of the reaction employing caesium carbonate as base, which has been shown in the literature to be successful in some similar reactions (although not while a maleic anhydride F-ring is present),[7] and so an alternative route had to be identified.

Previous work within the group had successfully cyclised a bis-methylated azaindole analogue **323** with a maleimide F-ring in place of the hydroxymaleimide to the indolocarbazole form **324** *via* photocyclisation.[2] The maleimide could then be Boc-protected **325** and converted to the hydroxymaleimide **326** under similar conditions to those used in this project to convert the maleic anhydrides (Scheme 4.3.19).




**Scheme 4.3.19**

Therefore, an attempt was made to cyclise 6,6'-[3,3'-(2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile **327** in a light-mediated reaction to form indolocarbazole **328**. Unfortunately, even after being irradiated with a high pressure mercury lamp for four days analysis by proton NMR showed only starting material to be present (Scheme 4.3.20).

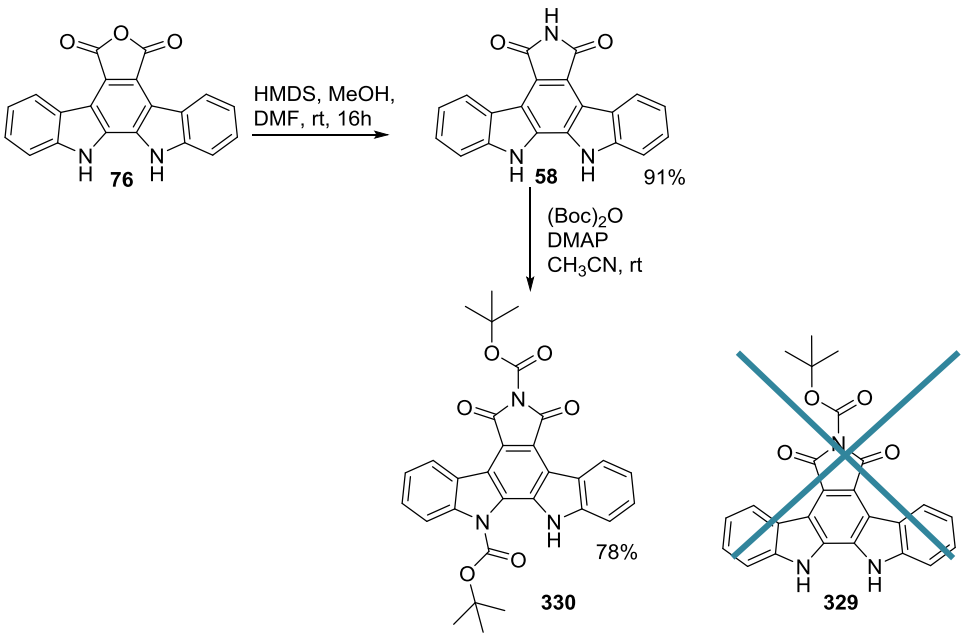



**Scheme 4.3.20**

Following on from Section 4.3.3.2 and the difficulties in indolocarbazole substitution, while there are no reports of bis-substitution of an indolocarbazole possessing a maleic anhydride F-ring in the literature, there are reports of a second substituent being added where different F-rings were in place. A benzylmaleimide is most commonly employed, however Slater *et al*. reported the alkylation of an indolocarbazole with a Boc-protected

maleimide F-ring and where the second indole was also Boc protected.[16] In this case, the double Boc protection of the unsubstituted maleimide **58** was achieved using sodium hydride so it was theorised that by using a weaker base, selective protection of the maleimide could be achieved to give **329** (Scheme 4.3.21).

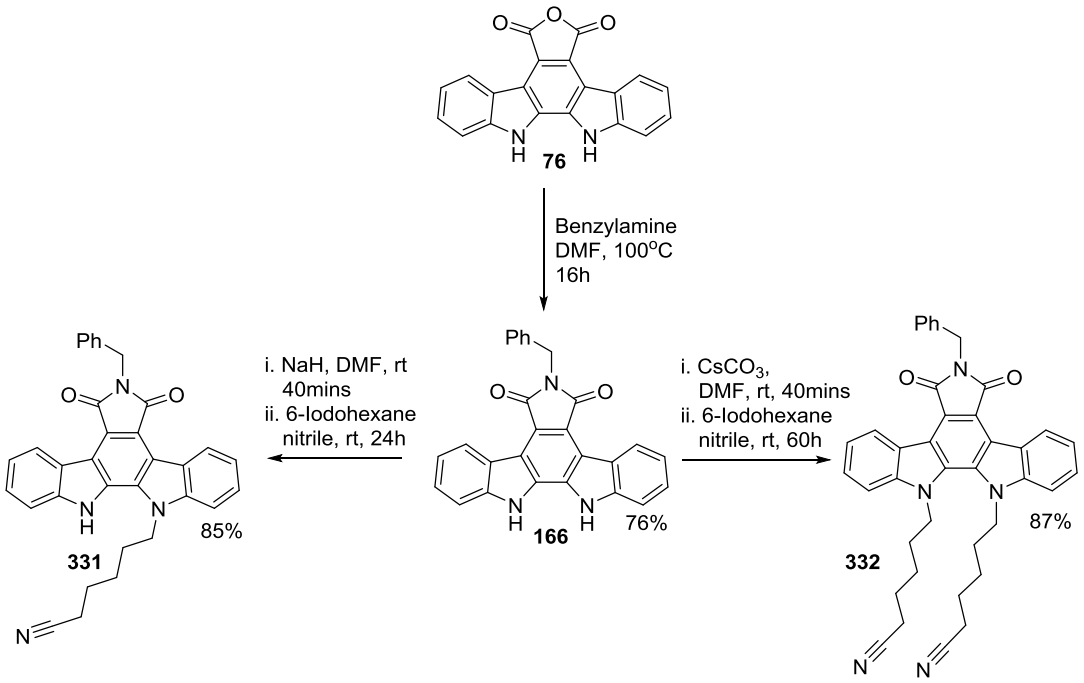
Maleic anhydride derivative **76** was treated with 1,1,1,3,3,3-hexamethyldisilazane and methanol in DMF to give the maleimide **58** as an orange solid in 91% yield. The maleimide was then suspended in acetonitrile and Boc anhydride and DMAP were added. After stirring for 2 hours, TLC analysis showed incomplete consumption of starting material and so a further 1 equivalent of Boc anhydride was added and the reaction was stirred for a further 1 hour. After work-up, proton NMR analysis showed the presence of two 9H singlets indicating the presence of two Boc groups. The reaction was repeated and this time worked up after 2 hours. Proton NMR analysis showed the presence of just 2 components – the original starting material **58** and di-Boc protected **330**. This indicates that the second Boc protection occurs very quickly after the first and so the mono-protected product **329** is unlikely to be successfully obtained using this method.




**Scheme 4.3.21**

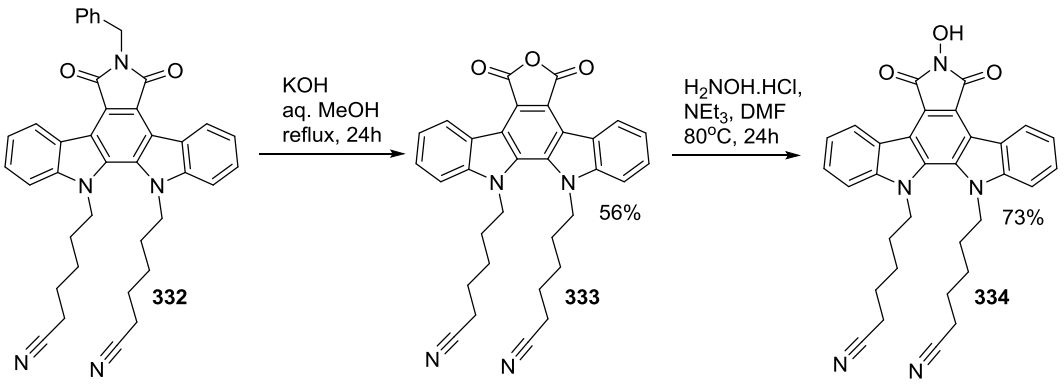
The benzylmaleimide **166** was then synthesised by heating the unsubstituted maleic anhydride **76** with benzylamine in DMF at 100 °C for 16 hours. The orange precipitate

was collected by vacuum filtration and washed with 1 M aqueous HCl to remove traces of DMF and benzylamine to give benzylmaleimide **166** in 76% yield. Alkylation using sodium hydride and iodohexanenitrile in DMF gave exclusively mono-substituted **331** in 85% yield. However, when the base was changed to caesium carbonate TLC analysis after 24 hours showed complete consumption of starting material and 2 spots, one of which corresponded to mono-alkylated **331**. The reaction was allowed to stir for a further 36 hours until TLC analysis showed just one component. Following work-up, the isolated orange solid was found to be desired product **332**, formed in 87% yield (Scheme 4.3.22).




**Scheme 4.3.22**

Attempts were made to convert the benzylmaleimide directly to the hydroxymaleimide using hydroxylamine hydrochloride and triethylamine in DMF, but even at a higher reaction temperature of 120 °C only starting material **332** was recovered. The benzylmaleimide **332** was then transformed back to the maleic anhydride **333** through treatment with potassium hydroxide in aqueous ethanol. After acidic work-up and purification using DCM/MeOH column chromatography, the maleic anhydride was isolated in 56% yield. The maleic anhydride was then converted to the hydroxymaleimide **334** using hydroxylamine hydrochloride and triethylamine in DMF, to give the product in 73% yield (Scheme 4.3.23).

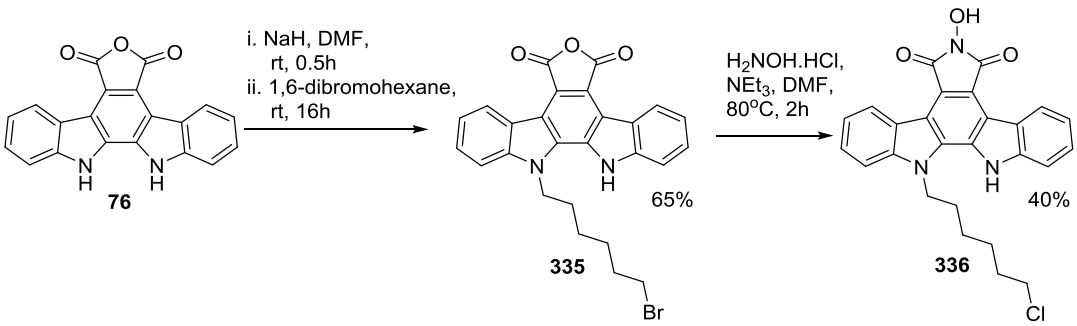



Scheme 4.3.23

### 4.3.4 Synthesis and derivatisation of 12-(6-bromohexyl)-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione

A bromohexyl chain was next introduced as this gave scope for further derivatisation. The alkylation procedure was altered slightly to reduce the possibility of dimer formation where two molecules of the indolocarbazole could attack either end of one 1,6-dibromohexane molecule. The unsubstituted maleic anhydride **76** was deprotonated as usual using excess sodium hydride in DMF and then this dark blue solution was transferred to a dropping funnel which was connected to a second flask containing a solution of 5 equivalents of dibromohexane and added slowly so there was always a large excess of dibromohexane compared to the dianion of the indolocarbazole. Following additional stirring for 16 hours and work-up, the alkylated product **335** was isolated in 65% yield (Scheme 4.3.24).

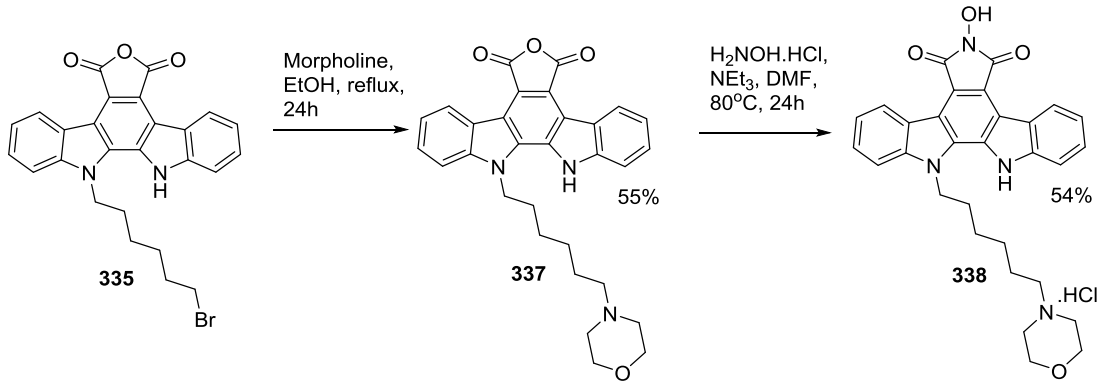
The maleic anhydride was then treated with hydroxylamine hydrochloride and triethylamine in DMF as usual to form the hydroxymaleimide. However, although the hydroxymaleimide moiety was formed successfully, analysis using mass spectrometry indicated the presence of an alkyl chloride rather than the expected alkyl bromide on the side chain. It is likely that the formation of this product was as a result of halide displacement caused by the excess of triethylammonium chloride which is formed as a by-product of the reaction, and 12-(6-chlorohexyl)-6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **336** was isolated as a red solid in 40% yield.




**Scheme 4.3.24**

A morpholine derivative was consequently prepared from bromide **335**. The maleic anhydride was suspended in ethanol, 10 equivalents of morpholine were added and the reaction was heated to reflux for 24 hours. The solvent was evaporated under reduced pressure and the residue dissolved in 10% methanol/DCM. The solution was washed with 1 M aqueous HCl to remove any remaining morpholine, followed by saturated aqueous sodium bicarbonate to convert the HCl salt back to the free amine. Initial attempts at purification using column chromatography with DCM/MeOH were unsuccessful even when a few drops of triethylamine were added to the eluent. Purification by recrystallisation from acetone was successful with the product **337** being isolated as a yellow solid in 55% yield (Scheme 4.3.25).

Conversion of maleic anhydride **337** to the corresponding hydroxymaleimide **338** was achieved under the usual conditions of hydroxylamine hydrochloride and triethylamine in DMF. The work-up was altered slightly with the reaction mixture being poured into water instead of 1 M aqueous HCl, in an attempt to isolate the free amine. Analysis using $^{1}$H NMR showed the absence of the characteristic hydroxy proton and so to confirm the transformation had occurred, the red solid was suspended in water and acidified using 1M aqueous HCl. The solid was collected by vacuum filtration and $^{1}$H NMR analysis now indicated the presence of the hydroxy group and also showed a broad singlet at 10.52 ppm indicating formation of the morpholine salt, which had been formed in 54% yield.




**Scheme 4.3.25**

Morpholine usually exists as rapidly interconverting chair forms where an axial proton becomes an equatorial proton and vice versa resulting in a single averaged peak being observed in the proton NMR spectrum. In other words, the axial and equatorial protons are equivalent by rapid exchange. This is observed for the maleic anhydride **337** (Figure 4.3.6) where just two peaks are observed (shown in green), one for the protons on the carbon by the oxygen and one for the protons on the carbon by the nitrogen. In hydroxymaleimide **338**, however, the morpholinium salt has been formed, stopping the interconversion. As a result, the axial and equatorial protons are now diastereotopic and appear as separate signals.

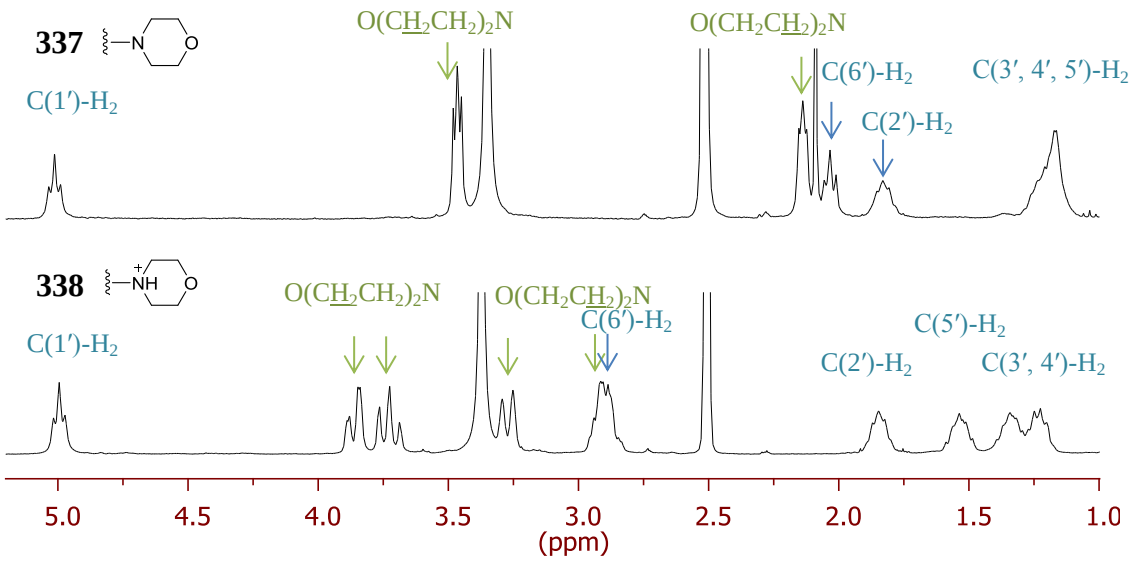



**Figure 4.3.6: Stacked proton NMR plots showing the alkyl region of morpholine** 337 **and morpholinium salt** 338

Salt formation also has a deshielding effect on the neighbouring C(6′)-$H_2$ protons and the morpholine $CH_2$ protons adjacent to the nitrogen due to the positively charged nitrogen, shifting the peaks between 0.8 and 1.0 ppm downfield. This effect can also be seen to a lesser extent on the C(5′)-$H_2$ protons and the protons neighbouring the oxygen of the morpholine ring.

A HSQC experiment confirms that the morpholine salt has two sets of equivalent carbons which show correlations to both their axial and equatorial protons (Figure 4.3.7).

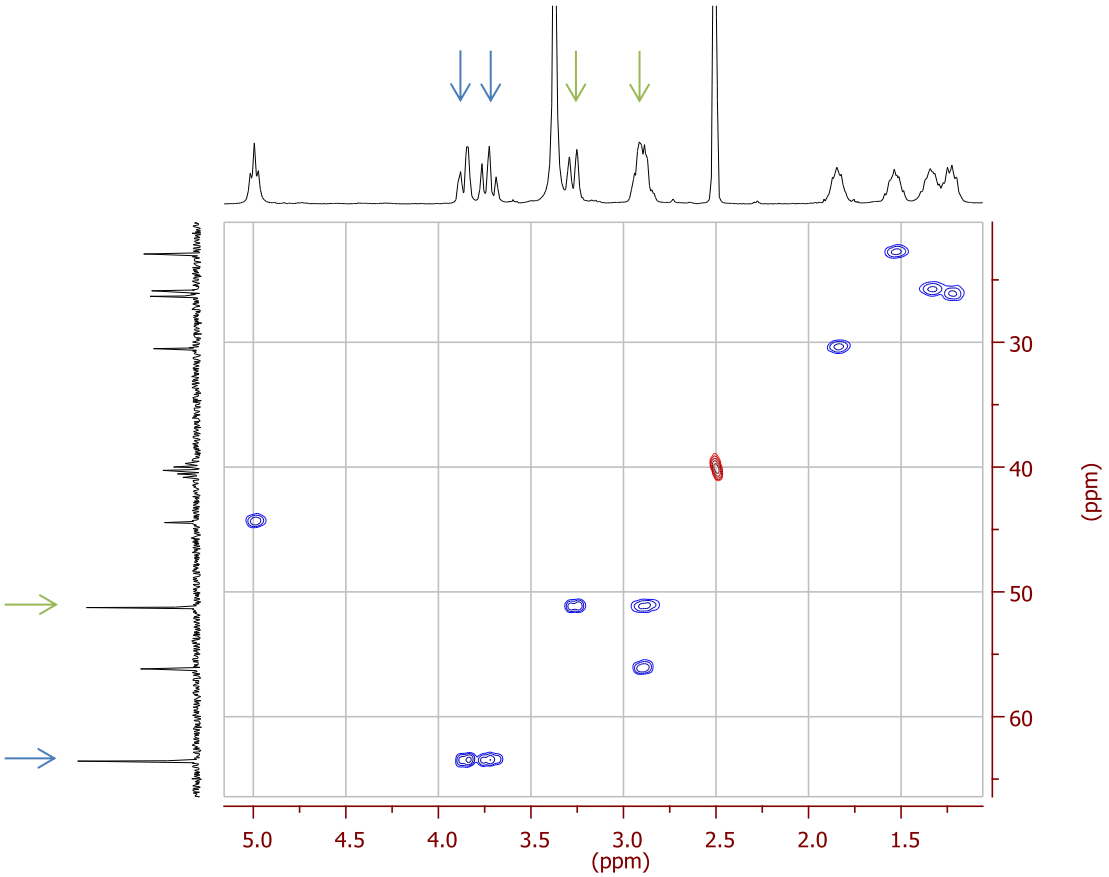


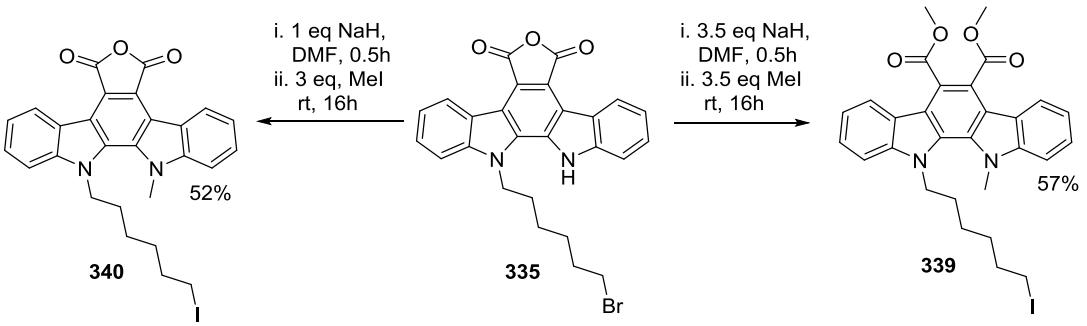

**Figure 4.3.7: HSQC 2D spectrum of alkyl region of morpholinium salt** 338

### *4.3.4.1 Methylation of 12-(6-bromohexyl)-12,13-dihydrofuro[3,4-c]indolo[2,3-a] carbazole-5,7-dione*

Following on from section 4.3.3.2, the effect of the addition of a methyl substituent on the unsubstituted indole unit of bromohexyl substituted maleic **335** was then

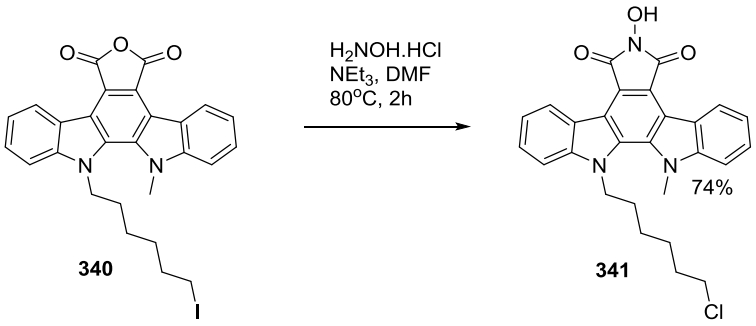
investigated. Maleic anhydride derivative **335** was suspended in DMF and treated with 2.5 equivalents of sodium hydride, followed by 2.5 equivalents of iodomethane after 40 minutes. After 16 hours of stirring, TLC analysis showed the presence of some remaining starting material and so a further 1 equivalent of sodium hydride followed by iodomethane were added. After a further 16 hours stirring, TLC analysis showed a single spot and so the reaction was quenched with water and, following work-up, gave a pale yellow solid. Analysis using proton NMR showed the presence of three 3H singlets indicating *N*-methylation and also ring opening of the maleic anhydride to form the diester **339**, which was isolated in 57% yield (Scheme 4.3.26). Carbon NMR analysis showed a peak at 8.8 ppm indicating the presence of an iodide instead of the expected bromide, which was confirmed by high resolution mass spectrometry. It is likely that the sodium iodide formed as a by-product in the reaction lead to the displacement of the bromide with an iodide in a Finkelstein reaction.

In a further attempt to form the maleic anhydride, the reaction was repeated with a reduced amount of sodium hydride, decreasing the number of equivalents from three to slightly over one, while keeping the equivalents of iodomethane the same. This time the maleic anhydride **340** was formed predominantly. Analysis using carbon NMR and high resolution mass spectrometry again indicated the conversion of the bromide to an iodide and the product was isolated in 52% yield as a bright yellow solid.




**Scheme 4.3.26**

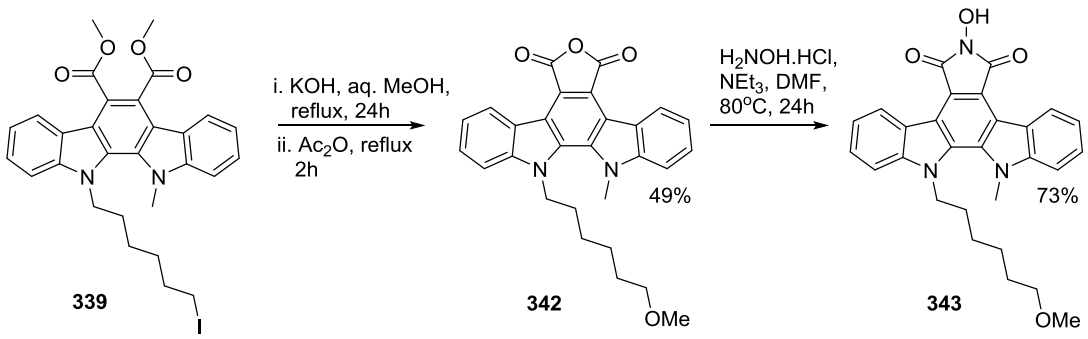
The maleic anhydride **340** was treated with hydroxylammonium chloride and triethylamine in DMF to form the hydroxymaleimide **341** with an alkyl chloride side chain, which was isolated in 74% yield (Scheme 4.3.27).




Scheme 4.3.27

Attempts were then made to convert diester **339** back to the maleic anhydride using the same conditions as for dimethyl 11,12-dimethyl-11,12-dihydroindolo[2,3-*a*]carbazole-5,6-dicarboxylate **188** with the expectation that the iodide would be converted to first a hydroxy group and then acylated with acetic anhydride in the ring closing step. The diester **339** was suspended in aqueous methanol and treated with potassium hydroxide and heated to reflux for 24 hours. A pale yellow solid was obtained after work-up which, due to solubility issues was used directly in the next step and not characterised. This material was suspended in acetic anhydride and heated to reflux for 2 hours before work-up and purification using column chromatography gave a bright yellow solid. Analysis using proton NMR showed the expected presence of a 3H singlet, however there was no extra carbonyl peak in the $^{13}C$ NMR indicating the presence of a methoxy group instead of the expected acetoxy group. This was confirmed using mass spectrometry and the methoxy compound **342** was isolated in 49% yield (Scheme 4.3.28).

The maleic anhydride **342** was transformed to the hydroxymaleimide **343** under the usual conditions in 73% yield.



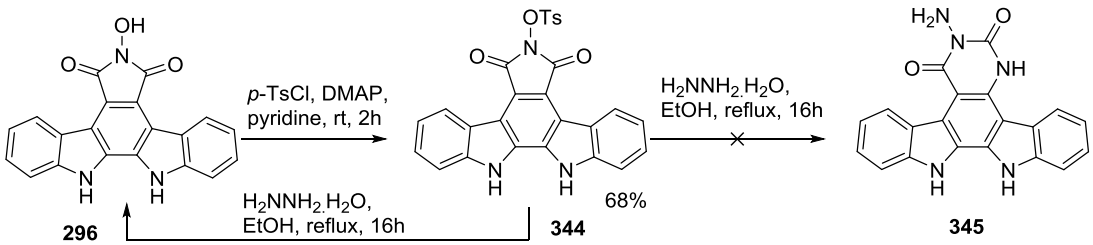

Scheme 4.3.28

### 4.3.5 Lossen rearrangement

Pursuing the development of the theme of F-ring modulation towards the synthesis of 6-membered rings, attention then turned to the use of a modified Lossen rearrangement of hydroxymaleimide indolocarbazoles. Previous work within the group had achieved this transformation by reacting hydrazine hydrate with the tosyl derivative of both a mono- and bisazaindolocarbazole to give the corresponding 3-amino uracils.[2] This section details the work towards the adaptation of this chemistry to include indolocarbazoles without a pyridine nitrogen and to synthesise a panel of novel uracil indolocarbazoles with varying substituents on the uracil F-ring and the indole nitrogens.

#### *4.3.5.1 Synthesis of 3-substituted uracils*

The original procedure involved initial activation of the hydroxyl group to the tosylate ester which was isolated and then used in the next step where it was treated with hydrazine hydrate in ethanol and heated to reflux for 16 hours. This procedure was attempted for unsubstituted hydroxymaleimide **296** with the crude tosyl intermediate **344** being formed in 68% yield. The presence of the tosyl group was confirmed by the characteristic peaks for the S=O stretch in the IR spectrum at 1327 and 1178 $cm^{-1}$. The disappearance of the hydroxy peak in the NMR section was also observed along with the appearance of a 3H singlet and four more aromatic protons. This material was found to be quite unstable on heating or on prolonged contact with water and so was not fully purified or characterised. Unfortunately, when treated with hydrazine hydrate in ethanol and heated to reflux for 16 hours only deprotected starting material **296** was recovered (Scheme 4.3.29).

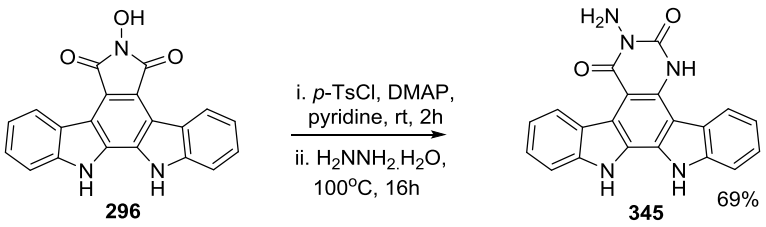



**Scheme 4.3.29**

The reaction was repeated using distilled ethanol, with flame-dried glassware and under nitrogen with the expectation that excluding as much water as possible would slow down degradation, giving more time for the hydrazine to attack. The reaction was also repeated with 10 equivalents of hydrazine hydrate. A final attempt was made using the original conditions but at a lower temperature of 60 °C. In all attempts, however, only hydroxymaleimide **296** was recovered; an unexpected result due to the similarity between this compound and the azaindole analogues which had previously been successful.

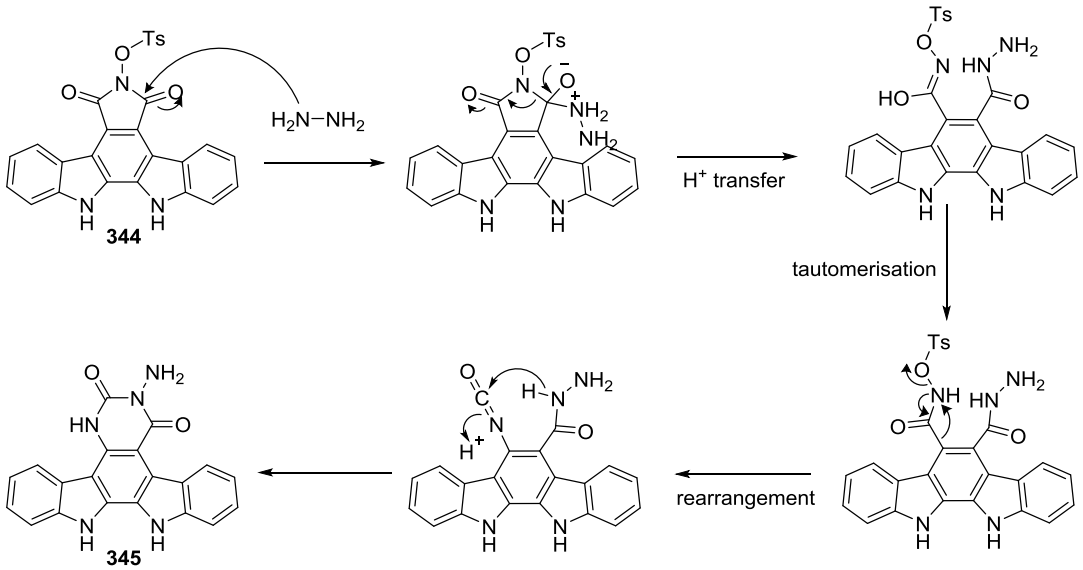
The procedure was then attempted from the mesylate ester with the expectation that the more stable intermediate would take longer to convert back to the hydroxymaleimide and also that the smaller group would result in less hindrance of the carbonyl group to allow faster attack of the hydrazine. However, again, only hydroxymaleimide **296** was recovered.

There are several reports in the literature of base-catalysed Lossen rearrangements, where the reaction is catalysed by a tertiary amine and so it was postulated that the pyridine and DMAP used in the tosylation reaction might be enough to catalyse the rearrangement. A suspension of hydroxymaleimide **296** was treated with DMAP and tosyl chloride as before and after stirring for 2 hours, hydrazine hydrate was added to the yellow solution and the reaction was heated to 80 °C for 16 hours. The solvent was then evaporated under reduced pressure and the residue suspended in ethanol. The grey precipitate was collected by vacuum filtration and washed with ethanol to give desired indolocarbazole with a 6-membered F-ring **345** in 69% yield (Scheme 4.3.30) as an off-white solid.



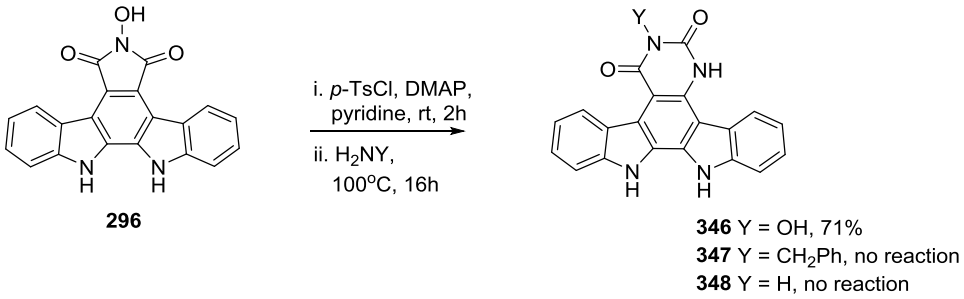

**Scheme 4.3.30**

The mechanism is proposed to proceed *via* initial nucleophilic attack of the hydrazine on one of the carbonyl groups, followed by ring opening to give a hydroxamic acid derivative on one side. This can then undergo Lossen rearrangement where the R group attached to the carbonyl migrates to the nitrogen as the tosylate ion leaves, forming an isocyanate intermediate. Nucleophilic attack of the amide nitrogen on the isocyanate reforms the ring, giving the uracil (Scheme 4.3.31).




**Scheme 4.3.31**

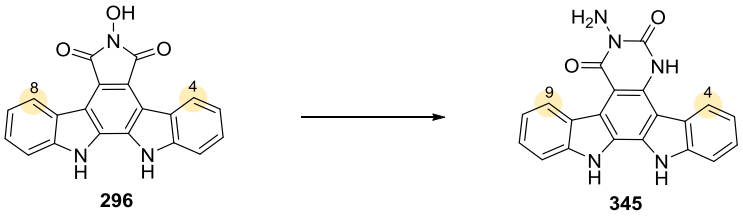
The reaction was then carried out under the same conditions with hydroxylamine hydrochloride replacing hydrazine hydrate to give 3-hydroxy uracil **346** in 71% yield (Scheme 4.3.32). The reaction was also attempted using benzylamine to form the benzyl uracil **347** and also using a 7N solution of ethanolic ammonia to form the unsubstituted uracil **348** but, in both cases, only hydroxymaleimide starting material **296** was recovered. This suggests that a strong nucleophile is needed to react with the tosyl intermediate first, before it degrades back to starting material.




**Scheme 4.3.32**

### *4.3.5.2 Spectroscopic properties*

The $^{1}$H NMR spectra of hydroxymaleimide **296** and amino uracil **345** are stacked for comparison (Figure 4.3.8). Conversion of the hydroxymaleimide to a uracil has a dramatic effect on the protons nearest the F-ring on the benzene ring (highlighted in orange). The shape of the 6-membered ring pushes the carbonyl group even closer to the C(9)-H proton, increasing the influence of the anisotropic effect and further deshielding the proton. Conversely, on the other side the carbonyl group has now moved further away from the C(4)-H proton reducing the effect and as a result, the proton is less deshielded. The other protons are affected to a much lesser extent.



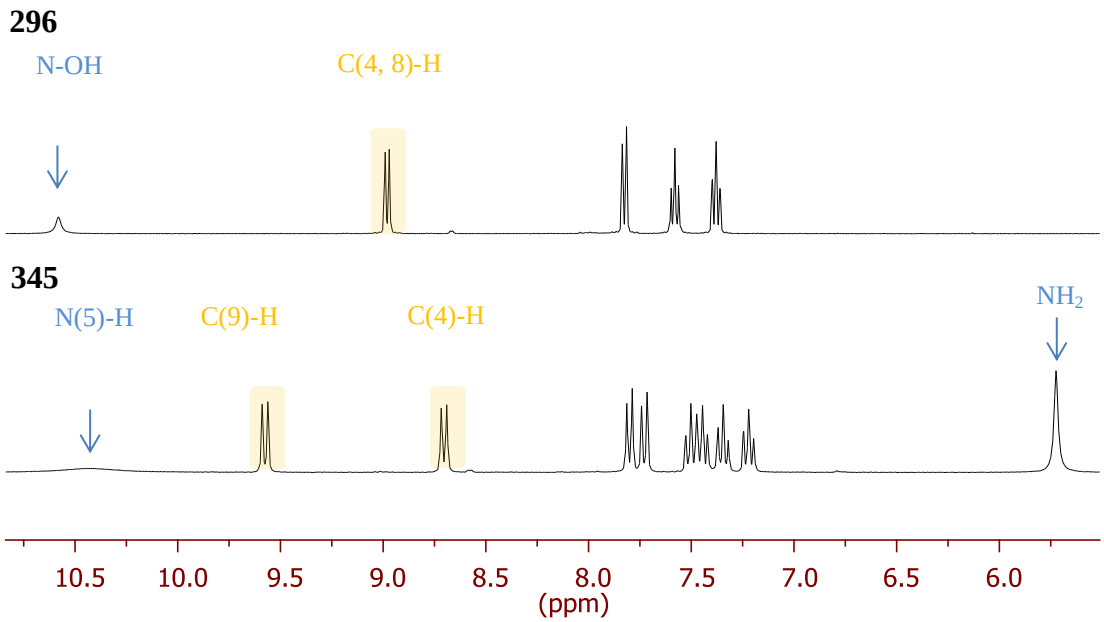
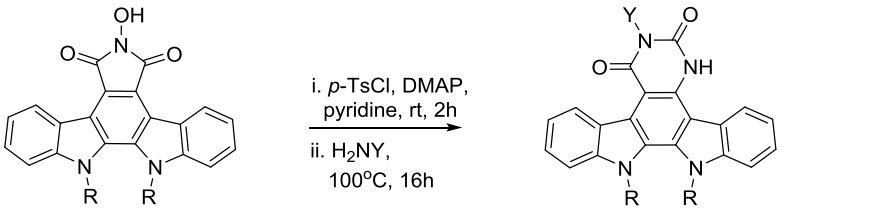




**Figure 4.3.8: Stacked $^{1}$H NMR spectra of** 296 **and** 345 **in DMSO-$d_6$ at 300 MHz**

The rearrangement was also carried out on the bis-methyl hydroxymaleimide **305** to form the 3-amino **349** and 3-hydroxy uracils **350** in 87% and 57% yields respectively (Scheme 4.3.33). The bis-hexanenitrile hydroxymaleimide **334** was also reacted with hydrazine hydrate to give the 3-amino uracil **351** in 61% yield. Due to a lack of hydroxymaleimide starting material **334** the 3-hydroxy uracil was not synthesised.



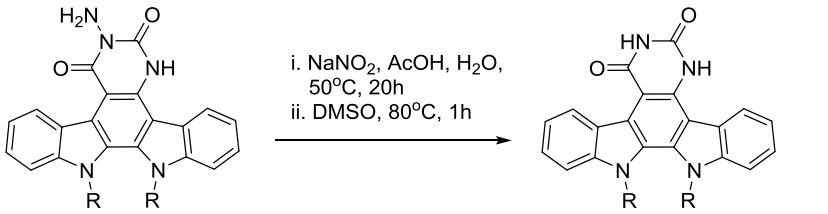

**305** R = Me
**334** R = $(CH_2)_5CN$

**349** R = Me, Y = $NH_2$, 87%
**350** R = Me, Y = OH, 57%
**351** R = $(CH_2)_5CN$, Y = $NH_2$, 61%

**Scheme 4.3.33**

### *4.3.5.3 Synthesis of unsubstituted uracils*

3-Amino uracil **345** was then deaminated to the unsubstituted analogue using nitrous acid. The amino uracil was initially treated with 5 equivalents of sodium nitrite in a 50:50 mixture of acetic acid and water and stirred at 50 °C for 16 hours. The crude proton NMR spectrum showed a mixture of products had formed with some starting material remaining. However, it was found that heating the DMSO-$d_6$ NMR sample in the NMR tube converted everything besides the unreacted starting material to the desired product **348**. As nitrous acid degrades quickly when heated, the reaction was repeated with the addition of a 2 further portions of sodium nitrite after 2 hours and again after 4 hours before being allowed to stir for a further 16 hours. This time, analysis of the heated proton NMR sample showed the reaction had gone to completion and so the remaining solid that had been collected by vacuum filtration from the reaction was suspended in DMSO and heated to 80 °C for 1 hour, resulting in a brown solution. Water was then added to precipitate the product which was collected by vacuum filtration in 85% yield. This reaction was repeated for the bis-methyl **349** and bis-hexanenitrile 3-amino uracils **351** to form uracils **352** and **353** in 86% and 61% yields, respectively (Scheme 4.3.34).

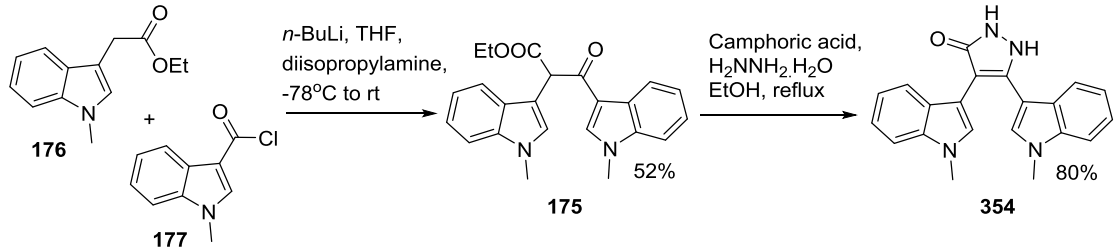



**Scheme 4.3.34**

## 4.4 Synthesis and derivatisation of novel methyl 2,3-di(1*H*-indol-3-yl)-3-oxopropanoates

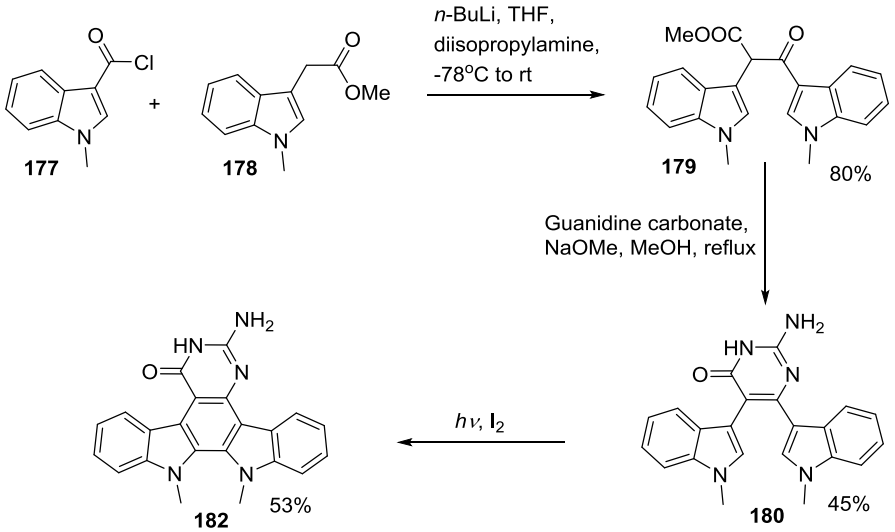
In 2006, Braña *et al*. reported the synthesis of β-keto ester **174** *via* base mediated coupling of ethyl ester **175** and acid chloride **176** in the presence of LDA. From this intermediate, a series of pyrazolones similar to **354** were formed through treatment with

the appropriate hydrazine in the presence of camphoric acid (Scheme 4.4.1).[17] The authors also reported that these bisindolyl pyrazolones were found to have a moderate anti-proliferative activity.




**Scheme 4.4.1**

Concurrently, work within our group developed a similar route, utilising methyl ester **177** in place of ethyl ester **175** and increasing the yield of β-keto ester **178** formation to 80%.[18] Condensation of this versatile intermediate with guanidine gave isocytosine **179** with photochemical cyclisation giving the indolocarbazole **181** (Scheme 4.4.2).[19] On assessment *via* the NCI-60 cell line screen, this indolocarbazole reduced growth to less than 50% in over 25 cell lines at a concentration 10 μM, with many displaying $GI_{50}$ values in the low micromolar range.



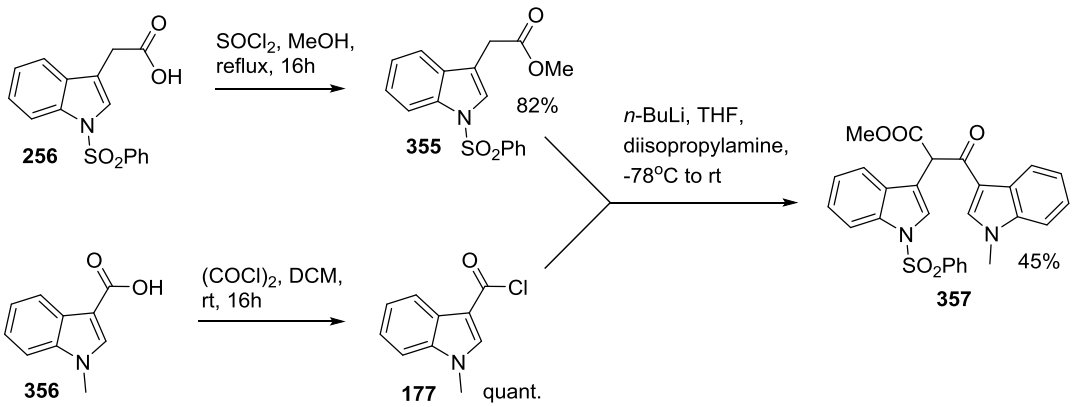

**Scheme 4.4.2**

The first of the two main goals for this section was to synthesise a fully aromatic indolocarbazole analogue of pyrazolone **354** as, to date, only bisindole analogues have

been reported. The second goal involved the incorporation of a hexane nitrile *N*-substituent into isocytosine **181**, due to the success of compounds with this side chain in the hydroxymaleimide series in the NCI-60 cell line screen.

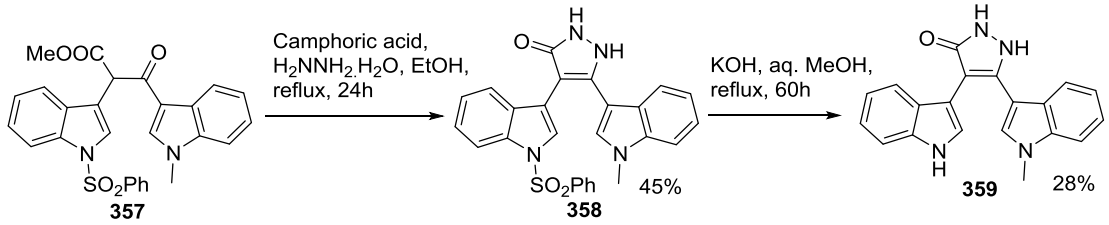
### 4.4.1 Synthesis of 13-methyl-5,6,12,13-tetrahydro-7*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*] carbazol-7-one

The methyl ester of benzenesulfonyl protected indole-3-acetic acid **256** was synthesised by treating a solution of the acid in distilled methanol with thionyl chloride to give the ester **355** as a pale yellow oil in 82% yield. The acid chloride **176** of *N*-methyl indole-3-carboxylic acid **356** was formed *via* treatment with oxalyl chloride as an off-white solid in quantitative yield. The β-keto ester **357** was then formed using the same methodology described by Braña *et al*. and previously used within the group. A solution of ester **355** in THF was added to a solution of LDA in THF under a nitrogen atmosphere while maintaining the temperature at -78 °C during addition and for a further 1 hour. A suspension of the acid chloride **176** in THF was then added and the reaction was allowed to warm slowly to room temperature over 16 hours. After work-up and purification using column chromatography followed by recrystallisation from ethyl acetate the β-keto ester **357** was isolated as a white solid in 45% yield (Scheme 4.4.3). In preparation for this reaction, the usual precautions to eliminate water from the reaction were observed e.g. flame-drying of apparatus, use of freshly distilled solvents etc. The LDA was prepared *in situ* as it was found this gave more consistent results than commercial LDA.




**Scheme 4.4.3**

The β-keto ester **357** was then reacted with hydrazine hydrate in a cyclocondensation reaction to form pyrazolone **358** in 45% yield (Scheme 4.4.4). Deprotection was initially attempted using potassium carbonate in refluxing aqueous methanol, but after 24 hours proton NMR analysis showed that only starting material was present. The stronger base potassium hydroxide was then employed. As the protected and deprotected materials overlapped completely on TLC analysis, the reaction had to be monitored by proton NMR analysis. After 24 hours, analysis showed a mixture of starting material and product and so the reaction was continued. After 48 hours, some starting materials still remained and some unwanted side products had begun to form. However, as the starting material and product would not be separable by column chromatography the reaction was continued until, after a total of 60 hours, the starting material had been completely consumed. After work-up and purification using column chromatography, the deprotected pyrazolone **359** was isolated as a light brown solid in 28% yield.

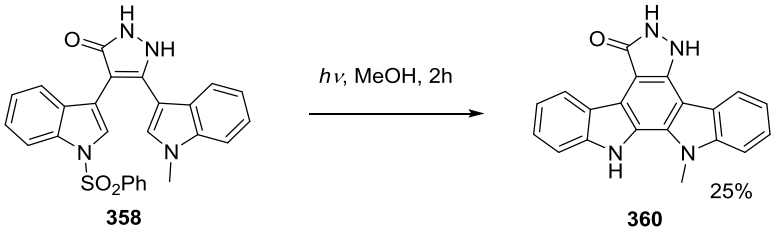



**Scheme 4.4.4**

Several other deprotecting strategies were attempted to increase the yield of this step. Deprotection using magnesium and ammonium chloride in methanol also gave a complex mixture of products that could not be separated. It was suspected that the strongly basic conditions of this reaction were too harsh for the pyrazolone and degradation of this was leading to the formation of side-products. Deprotection using milder TBAF in THF gave no reaction.

It has been reported in the literature that removal of a benzenesulfonyl group can be achieved in a light-mediated reaction.[20] As the end goal of this route was to synthesis the indolocarbazole **360** in a photocyclisation step, it was theorised that it might be possible that both transformations could be carried out simultaneously. In contrast to the photochemical reactions previously carried out in this project, the literature procedure

was carried out in the absence of iodine and, as deprotection was the original aim, initial attempts were carried out without iodine. Pyrazolone **358** was dissolved in methanol and irradiated using a standard high pressure mercury bulb for 2 hours, until TLC analysis indicated the complete consumption of starting material and the appearance of a new spot which fluoresced brightly when viewed under U.V. light, a characteristic of the indolocarbazoles. Following work-up and recrystallisation from MeOH/DCM, pyrazolone **360** was isolated in 25% yield (Scheme 4.4.5) and the absence of the C(2)-H proton signals from the $^{1}$H NMR spectrum confirmed cyclisation to the indolocarbazole.




**Scheme 4.4.5**

The $^{1}$H NMR spectra of bisindolyl pyrazolone **359** and the indolocarbazole analogue **360** are stacked for comparison (Figure 4.4.1). Again, the protons most affected by the transformation are those on the benzene rings which are closest to the F-ring, with the proton below the carbonyl group being particularly deshielded due to the anisotropic effect and is shifted downfield by approximately 1.6 ppm, consistent with other indolocarbazoles. The other protons are also shifted slightly downfield.

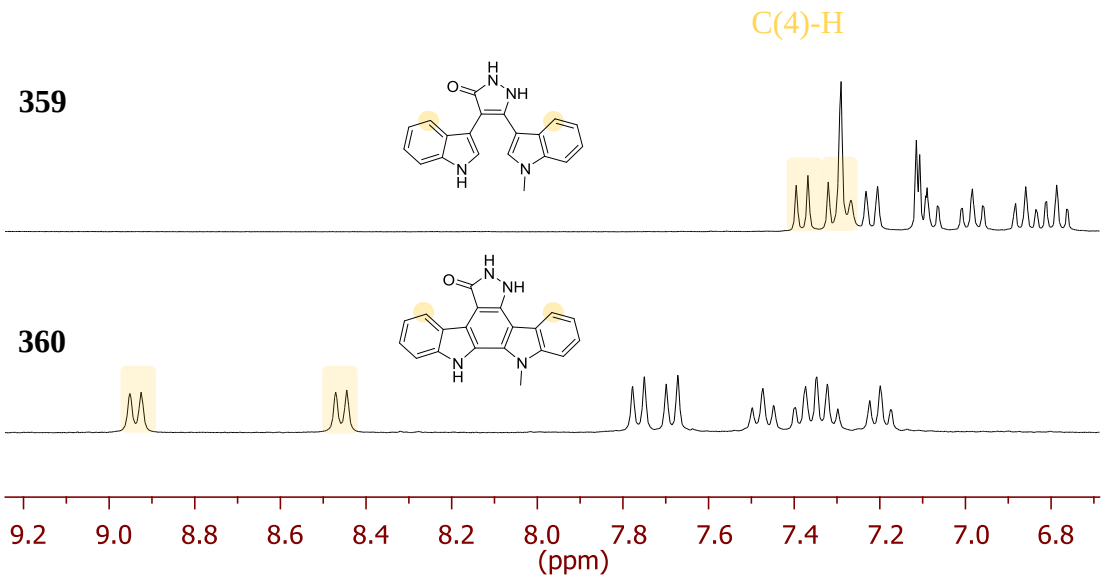
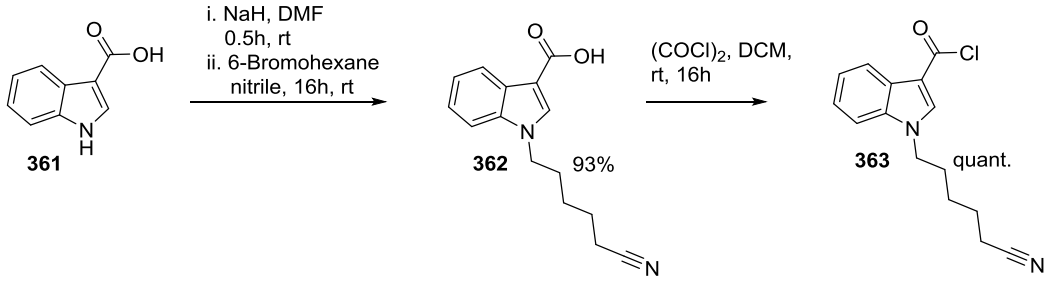



**Figure 4.4.1: Stacked proton NMR spectra for bisindole** 359 **and indolocarbazole** 360

### 4.4.2 Synthesis of 6-[6-amino-13-methyl-8-oxo-8,13-dihydroindolo[2,3-*a*]pyrimido [4,5-*c*]carbazol-14(7*H*)-yl]hexanenitrile

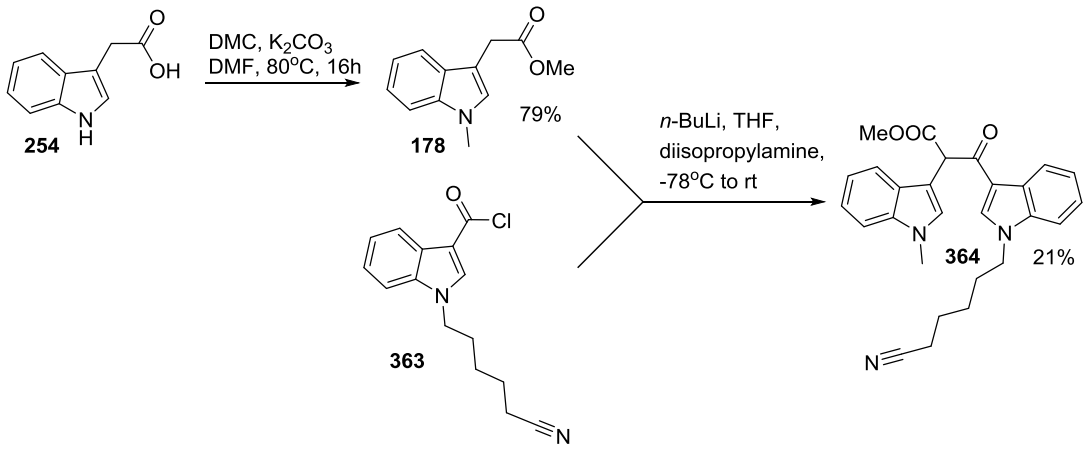
Due to the success of fully cyclised isocytosine **181** in NCI testing it was decided to synthesise a derivative of this compound with a hexane nitrile *N*-substituent replacing one of the methyl groups.

Indole-3-carboxylic acid **361** was alkylated using 2 equivalents of sodium hydride followed by 6-bromohexanenitrile to give alkylated product **362** in 93% yield as a white solid. This was then converted to the acid chloride **363** *via* treatment with oxalyl chloride (Scheme 4.4.6).




**Scheme 4.4.6**

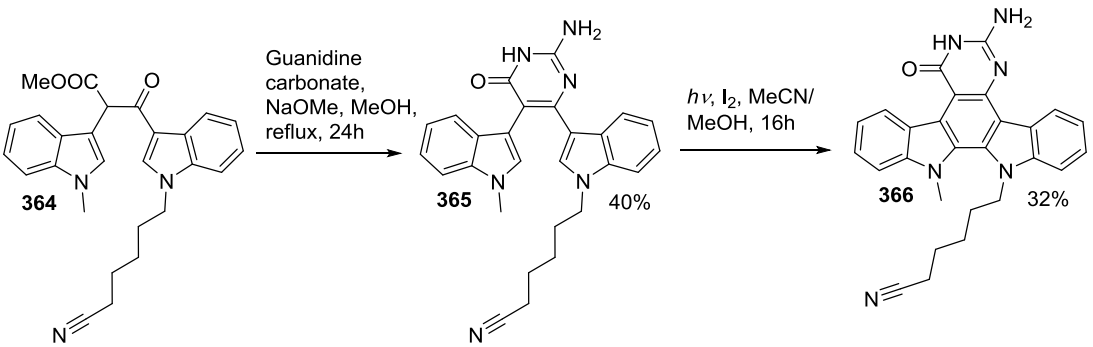
The *N*-methyl ester of indole-3-acetic acid **254** was synthesised following treatment with dimethyl carbonate with potassium carbonate in DMF giving the ester as a light brown oil **177** in 79% yield. The ester **177** and acid chloride **363** were then coupled using a similar procedure to that described above in Scheme 4.4.3, giving β-keto ester **364** as a pale yellow foamy solid in 21% yield (Scheme 4.4.7).




Scheme 4.4.7

To form the desired isocytosine, guanidine carbonate was initially stirred with sodium methoxide to liberate free guanidine from the salt and the β-keto ester **364** then underwent cyclocondensation with the guanidine in refluxing methanol. Following work-up and purification using column chromatography, the isocytosine **365** was formed in 40% yield as a pale yellow solid (Scheme 4.4.8).

Photocyclisation of the newly formed isocytosine was then achieved using identical conditions to bis-methyl analogue **181**, synthesised previously in the group. Isocytosine **365** was irradiated with a high pressure mercury lamp for 16 hours with a catalytic amount of iodine resulting in the formation of fully aromatic isocytosine **366** which was isolated in 32% yield.




Scheme 4.4.8

Although the formation of isocytosine **365** was confirmed by proton NMR and mass spectrometry data, the $^{13}C$ NMR appeared to be missing 4 quaternary carbon signals. NMR analysis of the sample was then run on a more powerful instrument at 150 MHz with an increased pulse time delay (τ) of 7 seconds resulting in the appearance of 4 weak, broad peaks at 103.2, 113.4, 159.0 and 163.7 ppm (Figure 4.4.2).

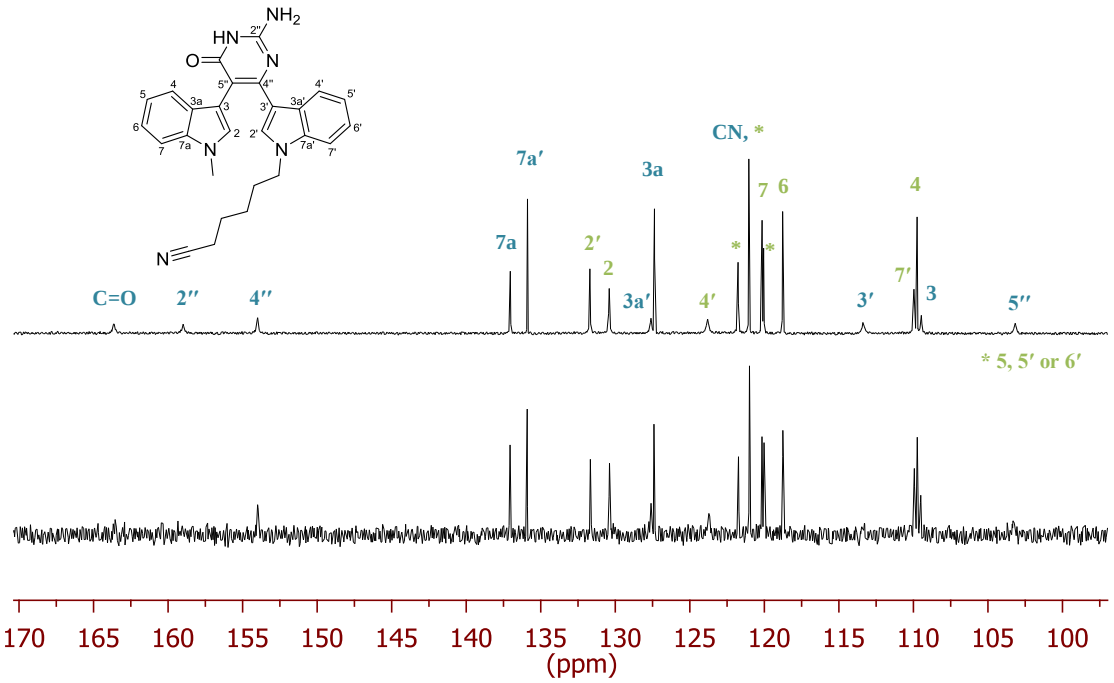



**Figure 4.4.2: Expansion of $^{13}C$ NMR spectra of 365 comparing spectrum run at 150 mHz and τ = 7 s (top) with original spectrum run at 75 mHz and τ = 1 s (bottom)**

2D NMR experiments were then carried out to identify the origin of these peaks. All CH signals were assigned with the aid of DEPT and HSQC experiments (5, 5′ and 6′ may be interchanged due to overlapping proton signals). A HMBC experiment was then used to assign the quaternary carbons (Figure 4.4.3 to Figure 4.4.6).

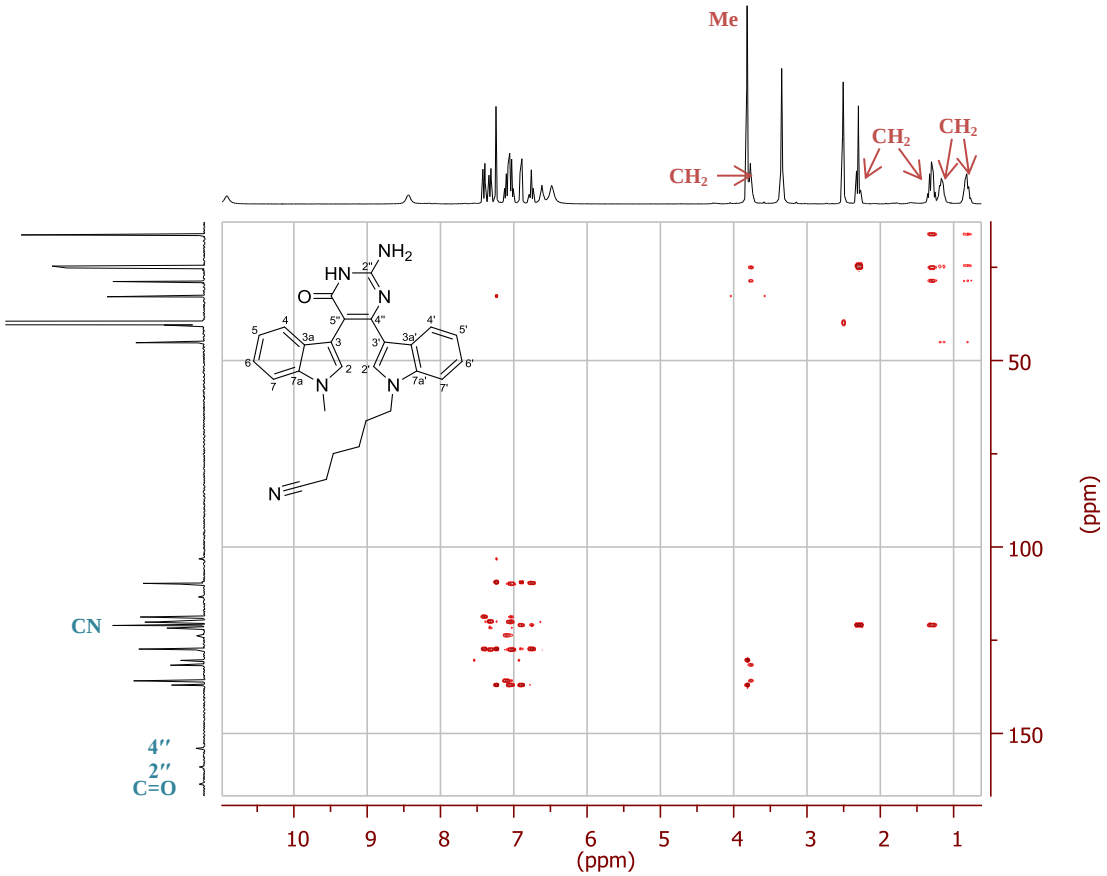



**Figure 4.4.3: 2D HMBC spectrum of** 365

The peak at 163.7 was assigned as the carbonyl carbon and the peak at 121.04 was assigned as the nitrile carbon as it showed a correlation to the neighbouring $CH_2$ protons. C(5″) of the isocytosine ring is identified by its correlation to C(2)-H. C(4″) and C(2″) of the isocytosine do not show any correlations, however it is possible to deduce that C(2″) is the most deshielded of the two as it is bonded to three heteroatoms whereas C(4″) is just bonded to one. The bridgehead carbon C(7a) is easily identified as it shows a strong correlation to the methyl protons, as well as showing correlations to C(2, 5, 6 and 7)-H. Similarly, C(7a′) shows a strong correlation to the neighbouring $CH_2$ protons, and also to C(2′, 5′ and 6′)-H (see expansion for aromatic region in Figure 4.4.4). The bridgehead carbon C(3a′) shows a correlation to C(2′, 5′ and 7′)-H, all of which are three bonds away, and also C(6′)-H at four bonds distance. The opposing bridgehead carbon C(3a) shows a correlation to C(2, 4, 6 and 7)-H (see expansion for 3a and 3a′ in Figure 4.4.5). C(3) shows a correlation to C(2)-H and also to C(7)-H (see expansion for 3 in Figure 4.4.6). On the other ring, C(3′) shows a correlation to C(2′)-H.

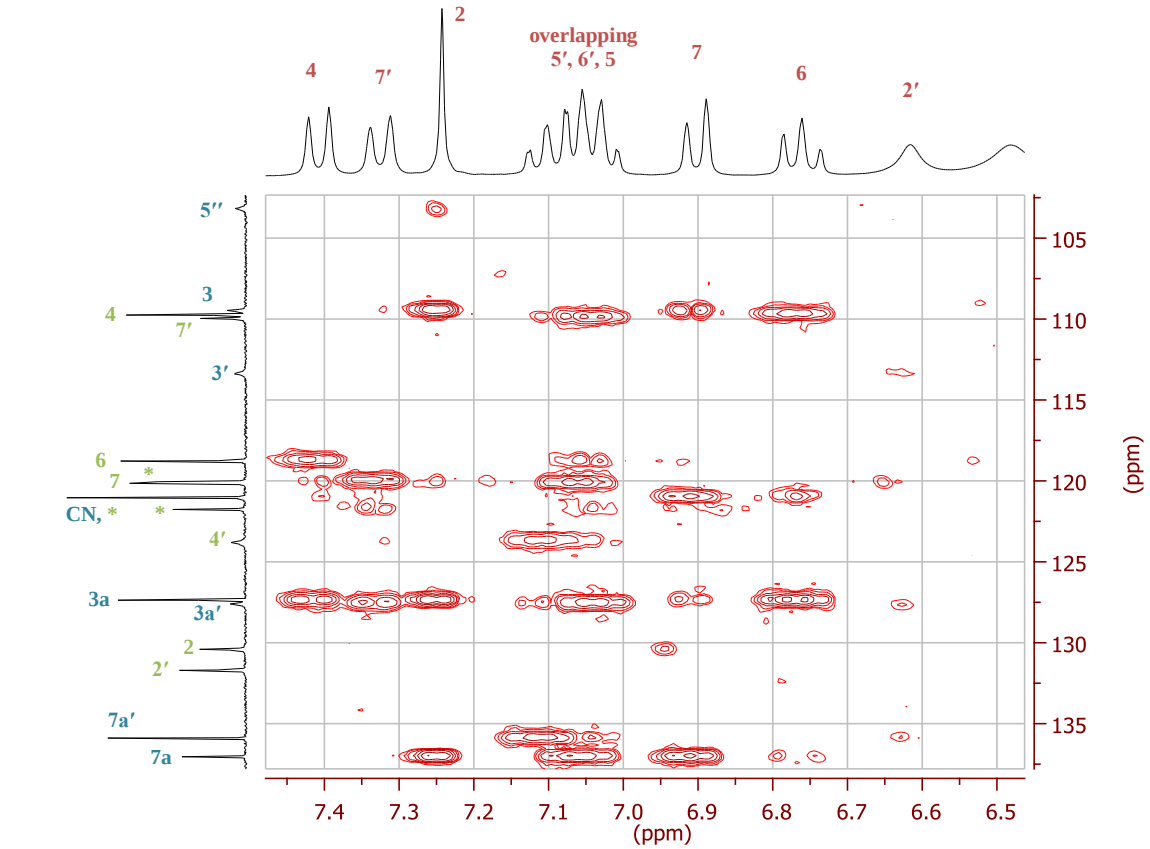



**Figure 4.4.4: Expansion of aromatic region in HMBC spectrum of** 365

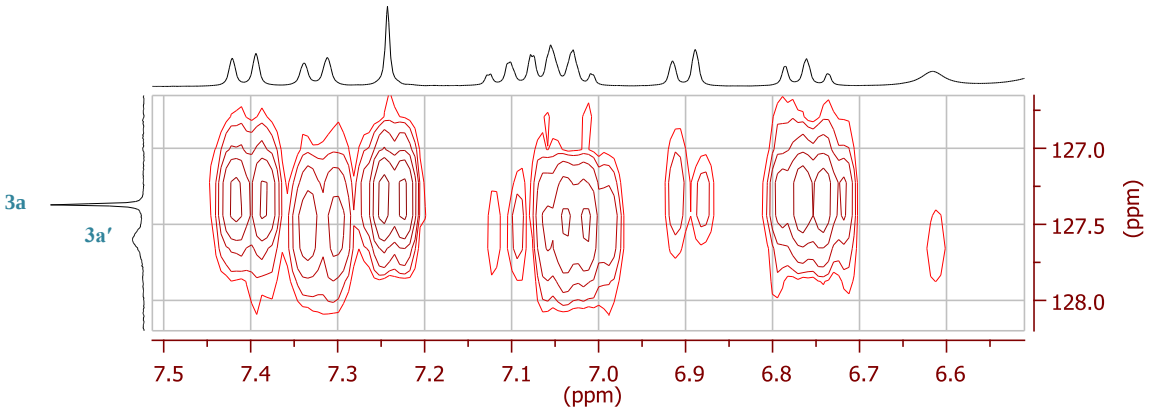



**Figure 4.4.5: Expansion of C(3a) and C(3a′) in HMBC spectrum of** 365

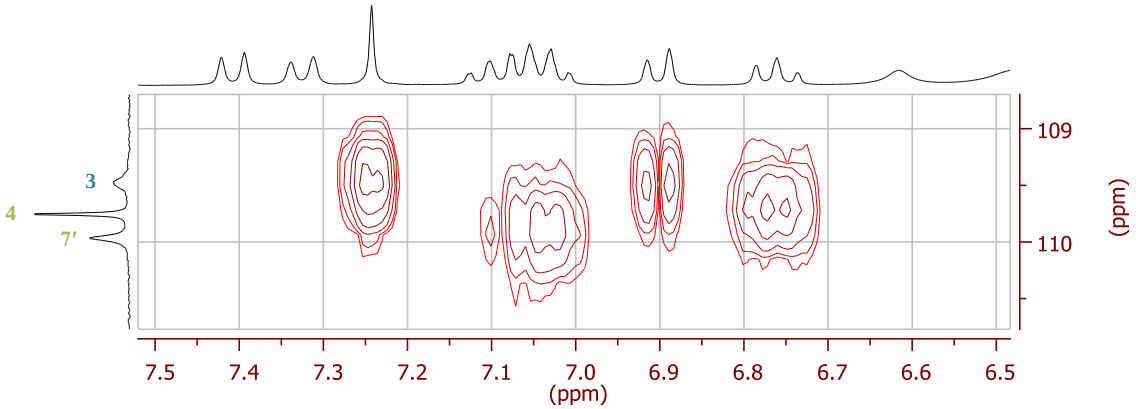



**Figure 4.4.6: Expansion of C(3) in HMBC spectrum of** 365

From the HBMC experiment it was shown that the weak, broad peaks corresponded to carbons on the isocytosine moiety or those of neighbouring carbons.

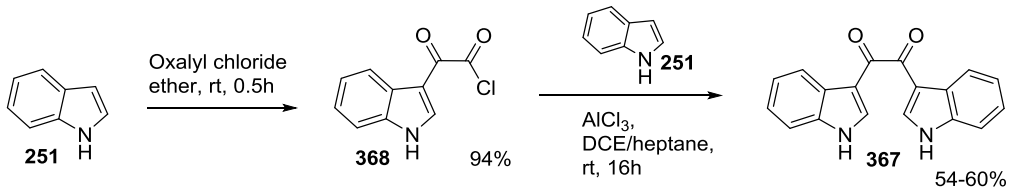
## 4.5 Routes towards 4,5-bisindolylimidazoles

A route towards the synthesis of imidazoles as novel F-ring heterocycles from a bisindolyl-1,2-ethanedione intermediate was also explored. The potential advantages of this route were the ability to use non-protected indoles and the very short synthesis to the dione intermediates.

### 4.5.1 Synthesis of 3,3'-(1*H*-imidazole-4,5-diyl)bis(1*H*-indole)

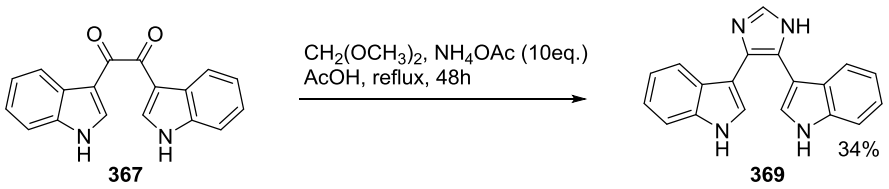
The dione intermediate **367** was formed by initially treating a solution of indole **251** in diethyl ether with oxalyl chloride. The yellow precipitate was collected after 30 minutes by vacuum filtration to give oxoacetyl chloride **368** in 94% yield. This reactive intermediate was always used immediately in the next step to avoid degradation. The oxoacetyl chloride **368** was then added portionwise to a suspension of aluminium chloride in a mixture of 1,2 dichloroethane and heptane, forming a dark red heterogeneous mixture. A solution of indole **251** in 1,2-dichloroethane was then added dropwise, with vigorous stirring to ensure mixing of the viscous reaction mixture and stirring was continued for 16 hours. It was found that on scale-up the yield of the reaction was reduced so the magnetic stirrer was replaced with a more efficient

overhead stirrer, which reliably maintained the yield of **367** in the range of 54-60% (Scheme 4.5.1).




**Scheme 4.5.1**

As part of our diversity orientated synthetic strategy, the dione intermediate could then be used to form a bisindolyl imidazole using a three-component reaction in one pot. Dione **367** was treated with 10 equivalents of ammonium acetate and one equivalent of formaldehyde dimethyl acetal in acetic acid which was heated to reflux for 48 hours. Column chromatography on silica gel using DCM/methanol as eluent was unsuccessful, due to the very long elution time leading to coelution of multiple products. Column chromatography on alumina, however, proved successful with imidazole **369** being isolated as a dark brown solid in 34% yield (Scheme 4.5.2). Attempts to increase the yield by increasing the number of equivalents of formaldehyde dimethyl acetate from one equivalent to five were unsuccessful with the yield remaining the same. The reaction was also repeated at a lower temperature of 100 °C in an attempt to reduce the number of side products formed, however only starting material was recovered.




**Scheme 4.5.2**

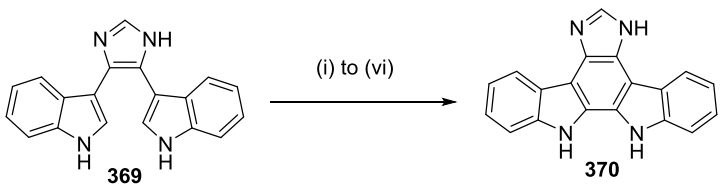
## 4.5.2 Derivatives of 3,3'-(1*H*-imidazole-4,5-diyl)bis(1*H*-indole)

### *4.5.2.1 Oxidative cyclisations*

The next target was the fully aromatic analogue **370**. Initially cyclisation attempts were focussed on the direct cyclisation of novel imidazole **369**. This was initially attempted using the photocyclisation method, which had proved successful for the cyclisation of other bisindolyl compounds. Irradiation of a solution of **369** in a mixture of acetonitrile and methanol with a catalytic amount of iodine for 16 hours gave a complex mixture of products and, although analysis using mass spectrometry showed that some of the desired product **370** had formed, isolation using column chromatography on silica gel or alumina was unsuccessful due to coelution of multiple products.

As the addition of iodine made the already dark coloured solution much darker, it was suspected that insufficient light was passing through the reaction. These cyclisation reactions can sometimes be successful in the absence of iodine and so the reaction was repeated without the iodine catalyst. Although analysis of the crude material by proton NMR suggested a higher conversion of the bisindolyl compound **369** to the indolocarbazole **370** purification was, again, unsuccessful. Attempted cyclisation of the compound using heat in the absence of the light source was also unsuccessful and only starting material was recovered in this case.

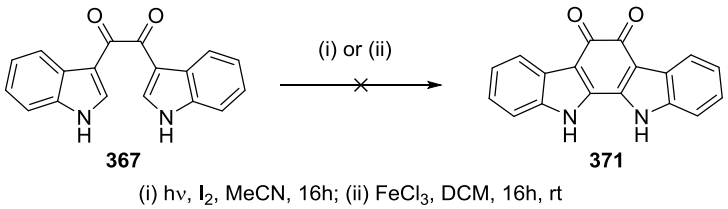
Attention then turned to a palladium based method. Palladium (II) acetate was heated to reflux in a solution of the bisindolyl imidazole **369** in acetic acid for 3 hours. Analysis using proton NMR and mass spectrometry indicated the desired product had formed but, again, the same issues with isolation arose. Two more oxidative reactions were attempted using manganese dioxide and using DDQ but unfortunately similar complex mixtures were formed.



| Reaction No. | Oxidant | Stoichiometry | Conditions | Product |
|---|---|---|---|---|
| i | hν/$I_2$ | 0.1 eq. | $CH_3CN$/MeOH (1:1) reflux | complex mixture |
| ii | hν | - | $CH_3CN$/MeOH (1:1) reflux | complex mixture |
| iii | Δ | - | $CH_3CN$/MeOH (1:1) reflux | starting material |
| iv | $Pd(OAc)_2$ | 1 eq. | AcOH, 120 °C | complex mixture |
| v | $MnO_2$ | 10 eq. | Acetone, r.t. | complex mixture |
| vi | DDQ | 1.2 eq. | TFA, reflux | complex mixture |

**Table 4.5.1: Summary of cyclisation study for synthesising indolocarbazole** 370 **from bisindolyl** 369

Oxidation of dione **367** to form indolocarbazole **371** was also attempted as it was envisaged that the imidazole forming reaction would be more successful if the two carbonyl groups were fixed in the same orientation. However, attempted cyclisations with either a photochemical reaction or using iron (III) chloride were both unsuccessful (Scheme 4.5.3).




**367** **371**

(i) hν, $I_2$, MeCN, 16h; (ii) $FeCl_3$, DCM, 16h, rt

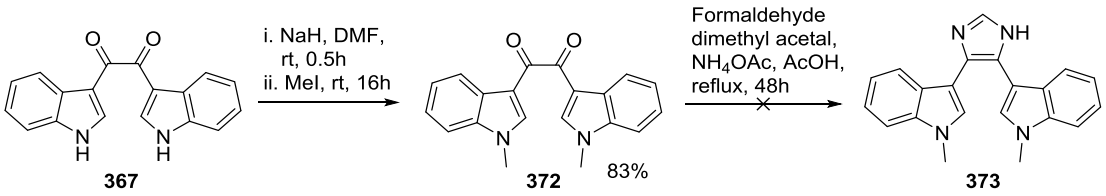
**Scheme 4.5.3**

### *4.5.2.2 N-substitution*

Work then turned towards the synthesis of novel imidazole compounds with *N*-substituted indoles with the intention of probing the effects of this change on the biological activity. A bis-methyl substituted dione **372** was first synthesised by treating the unsubstituted dione **367** with sodium hydride followed by iodomethane. Following

work-up and purification using column chromatography gave dione **372** as a brown solid in 83% yield (Scheme 4.5.4).

This *N*-methylated dione was then treated with formaldehyde dimethyl acetal and ammonium acetate in acetic acid in an attempt to form the imidazole **373**, but only starting material was recovered from the reaction. The reaction was repeated using a fresh bottle of formaldehyde dimethyl acetal; however the same result was obtained. It is possible that the presence of the two methyl groups prevents the compound from achieving the correct orientation for the reaction to occur.




**Scheme 4.5.4**

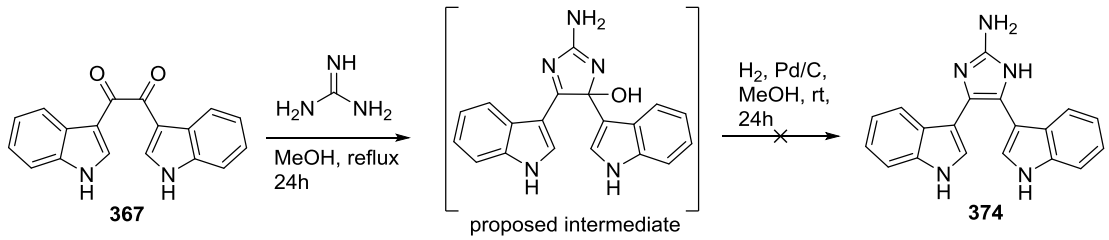
To further demonstrate the capricious nature of this reaction, the conversion of the unsubstituted dione **367** to the imidazole **369** was unsuccessful when the reaction was repeated some time later. Attempts were then made using fresh reagents, with a longer reaction time and with dried solvent; however, each time a complex mixture along with some starting material was recovered, proving that more robust conditions were required.

### 4.5.3 Alternative routes

As imidazoles formed by the three-component condensation reaction were prone to difficulties with isolation and proved unsuccessful with *N*-substituted indoles, several other routes to imidazoles were explored.

#### *4.5.3.1 Reaction with guanidine*

A procedure to form an amino imidazole from an aromatic (non-indole) diketone precursor through condensation with guanidine *via* a proposed hydroxy imidazole intermediate has been reported by Nishimura *et al*.[21] Guanidine was first liberated from its carbonate salt by treatment with sodium hydroxide. The isolated guanidine was then added to a solution of dione **367** in methanol and the reaction was heated to reflux for 16 hours. The precipitated solid was collected using vacuum filtration and due to a lack of solubility was used without further purification or characterisation in the next step. This intermediate was then suspended in methanol and 10% Pd/C was added and the suspension was hydrogenated at 10 psi for 24 hours. After filtration through Celite to remove the remaining catalyst, the reaction mixture was evaporated to dryness under reduced pressure. Analysis using proton NMR and mass spectrometry showed none of the desired product **374** had formed (Scheme 4.5.5).

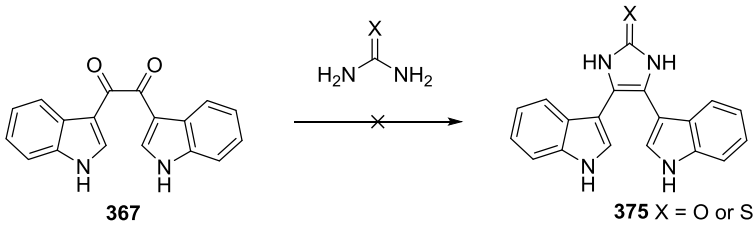



**Scheme 4.5.5**

#### *4.5.3.2 Condensation with ureas*

Work towards the condensation of dione **367** with urea was then undertaken. Several different reaction additives were selected from the literature including camphorsulfonic acid, *p*-tosic acid, zinc triflate and pyridine (Table 4.5.2) but in all cases only starting material was recovered with none of the desired product formed **375**. The reaction was

also attempted using the more nucleophilic thiourea using trifluoroacetic acid and using sodium methoxide but, again, only starting material was recovered.




**367** **375** X = O or S

| Reaction No. (X) | Stoichiometry (eq. of urea) | Additive | Conditions | Product |
|---|---|---|---|---|
| **1 (O)** | 1.3 | CSA (1 eq.) | Ethanol reflux 24 hours | starting material |
| **2 (O)** | 5 | *p*-TsOH (1 eq.), | Acetonitrile, reflux, $N_2$, 4 Å mol. sieves, 24 hours | starting material |
| **3 (O)** | 1.5 | $Zn(OTf)_2$, (0.2 eq.) | Acetonitrile, reflux, $N_2$, 24 hours | starting material |
| **4 (O)** | 5 | - | Pyridine, reflux, 24 hours | starting material |
| **5 (S)** | 5 | TFA (cat.) | THF, reflux, $N_2$, 24 hours | starting material |
| **6 (S)** | 5 | NaOMe (10 eq.) | Methanol, reflux, $N_2$, 24 hours | starting material |

**Table 4.5.2: Reaction conditions for the reaction of dione** 367 **with urea or thiourea**

## 4.6 Summary of synthetic work

In this work, a panel of 42 novel bisindolylmaleimide and indolocarbazole derivatives were prepared for biological evaluation. In the bisindolylmaleimide series, substitution on the indole nitrogens of maleic anhydride intermediates led to a large panel of novel hydroxymaleimides with substituents incorporating either lipophilicity or hydrophilicity (Figure 4.6.1). Development of two other routes involving a β-keto ester or dione intermediate led to the synthesis of bisindolylmaleimides with pyrazolone and imidazole F-rings, respectively.

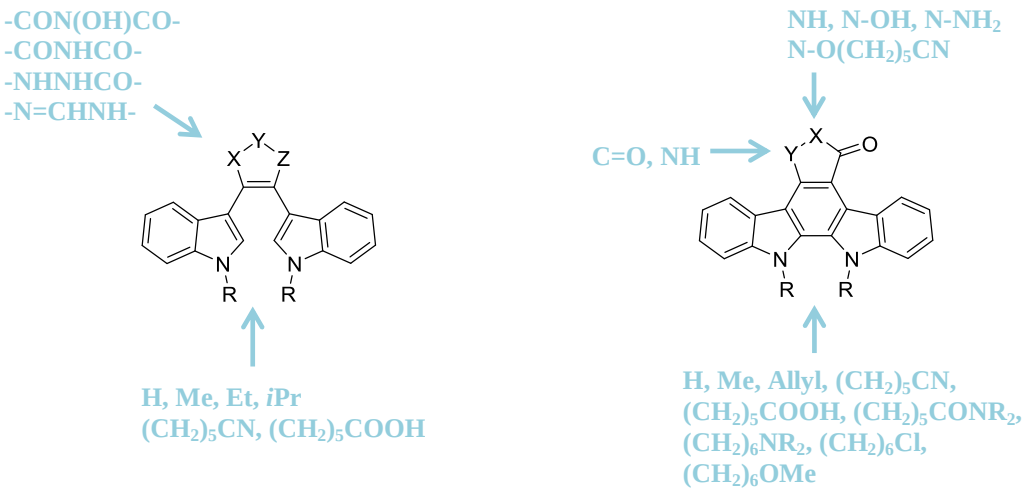



**Figure 4.6.1: Novel five-membered bisindolylmaleimide and indolocarbazole derivatives.**

Expanding on these routes to form the fully cyclised indolocarbazole analogues resulted in a panel of fifteen novel indolocarbazole derivatives with hydroxymaleimide F-rings and a diverse range of *N*-substituents, as well as a novel pyrazolone derivative (Figure 4.6.1).

The successful modified Lossen rearrangement resulted in the synthesis of eight novel indolocarbazoles with uracil F-rings and a number of different *N*-substituents (Figure 4.6.2). Synthesis of both a novel bisindolyl and indolocarbazole derivative with an isocytocine F-ring from the β-keto ester route was also achieved.

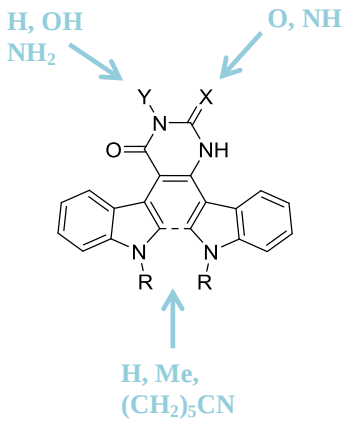



**Figure 4.6.2: Novel six membered indolocarbazoles**

The results of the biological evaluation of these compounds are discussed in Chapter 5.

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# 5 Biological Results and Discussion

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## 5.1 NCI-60 Cell Screen

In total, a panel of 42 novel potential anticancer agents were synthesised, 17 bisindolylmaleimide analogues and 25 indolocarbazoles. Anticancer activity is the most extensively studied and reported biological effect of these compounds and given the importance of anticancer medicine in modern times, this is the area that was focussed on in the evaluation of this panel of compounds.[1,2]

Prospective candidates were initially submitted to the NCI 60-cell line screen, a screening program which is run by the Developmental Therapeutics Program (DTP) of the NCI. Each compound is screened for activity against 60 human cancer cell lines including lines from cancers such as leukaemia, non-small cell lung cancer, melanoma, and cancers of the colon, CNS, ovary, kidney, prostate and breast. This gives an overall picture of the cytotoxicity of the compounds as well as any specificity of a compound towards individual cell lines. The compounds are initially tested at a single dose with a concentration of 10 μM, and any compounds with significant activity are taken forward to a five dose assay where they are screened at concentrations of 100 μM, 10 μM, 1 μM, 100 nM and 10 nM. The dose response data is then used to calculate three concentration parameters – $GI_{50}$, TGI and $LC_{50}$.

The screen was originally set up to identify compounds with growth-inhibitory or cytotoxic effects; however, it was soon found that further information could also be obtained. The patterns of relative sensitivity and resistance of known drugs tested gave an indication to their mechanism of action. As a result, the COMPARE algorithm was developed by Paull *et al*. which could be used to predict the mode of action of potential chemotherapeutic agents.[3] This is achieved by specifying the desired compound using its NCI accession number and the algorithm then ranks the entire database of tested compounds in order of similarity to the original "seed" compound based on patterns of activity. Compounds high in this ranking may possess a similar mechanism of action to that of the seed compound.

### 5.1.1 Single dose results

In this section, the results of the single dose assays are discussed for all bisindolyl and indolocarbazole compounds tested. Although the results are not as detailed as for the five dose assay, the single dose screen allows for a higher throughput of compounds and is a valuable tool for the identification of novel chemotherapeutic agents. The results are displayed as an overall mean graph of cell growth (Figure 5.1.1). The 60 cell lines are listed along the left along with the specific growth percent of each cell line measured. The central line (marked 0 on the graph) represents the mean growth across all cell lines (in this case 77.99% growth). The bars indicate deviation from the mean growth for individual cell lines, with bars extending to the right indicating selectivity for that cell line. Single dose mean graphs for all the derivatives tested can be found in the appendices.

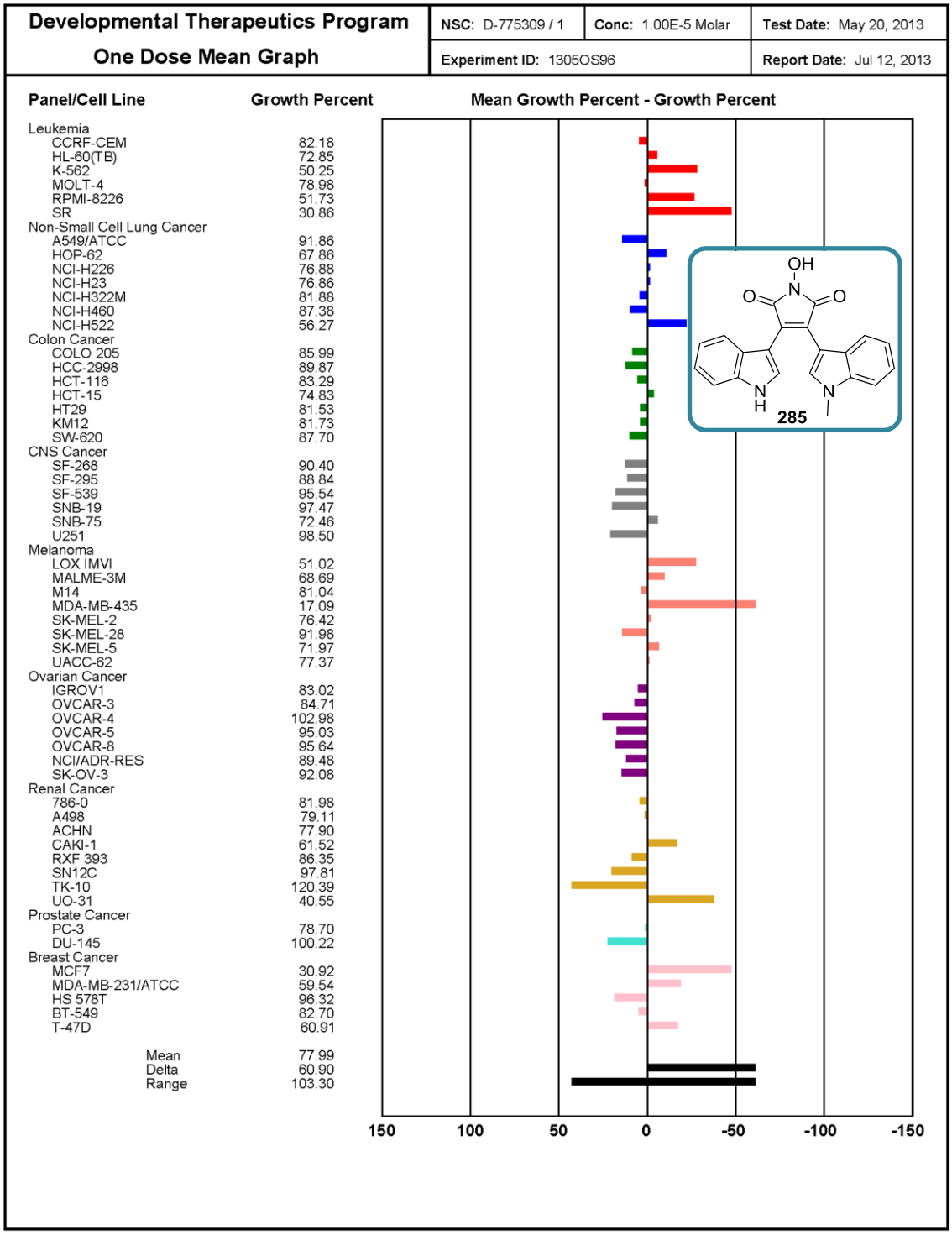
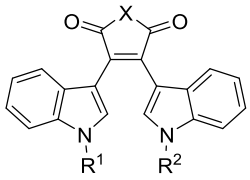



**Figure 5.1.1: Single dose data for hydroxymaleimide** 285

### *5.1.1.1 Bisindolylmaleimide derivatives*

A total of 11 related novel bisindolylmaleimide analogues were submitted for screening and the mean growth percent (mean) and deviation from the mean (delta) are displayed in Table 5.1.1.




| Compound | NSC | $R^1$ | $R^2$ | X | Mean (%) | Delta (%) |
|---|---|---|---|---|---|---|
| **285** | 775309 | Me | H | N-OH | 78.0 | 60.9 |
| **286** | 774887 | Me | Me | N-OH | 97.6 | 24.8 |
| **290** | 775308 | *i*Pr | H | N-OH | 94.4 | 37.5 |
| **291** | 775307 | *i*Pr | Me | N-OH | 90.8 | 34.4 |
| **281** | 776690 | $(CH_2)_5CN$ | H | N-OH | 85.6 | 42.4 |
| **288** | 776694 | $(CH_2)_5CN$ | Me | N-OH | 90.8 | 40.0 |
| **283** | 776692 | $(CH_2)_5COOH$ | H | N-OH | 97.9 | 36.9 |
| **289** | 776691 | $(CH_2)_5COOH$ | Me | N-OH | 96.4 | 33.1 |
| **282** | 776693* | $(CH_2)_5CN$ | $(CH_2)_5CN$ | N-OH | 67.5 | 52.4 |
| **327** | 781333 | $(CH_2)_5CN$ | $(CH_2)_5CN$ | NH | 86.9 | 39.7 |
| **284** | 781334 | $-(CH_2)_6-$ | | N-OH | 103.3 | 24.0 |

*Five dose data available

**Table 5.1.1: Mean growth over 60-cell panel on treatment with bisindolylmaleimide derivatives**

Compound **285** with one methyl *N*-substituent and one unsubstituted indole showed some activity with a mean growth of 78.0% (Figure 5.1.1). Selectivity was also observed for leukaemia cell lines (growth reduced to 30.9% in SR) and also for melanoma cell line MDA-MB-435 (17.1%), renal cancer cell line UO-31 (40.6%) and breast cancer cell line MCF7 (30.9%). Addition of a second methyl substituent significantly reduced activity with compound **286** being almost completely inactive; although some selectivity was observed for leukaemia cell line SR (75.4%), melanoma cell line LOX IMVI (72.8%) and renal cancer cell line UO-31 (74.2%). These results are similar to those for mono azaindolyl **376** (Figure 5.1.2), synthesised previously

within the group, which had a mean growth of 97.8% and also showed selectivity for SR (53.1%), LOX IMVI (83.0%) and UO-31 (66.0%).[4]

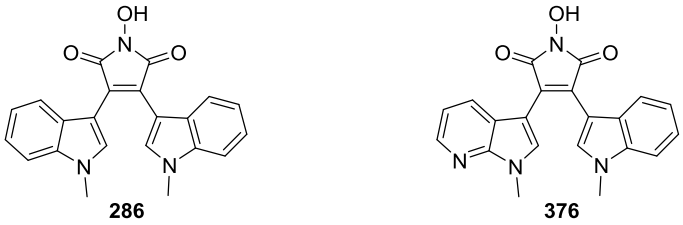



**Figure 5.1.2: Structures of compounds** 286 **and** 376

Compounds with an isopropyl substituent (**290** and **291**) were mostly inactive apart from a similar selectivity towards LOX IMVI (58.8% and 61.5%, respectively) and UO-31 (67.9% and 56.4%, respectively) and for most leukaemia cell lines.

Compounds with one extended alkyl chain with a nitrile or carboxylic acid functional group also displayed little activity with nitriles **281** and **288** being slightly more active than acids **283** and **289**. These compounds again displayed selectivity towards LOX IMVI (between 53.3% and 80.4%) and UO-31 (between 50.8 and 63.2%) and compound **281** also showed selectivity towards leukaemia cell line RPMI-8226 (43.5%) and non-small cell lung cancer cell line HOP-92 (48.0%).

Interestingly, addition of a second nitrile chain significantly increased activity with compound **282** displaying a mean growth of 67.5% and being selected to progress to five dose screening (Figure 5.1.3). This derivative exhibited considerable activity towards a number of individual cell lines, including leukaemia cell lines HL-60(TB) (15.1%) and SR (17.8%), melanoma cell lines LOX IMVI (30.4%) and MDA-MB-435 (16.0%), renal cancer cell line UO-31 (43.8%) and breast cancer cell lines MCF7 (39.9%) and MDA-MB-231/ATCC (40.3%). Replacement of the hydroxymaleimide moiety with an unsubstituted maleimide reduced activity, with compound **327** showing a mean growth of 86.9%.

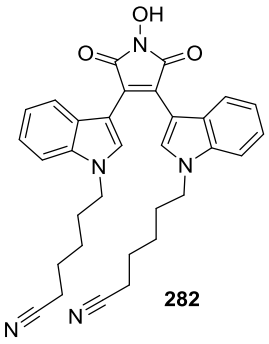



**Figure 5.1.3: Structure of hydroxymaleimide** 282

The derivative with nitrogens linked by a 6-carbon chain **284** was found to be almost completely inactive with a mean growth of 103.3% and little deviation from the mean growth across the panel. This was a rather disappointing result as maleimide analogue **377** shows significant inhibition of a number of kinases including PKC$\alpha$ ($K_i$ = 0.3 μM), p70 S6K ($K_i$ = 0.9 μM) and GSK3$\beta$ ($K_i$ = 0.7 μM), and compound **284** is also an analogue of ruboxistaurin **28**, a compound with significant biological activity (Figure 5.1.4).[5,6]

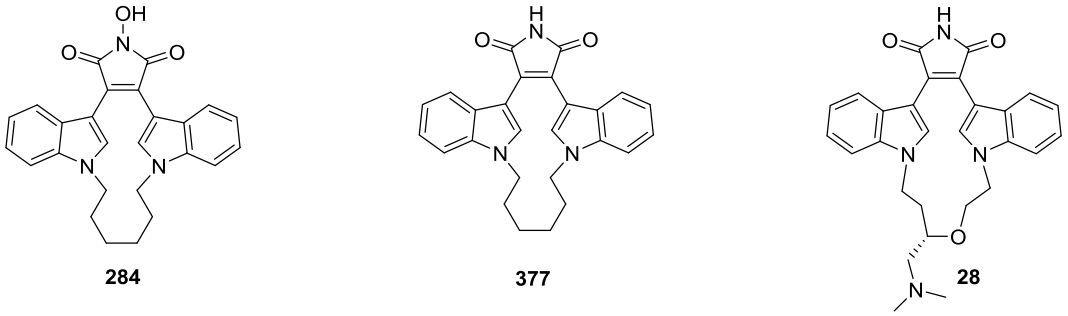
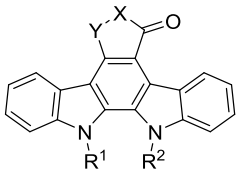



**Figure 5.1.4: Structures of** 284**,** 377 **and** 28

### *5.1.1.2 Indolocarbazoles with 5-membered F-rings*

A total of 15 indolocarbazoles with 5-membered F-rings were screened for activity. Of these, 9 were found to have significant enough activity to progress to five dose testing. The mean growths of a panel of derivatives with methyl or allyl substituents are displayed in Table 5.1.2.

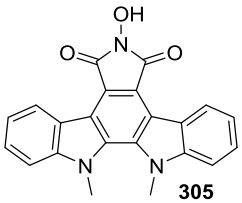


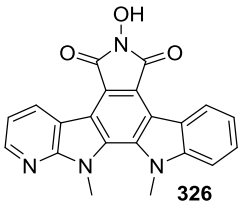

| Compound | NSC | $R^1$ | $R^2$ | X | Y | Mean (%) | Delta (%) |
|---|---|---|---|---|---|---|---|
| **305** | 776701* | Me | Me | N-OH | C=O | 32.9 | 79.5 |
| **306** | 783510* | Allyl | Allyl | N-OH | C=O | 18.4 | 116.3 |
| **298** | 774888 | Me | H | N-OH | C=O | 76.8 | 68.0 |
| **299** | 781337 | Me | H | $N\text{-}NH_2$ | C=O | 89.0 | 32.0 |
| **321** | 781338 | Me | H | $N\text{-}O(CH_2)_5CN$ | C=O | 52.6 | 90.1 |
| **360** | 785683* | Me | H | NH | NH | 23.4 | 76.2 |

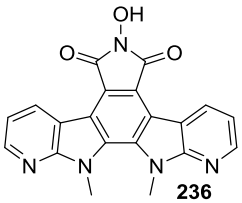
*Five dose data available

**Table 5.1.2: Mean growth over 60-cell panel on treatment with indolocarbazoles with methyl or allyl substituents**

Bis-methyl derivative **305** was found to be considerably more active than its bisindolyl counterpart **286**. In addition to the low mean growth percent displayed by **305**, significant selectivity was also observed against a number of cell lines including leukaemia cell line MOLT-4, non-small cell lung cancer cell line NCI-H522, melanoma cell lines LOX IMVI and SK-MEL-5 and breast cancer cell line MDA-MB-468 (Table 5.1.3). This compound was more active overall than monoaza **326** but less active than the bisaza analogue **236**, both synthesised previously within the group.[4] Interestingly, the patterns of selectivity also differed between the three compounds, indicating different biological targets.




| Compound No. | Mean growth | Selected cell lines | | | | | |
|---|---|---|---|---|---|---|---|
| | | MOLT-4 | NCI-H522 | LOX IMVI | SK-MEL-5 | MDA-MB-468 | 786-0 |
| **305** | 32.9% | -28.0% | -46.6% | -37.4% | -27.8% | -12.2% | 26.6% |
| **326** | 37.7% | -8.6% | 4.02 | 8.3% | 9.0% | 41.5% | - |
| **236** | 20.0% | -33.0 | 4.9% | 0.6% | - | -14.9% | -24.8% |

**Table 5.1.3: Comparison of single dose data for compounds** 305**,** 326 **and** 236

Changing from methyl to allyl substituents (**306**, Figure 5.1.5) further improved activity compared to **305**, with selectivity towards MOLT-4 (-10.4%), NCI-H522 (-62.5%), LOX IMVI (-81.2%) and SK-MEL-5 (-48.5%) being maintained. This compound also showed remarkable activity towards non-small cell lung cancer cell line NCI-H226 (-97.9%) and renal cancer cell line CAKI-1 (-70.6%). Both of these derivatives were selected for five dose testing.

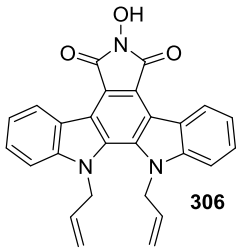
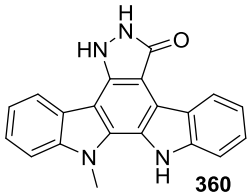



**Figure 5.1.5: Structure of indolocarbazole** 306

Compounds **298**, **299**, **321** and **360** all have identical *N*-substituents allowing for a comparison of the effect of differing F-rings on biological activity. Hydroxymaleimide **298** displayed only moderate activity with some selectivity towards leukaemia cell lines CCRF-CEM (8.8%) and SR (9.1%), a surprising result based on the potent activity of its bis-methyl counterpart **305**. Changing from a hydroxy to an amino group (**299**) further decreased both overall activity and selectivity. Addition of a 6-hexanenitrile chain to the hydroxymaleimide to give compound **321** suprisingly increased activity considerably compared to hydroxymaleimide **298,** while maintaining a similar selectivity pattern.

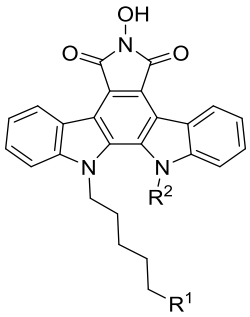
COMPARE analysis gave a correlation of 0.693, indicating that the mode of biological action is not altered significantly by the addition of a bulky chain to this position. Compound **321** also showed remarkable activity towards breast cancer cell line MDA-MB-468 (-37.5%). Removal of the maleimide *N*-substituent combined with replacement of one carbonyl group with a nitrogen gave pyrazolone **360** (Figure 5.1.6) which greatly improved activity resulting in a mean growth of 23.4%. The selectivity pattern was quite different to that of hydroxymaleimide **298** with COMPARE analysis giving a correlation of just 0.357, indicating a different mode of action. Highest activity was seen in colon cancer cell line HCT-116 (-52.8%), melanoma cell lines LOX IMVI (-30.3%) and SK-MEL-5 (-18.1%) and renal cancer cell lines RXF 393 (-36.7%) and UO-31 (-26.9%). Pyrazolone **360** was also accepted for five dose screening.




**360**

**Figure 5.1.6: Structure of pyrazolone** 360

A panel of hydroxymaleimide indolocarbazoles with functionalised six-carbon chains were also screened and their mean growths are displayed in Table 5.1.4.




| Compound | NSC | $R^1$ | $R^2$ | Mean (%) | Delta (%) |
|---|---|---|---|---|---|
| **308** | 781336* | CN | H | -7.0 | 90.0 |
| **322** | 781335* | CN | Me | 7.6 | 87.9 |
| **334** | 783507 | CN | $(CH_2)_5CN$ | 76.4 | 75.6 |
| **343** | 783508 | $CH_2OMe$ | Me | 65.9 | 61.7 |
| **336** | 783509* | $CH_2Cl$ | H | 4.2 | 74.5 |
| **338** | 785682* | $CH_2N(CH_2CH_2)_2O.(HCl)$ | H | 63.0 | 151.4 |
| **314** | 783514* | $CON(CH_2CH_2)_2O$ | H | 41.7 | 68.2 |
| **315** | 783515* | $CON(CH_2CH_3)_2$ | H | 35.1 | 101.7 |
| **316** | 783513 | $CO_2Me$ | H | 40.0 | 105.0 |

*Five dose data available

**Table 5.1.4: Mean growth over 60-cell panel on treatment with hydroxymaleimide series of indolocarbazoles (long chain)**

Nitrile derivative **308** (Figure 5.1.7) was found to have the highest activity in the single dose screen of the compounds tested in this project, with a mean growth of -7%. This compound showed remarkable cytotoxicity against CNS cancer cell line SF-539 (-95.4%) and melanoma cell line SK-MEL-5 (-97.0%) which are the most potent in single cell lines of all the derivatives tested. The compound also displayed selectivity for a number of other cell lines, such as non-small cell lung cancer cell line NCI-H522 (-53.9%), melanoma cell line LOX IMVI (-81.0%) and the mean growth of five renal cancer cell lines were reduced to below -30%. Addition of a methyl group to the unsubstituted indole to give compound **322** reduced activity slightly while a similar pattern of selectivity was maintained. This analogue also displayed selectivity for SF-539 (-33.0%), SK-MEL-5 (-80.3%), NCI-H522 (-51.9%), LOX IMVI (-66.7%) and several renal cancer cell lines (three lines below -20%).

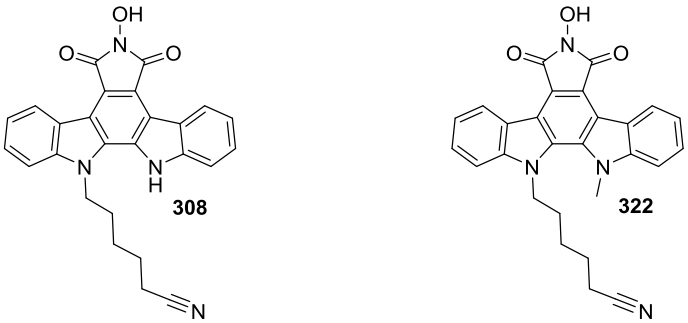



**Figure 5.1.7: Structures of** 308 **and** 322

The addition of a second hexane nitrile substituent to give derivative **334** greatly reduced activity to 76.4% mean growth. This was a disappointing result as its bisindolyl counterpart **282** was the most active of the bisindolylmaleimide analogues with a mean growth of 67.5% (Table 5.1.1). Some selectivity was still observed against several cell lines including melanoma cell line LOX IMVI (24.1%) and renal cancer cell line CAKI-1 (0.9%).

Replacement of the nitrile with a methyl ether to give compound **343** led to a reduction in activity with a mean growth of 66% instead of 8% for nitrile **322**. Compound **343** still showed selectivity for leukaemia cell lines (59.7% to 10.9% growth) as well as melanoma cell line LOX IMVI (5.8%) and renal cancer cell line CAKI-1 (4.2%).

Chloride **336** (Figure 5.1.8) was another very active derivative which displayed cytotoxicity towards a number of cell lines and had an overall mean growth of just 4.2%. Although possessing an alkyl halide functionality which could allow this analogue to work as an alkylating agent, the large delta value of 74.5% suggests that its biological effects are affected through a more selective mechanism. The most potent selectivity was observed in leukaemia cell line HL-60(TB) (-41.7%) and melanoma cell lines LOX IMVI (-70.2%) and SK-MEL-5 (-69.5%). Replacement of the chloride with a morpholine salt forming **338** increased mean growth to 63.0% but greatly increased selectivity for individual cell lines with a range of 151.4%. This derivative showed remarkable selectivity for leukaemia cell lines K-562 (-32.9%) and SR (-26.2%), colon cancer cell line HCT-116 (-16.0%), CNS cancer cell line U251 (-23.2%) and melanoma cell line LOX IMVI (-88.4%).

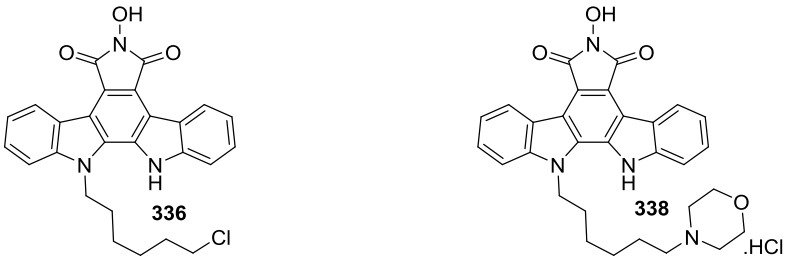



**Figure 5.1.8: Structures of compounds** 336 **and** 338

The addition of a carbonyl group to amide **338** to form amide **314** (Figure 5.1.9) caused an overall increase in activity with the mean growth dropping to 41.7%. The selectivity profile was very different to its amine counterpart **338** with LOX IMVI being the only cell line to which both compounds showed cytotoxic effects (-16.8% for **314**). The amide also displayed cytotoxic effects against leukaemia cell line MOLT-4 (-14.9%), ovarian cancer cell line IGROV1 (-26.5%) and renal cancer cell line CAKI-1 (-22.9%). Diethyl amide derivative **315** maintained a similar level of activity to **314** at 35.1% growth; however, the selectivity profile was quite different with COMPARE analysis giving a correlation of just 0.554 between the two compounds. While selectivity was retained for cell lines LOX IMVI (-59.0%) and CAKI-1 (-59.0%), **315** was also particularly active against non-small cell lung cancer cell line NCI-H522 (-66.5%) and renal cancer cell lines ACHN (-64.9%) and UO-31 (-50.7%). Interestingly, the activity profile of methyl ester **316** was very similar to that of diethyl amide **315**, with selectivity being retained for cell lines NCI-H522 (-60.0%), LOX IMVI (-65.0%), ACHN (-18.5%) and CAKI-1 (-16.8%). COMPARE analysis gave a correlation of 0.869, indicating that a similar mode of action was likely.

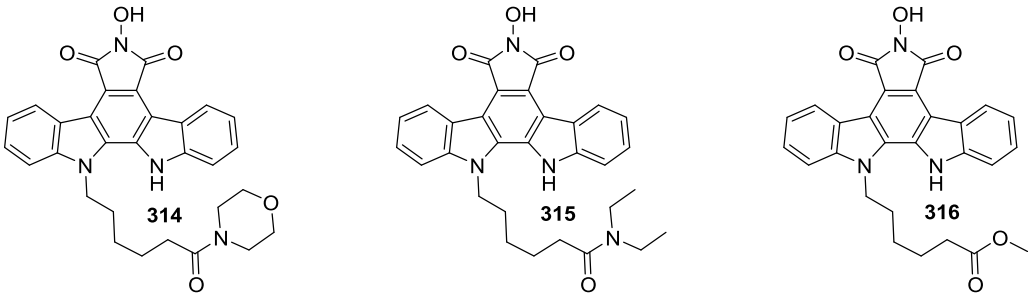
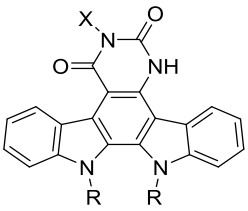



**Figure 5.1.9: Structures of** 314, 315 **and** 316

Six of the nine compounds in this panel progressed to five dose testing.

### *5.1.1.3 Indolocarbazoles with uracil F-rings*

The biological activity of a panel of eight indolocarbazoles with uracil F-rings was also assessed and the results are displayed in Table 5.1.5.




| Compound | NSC | X | R | Mean (%) | Delta (%) |
|---|---|---|---|---|---|
| **345** | 778802 | $NH_2$ | H | 88.8 | 32.3 |
| **349** | 778803 | $NH_2$ | Me | 84.5 | 70.3 |
| **351** | 783511 | $NH_2$ | $(CH_2)_5CN$ | 88.5 | 34.2 |
| **346** | 778801 | OH | H | 44.6 | 52.9 |
| **350** | 778804* | OH | Me | 30.3 | 42.9 |
| **348** | 778805 | H | H | 35.3 | 45.4 |
| **352** | 781339* | H | Me | 30.9 | 69.4 |
| **353** | 783512 | H | $(CH_2)_5CN$ | 47.2 | 55.1 |

*Five dose data available

**Table 5.1.5: Mean growth over 60-cell panel on treatment with uracil series of indolocarbazoles**

Overall, amino derivatives **345**, **349** and **351** were inactive with mean growths of 84-89% and little deviation from the mean growth across the panel. The only exception was compound **349** which showed considerable activity against ovarian cancer cell line OVCAR-4 (14.2%).

Hydroxy derivatives **346** and **350** were significantly more active than the amino analogues, with the presence of *N*-methyl substituents increasing activity compared to unsubstituted indole nitrogens. Compound **346** with free indole nitrogens reduced growth to 44.6% and displayed cytotoxic activity towards non-small cell lung cancer cell line HOP-92 (-8.3%) and also selectivity for the leukaemia cell lines (all between 5.6% and 26.5%), non-small cell lung cancer cell line NCI-H522 (4.3%) and melanoma cell line LOX IMVI (10.0%). The highest activity for bis-methyl **350** (Figure 5.1.10)

was observed against leukaemia cell lines RPMI-8226 (-12.6%) and SR (4.5%), non-small cell lung cancer cell lines HOP-92 (-2.9%) and NCI-H23 (-2.7%), colon cancer cell line HCT-116 (0.5%) and melanoma cell lines LOX IMVI (-4.9%) and SK-MEL-5 (2.7%). Interestingly, this compound reduced overall growth to 30.3% and so was slightly more active than its hydroxymaleimide counterpart **305** (mean growth 32.9%) and was the most active of the uracil compounds tested. Derivative **350** was also selected for five dose testing.

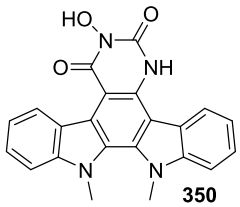
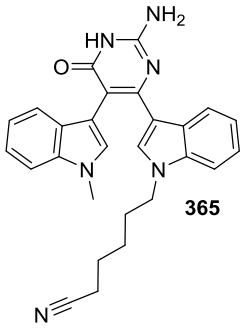



**Figure 5.1.10: Structure of hydroxy uracil** 350

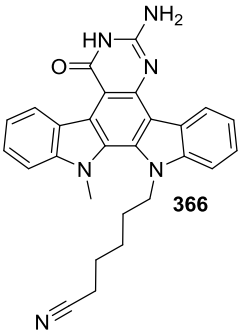
Unsubstituted uracil compounds **348**, **352** and **353** had similar levels of activity to the hydroxy uracil derivatives. Compound **348** which had unsubstituted indole nitrogens reduced mean growth to 35.3%, and so was more active than its hydroxy counterpart. Selectivity was observed for leukaemia cell line RPMI-8226 (6.0%), non-small cell lung cancer cell line HOP-92 (5.7%), renal cancer cell line A498 (-10.1%) and breast cancer cell line MDA-MB-468 (6.0%). The addition of *N*-methyl substituents (**352**) again improved activity, although disappointingly this unsubstituted uracil was no more active than its hydroxy analogue. However, with a mean growth of 30.9% it was deemed active enough to progress to the five dose assay. Cytotoxic effects were seen against several cell lines including non-small cell lung cancer cell line HOP-92 (-4.4%), CNS cancer cell line SNB-75 (-38.4%), ovarian cancer cell line OVCAR-4 (-10.1%), renal cancer cell line A498 (-4.1%) and breast cancer cell line HS 578T (-7.3%). Addition of hexanenitrile *N*-substituents again reduced activity to 47.2% mean growth, although significant selectivity was displayed for a number of cell lines including CNS cancer cell line SF-539 (-0.2%), renal cancer cell line 786-0 (0.2%) and breast cancer cell line MDA-MB-231/ATCC (-7.9%).

### *5.1.1.4 Analogues with other F-rings*

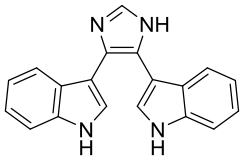
Analogues with two other F-ring types were synthesised during this project, one with an imidazole and two with an isocytosine and the results are displayed in Table 5.1.6.




**365**




**366**

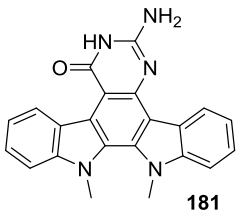



**369**

| Compound | NSC | Mean (%) | Delta (%) |
|---|---|---|---|
| **365** | 778800 | 98 | 37 |
| **366** | 785684 | 76 | 43 |
| **369** | 763911 | 78 | 48 |

**Table 5.1.6: Mean growth over 60-cell panel on treatment with analogues with other F-rings**

Bisindolyl derivative **365** showed very little activity, with modest selectivity observed for only one cell line – non-small cell lung cancer cell line HOP-92 (61.2%). Conversion to the indolocarbazole (**366**) improved activity, with mean growth being reduced to 76.4%. Disappointingly, this compound was less active than bis-methyl derivative **181** (Figure 5.1.11), synthesised previously within the group (mean growth 58.9%),[7] despite the presence of a hexanenitrile substituent which had caused a dramatic increase in activity in the hydroxymaleimide series. The highest selectivity for compound **366** was observed for leukaemia cell line SR (35.5%) and renal cancer cell line RXF 393 (33.5%).




**181**

**Figure 5.1.11: Structure of** 181

Imidazole **369** was the second most active bisindolyl derivative tested in this project with a mean growth of 77.7%. This compound displayed good selectivity against CNS cancer cell line SF-295, as well as moderate selectivity against a number of other cell lines such as renal cancer cell lines CAKI-1 (48.7%) and UO-31 (52.5%) and breast cancer cell line MDA-MB-231/ATCC (49.0%).

### 5.1.2 Five dose results

A total of 12 compounds were selected to progress to five dose testing where they were screened at concentrations of 100 μM, 10 μM, 1 μM, 100 nM and 10 nM. The concentration parameters $GI_{50}$ (concentration to inhibit cell growth by 50%) and $LC_{50}$ (concentration required for 50% cell death) are extracted from concentration response curves by linear extrapolation. TGI (total growth inhibition) is read as the x-axis intercept from the five different drug concentrations.[8] Concentration on the x-axis is given as $\log_{10}$ of the sample concentration in moles, i.e. -6 is $1 \times 10^{-6}$ M or 1 μM.

#### *5.1.2.1 Five dose data for bisindolyl derivative **282***

Bis-hexanenitrile derivative **282** was the only bisindolyl compound selected for the five dose screen. Although this compound had the highest mean growth of the compounds that went forward to five dose testing, low micromolar $GI_{50}$ values were observed for a number of cell lines, especially in leukaemia and melanoma cancer types (Figure 5.1.12). Most cell lines required a concentration of greater than 100 μM for the death of 50% of cell population ($LC_{50}$), with only 2 cell lines, SK-MEL-5 and UACC-62, showing any appreciable cytotoxicity (Table 5.1.7).

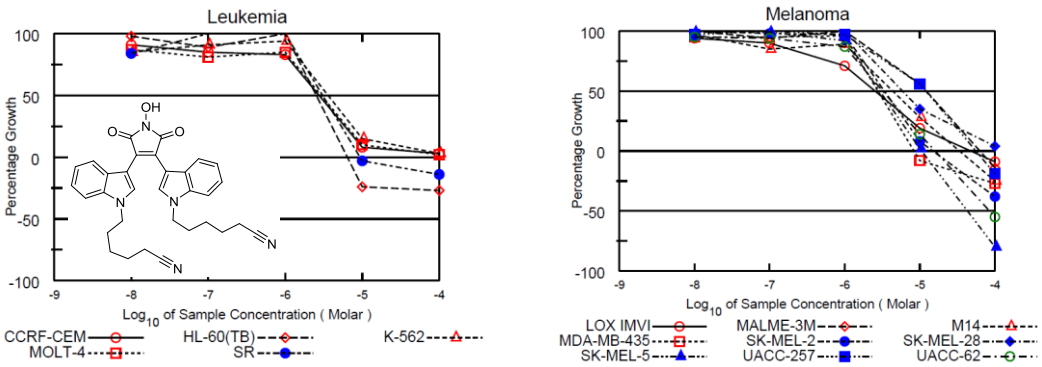



**Figure 5.1.12: Dose response curves for bisindolyl derivative** 282

| Cancer type | Cell line | $GI_{50}$ (μM) | TGI (μM) | $LC_{50}$ (μM) |
|---|---|---|---|---|
| Leukaemia | HL-60(TB) | 2.54 | 6.44 | >100 |
| | SR | 3.55 | 9.48 | >100 |
| Melanoma | MDA-MB-435 | 2.74 | 8.31 | >100 |
| | SK-MEL-5 | 2.92 | 10.6 | 42.8 |
| | UACC-62 | 3.23 | 16.0 | 84.4 |

**Table 5.1.7: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 282

### *5.1.2.2 Five dose data for indolocarbazoles **305** and **306***

$GI_{50}$ values were observed at sub micromolar concentrations in a third of all cell lines for bis-methyl derivative **305**, with the greatest activity observed in the leukaemia cell line SR, which had a $GI_{50}$ value of just 73 nM (Table 5.1.8). Little cytotoxicity is observed across the panel, however, with all $LC_{50}$ values being greater than 100 μM apart from CNS cancer cell line SNB-75.

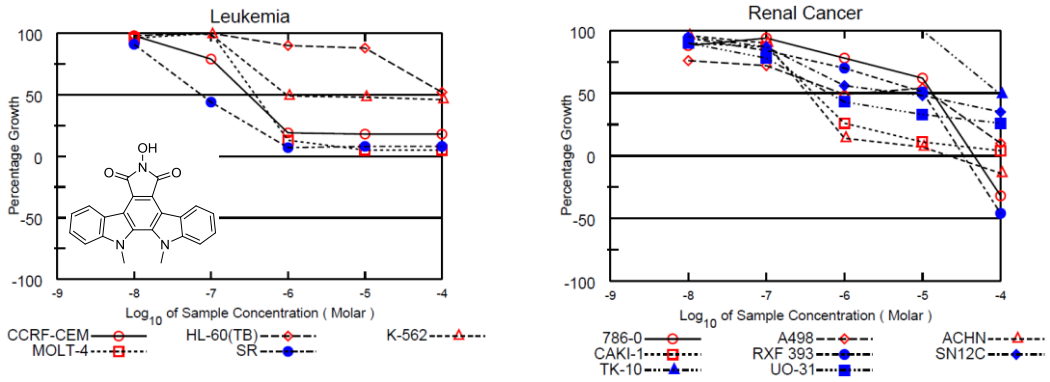



**Figure 5.1.13: Dose response curves for** 305

| Cancer type | Cell line | $GI_{50}$ (μM) | TGI (μM) | $LC_{50}$ (μM) |
|---|---|---|---|---|
| Leukaemia | CCRF-CEM | 0.302 | >100 | >100 |
| | MOLT-4 | 0.376 | >100 | >100 |
| | SR | 0.073 | >100 | >100 |
| Renal Cancer | ACHN | 0.334 | 21.1 | >100 |
| | CAKI-1 | 0.402 | >100 | >100 |
| CNS Cancer | SNB-75 | 11.3 | 30.0 | 79.2 |

**Table 5.1.8: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 305

Monoaza derivative **326** showed similar dose-response curves to **305**, with a similar number of sub micromolar $GI_{50}$ values and with no $LC_{50}$ values less than 100 μM.[4] However, bisaza derivative **236** was much more cytotoxic at higher concentrations with a quarter of all cell lines possessing $LC_{50}$ values less than 100 μM and melanoma cell line SK-MEL-5 displaying the lowest at 813 nM. This compound was also more active at lower concentrations with almost all cell lines having $GI_{50}$ values of less than 100 nM.

Despite being similar in structure to **305**, bis-allyl derivative **306** exhibited much greater cytotoxicity at higher concentrations, with $LC_{50}$ values for many cell lines in the low micromolar range. However, a marked drop-off in activity was observed between the 10 μM and 1 μM levels, with only 4 cell lines possessing sub micromolar $GI_{50}$ values (Table 5.1.9). Interestingly, both derivative **305** and **306** were most selective for leukaemia cell line SR with $GI_{50}$ values of 73 nM and 363 nM, respectively. COMPARE analysis gave a correlation of 0.633 indicating only a moderate similarity in their biological mode of action.

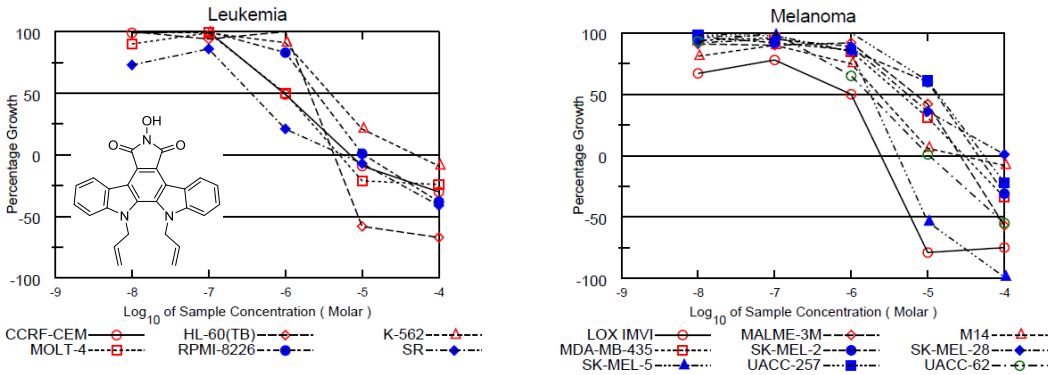



**Figure 5.1.14: Dose response curves for** 306

| Cancer type | Cell line | $GI_{50}$ (μM) | TGI (μM) | $LC_{50}$ (μM) |
|---|---|---|---|---|
| Leukaemia | CCRF-CEM | 0.971 | 7.10 | >100 |
| | HL-60(TB) | 2.09 | 4.31 | 8.86 |
| | SR | 0.363 | 5.67 | >100 |
| Melanoma | LOX IMVI | 0.967 | 2.43 | 5.95 |
| | SK-MEL-5 | 1.79 | 4.10 | 9.38 |
| Breast Cancer | MCF7 | 0.674 | 29.9 | >100 |

**Table 5.1.9: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 306

Pyrazolone **360** exhibited selective cytotoxicity at higher concentrations with two cell lines, SF-539 and RXF 393 (Table 5.1.10), possessing low micromolar $LC_{50}$ values despite the majority having values greater than 100 µM. Most cell lines were found to have low micromolar $GI_{50}$ values, although the three cell lines KM12, SNB-75 and HS578T displayed sub micromolar values.

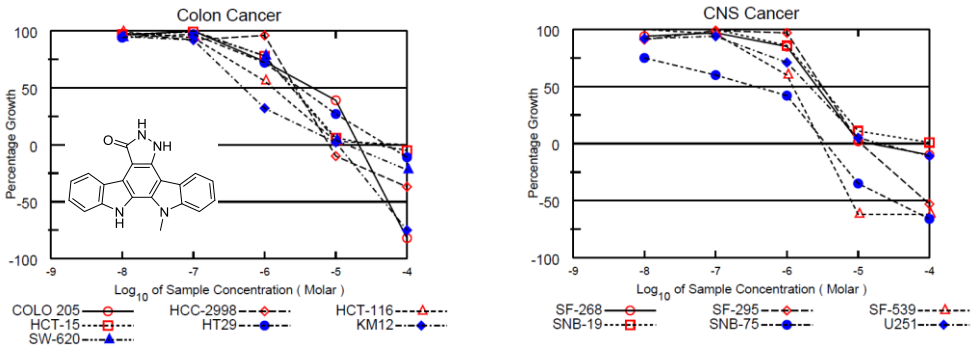



**Figure 5.1.15: Dose-response curves for 360**

| Cancer type | Cell line | $GI_{50}$ (µM) | TGI (µM) | $LC_{50}$ (µM) |
|---|---|---|---|---|
| Colon cancer | HCC-2998 | 2.74 | 8.10 | >100 |
| | KM12 | 0.493 | 10.5 | 47.2 |
| CNS cancer | SF-539 | 1.22 | 3.12 | 7.99 |
| | SNB-75 | 0.355 | 3.53 | 30.9 |
| Renal cancer | RXF 393 | 1.51 | 3.57 | 8.49 |
| Breast cancer | HS 578T | 0.993 | 5.88 | >100 |

**Table 5.1.10: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 360

### *5.1.2.3 Five dose data for other 5-membered F-ring indolocarbazoles*

With the lowest mean growth of any of the compounds in the single dose screen, nitrile derivative **308** was a clear candidate for further biological evaluation. Notable cytotoxic activity was observed at higher concentrations with several cell lines possessing low micromolar $LC_{50}$ values (Table 5.1.11). However, there is again a steep decrease in activity between the 10 µM and 100 nM levels. Notable selectivity was observed for non-small cell lung cancer cell line NCI-H522 compared to other cell lines of this cancer type, as in the single dose results.

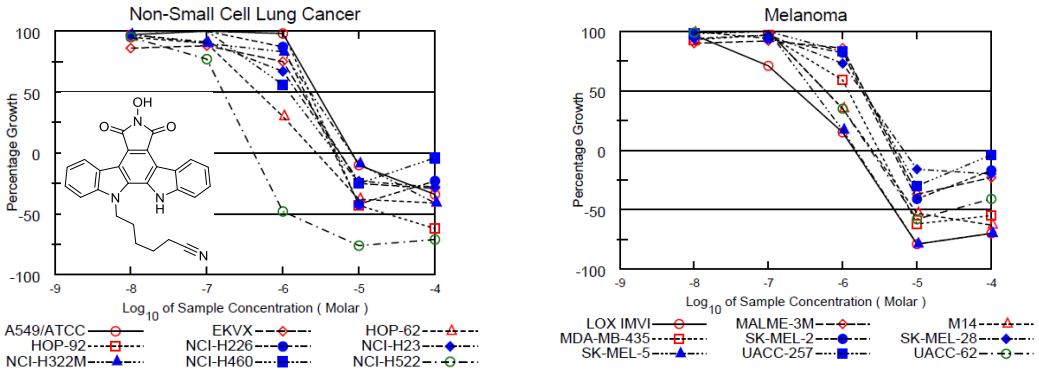



**Figure 5.1.16: Dose response curves for** 308

| Cancer type | Cell line | $GI_{50}$ (μM) | TGI (μM) | $LC_{50}$ (μM) |
|---|---|---|---|---|
| Non-small cell lung cancer | NCI-H522 | 0.164 | 0.411 | 1.16 |
| Melanoma | LOX IMVI | 0.240 | 1.44 | 4.88 |
| | M14 | 0.595 | 2.51 | 9.18 |
| | MDA-MB-435 | 1.19 | 3.09 | 8.02 |
| | SK-MEL-5 | 0.403 | 1.50 | 4.96 |

**Table 5.1.11: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 308

Addition of a methyl group to form indolocarbazole **322** displayed a similar dose response curve to derivative **308**. Many cell lines showed cytotoxic activity with low micromolar $LC_{50}$ values but activity rapidly diminished with decreasing concentration (Table 5.1.12). Compound **308** and **322** both show selectivity for melanoma cell lines LOX IMVI and SK-MEL-5 which agrees with the single dose assay.

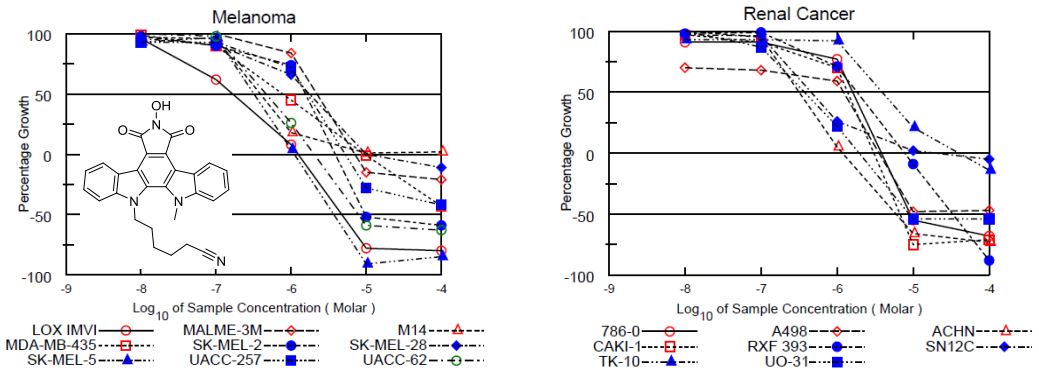



**Figure 5.1.17: Dose response curves for** 322

| Cancer type | Cell line | $GI_{50}$ (μM) | TGI (μM) | $LC_{50}$ (μM) |
|---|---|---|---|---|
| Melanoma | LOX IMVI | 0.167 | 1.24 | 4.74 |
| | SK-MEL-5 | 0.319 | 1.09 | 3.70 |
| | UACC-62 | 0.465 | 2.03 | 7.85 |
| Renal Cancer | ACHN | 0.317 | 1.19 | 5.97 |
| | UO-31 | 0.374 | 1.97 | 8.93 |

**Table 5.1.12: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 322

Chloride **336** also had a similar growth response curve with significant cytotoxicity at higher concentrations which dropped off steeply towards the lower concentrations (Table 5.1.13). The curves were more atypical at lower concentrations with many cell lines showing an increase in activity at 100 nM compared to 1 μM (Figure 5.1.18). Due to this phenomenon, it was not possible to calculate $GI_{50}$ values for some cell lines. Again, this compound showed selectivity for cell lines LOX IMVI and SK-MEL-5, as in the single dose screen.

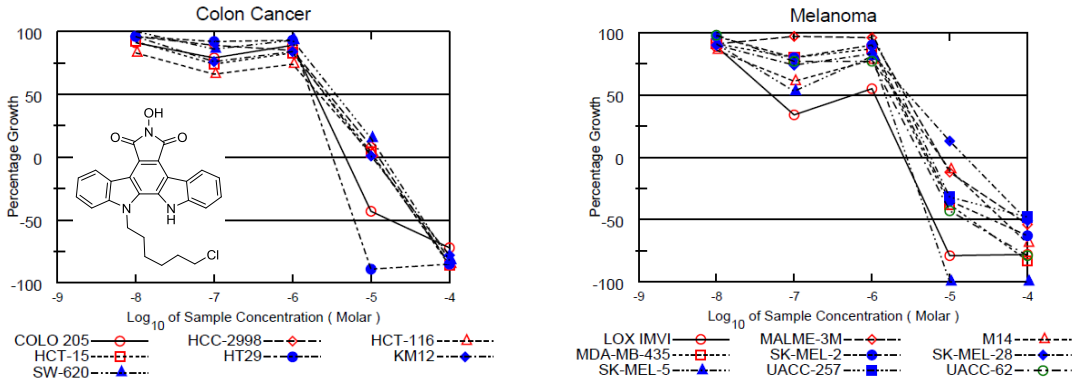



**Figure 5.1.18: Dose response curves for** 336

| Cancer type | Cell line | $GI_{50}$ (μM) | TGI (μM) | $LC_{50}$ (μM) |
|---|---|---|---|---|
| Colon | COLO 205 | 1.97 | 4.70 | 16.9 |
| | HT29 | 1.73 | 3.25 | 6.12 |
| Melanoma | LOX IMVI | - | 2.59 | 6.11 |
| | SK-MEL-5 | 1.48 | 2.80 | 5.29 |
| | UACC-62 | 1.69 | 4.42 | 16.1 |

**Table 5.1.13: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 336

Morpholine salt **338** displayed interesting selectivity towards individual cell lines especially at the 10 μM level (Figure 5.1.19). Percentage growth at this concentration ranged from -89% (LOX IMVI) to 102% (OVCAR-5), which correlates well with single dose data. Although many cell lines experienced a sharp drop-off in activity between the 10 μM and 1 μM levels, selectivity for a small number of cell lines remains especially for LOX IMVI (percentage growth at 1 μM = -12%). In fact **338** still completely inhibits growth (TGI) of LOX IMVI cells at 736 nM, while all other cell lines require a concentration of at least 2.85 μM to completely inhibit growth (Table 5.1.14).

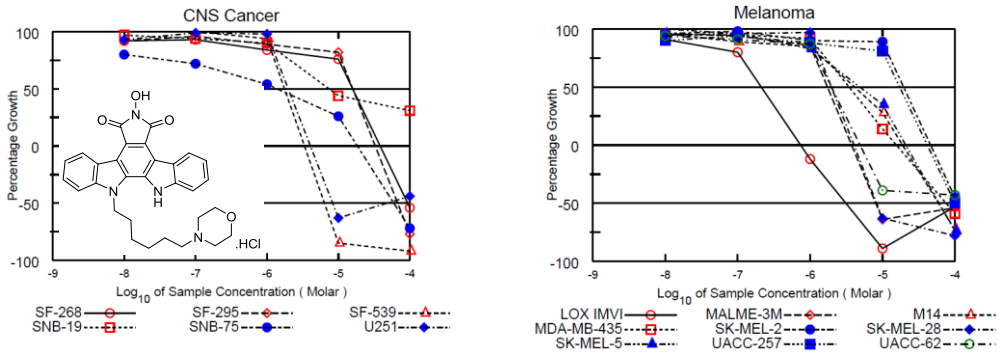



**Figure 5.1.19: Dose response curves for** 338

| Cancer type | Cell line | $GI_{50}$ (μM) | TGI (μM) | $LC_{50}$ (μM) |
|---|---|---|---|---|
| CNS cancer | SF-539 | 1.77 | 3.36 | 6.38 |
| | SNB-75 | 1.44 | 18.5 | 59.9 |
| Melanoma | LOX IMVI | 0.210 | 0.736 | 3.11 |
| | MALME-3M | 1.74 | 3.76 | 8.10 |
| | SK-MEL-28 | 1.96 | 4.02 | 8.25 |
| Renal cancer | UO-31 | 1.38 | 2.85 | 5.86 |

**Table 5.1.14: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 338

Morpholine amide **314** again displayed cytotoxic activity in a number of cell lines with several having low micromolar $LC_{50}$ values (Table 5.1.15). However, a sharp drop-off in activity, similar to that in other compounds in this series, was observed between the 10 μM and 1 μM levels. No sub-micromolar $GI_{50}$ values were observed.

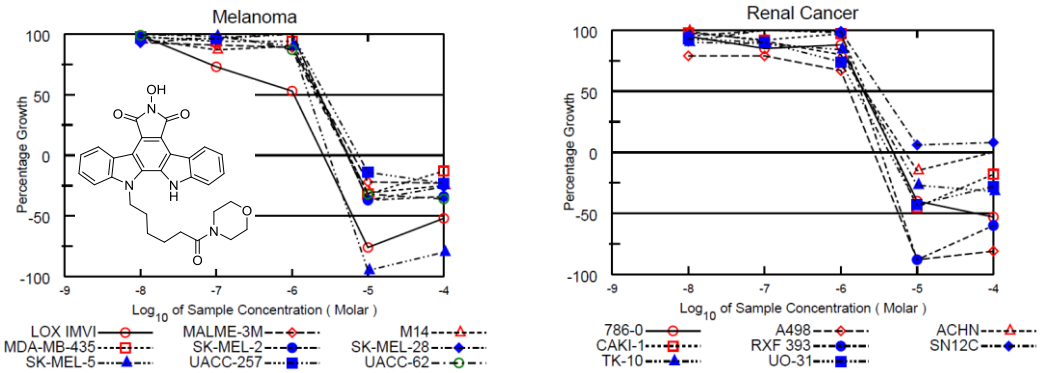



**Figure 5.1.20: Dose response curves for** 314

| Cancer type | Cell line | $GI_{50}$ (µM) | TGI (µM) | $LC_{50}$ (µM) |
|---|---|---|---|---|
| Melanoma | LOX IMVI | 1.05 | 2.57 | 6.27 |
| | SK-MEL-5 | 1.64 | 3.06 | 5.71 |
| Renal Cancer | A498 | 2.07 | 2.70 | 5.66 |
| | RXF 393 | 1.81 | 3.37 | 6.25 |

**Table 5.1.15: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 314

For diethyl amide **315**, significant selectivity was observed for colon cancer cell lines COLO 205 and HT29 and melanoma cell lines LOX IMVI and SK-MEL-5 at higher concentrations, with selectivity for LOX IMVI being retained to micromolar levels (Figure 5.1.21). Again, there was a considerable decrease in activity between the 10 µM and 1 µM levels.

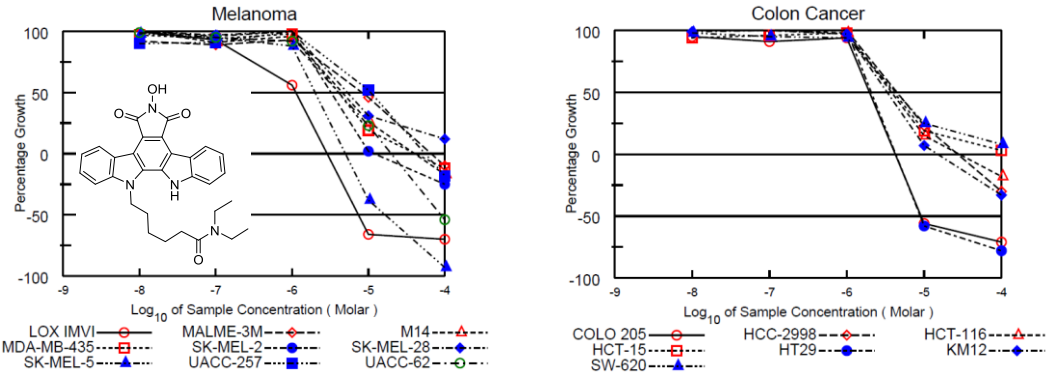



**Figure 5.1.21: Dose response curves for** 315

| Cancer type | Cell line | $GI_{50}$ (μM) | TGI (μM) | $LC_{50}$ (μM) |
|---|---|---|---|---|
| Colon Cancer | COLO 205 | 1.97 | 4.23 | 9.09 |
| | HT29 | 2.21 | 4.44 | 8.91 |
| Melanoma | LOX IMVI | 1.13 | 2.88 | 7.38 |
| | SK-MEL-5 | 2.00 | 5.02 | 16.7 |

**Table 5.1.16: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 315

### *5.1.2.4 Five dose data for indolocarbazoles with 6-membered F-rings*

Although less cytotoxicity was observed for hydroxyuracil **350** compared to the hydroxymaleimide series, cytostatic effects were evident at considerably lower concentrations with several cell lines possessing sub micromolar $GI_{50}$ values (Table 5.1.17). Greatest activity was seen in melanoma cell lines LOX IMVI and SK-MEL-5 and a number of non-small cell lung cancer cell lines, which is consistent with the single dose screen.

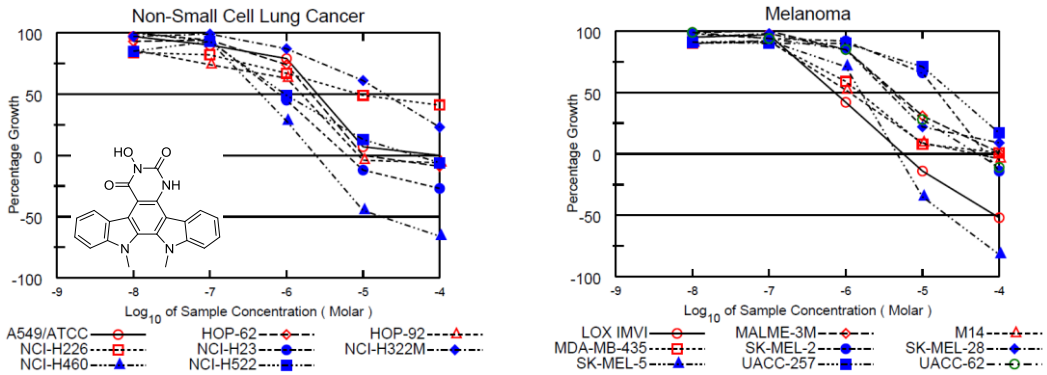



**Figure 5.1.22: Dose response curves for** 350

| Cancer type | Cell line | $GI_{50}$ (μM) | TGI (μM) | $LC_{50}$ (μM) |
|---|---|---|---|---|
| Non-small cell lung cancer | NCI-H23 | 0.787 | 6.18 | >100 |
| | NCI-H460 | 0.457 | 2.39 | 16.9 |
| | NCI-H522 | 0.934 | 47.7 | >100 |
| Melanoma | LOX IMVI | 0.710 | 5.58 | 86.5 |
| | SK-MEL-5 | 1.58 | 4.65 | 20.6 |

**Table 5.1.17: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 350

Despite having the same mean growth in the single dose assay as hydroxyuracil **350**, uracil **352** showed much more selectivity towards individual cell lines. $LC_{50}$ values ranged from >100 μM down to low micromolar values (Table 5.1.18). Excellent growth inhibition was observed against renal cancer cell line A498 which displayed a $GI_{50}$ value of just 48 nM, the lowest of any cell line for any of the compounds tested in this project. Most other cell lines possessed low micromolar $GI_{50}$ values.

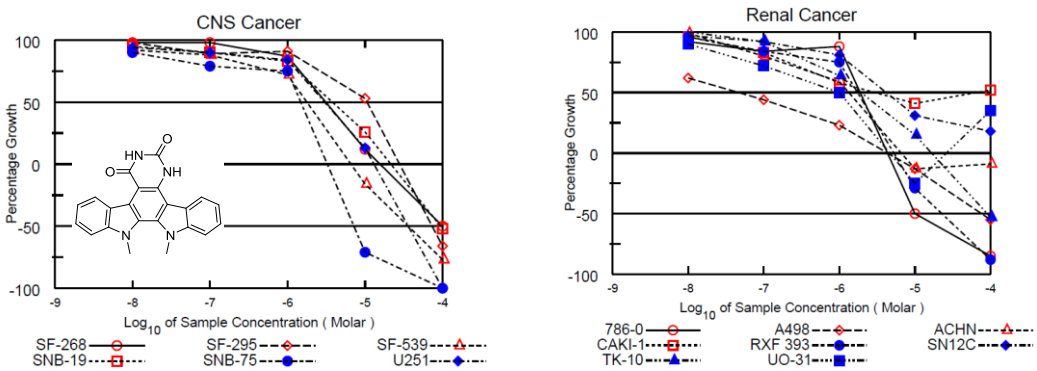



**Figure 5.1.23: Dose response curves for** 352

| Cancer type | Cell line | $GI_{50}$ (μM) | TGI (μM) | $LC_{50}$ (μM) |
|---|---|---|---|---|
| CNS Cancer | SF-539 | 1.78 | 6.61 | 36.3 |
| | SNB-75 | 1.49 | 3.27 | 7.19 |
| Renal Cancer | A498 | 0.048 | 4.37 | 76.8 |
| | UO-31 | 0.981 | - | >100 |

**Table 5.1.18: Selected $GI_{50}$, TGI and $LC_{50}$ values for** 352

### 5.1.3 Possible Modes of Action

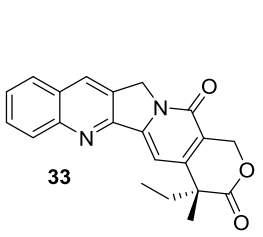
A number of cell lines consistently appeared to be most active across the panel of compounds and information on the known gene mutations and turnover rates of these cell lines was obtained.[9] Cyclin-dependent kinase inhibitor 2 A (CDK2NA) mutations were very common, being present in almost all of the most affected cell lines, and BRAF and TP53 mutations were also common (Table 5.1.19). No correlation between activity and turnover rate was observed.

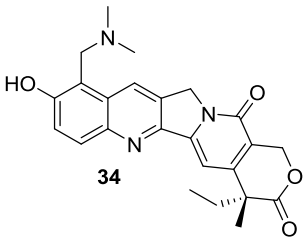
| Cell line | Gene mutations |
|---|---|
| **SR** | CDK2NA |
| **UO-31** | CDK2NA |
| **LOX IMVI** | BRAF, CDK2NA |
| **HOP-92** | CDK2NA, TP53 |
| **NCI-H522** | TP53 |
| **SK-MEL-5** | BRAF, CDK2NA |
| **CAKI-1** | CDK2NA |

**Table 5.1.19: Most commonly inhibited cell lines with their known gene mutations**

CDK2NA is a tumour suppressor gene which encodes an inhibitor of cyclin dependent kinases, indicating that derivatives which affect cells with this mutation could be active as inhibitors of a CDK kinase. BRAF is a proto-oncogene encoding a serine/threonine-protein kinase inhibitor known as B-RAF, which is involved in the MAPK/ERK signalling pathway. Drugs that treat cancers possessing a BRAF mutation such as vemurafenib and dabrafenib have been approved, and work by interrupting certain steps in this pathway. TP53 encodes a major tumour suppressor protein, p53, which is critical for the prevention of cancer formation by initiating apoptosis when DNA damage is irreparable.

Investigation using COMPARE analysis demonstrates a high correlation between bis-methyl hydroxymaleimide indolocarbazole **305** and both camptothecin **33** and its derivative, topotecan **34**, indicating a similar biological mode of action (Table 5.1.20). As camptothecin and its derivatives are known topo I inhibitors, this suggests that this indolocarbazole could interact with the same enzyme.




| Seed (NSC no.) | Target | Correlation |
|---|---|---|
| **305** (776701) | topotecan | 0.723 |
| **305** (776701) | camptothecin | 0.780 |
| **308** (781336) | topotecan | 0.679 |
| **305** (776701) | **350** (778804) | 0.672 |
| **350** (778804) | topotecan | 0.334 |

**Table 5.1.20: COMPARE analysis of indolocarbazole derivatives with camptothecin** 33 **and topotecan** 34

The hydroxymaleimide with a hexanenitrile *N*-substituent (**308**) also displayed a similarly high correlation to topotecan, again indicating action through topo I inhibition. Interestingly, hydroxy uracil **350** showed a low correlation to topotecan, despite demonstrating a high correlation to hydroxymaleimide **305**, suggesting that changing the F-ring from a hydroxymaleimide to a hydroxy uracil removes the ability of the compound to act as a topo I inhibitor. This is an interesting result as the addition of bulky groups to a maleimide F-ring usually has no adverse effect on anti-topo I activity, as can be seen from rebeccamycin derivative edotecarin **46** (Figure 5.1.24) which has undergone several clinical trials and functions through topo I inhibition.

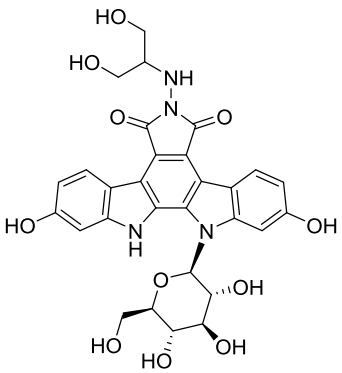


**Figure 5.1.24: Structure of edotecarin** 46

## 5.2 Kinase Screen

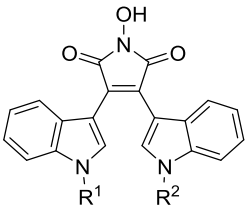
Kinase screening was carried out in collaboration with the Kinase Inhibitor Specialised Screening Facility (KISSf) in Station Biologique de Roscoff, France. Since 2001, KISSf has offered screening services for the assessment of the biological properties of small molecules using a panel of selected disease-related protein kinases. For potential anticancer agents, the panel consists of the following kinases: Ld_TLK, Hs_HASPIN, Hs_AURKB, Hs_RIPK3, Hs_CDK2/CyclinA, Hs_CDK5/p25, Hs_CDK9/CyclinT, Mm_CLK1, Hs_DYRK1A, Ssc_GSK3α/β, Pf_GSK3, Hs_PIM1 and Ssc_CK1δ/ε.

### 5.2.1 Results

To date the results of the kinase screen are available for seven compounds from the bisindolyl panel and are displayed in Table 5.2.1, with remaining compounds currently undergoing assessment.

Inhibitory action for individual kinases is observed to a certain degree for all the compounds in the panel, except for macrocyclic **284** which shows no activity at the levels screened in this assay. This was unsurprising as this derivative was also completely inactive in the NCI screen, although it is related to ruboxistaurin. Ssc_GSK3α/β was the most commonly inhibited kinase, with the other six derivatives possessing $IC_{50}$ values of between 1 and 3 μM. Inhibition of GSK-3*β* has been shown to have effects such as the reduction of apoptosis, making it potentially useful for the protection of Alzheimer's disease and protection against cell death.[10,11] More recently, a group of bisindolylmaleimide GSK-3*β* inhibitors have been used to show the involvement of GSK-3*β* in the regulation of murine embryonic stem cell self-renewal.[12] Although the activity of these derivatives is modest, this could be improved upon in future SAR studies.

PIM1 kinase was also inhibited by the six derivatives, with $IC_{50}$ values of less than 10 μM. PIM1 is mainly involved in cell cycle progression, apoptosis and transcriptional activation, and also more general signal transduction pathways and is highly expressed in cell cultures isolated from human tumours.[13]




| | 285 | 286 | 287 | 290 | 291 | 292 | 284 |
|---|---|---|---|---|---|---|---|
| $R^1$ | Me | Me | Me | *i*Pr | *i*Pr | *i*Pr | $-(CH_2)_6-$ |
| $R^2$ | H | Me | Et | H | Me | Et | |
| Mean Growth (10 µM)* | 78.0% | 97.6% | - | 94.4% | 90.8% | - | 103.3% |
| Kinase | $IC_{50}$ (µM) | | | | | | |
| Ld_TLK | >10 | >10 | >10 | >10 | >10 | >10 | >10 |
| Hs_HASPIN | >10 | >10 | >10 | >10 | >10 | >10 | >10 |
| Hs_AURKB | >10 | >10 | >10 | >10 | >10 | >10 | >10 |
| Hs_RIPK3 | >10 | >10 | >10 | >10 | >10 | >10 | >10 |
| Hs_CDK2/CyclinA | >10 | >10 | >10 | >10 | **6.0** | >10 | >10 |
| Hs_CDK5/p25 | >10 | >10 | >10 | >10 | **8.0** | >10 | >10 |
| Hs_CDK9/CyclinT | **8** | >10 | **9.2** | >10 | **5.0** | **6** | >10 |
| Mm_CLK1 | **2** | >10 | >10 | >10 | >10 | >10 | >10 |
| Hs_DYRK1A | >10 | >10 | >10 | >10 | >10 | >10 | >10 |
| Ssc_GSK3α/β | **1** | **3** | **1.9** | **3.0** | **1.2** | **2** | >10 |
| Pf_GSK3 | >10 | >10 | >10 | >10 | >10 | >10 | >10 |
| Hs_PIM1 | **3** | **8** | **6.5** | **5.6** | **7.0** | **8** | >10 |
| Ssc_CK1δ/ε | >10 | >10 | >10 | >10 | >10 | >10 | >10 |

*From NCI single dose screen

**Table 5.2.1: $IC_{50}$ values of bisindolyl compounds in kinase inhibition assay**

Although with the limited number of compounds screened so far it is difficult to predict the structure-activity relationships, the results of the remaining compounds in this kinase assay are eagerly awaited, especially for a number of the indolocarbazole derivatives which displayed significant activity and selectivity in the NCI screen.

## 5.3 Conclusion

A total of 37 bisindole and indolocarbazole compounds have been submitted to the NCI for screening to date, with 12 compounds judged to have sufficient activity or selectivity to progress to five dose testing. Although not as detailed as the five dose assay, the single dose screen provided much information on the chemotherapeutic potential of these compounds, with a number of promising leads for future work. Little anticancer activity was observed in the compounds tested from the bisindole series, with only bis-hexanenitrile derivative **282** being selected for five dose screening. The indolocarbazoles were generally much more active, with highly cytotoxic activity being displayed by several derivatives against a number of cell lines and with eleven in total progressing to the five dose screen.

It was apparent that both F-ring modulation and variation of *N*-substituents had a profound effect on the anticancer activity of these compounds. Removal of the F-ring hydroxy group combined with replacement of one carbonyl to form pyrazolone **360** caused a considerable increase in activity compared to its hydroxymaleimide counterpart **298**. Also the addition of an extra nitrogen atom to the maleimide F-ring formed a series of uracil derivatives with significantly different antiproliferative profiles. Similarly, very small changes to the *N*-substituents greatly impacted on the single dose data obtained for different candidates. For example, switching from a hexanenitrile (**308**) to a hexylmorpholine (**338**) substituent causes an increase in mean growth from -7% to 63%.

The results from five dose assays on the twelve selected derivatives provided further information on biological activity of these compounds. Many of the compounds are highly cytotoxic towards specific cell lines at higher concentrations but suffer from a drop-off in potency at lower concentrations. However, this decrease in activity is an issue which could be addressed with further SAR studies. Despite this, most compounds displayed sub-micromolar $GI_{50}$ values in a number of cell lines with two compounds (hydroxymaleimide **305** and uracil **352**) exhibiting $GI_{50}$ values of <100 nM against one of the cell lines.

Analysis using the COMPARE algorithm showed a strong correlation between two of the hydroxymaleimide analogues (**305** and **308**) and topotecan, indicating the ability of these compounds to act as topo I inhibitors. Interestingly, this correlation was not retained for the uracil derivatives, which indicated that changing from a five-membered to a six-membered F-ring can switch the mechanism of action of these compounds while maintaining anticancer activity. Examination of the known gene mutations in the cancer cell lines most commonly affected by the compounds tested showed that cell lines containing a CDK2NA, BRAF or TP53 mutation were most commonly affected.

To date, seven bisindolylmaleimide derivatives have undergone kinase screening against a panel of kinases associated with cancer, with six of the compounds showing kinase inhibition at low micromolar concentrations for certain kinases. These results are interesting given the low cytotoxicity observed in the NCI screen indicating that this type of compound could be used to regulate certain kinase activities without affecting cell growth.

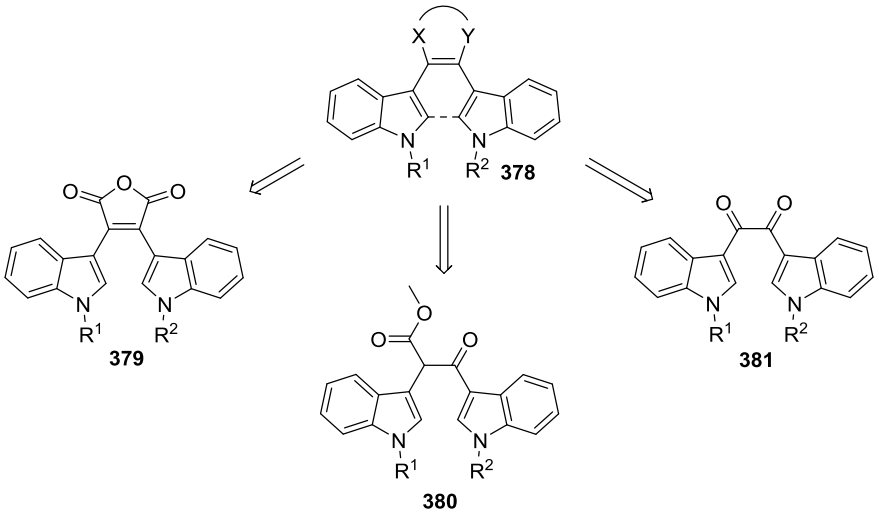
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# 6 Current Perspectives

## 6.1 Overview

The overall objective of this project was to produce selective and highly potent novel anticancer agents of structure **378**. Three synthetic routes were developed involving maleic anhydride **379**, β-keto ester **380**, or dione **381** intermediates in order to advance our goal of diversity-oriented synthesis. In total, a panel of 42 novel potential anticancer agents were synthesised of the bisindolylmaleimide and indolocarbazole structures.




**Scheme 6.1.1**

A route was designed from a maleic anhydride intermediate, formed using Perkin-type methodology and this route was utilised to synthesise a panel of both symmetrically and unsymmetrically substituted bisindolyl derivatives. Progression of this synthetic strategy included the addition of an oxidative cyclisation step to form an indolocarbazole intermediate, which was subsequently used to synthesise a panel of both symmetrically and unsymmetrically substituted indolocarbazoles, later to prove of considerable biological interest. Previous work in the field focussed on routes where the cyclisation to the indolocarbazole is the final step in the synthesis. This path is limited as the cyclisation of flexible bisindolyl precursors often proves to be a challenging transformation. The strategy reported in this thesis overcomes this issue by achieving this conversion earlier in the route to form a highly versatile indolocarbazole intermediate from which a wide range of derivatives with both 5- and 6-membered F-rings were formed.

A reliable, high-yielding Lossen rearrangement step was developed to form the novel six-membered rings in this route from hydroxymaleimide derivatives, the first time this type of rearrangement has been achieved with such a sterically hindered substrate. This methodology was used to synthesise a panel of novel indolocarbazoles with uracil headgroups which is also the first time the synthesis of a panel of indolocarbazoles with 6-membered F-rings has been accomplished.

This route was also adapted to synthesise derivatives with mono-*N*-substitution and both symmetrical and asymmetrical bis-*N*-substitution to incorporate a wide range of functional groups. Derivatives with alkyl substituents were synthesised to investigate the effects of added lipophilicity and derivatives with more polar side-chains were synthesised to investigate the effects of additional hydrophilicity and solubility.

A second route was utilised to expand the theme of F-ring modulation to synthesise both bisindolyl and indolocarbazole analogues with a pyrazolone or isocytosine headgroup. Further advancement of a route containing a β-keto ester intermediate, previously developed within the group, was achieved to include a nitrile chain and a cleavable protecting group.[1,2] This resulted in an indolocarbazole with a pyrazolone headgroup being synthesised for the first time and also the first synthesis of pyrazolone analogues with an unsubstituted indole nitrogen. The first synthesis of an indolocarbazole with an isocytosine headgroup and a non-methyl *N*-substituent was also accomplished. Work on a further route involving a dione intermediate resulted in the first isolation of a bisindolyl imidazole derivative.

As the vast majority of compounds reported in the literature possess either a lactam or maleimide F-ring, modification of this F-ring was one of the main aims of this project. Work towards the replacement of this key H-bonding structure within these molecules resulted in the formation of analogues with ten different F-rings possessing a range of H-bonding motifs (Figure 6.1.1). Heterocyclic elaboration of this ring aimed to expand the H-bonding network within the ligand-enzyme complex and exploit discrete differences in the active sites of protein kinases. This was undertaken to probe the molecular space within proposed binding pockets and to discover key new features of the SAR of these indolocarbazole analogues.

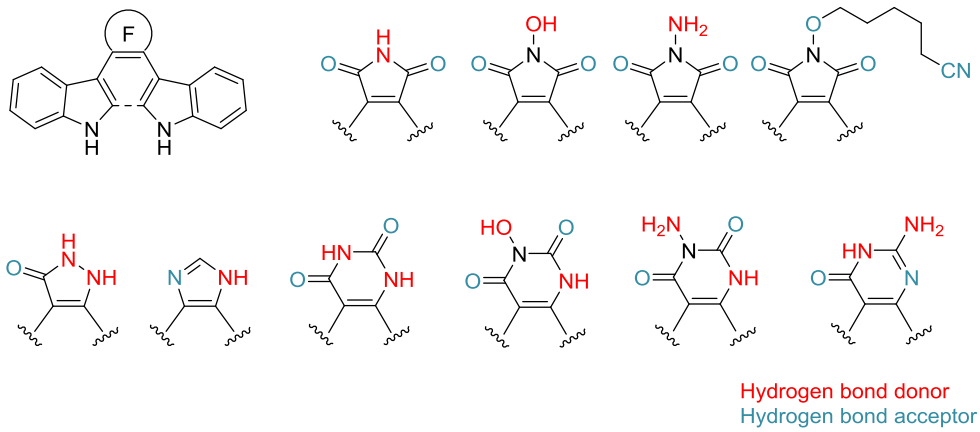
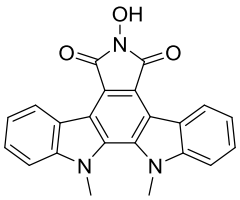


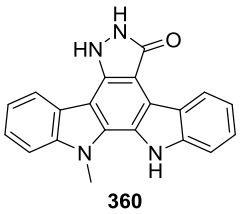

**Figure 6.1.1: F-rings containing novel H-bonding motifs**

Preliminary biological evaluation of both bisindole and indolocarbazole derivatives was achieved using the NCI-60 cell screen. This has led to the identification of several lead compounds with high cytotoxicity and excellent selectivity profiles. Of the 37 compounds submitted for the single dose screen, 12 were selected to progress to five dose testing, representing a very high success rate. Lead compounds from the NCI results include hydroxymaleimide **305** and uracil **352** which possessed nanomolar $GI_{50}$ values against specific cancer cell lines, and hydroxymaleimides **308** and **336**, and pyrazolone **360** which displayed low mean growths across the panel and also selective cytotoxicity (Figure 6.1.2).



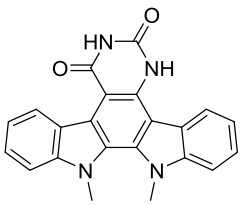
305

| Mean growth (10 µM) = 32.9% |
| --- |
| Leukaemia SR $GI_{50}$ = 73 nM |



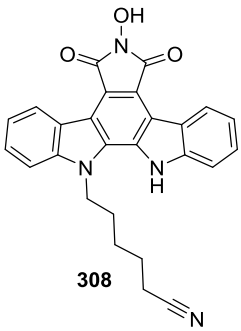
360

| Mean growth (10 µM) = 23.4% |
| --- |
| Selective cytotoxicity |
| CNS SF-539 $LC_{50}$ = 7.99 µM |
| Renal RXF 393 $LC_{50}$ = 8.49 µM |



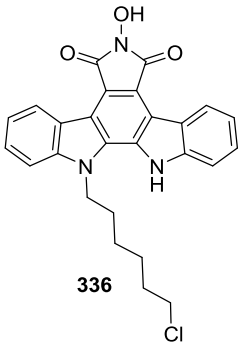
352

| Mean growth (10 µM) = 35.3% |
| --- |
| Renal cancer A498 $GI_{50}$ = 48 nM |



308

| Mean growth (10 µM) = -7.0% |
| --- |
| Selective cytotoxicity |
| NSCLC NCI-H522 $LC_{50}$ = 1.16 µM |
| Submicromolar $GI_{50}$ values |



336

| Mean growth (10 µM) = 4.2% |
| --- |
| Selective cytotoxicity |
| Melanoma LOX IMVI $LC_{50}$ = 6.11 µM |
| SK-MEL-5 $LC_{50}$ = 5.29 µM |

**Figure 6.1.2: Novel indolocarbazole derivatives with anticancer activity**

Evaluation against a panel of kinases has also been completed for a number of the bisindole derivatives which has demonstrated noteworthy selectivity towards GSK3α/β and PIM1 kinases, with low micromolar $IC_{50}$ values being observed for these enzymes.

## 6.2 Future Work

Solubility is a significant issue in the future development of indolocarbazoles as clinical candidates. This problem could be addressed by adding solubilising chains on the indole nitrogens and also on the hydroxy group on the hydroxymaleimide headgroup as

initiated in this work. As the addition of a hexanenitrile chain to the hydroxymaleimide was seen to improve activity, this could be a very useful site for the introduction of solubilising groups. The formation of *N*-glycosidic analogues of compound classes in this work to mimic staurosporine and rebeccamycin would also be worthwhile to evaluate the effect on their solubility and biological properties.

Pyrazolone derivative **360** displayed a much lower mean growth in the NCI-60 cell screen compared to its hydroxymaleimide counterpart, providing a lead compound for future work. As the incorporation of a hexanenitrile chain greatly increased activity compared to a methyl substituent, it is envisaged that a side chain would increase potency and solubility (Figure 6.2.1). The synthesis of the regioisomers of these compounds would also be worthwhile as an opportunity to expand the SAR study.

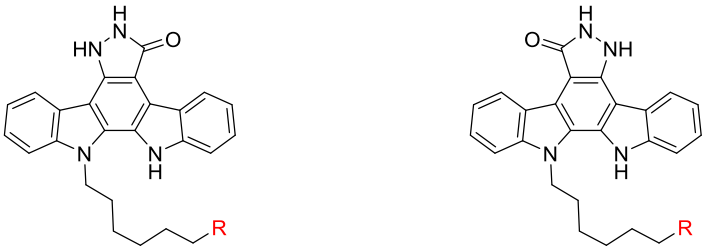


**Figure 6.2.1**

The incorporation of an amine salt functionality to the extended chain *N*-substituent on the hydroxymaleimide indolocarbazole template conferred admirable selectivity towards a number of cancer cell lines with the NCI-60 cell screen. It is envisaged that further SAR study would examine the use of other amines in place of the morpholine moiety (Figure 6.2.2).

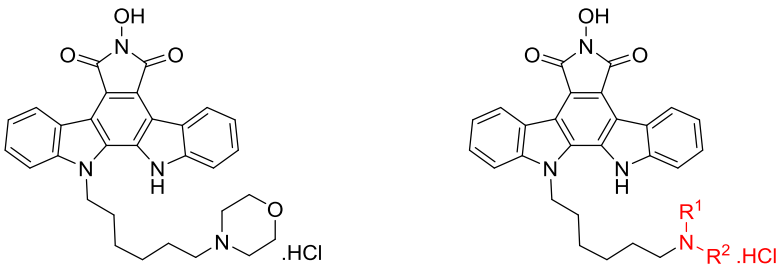
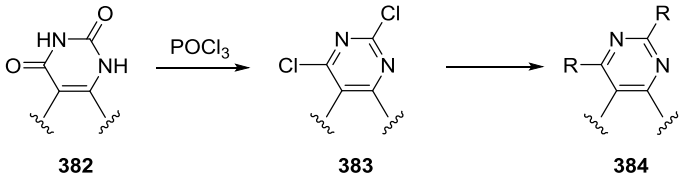


**Figure 6.2.2**

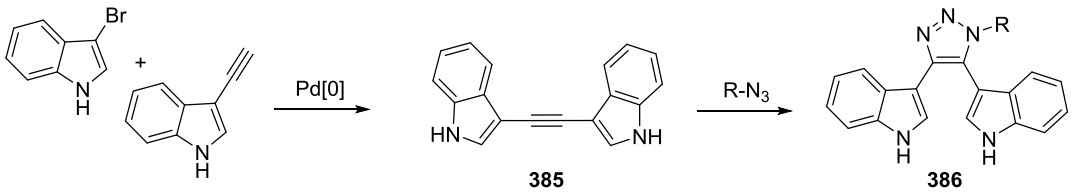
Given the interesting and potent activity of uracil **352** in NCI screening, it should also function as a lead compound for future work. To expand the panel of indolocarbazoles with 6-membered F-rings, the uracil **382** could be converted to a dichloropyrimidine **383** which could then be reacted with a range of different nucleophiles to form a series of substituted pyrimidines **384** (Scheme 6.2.1).




**382** **383** **384**

**Scheme 6.2.1: Proposed synthesis of novel pyrimidine derivatives**

Much potential remains in the field of indolocarbazole chemistry for further F-ring modulation in order to diversify H-bonding networks between the molecule and the enzyme active site which can lead to derivatives with unique bioactivity profiles. For example, the synthesis of a bisindolyl ethyne intermediate **385** could allow access to a variety of novel F-rings through reaction with different 1,3-dipoles such as triazoles **386** through reaction with azides (Scheme 6.2.2).




**385** **386**

**Scheme 6.2.2: Proposed synthesis of bisindolyl derivatives *via* 1,3 dipolar cycloaddition**

Due to time constraints, some of the biological results from the kinase screen were not available for inclusion in this thesis. This testing is currently underway and the results for the remaining compounds are keenly anticipated. As a number of these derivatives were highly active in NCI screening, it is envisaged that these results will provide further insight into the biological mode of action of these compounds.

Overall, this project has investigated many different aspects of both the indolocarbazole and bisindolylmaleimide pharmacophores and their potential as anticancer agents. It is interesting to note that on COMPARE analysis hydroxymaleimide **305,** pyrazolone **360**

or uracil **352** showed low correlations to the current indolocarbazoles in the NCI database, including similar compounds synthesised previously within the group even though the potency is upheld. This further demonstrates how small changes to the structure of the molecules can cause significant effects in the mode of ligand interaction with target enzymes. It is evident from the biological results obtained to date that huge potential exists within this class of compounds as chemotherapeutic agents and combined results from NCI-60 cell line, topo I and kinase screening should be used for the rational design of next-generation indolocarbazole derivatives, hopefully ultimately leading to new cancer treatments in the clinic.

## References

1. L. T. Pierce, *PhD Thesis, University College Cork*, 2011.
2. M. M. Cahill, *PhD Thesis, University College Cork*, 2012.

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## 7.1 General Procedures

All solvents were distilled prior to use by the following methods: dichloromethane was distilled from phosphorus pentoxide, ethyl acetate was distilled from potassium carbonate, hexane was distilled prior to use, methanol was distilled from magnesium in the presence of iodine and stored over 3 Å molecular sieves, tetrahydrofuran was freshly distilled from sodium and benzophenone. Anhydrous dimethylformamide was obtained from Sigma or Acros and stored under nitrogen. Molecular sieves were dried by heating to 140 °C for 24 hours. Organic phases were dried over anhydrous magnesium sulfate.

All commercial reagents were used without further purification unless stated otherwise. Commercially obtained *n*-butyllithium was titrated prior to use using the Gilman double titration[1,2] as follows: *n*-butyllithium solution (1.0 mL) was added dropwise *via* syringe to a 100 mL conical flask containing water (25 mL). A second flask, containing dry dibromoethane (~2 mL, dried over molecular sieves) stoppered with a SubaSeal and flushed with nitrogen with provision for pressure release was also treated dropwise with *n*-butyllithium (1.0 mL). Two drops of phenolphthalein were added to the first flask and the solution was titrated with 0.1 M aqueous HCl (standard solution) until the pink colour permanently disappeared. The SubaSeal was removed from the second flask and water (~25 mL) was added, along with 2 drops of phenolphthalein and titrated as before. (Note: this flask was biphasic and required constant swirling.) The first titre is total base (*n*-BuLi + inorganic base) and the second is the free base (inorganic base). Thus, (titre 1 – titre 2) × molarity of HCl = the molarity of the *n*-butyllithium solution.

In reactions with *n*-butyllithium the glassware was flame-dried under nitrogen atmosphere prior to use. For smaller quantities (<10 mL) glass syringes were used to transfer reagents. For larger quantities (>10 mL) an addition funnel was calibrated to the desired volume prior to flame-drying and the reagent was transferred directly from the bottle to the addition funnel *via* cannula.

Low temperature reactions were carried out with cooling mixtures as follows: -10 to -15 °C, ice and salt; -60 to -80 °C, acetone and liquid nitrogen. All low temperature

reactions were carried out in a three-necked round-bottomed flask, fitted with a low temperature thermometer. The temperatures reported are internal temperatures.

Photochemical reactions were carried out using a Philips HPL-N 80W/542 mercury lamp. The reaction flask was fitted with a condenser and the lamp was placed as close as possible to the flask. The entire apparatus was wrapped in aluminium foil prior to switching on the lamp.

$^{1}$H (300 MHz) and $^{13}$C (75 MHz) NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer. $^{1}$H (400 MHz) NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. $^{13}$C (125 MHz) NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer. $^{1}$H (600 MHz) and $^{13}$C (150 MHz) NMR spectra were recorded on a BrukerAvance III 600 MHz spectrometer equipped with a dual CH cryoprobe. All spectra were recorded at 20 °C in deuterated dimethylsulfoxide (DMSO-$d_6$) or deuterated chloroform ($CDCl_3$) using tetramethylsilane (TMS) as an internal standard unless otherwise stated. Chemical shifts ($\delta_H$ and $\delta_C$) are reported in parts per million (ppm) relative to the reference peak and coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns in $^{1}$H spectra are designated as s (singlet), bs (broad singlet), d (doublet), bd (broad doublet), t (triplet), q (quartet), quin (quintet), sept (septet), dd (doublet of doublets), dt (doublet of triplets), ddd (doublet of doublet of doublets), ddt (doublet of doublet of triplets) and (m) multiplet. COSY (Correlation Spectroscopy) and HMBC (Heteronuclear Multiple-Bond Correlation) experiments were used to assign ambiguous $^{1}$H NMR spectra. Carbon spectra were assigned (CH, $CH_2$, $CH_3$) with the aid of DEPT (Distortionless Enhancement by Polarisation Transfer) experiments.

Infrared spectra were recorded as thin films on sodium chloride plates for oils or as potassium bromide (KBr) discs for solids on a Perkin Elmer Spectrum 100 FT-IR spectrometer or a Perkin Elmer Spectrum One FT-IR spectrometer.

Nominal mass spectra were recorded on a Waters Quattro Micro triple quadrupole spectrometer (QAA1202) in ESI mode using 50% acetonitrile – water containing 0.1% formic acid as eluent. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier Time of Flight spectrometer (KD160) in ESI mode using 50% acetonitrile

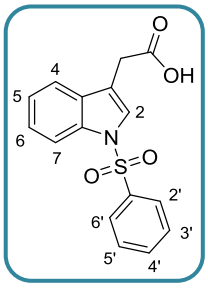
– water containing 0.1% formic acid as eluent. Samples (max. 1 mg) were dissolved in acetonitrile, methanol or 1:1 acetonitrile – methanol.

Melting points were measured on a uni-melt Thomas Hoover capillary melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck 60 PF254) or aluminium oxide TLC plates (Sigma). Visualisation was achieved by UV light detection (254 and 366 nm). Wet flash column chromatography was performed using Merck $PF_{254}$ silica gel unless otherwise stated.

## 7.2 Synthesis and derivatisation of 1-hydroxy-3,4-di(1*H*-indol-3-yl)-1*H*-pyrrole-2,5-diones

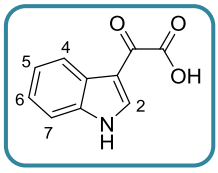
### 7.2.1 Synthesis of maleic anhydride intermediates

**2-[1-(Phenylsulfonyl)-1*H*-indol-3-yl]acetic acid 256**[3]



To a stirred solution of indol-3-acetic acid **254** (20.011 g, 114 mmol) in THF (200 mL) under a nitrogen atmosphere at -70°C was added *n*-butyllithium (2.5 M in hexanes, 96 mL, 240 mmol) while maintaining the temperature below -70 °C. The mixture was stirred for 1 hour at this temperature before a solution of benzenesulfonyl chloride (14.6 mL, 20.2 g, 240 mmol) in THF (50 mL) was added slowly. Following addition, the reaction vessel was allowed to warm to room temperature over ~1 hour and stirred at this temperature for a further 16 hours. Water (10 mL) was then added to quench the reaction and the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (200 mL) and the organic layer was then washed with 1 M aqueous HCl (100 mL × 2), followed by water (120 mL), before being dried over anhydrous magnesium sulfate and the solvent evaporated under reduced pressure to form a brown solid. Recrystallisation from ethyl acetate gave product as an off-white solid (32.210 g, 90%): m.p. 176-178 $^{o}$C; (Lit. 171-173 °C)[3]; $\nu_{max}$/cm$^{-1}$ (KBr) 3388, 3055, 1710, 1448, 1362, 1173; $\delta_H$ (300 MHz, DMSO-$d_6$) 3.71 [2H, s, $CH_2$], 7.26 [1H, t, *J* 7.5, C(6)-H], 7.36 [1H, ddd, *J* 7.5, 7.2, 1.1, C(5)-H], 7.53-7.61 [3H, m, C(3′, 5′, 7)-H], 7.69 [1H, m, C(4′)-H], 7.74 [1H, s, C(2)-H], 7.92-7.98 [3H, m, C(2′, 6′, 4)-H], 12.47 [1H, bs, OH]; m/z (ESI$^-$) 314.1 [(M-H)$^-$ 100%].

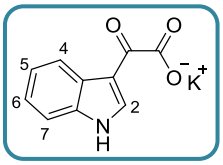
**2-(1*H*-Indol-3-yl)-2-oxoacetic acid 252**[4]



To a solution of indole **251** (10.021 g, 85.5 mmol) in diethyl ether (150 mL) was added oxalyl chloride (11.0 mL, 16.3 g, 128 mmol) dropwise over 10 minutes and the yellow suspension was allowed to stir for a further 30 minutes. Saturated aqueous sodium bicarbonate (20 mL) was then added with caution and the biphasic mixture was stirred for 16 hours. The product was

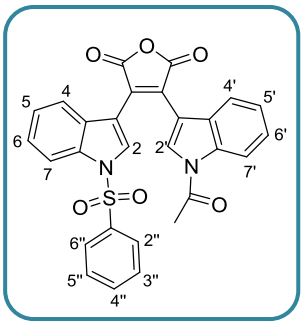
then collected using vacuum filtration, washed with diethyl ether and dried overnight at 40 °C to give a yellow solid (14.167 g, 88%): m.p. 193-196 °C; (Lit. 218 °C decomp.)[4]; $\nu_{max}/cm^{-1}$ (KBr) 3549, 3195, 1719, 1611, 1268; $\delta_H$ (300 MHz, DMSO-$d_6$) 7.26 [1H, ddd, *J* 9.1, 7.1, 1.7, C(5)-H], 7.29 [1H, ddd, *J* 9.3, 7.1, 1.9, C(6)-H], 7.52-7.58 [1H, m, C(7)-H], 8.14-8.20 [1H, m, C(4)-H], 8.42 [1H, d, *J* 3.3, C(2)-H], 12.36 (1H, bs, N-H); m/z ($ESI^+$) 190.3 $[(M+H)^+$ 100%].

**Potassium 2-(1*H*-indol-3-yl)-2-oxoacetate 253**[5]



A solution of 2-(1*H*-indol-3-yl)-2-oxoacetic acid **252** (14.205 g, 75.1 mmol) in ethanol (280 mL) was treated with potassium hydroxide (85 wt. %, 4.958 g, 75.1 mmol) and stirred at room temperature for 4 hours. The product was then collected using vacuum filtration and dried overnight at 40 °C to give a white solid (14.507 g, 85%): m.p. 291-293 °C (decomposition); $\nu_{max}/cm^{-1}$ (KBr) 3649, 3392, 1632, 1614, 1114; $\delta_H$ (300 MHz, DMSO-$d_6$) 7.12 [1H, ddd, *J* 8.6, 7.1, 1.5, C(5)-H], 7.16 [1H, ddd, *J* 8.8, 7.1, 1.7, C(6)-H], 7.46 [1H, d, *J* 6.7, C(7)-H], 8.13 [1H, s, C(2)-H], 8.17 [1H, d, *J* 7.7, C(4)-H], 12.12 (1H, bs, N-H); m/z ($ESI^-$) 188.4 $[(M)^-$ 70%].

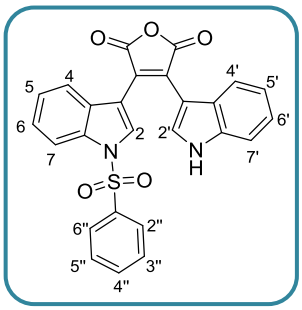
**3-(1-Acetyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione 257**



A mixture of potassium 2-(1*H*-indol-3-yl)-2-oxoacetate **253** (3.677 g, 15.8 mmol), 2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]acetic acid **256** (5.020 g, 15.8 mmol) and acetic anhydride (30 mL) was stirred at 80 °C for 24 hours. The yellow suspension was then cooled to room temperature and filtered. The solid collected was stirred in boiling ethyl acetate for 30 minutes before hot filtration removed a white side product. The yellow filtrate was concentrated to dryness giving a yellow crystalline solid (2.426 g). The acetic anhydride solution from the initial filtration was concentrated under reduced pressure. The residue was then washed with saturated sodium bicarbonate solution (50 mL) and extracted into ethyl acetate (3 × 30 mL). The organic layer was filtered before being washed with saturated sodium bicarbonate solution (30 mL), followed by water (30 mL) and brine (30 mL) and then dried over anhydrous magnesium sulfate, filtered and the solvent

evaporated under reduced pressure. The crude residue was recrystallised from ethyl acetate to give additional product (0.544 g, combined yield 2.970 g, 40%): m.p. 223-225 °C; $\nu_{max}/cm^{-1}$ (KBr) 3139, 1830, 1762, 1718, 1373, 1176; $\delta_H$ (300 MHz, DMSO-$d_6$) 2.69 (3H, s, $CH_3$), 6.44 [1H, t, *J* 7.4, C(6′)-H], 6.59 [1H, d, *J* 7.8, C(7′)-H], 6.95 [1H, t, *J* 7.3, C(6)-H], 7.03 [1H, d, *J* 7.6, C(7)-H], 7.16 [1H, t, *J* 7.8, C(5′)-H], 7.27 [1H, t, *J* 7.7, C(5)-H], 7.61-7.71 [2H, m, C(3″, 5″)-H], 7.75-7.85 [1H, t, *J* 7.4, C(4″)-H], 7.93 [1H, d, *J* 8.6, C(4)-H], 7.98-8.06 [2H, m, C(2″, 6″)-H], 8.19 [1H, s, C(2)-H], 8.26 [1H, d, *J* 8.3, C(4′)-H], 8.30 [1H, s, C(2′)-H]; $\delta_C$ (75 MHz, DMSO-$d_6$) 23.9 ($CH_3$), 109.3 (C, aromatic C), 111.2 (C, aromatic C), 113.1 (CH, aromatic CH), 115.9 (CH, aromatic CH), 120.5 (CH, aromatic CH), 121.6 (CH, aromatic CH), 123.1 (CH, aromatic CH), 123.7 (CH, aromatic CH), 125.4 (CH, aromatic CH), 125.7 (CH, aromatic CH), 126.6 (C, aromatic C), 126.9 (2CH, 2 × aromatic CH), 127.6 (C, aromatic C), 129.6 (CH, aromatic CH), 130.1 (2CH, 2 × aromatic CH, C, aromatic C), 130.8 (CH, aromatic CH), 131.4 (C, aromatic C), 133.6 (C, aromatic C), 134.7 (C, aromatic C), 135.2 (CH, aromatic CH), 136.4 (C, aromatic C), 164.9 (C=O), 165.1 (C=O), 169.7 (C=O); m/z ($ESI^+$) 511.1 [$(M+H)^+$ 10%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{28}H_{19}N_2O_6S)^+$ 511.0964. Found 511.0962.

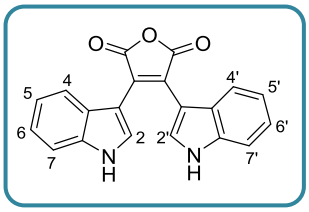
**3-(1*H*-Indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione 267**




3-(1-Acetyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol -3-yl]furan-2,5-dione **257** (0.599 g, 1.17 mmol) was suspended in methanol (20 mL) and water (5 mL) and potassium carbonate (0.812 g, 5.85 mmol) was added. The mixture was stirred for 1 hour eventually forming an orange solution. The solvent was then removed under reduced pressure and the residue dissolved in water (20 mL) before being acidified with 10% aqueous HCl to pH 2 and extracted into ethyl acetate (2 × 20 mL). The combined organic layers were washed with water (3 × 20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give an orange solid (0.517 g, 92%): m.p. 220-221 °C; $\nu_{max}/cm^{-1}$ (KBr); 3391, 1819, 1746, 1537, 1381, 1177, 1131; $\delta_H$ (300 MHz, DMSO-$d_6$) 6.16 [1H, ddd, *J* 8.1, 7.1, 0.9, C(6′)-H], 6.37 [1H, d, *J* 8.0, C(7′)-H], 6.88-6.99 [3H, m, C(5′, 6, 7)-H], 7.25 [1H, ddd, *J* 8.4, 6.9, 1.6, C(5)-H], 7.34 [1H, d, *J* 8.1, C(4)-H], 7.62-7.72 [2H, m, C(3″, 5″)-H], 7.81 [1H, m, C(4″)-H], 7.95 [1H, d, *J* 8.5, C(4′)-H],

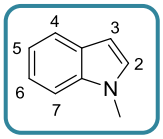
8.02-8.07 [2H, m, C(2″, 6″)-H], 8.09 [1H, s, C(2)-H], 8.12 [1H, s, C(2′)-H], 12.18 (1H, bs, N-H) $\delta_H$ (300 MHz, DMSO-$d_6$); $\delta_c$ (75 MHz, DMSO-$d_6$) 104.6 (C, aromatic C), 112.1 (C, aromatic C), 112.4 (CH, aromatic CH), 113.1 (CH, aromatic CH), 120.2 (CH, aromatic CH), 120.4 (CH, aromatic CH), 121.7 (CH, aromatic CH), 122.3 (C, aromatic C), 122.4 (CH, aromatic CH), 123.4 (CH, aromatic CH), 124.4 (C, aromatic C), 125.4 (CH, aromatic CH), 126.9 (2CH, 2 × aromatic CH), 128.3 (C, aromatic C), 128.4 (CH, aromatic CH), 130.0 (2CH, 2 × aromatic CH), 132.3 (CH, aromatic CH), 133.5 (C, aromatic C), 134.7 (C, aromatic C), 135.0 (CH, aromatic CH), 136.2 (C, aromatic C), 136.6 (C, aromatic C), 165.5 (C=O), 165.9 (C=O); m/z (ESI$^+$) 469.1 [$(M+H)^+$ 70%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{26}H_{17}N_2O_5S)^+$ 469.0858. Found 469.0850.

**3,4-Di(1*H*-indol-3-yl)furan-2,5-dione 255**[6]



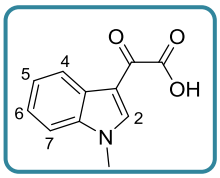

A suspension of 3-(1-acetyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **257** (3.611 g, 7.07 mmol) in a mixture of methanol (100 mL) and water (25 mL) was treated with potassium hydroxide (85 wt. %, 2.774 g, 42.4 mmol) and the mixture was heated to reflux for 24 hours. The reaction mixture was allowed to cool to room temperature and the solvent evaporated under reduced pressure. The residue was acidified to pH 2 using 20% aqueous HCl and extracted using ethyl acetate (100 mL). The organic layer was washed once with 1 M aqueous HCl (50 mL), followed by water (2 × 50 mL) and brine (50 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give a red foamy residue. The residue was dissolved in chloroform, allowed to crystallise and the red crystalline solid was collected by vacuum filtration (1.981 g, 85%): m.p. 227-229 °C; (Lit. 227-230 °C)[6]; $\nu_{max}$/cm$^{-1}$ (KBr) 3389, 1819, 1750, 1530, 1427, 1253; $\delta_H$ (300 MHz, DMSO-$d_6$) 6.71 [t, *J* 7.4, C(6, 6′)-H], 6.86 [d, *J* 7.9, C(7, 7′)-H], 7.04 [t, *J* 7.6, C(5, 5′)-H], 7.43 [d, *J* 8.2, C(4, 4′)-H], 7.85 [d, *J* 2.6, C(2, 2′)-H], 11.92 [2H, s, N(1, 1′)-H]; m/z (ESI$^-$) 327.4 [$(M-H)^-$ 100%].

**1-Methyl-1*H*-indole 148**[7]



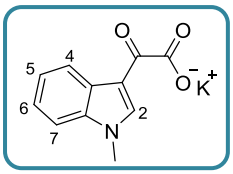
To a stirred solution of indole **251** (2.002 g, 17.1 mmol) in anhydrous DMF (90 mL) was added sodium hydride (60 wt. % oil dispersion, 1.367 g, 34.2 mmol) at 0°C under nitrogen and the mixture was stirred for 30 minutes. Methyl iodide (1.80 mL, 4.10 g, 28.9 mmol) was then added before the mixture was allowed to warm to room temperature and stirred for a further 3 hours. Water (120 mL) was then cautiously added and the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with 1 M aqueous HCl (3 × 50 mL) followed by water (2 × 50 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated to give a pale yellow oil (2.137 g, 95%) which was used without further purification: $\nu_{max}/cm^{-1}$ (NaCl) 3054, 2917, 1614, 1513, 1316, 1242; $\delta_H$ (300 MHz, $CDCl_3$) 3.78 [3H, s, N-$CH_3$], 6.48 [1H, dd, *J* 3.1, 0.8, C(3)-H], 7.03 [1H, d, *J* 3.1, C(2)-H], 7.10 [1H, ddd, *J* 8.0, 7.0, 1.1, C(5)-H], 7.22 [1H, ddd, *J* 8.0, 7.2, 1.1, C(6)-H], 7.32 [1H, dd, *J* 8.1, 0.7, C(7)-H], 7.63 [1H, dt, *J* 7.9, 0.9, C(4)-H]; m/z ($ESI^+$) 132.2 $[(M+H)^+$ 100%].

**2-(1-Methyl-1*H*-indol-3-yl)-2-oxoacetic acid 259**[8]



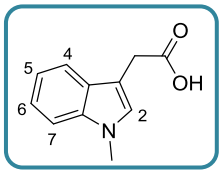
To a solution of 1-methyl-1*H*-indole **148** (4.503 g, 34.3 mmol) in diethyl ether (60 mL) was added oxalyl chloride (4.40 mL, 6.51 g, 51.5 mmol), dropwise, over 10 minutes. The yellow suspension was then stirred for a further 30 minutes. Saturated sodium bicarbonate solution (10 mL) was added with caution and the biphasic mixture was stirred for 16 hours. The product was collected using vacuum filtration, washed with diethyl ether and dried overnight at 40 °C to give a pale yellow solid (5.158 g, 74%): m.p. 157-158 °C; $\nu_{max}/cm^{-1}$ (KBr) 3249, 3145, 1754, 1619, 1521, 1308, 1210; $\delta_H$ (400 MHz, DMSO-$d_6$) 3.92 (s, $CH_3$), 7.32 [1H, ddd, *J* 8.4, 7.2, 1.1, C(5,)-H], 7.36 [1H, ddd, *J* 8.6, 7.2, 1.4, C(6)-H], 7.61 [1H, d, *J* 7.3, C(7)-H], 8.20 [1H, dd, *J* 7.0, 1.4, C(4)-H], 8.49 [1H, s, C(2)-H], 13.89 (1H, bs, COOH); m/z ($ESI^+$) 204.3 $[(M+H)^+$ 100%].

**Potassium 2-(1-methyl-1*H*-indol-3-yl)-2-oxoacetate 261**



A solution of 2-(1-methyl-1*H*-indol-3-yl)-2-oxoacetic acid **259** (5.011 g, 24.6 mmol) in ethanol (120 mL) was treated with potassium hydroxide (85 wt. %, 1.620 g, 24.6 mmol) and stirred at room temperature for 4 hours. The product was then collected using vacuum filtration and dried overnight at 40 °C to give a white solid (5.646 g, 95%): m.p. >300 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3420, 3047, 1623, 1520, 1361, 1204, 1084; $\delta_H$ (400 MHz, DMSO-$d_6$) 3.84 (1H, s, $CH_3$), 7.18 [1H, t, *J* 7.4, C(5)-H], 7.24 [1H, t, *J* 7.5, C(6)-H], 7.49 [1H, d, *J* 8.0, C(7)-H], 8.12 [1H, s, C(2)-H], 8.18 [1H, d, *J* 7.5, C(4)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 32.9 ($CH_3$), 110.3 (CH, aromatic CH), 113.0 (C, aromatic C), 121.3 (CH, aromatic CH), 121.4 (CH, aromatic CH), 122.3 (CH, aromatic CH), 126.3 (C, aromatic C), 137.0 (C, aromatic C), 139.2 (CH, aromatic CH), 169.7 (C=O), 193.1 (C=O); m/z (ESI$^+$) 204.3 [(M+2H)$^+$ 20%]. HRMS (ESI$^+$): Exact mass calculated for $(C_{11}H_{10}NO_3)^+$ 204.0654. Found 204.0661.

**2-(1-Methyl-1*H*-indol-3-yl)acetic acid 150**[9]

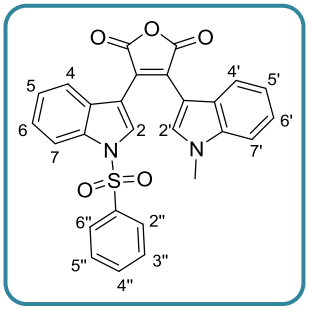


To a stirred suspension of sodium hydride (60 wt.% oil suspension, 7.202 g, 180 mmol) in dry THF (200 mL) at 0 °C under a nitrogen atmosphere was added dropwise a solution of indole-3-acetic acid **254** (10.211 g, 58.3 mmol) in dry THF (50 mL). The mixture was stirred for 30 minutes at this temperature before a solution of iodomethane (5.60 mL, 12.77 g, 90.0 mmol) in THF (30 mL) was added. The mixture was stirred for 16 hours while warming slowly to room temperature. Methanol (10 mL) was then added to quench the reaction, followed by water (150 mL). The aqueous solution was washed with diethyl ether (100 mL) before being acidified to pH 2 using 10% HCl. The aqueous layer was extracted with DCM (3 × 100 mL) and the combined DCM layers were washed with water (150 mL), before being dried over anhydrous magnesium sulfate and filtered. The solvent was reduced to approximately 50 mL before hexane was added until the solution remained cloudy and the mixture was allowed to stand for 16 hours. The precipitate was then collected to give desired compound as a light brown solid (6.751 g, 64%): m.p. 126-127 °C; (Lit. 127-128 °C)[9]; $\nu_{max}$/cm$^{-1}$ (KBr) 2927, 2676, 1698, 1474, 1412, 1304, 941; $\delta_H$ (400 MHz, DMSO-$d_6$) 3.64 [2H, s, $CH_2$], 3.75 [3H, s, $CH_3$], 7.03 [1H, ddd, *J* 8.0, 7.1,

1.0, C(5)-H], 7.15 [1H, ddd, *J* 8.2, 7.0, 1.1, C(6)-H], 7.22 [1H, s, C(2)-H], 7.39 [1H, d, *J* 8.1, C(7)-H], 7.51 [1H, dt, *J* 7.9, 0.9, C(4)-H], 12.16 [1H, bs, COOH]; m/z ($ESI^+$) 190.3 [$(M+H)^+$ 100%].

**3-(1-Methyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione 263**[3]

Method A



A mixture of potassium 2-(1-methyl-1*H*-indol-3-yl)-2-oxoacetate **261** (4.605 g, 19.0 mmol) and 2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]acetic acid **256** (6.002 g, 19.0 mmol) were suspended in acetic anhydride (30 mL) and heated to 80 °C for 24 hours. The resulting orange suspension was allowed to cool and the solvent evaporated under reduced pressure. The residue was washed with saturated aqueous sodium bicarbonate (60 mL) before being extracted into ethyl acetate (2 × 40 mL). The organic layer was washed with saturated aqueous sodium bicarbonate (4 × 30 mL), followed by water (40 mL), before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude material was purified using column chromatography on silica gel with 20% ethyl acetate/hexane to give an orange foamy residue. The residue was dissolved in chloroform (20 mL) and diethyl ether was added until an orange solid precipitated which was collected by vacuum filtration (2.822 g, 31%): m.p. 185-187 °C; (Lit. >200 °C decomp.)[3]; $\nu_{max}/cm^{-1}$ (KBr) 3418, 2924, 1822, 1756, 1634, 1374, 1176; $\delta_H$ (300 MHz, DMSO-$d_6$) 3.92 (3H, s, $CH_3$), 6.16 [1H, t, *J* 7.6, C(6′)-H], 6.29 [1H, d, *J* 8.0, C(7′)-H], 6.92 [1H, ddd, *J* 7.9, 7.2, 0.7, C(6)-H], 6.98-7.05 [2H, m, C(5′, 7)-H], 7.26 [1H, ddd, *J* 8.4, 7.2, 1.3, C(5)-H], 7.45 [1H, d, *J* 8.2, C(4′)-H], 7.64-7.71 [2H, m, C(3″,5″)-H], 7.82 [1H, m, C(4″)-H], 7.96 [1H, d, *J* 8.3, C(4)-H], 8.04-8.09 [2H, m, C(2″,6″)-H], 8.11 [1H, s, C(2)-H], 8.21 [1H, s, C(2′)-H]; m/z ($ESI^+$) 483.1 [$(M+H)^+$ 50%].

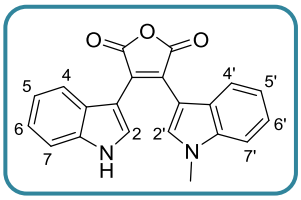
Method B

To a solution of 3-(1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **267** (0.101 g, 0.216 mmol) in anhydrous DMF (8 mL) in a flame-dried flask under nitrogen was added sodium hydride (0.010 g, 0.260 mmol) and the mixture was stirred

for 30 minutes at room temperature. Iodomethane (0.02 mL, 0.05 g, 0.34 mmol) was then added and the mixture was stirred for a further 4 hours. The reaction mixture was then poured into water (30 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with 1 M aqueous HCl (3 × 20 mL), followed by water (20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give desired product as an orange foamy solid (0.87 g, 84%). Analysis was identical to that given above.

**3-(1*H*-Indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione 265**[3]

Method A



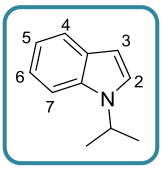

To a stirred suspension of 3-(1-methyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **263** (3.930 g, 8.14 mmol) in methanol (100 mL) and water (25 mL) was added potassium hydroxide (2.691 g, 4.07 mmol) and the resulting solution was heated to reflux for 24 hours. The reaction mixture was then cooled before the solvent was evaporated under reduced pressure. The residue was dissolved in water (40 mL) and acidified to pH 2 with 10% aqueous HCl before being extracted into ethyl acetate (2 × 40 mL). The organic layer was washed with water (3 × 30 mL), followed by brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure giving a red solid (2.634 g, 95%): m.p. 251-252 °C; (Lit. 246-248 °C)[3]; $\nu_{max}$/cm$^{-1}$ (KBr) 3372, 3037, 1819, 1747, 1529, 1248; $\delta_H$ (300 MHz, DMSO-$d_6$) 3.89 (3H, s, $CH_3$), 6.67-6.79 [3H, m, C(6, 6′, 7′)-H], 6.89 [1H, d, C(7)-H], 7.04 [1H, t, *J* 7.4, C(5)-H], 7.09 [1H, t, *J* 7.5, C(5′)-H], 7.43 [1H, d, *J* 8.2, C(4)-H], 7.49 [1H, d, *J* 8.3, C(4′)-H], 7.83 [1H, d, *J* 2.8, C(2)-H], 7.95 [1H, s, C(2′)-H], 11.93 (1H, bs, NH); m/z (ESI$^+$) 343.2 [(M+H)$^+$ 10%].

Method B

A suspension of potassium 2-(1*H*-indol-3-yl)-2-oxoacetate **253** (6.823 g, 26.6 mmol), 2-(1-methyl-1*H*-indol-3-yl)acetic acid **150** (5.030 g, 26.6 mmol) and acetic anhydride (30 mL) was stirred at 80 °C for 24 hours. The yellow suspension was then cooled to room temperature and the solvent evaporated under reduced pressure. The residue was then washed with saturated aqueous sodium bicarbonate (50 mL) and extracted into ethyl acetate (2 × 30 mL). The organic layer was washed with saturated aqueous sodium

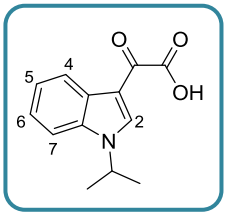
bicarbonate (30 mL), followed by water (30 mL) and brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was then purified using column chromatography on silica gel with 10% ethyl acetate/hexane to give the desired product as a red solid (1.849 g, 18%). Analysis was identical to that given above.

**1-Isopropyl-1*H*-indole 258**[10]




To a stirred solution of indole **251** (4.000 g, 34.1 mmol) in anhydrous DMF (70 mL), at 0 °C under nitrogen was added sodium hydride (60 wt. % oil dispersion, 2.728 g, 68.2 mmol) and the suspension was stirred for 30 minutes at this temperature. Isopropyl iodide (8.53 mL, 14.53 g, 85.4 mmol) was then added to the solution which was then allowed to warm to room temperature and stirred for 16 hours. Water (70 mL) was added and the mixture was extracted with ethyl acetate (25 mL × 2). The combined organic layers were washed using 1 M aqueous HCl (25 mL × 3) and water (25 mL × 2) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was then purified using column chromatography on silica gel with 10% ethyl acetate/hexane to give desired product as a pale yellow oil (3.220 g, 59%) which was used without further purification: $\nu_{max}$/cm$^{-1}$ (NaCl) 2975, 1462, 1314, 1300, 1223, 739; $\delta_H$ (300 MHz, $CDCl_3$) 1.52 [6H, d, *J* 6.7, CH(C<u>H</u>$_3$)$_2$], 4.68 [1H, sept, *J* 6.7, C<u>H</u>(CH$_3$)$_2$], 6.51 [1H, dd, *J* 3.2, 0.6, C(3)-H], 7.09 [1H, ddd, *J* 8.0, 7.1, 1.1, C(5)-H], 7.19 [1H, ddd, *J* 8.3, 7.2, 1.2, C(6)-H], 7.22 [1H, d, *J* 3.2, C(2)-H], 7.38 [1H, dd, *J* 8.2, 0.6, C(7)-H], 7.63 [1H, dt, *J* 7.9, 1.0, C(4)-H]; m/z (ESI$^+$) 160.4 [(M+H)$^+$ 100%].

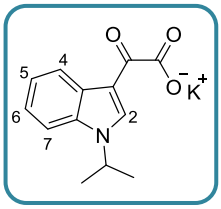
**2-(1-Isopropyl-1*H*-indol-3-yl)-2-oxoacetic acid 260**




To a solution of 1-isopropyl-1*H*-indole **258** (2.894 g, 18.0 mmol) in diethyl ether (50 mL) was added oxalyl chloride (2.33 mL, 3.45 g, 27.2 mmol) dropwise, over 10 minutes. The solution was then stirred for a further 30 minutes. Saturated aqueous sodium bicarbonate (10 mL) was then added with caution and the biphasic mixture was stirred for 16 hours. The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 15 mL). The combined organic layers were washed with water before being dried over

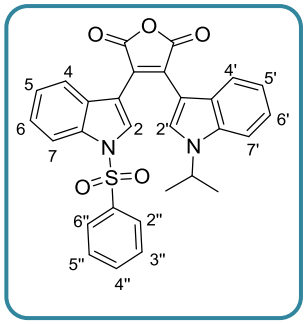
anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give the desired product as a yellow solid (3.790 g, 90%): m.p. 113-115 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3287, 2988, 1760, 1605, 1321, 1199, 740; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.54 [6H, d, *J* 6.7, CH(C<u>H</u>$_3$)$_2$], 4.88 [1H, sept, *J* 6.7, C<u>H</u>(CH$_3$)$_2$], 7.31 [1H, ddd, *J* 8.5, 7.2, 1.4, C(5)-H], 7.35 [1H, ddd, *J* 8.6, 7.1, 1.6, C(6)-H], 7.72 [1H, d, *J* 7.4, C(7)-H], 8.22 [1H, d, *J* 6.8, C(4)-H], 8.42 [1H, s, C(2)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 21.9 (2 × CH$_3$), 48.2 [<u>C</u>H(CH$_3$)$_2$], 111.4 (CH, aromatic CH), 111.6 (C, aromatic C), 121.4 (CH, aromatic CH), 123.1 (CH, aromatic CH), 123.7 (CH, aromatic CH), 126.3 (C, aromatic C), 136.1 (C, aromatic C), 136.5 (CH, aromatic CH), 165.2 (C=O), 180.3 (C=O); m/z (ESI$^+$) 232.3 [(M+H)$^+$ 100%]; HRMS (ESI$^+$): Exact mass calculated for (C$_{13}$H$_{14}$NO$_3$)$^+$ 232.0974. Found 232.0970.

**Potassium 2-(1-isopropyl-1*H*-indol-3-yl)-2-oxoacetate 262**



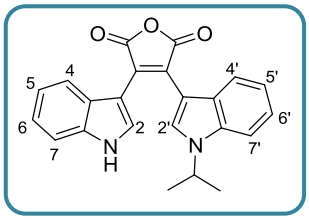

A solution of 2-(1-isopropyl-1*H*-indol-3-yl)-2-oxoacetic acid **260** (3.499 g, 15.1 mmol) in ethanol (50 mL) was treated with potassium hydroxide (85 wt. %, 0.993 g, 15.1 mmol) and the mixture was stirred at room temperature for 4 hours. The solvent was removed under reduced pressure and the residue dried overnight at 40 °C to give the desired product as a pale pink solid (3.961 g, 97%): m.p. 80-84 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3456, 2981, 1649, 1607, 1301, 1203, 738; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.48 [d, 6H, *J* 6.6, CH(C<u>H</u>$_3$)$_2$], 4.80 [1H, sept, *J* 6.7, C<u>H</u>(CH$_3$)$_2$], 7.17 [1H, ddd, *J* 8.2, 7.2, 1.1, C(5)-H], 7.22 [1H, ddd, *J* 8.5, 7.1, 1.4, C(6)-H], 7.58 [1H, d, *J* 7.6, C(7)-H], 8.14 [1H, s, C(2)-H], 8.19 [1H, d, *J* 7.3, C(4)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 22.2 (2 × CH$_3$), 47.2 [<u>C</u>H(CH$_3$)$_2$], 110.5 (CH, aromatic CH), 113.4 (C, aromatic C), 121.4 (CH, aromatic CH), 121.5 (CH, aromatic CH), 122.2 (CH, aromatic CH), 126.5 (C, aromatic C), 134.0 (CH, aromatic CH), 135.8 (C, aromatic C), 169.8 (C=O), 193.1 (C=O); m/z (ESI$^+$) 232.3 [(M+2H)$^+$ 100%]; HRMS (ESI$^+$): Exact mass calculated for (C$_{13}$H$_{17}$N$_2$O$_3$)$^+$ 249.1239 [(M+NH$_4$+H)$^+$]. Found 249.1239.

**3-(1-Isopropyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione 264**



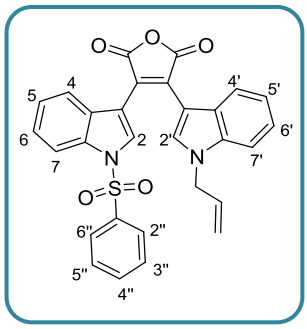
A mixture of potassium 2-(1-isopropyl-1*H*-indol-3-yl)-2-oxoacetate **262** (3.502 g, 13.0 mmol), 2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]acetic acid **256** (4.096 g, 13.0 mmol) and acetic anhydride (30 mL) was stirred at 80 °C for 24 hours. The yellow suspension was then allowed to cool to room temperature and the solvent was evaporated under reduced pressure. The residue was washed with saturated aqueous sodium bicarbonate (50 mL) and extracted into ethyl acetate (2 × 30 mL). The organic layer was washed with saturated aqueous sodium bicarbonate (30 mL), followed by water (30 mL) and brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was then purified using column chromatography on silica gel with 10% ethyl acetate/hexane to give a yellow foamy residue which was dissolved in chloroform (15 mL) and diethyl ether was added until a yellow solid precipitated which was collected by vacuum filtration (1.582 g, 24%): m.p. 102-105 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3561, 3135, 2977, 1821, 1755, 1631, 1536, 1372, 1185; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.35 [6H, d, *J* 6.6, CH(C<u>H</u>$_3$)$_2$], 4.80 [1H, sept, *J* 6.6, C<u>H</u>(CH$_3$)$_2$], 6.39 [1H, t, *J* 7.6, C(6′)-H], 6.70 [1H, d, *J* 8.0, C(7′)-H], 6.83-6.93 [2H, m, C(6, 7)-H], 7.05 [1H, t, *J* 7.7, C(5′)-H], 7.26 [1H, ddd, *J* 8.3, 6.9, 1.5, C(5)-H], 7.54 [1H, d, *J* 8.4, C(4′)-H], 7.63-7.69 [2H, m, C(3″, 5″)-H], 7.80 [1H, m, C(4″)-H], 7.91 [1H, s, C(2)-H], 7.95 [1H, d, *J* 8.3, C(4)-H], 8.04-8.07 [2H, m, C(6″, 2″)-H], 8.15 [1H, s, C(2′)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 22.0 (2 × CH$_3$), 47.5 [C<u>H</u>(CH$_3$)$_2$], 104.1 (C, aromatic C), 110.9 (CH, aromatic CH), 112.0 (C, aromatic C), 113.2 (CH, aromatic CH), 120.7 (CH, aromatic CH), 121.0 (CH, aromatic CH), 121.7 (CH, aromatic CH), 122.5 (CH, aromatic CH), 122.8 (C, aromatic C), 123.4 (CH, aromatic CH), 125.0 (C, aromatic C), 125.4 (CH, aromatic CH), 126.9 (2CH, 2 × aromatic CH), 127.6 (C, aromatic C), 128.6 (CH, aromatic CH), 130.0 (2CH, 2 × aromatic CH), 130.9 (CH, aromatic CH), 133.7 (C, aromatic C), 134.2 (C, aromatic C), 135.0 (CH, aromatic CH), 135.7 (C, aromatic C), 136.6 (C, aromatic C), 165.5 (C=O), 165.8 (C=O); m/z (ESI$^+$) 511.1 [(M+H)$^+$ 50%]; HRMS (ESI$^+$): Exact mass calculated for (C$_{29}$H$_{23}$N$_2$O$_5$S)$^+$ 511.1328. Found 511.1310.

**3-(1*H*-Indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione 266**



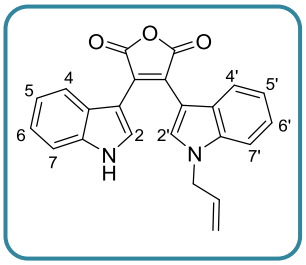
To a stirred suspension of 3-(1-isopropyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **264** (1.353 g, 2.37 mmol) in a mixture of methanol (60 mL) and water (15 mL) was added potassium hydroxide (85 wt. %, 1.053 g, 15.9 mmol). The mixture was then heated to reflux for 24 hours before being allowed to cool and the solvent evaporated under reduced pressure. The residue was acidified to pH 2 using 10% aqueous HCl and extracted with ethyl acetate (3 × 25 mL). the combined organic layers were washed with water (3 × 30 mL) and brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give the desired product as red solid (0.764g, 87%): m.p. 186-188 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3118, 2971, 1812, 1745, 1627, 1529, 1247; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.38 [6H, d, *J* 6.7, CH(C<u>H</u>$_3$)$_2$], 4.81 [1H, sept, *J* 6.6, C<u>H</u>(CH$_3$)$_2$], 6.68-6.71 [2H, m, C(6, 7)-H], 6.85 [1H, ddd, *J* 8.1, 6.9, 0.7, C(6′)-H], 7.03-7.17 [3H, m, C(5, 5′, 7′)-H], 7.44 [1H, d, *J* 8.1, C(4)-H], 7.59 [1H, d, *J* 8.2, C(4′)-H], 7.75 [1H, s, C(2′)-H], 7.92 [1H, d, *J* 2.9, C(2)-H], 11.94 (1H, bs, N-H); $\delta_c$ (75 MHz, DMSO-$d_6$) 22.1 (2 × CH$_3$), 47.2 (CH), 104.4 (C, aromatic C), 104.8 (C, aromatic C), 110.6 (CH, aromatic CH), 112.2 (CH, aromatic CH), 119.9 (CH, aromatic CH), 120.2 (CH, aromatic CH), 121.4 (CH, aromatic CH), 121.6 (CH, aromatic CH), 122.1 (2CH, 2 × aromatic CH), 124.2 (C, aromatic C), 125.6 (C, aromatic C), 127.2 (C, aromatic C), 128.5 (C, aromatic C), 129.5 (CH, aromatic CH), 130.9 (CH, aromatic CH), 135.5 (C, aromatic C), 136.3 (C, aromatic C), 166.3 (C=O), 166.5 (C=O); m/z (ESI$^-$) 369.3 [(M-H)$^-$ 100%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{23}H_{19}N_2O_3)^+$ 371.1396. Found 371.1395.

**3-(1-Allyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione 268**



To a solution of 3-(1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **267** (0.202 g, 0.431 mmol) in anhydrous DMF (20 mL) in a flame-dried flask under nitrogen was added sodium hydride (60 wt. % oil dispersion, 0.019 g, 0.474 mmol) and the solution was stirred for 30 minutes at room temperature. Allyl bromide (0.04 mL, 0.06 g, 0.52 mmol) was then added and the mixture was stirred for a further 4 hours. The reaction mixture was poured into water (60 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layer was washed with 1 M aqueous HCl (3 × 30 mL), followed by water (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give an orange foamy residue. The residue was dissolved in chloroform (2 mL) and diethyl ether was added until an orange solid precipitated which was collected by vacuum filtration (0.180 g, 82%): m.p. 157-159 °C; $\nu_{max}/cm^{-1}$ (KBr) 3128, 2926, 1819, 1751, 1636, 1540, 1252, 1176, 923; $\delta_H$ (300 MHz, $CDCl_3$) 4.78 (2H, d, *J* 5.4, C$\underline{H}_2$-CH=CH$_2$), 5.14 [1H, d, *J* 17.0, CH$_2$-CH=C$\underline{H}_2$ (trans)], 5.29 [1H, d, *J* 10.2, CH$_2$-CH=C$\underline{H}_2$ (cis)], 6.00 (1H, ddt, *J* 17.0, 10.2, 5.3, CH$_2$-C$\underline{H}$=CH$_2$), 6.49 [1H, t, *J* 7.5, C(6′)-H], 6.61 [1H, d, *J* 8.1, C(7′)-H], 6.88 [1H, t, *J* 7.5, C(6)-H], 6.97 [1H, d, *J* 7.7, C(7)-H], 7.08 [1H, t, *J* 7.7, C(5′)-H], 7.22 [1H, t, *J* 7.8, C(5)-H], 7.28 [1H, d, *J* 8.2, C(4′)-H], 7.49 [2H, t, *J* 7.9, C(3″, 5″)-H], 7.61 [1H, t, *J* 7.5, C(4″)-H], 7.88-7.94 [2H, m, C(2″, 6″)-H], 7.93 [1H, s, C(2)-H], 7.97 [1H, d, *J* 8.4, C(4)-H], 8.04 [1H, s, C(2′)-H]; $\delta_c$ (75 MHz, $CDCl_3$) 49.5 (C$\underline{H}_2$-CH=CH$_2$), 105.1 (C, aromatic C), 110.5 (CH, aromatic CH), 111.9 (C, aromatic C), 113.5 (CH, aromatic CH), 118.8 (CH=C$\underline{H}_2$), 121.5 (CH, aromatic CH), 121.9 (CH, aromatic CH), 122.1 (CH, aromatic CH), 123.15 (C, aromatic C), 123.20 (CH, aromatic CH), 123.4 (CH, aromatic CH), 125.4 (CH, aromatic CH), 125.5 (C, aromatic C), 127.1 (2CH, 2 × aromatic CH), 128.2 (C, aromatic C), 129.0 (CH, aromatic CH), 129.6 (2CH, 2 × aromatic CH), 131.8 (CH, aromatic CH), 133.3 (C, aromatic C), 134.28 (CH, aromatic CH), 134.30 (CH, aromatic CH), 134.4 (C, aromatic C), 136.6 (C, aromatic C), 137.7 (C, aromatic C), 165.6 (C=O), 166.1 (C=O); m/z ($ESI^+$) 509.1 [$(M+H)^+$ 30%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{29}H_{21}N_2O_5S)^+$ 509.1171. Found 509.1157.

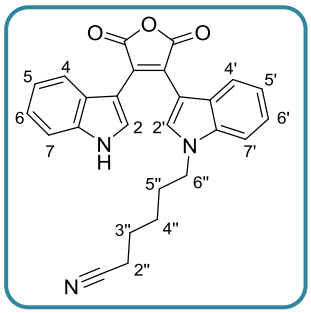
**3-(1-Allyl-1*H*-indol-3-yl)-4-(1*H*-indol-3-yl)furan-2,5-dione 269**



A stirred suspension of 3-(1-allyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]furan-2,5-dione **268** (0.280 g, 0.551 mmol) in methanol (20 mL) and water (5 mL) was treated with potassium hydroxide (85 wt. %, 0.223 g, 3.30 mmol) and the resulting solution was heated to reflux for 24 hours. The reaction mixture was then allowed to cool before the solvent was evaporated under reduced pressure. The residue was dissolved in water (20 mL) and acidified to pH 2 with 10% aqueous HCl before being extracted into ethyl acetate (2 × 20 mL). The combined organic layers were washed with water (3 × 30 mL), followed by brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure giving a red solid (0.192 g, 87%): m.p. 174-175 °C; $\nu_{max}/cm^{-1}$ (KBr) 3384, 2924, 1819, 1746, 1528, 1250, 742; $\delta_H$ (300 MHz, DMSO-$d_6$) 4.94 (2H, bd, *J* 5.3, C$\underline{H}_2$-CH=C$H_2$), 5.04 (1H, dd, *J* 17.1, 1.5, CH=C$\underline{H}_2$ trans), 5.19 (1H, dd, *J* 10.3, 1.5, CH=C$\underline{H}_2$ cis), 6.00 (1H, ddt, *J* 17.0, 10.4, 5.3, C$\underline{H}$=C$H_2$), 6.69 [1H, ddd, *J* 8.0, 7.0, 1.0, C(5)-H], 6.75 [1H, ddd, *J* 8.0, 7.1, 0.9, C(5′)-H], 6.82 [1H, d, *J* 8.0, C(7)-H], 6.90 [1H, d, *J* 7.8, C(7′)-H], 7.04 [1H, ddd, *J* 8.1, 7.1, 1.2, C(6)-H], 7.08 [1H, ddd, *J* 8.2, 7.1, 1.1, C(6′)-H], 7.43 [1H, d, *J* 8.1, C(4)-H], 7.46 [1H, d, *J* 8.3, C(4′)-H], 7.87 [1H, s, C(2′)-H], 7.89 [1H, d, *J* 2.6, C(2)-H], 11.93 (1H, bs, NH); $\delta_c$ (75 MHz, DMSO-$d_6$) 48.3 (N$\underline{C}H_2$), 104.5 (C, aromatic C), 104.9 (C, aromatic C), 110.8 (CH, aromatic CH), 112.1 (CH, aromatic CH), 117.0 (CH=$\underline{C}H_2$), 119.8 (CH, aromatic CH), 120.1 (CH, aromatic CH), 121.1 (CH, aromatic CH), 121.4 (CH, aromatic CH), 122.1 (CH, aromatic CH), 122.2 (CH, aromatic CH), 124.8 (C, aromatic C), 125.6 (C, aromatic C), 127.2 (C, aromatic C), 128.5 (C, aromatic C), 130.6 ($\underline{C}$H=$CH_2$), 133.0 (CH, aromatic CH), 133.5 (CH, aromatic CH), 135.9 (C, aromatic C), 136.1 (C, aromatic C), 166.38 (C=O), 166.43 (C=O); m/z (ESI$^+$) 369.1 [$(M+H)^+$ 80%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{23}H_{17}N_2O_3)^+$ 369.1239. Found 369.1225.

### 7.2.2 Derivatisation of maleic anhydride intermediates

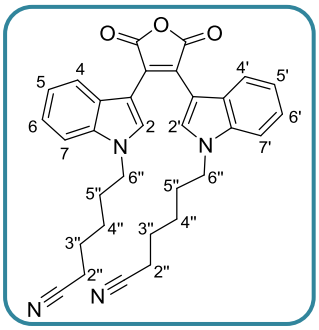
**6-{3-[4-(1*H*-Indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanenitrile 270**



To a solution of 3,4-di(1*H*-indol-3-yl)furan-2,5-dione **255** (5.004 g, 15.2 mmol) in anhydrous DMF (80 mL) at 0°C under a nitrogen atmosphere was added sodium hydride (60 wt. % oil dispersion, 0.640 g, 16.0 mmol). The dark purple solution was stirred for 30 minutes while warming to room temperature before 6-bromohexanenitrile (2.62 mL, 3.47 g, 19.7 mmol) was added. The solution was stirred for 16 hours before being poured into water (200 mL) and extracted with ethyl acetate (3 × 60 mL). The combined organic layers were washed with 1 M aqueous HCl (2 × 60 mL) followed by water (60 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude material was purified using column chromatography on silica gel with 20:80 – 30:70 ethyl acetate/hexane to give 2 fractions found to be the mono alkylated product **270** and also dialkylated 6,6'-[3,3'-(2,5-dioxo-2,5-dihydrofuran-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile **271**.

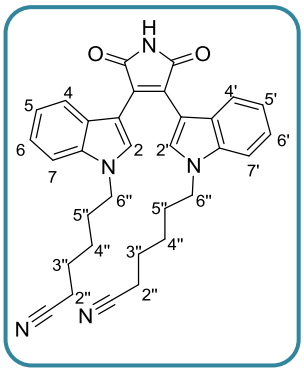
6-{3-[4-(1*H*-Indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanenitrile **270** was isolated as a red solid (0.953 g, 15%): m.p. 102-104 °C; $\nu_{max}/cm^{-1}$ (KBr) 3391, 2935, 2246, 1819, 1748, 1529, 1251, 743; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.32 [2H, m, C(4″)-$H_2$], 1.57 [2H, quin, *J* 7.3, C(3″)-$H_2$], 1.74 [2H, quin, *J* 7.3, C(5″)-$H_2$], 2.45 [2H, t, *J* 7.1, C(2″)-$H_2$], 4.26 [2H, t, *J* 7.0, C(6″)-$H_2$], 6.69 [1H, ddd, *J* 7.9, 6.9, 0.9, C(6)-H], 6.74-6.82 [2H, m, C(6′, 7)-H], 6.95 [1H, d, *J* 7.8, C(7′)-H], 7.04 [1H, ddd, *J* 8.2, 7.1, 1.2, C(5)-H], 7.10 [1H, ddd, *J* 8.2, 7.2, 1.1, C(5′)-H], 7.43 [1H, d, *J* 8.2, C(4)-H], 7.55 [1H, d, *J* 8.2, C(4′)-H], 7.86 [1H, s, C(2′)-H], 7.89 [1H, s, C(2)-H], 11.92 (1H, bs, NH); $\delta_c$ (75 MHz, DMSO-$d_6$) 16.0 ($CH_2$), 24.3 ($CH_2$), 25.2 ($CH_2$), 28.7 ($CH_2$), 45.6 ($CH_2$), 104.2 (C, aromatic C), 104.9 (C, aromatic C), 110.5 (CH, aromatic CH), 112.1 (CH, aromatic CH), 119.8 (CH, aromatic CH), 120.0 (CH, aromatic CH), 120.5 (CN), 121.2 (CH, aromatic CH), 121.4 (CH, aromatic CH), 122.1 (CH, aromatic CH), 122.2 (CH, aromatic CH), 124.7 (C, aromatic C), 125.5 (C, aromatic C), 127.4 (C, aromatic C), 128.2 (C, aromatic C), 130.6 (CH, aromatic CH), 133.0 (CH, aromatic CH), 135.9 (C,

aromatic C), 136.2 (C, aromatic C), 166.36 (C=O), 166.43 (C=O); m/z ($ESI^+$) 424.2 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{26}H_{22}N_3O_3)^+$ 424.1661. Found 424.1659.



6,6'-(3,3'-(2,5-Dioxo-2,5-dihydrofuran-3,4-diyl)bis(1*H*-indole-3,1-diyl))dihexanenitrile **271** was isolated as a red solid (1.658 g, 21%): m.p. 120-122 °C; $\nu_{max}/cm^{-1}$ (KBr) 3435, 2939, 2243, 1815, 1749, 1525, 1257, 747; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.27-1.40 [4H, m, 2 × C(4″)-$H_2$], 1.57 [4H, quin, *J* 7.4, 2 × C(3″)-$H_2$], 1.76 [4H, quin, *J* 7.3, 2 × C(5″)-$H_2$], 2.45 [4H, t, *J* 7.0, 2 × C(2″)-$H_2$], 4.27 [4H, t, 2 × C(6″)-$H_2$], 6.75 [2H, t, *J* 7.4, C(6, 6′)-H], 6.87 [2H, d, *J* 7.9, C(7, 7′)-H], 7.10 [2H, t, *J* 7.7, C(5, 5′)-H], 7.55 [2H, d, *J* 8.3, C(4, 4′)-H], 7.90 [2H, s, C(2, 2′)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 16.0 (2 × $CH_2$), 24.3 (2 × $CH_2$), 25.1 (2 × $CH_2$), 28.7 (2 × $CH_2$), 45.6 (2 × $CH_2$), 104.2 (2C, 2 × aromatic C), 110.6 (2CH, 2 × aromatic CH), 120.0 (2CH, 2 × aromatic CH), 120.5 (2 × CN), 121.5 (2CH, 2 × aromatic CH), 122.2 (2CH, 2 × aromatic CH), 125.3 (2C, 2 × aromatic C), 127.6 (2C, 2 × aromatic C), 133.1 (2CH, 2 × aromatic CH), 136.0 (2C, 2 × aromatic C), 166.3 (2 × C=O); m/z ($ESI^+$) 519.2 [$(M+H)^+$ 10%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{32}H_{31}N_4O_3)^+$ 519.2396. Found 519.2401.

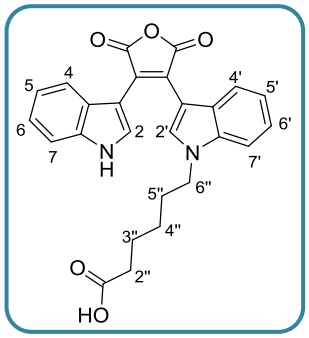
**6,6'-[3,3'-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile 327**



To a solution of 6,6'-[3,3'-(2,5-dioxo-2,5-dihydrofuran-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile **271** (1.302 g, 2.51 mmol) in anhydrous DMF (10 mL) was added 1,1,1,3,3,3 hexamethyldisilazane (3.53 mL, 0.77 g, 28.4 mmol), followed by methanol (0.34 mL, 0.27 g, 8.4 mmol) and the reaction mixture was stirred at room temperature for 8 hours. Water (40 mL) was then added and the product extracted into ethyl acetate (3 × 30 mL). The combined organic layers were washed with water (3 × 40 mL) followed by brine (40 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give a red solid (1.165 g, 90%): m.p. 82-84 °C; $\nu_{max}/cm^{-1}$ (KBr) 3274, 2936, 2244, 1704, 1533, 1334, 1160, 743;

$\delta_H$ (300 MHz, $CDCl_3$) 1.39-1.51 [4H, m, 2 × C(4″)-$H_2$], 1.65 [4H, quin, *J* 7.3, 2 × C(3″)-$H_2$], 1.87 [4H, quin, *J* 7.3, 2 × C(5″)-$H_2$], 2.30 [4H, t, *J* 7.0, 2 × C(2″)-$H_2$], 4.15 [4H, t, *J* 7.0, 2 × C(6″)-$H_2$], 6.76 [2H, t, *J* 7.4, C(6, 6′)-H], 6.99 [2H, d, *J* 8.0, C(7, 7′)-H], 7.11 [2H, t, *J* 7.6, C(5, 5′)-H], 7.28 [2H, d, *J* 8.3, C(4, 4′)-H], 7.43 (1H, s, NH), 7.63 [1H, s, C(2, 2′)-H]; $\delta_c$ (75 MHz, $CDCl_3$) 17.0 (2 × $CH_2$), 25.0 (2 × $CH_2$), 25.9 (2 × $CH_2$), 29.2 (2 × $CH_2$), 46.3 (2 × $CH_2$), 105.9 (2C, 2 × aromatic C), 109.5 (2CH, 2 × aromatic CH), 119.4 (2 × CN), 120.1 (2CH, 2 × aromatic CH), 122.3 (2CH, 2 × aromatic CH), 122.4 (2CH, 2 × aromatic CH), 126.2 (2C, 2 × aromatic C), 127.8 (2C, 2 × aromatic C), 131.7 (2CH, 2 × aromatic CH), 136.2 (2C, 2 × aromatic C), 172.3 (2 × C=O); m/z ($ESI^+$) 518.2 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{32}H_{32}N_5O_2)^+$ 518.2556. Found 518.2554.

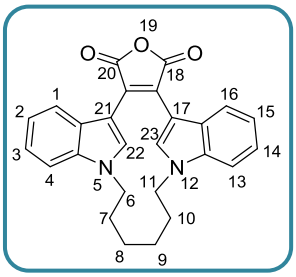
**6-{3-[4-(1*H*-Indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanoic acid 278**




6-{3-[4-(1*H*-Indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1-indol-1-yl}hexanenitrile **270** (0.402 g, 0.950 mmol) was dissolved in a mixture of methanol (20 mL) and 10% aqueous potassium hydroxide solution (20 mL). The solution was heated to reflux for 16 hours before being allowed to cool and the solvent evaporated under reduced pressure. The residue was dissolved in water (20 mL) and acidified to pH 2 using 20% aqueous HCl, and then extracted with ethyl acetate (20 mL × 2). The organic layer was washed with water (20 mL × 2) followed by brine (20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give desired compound as a red solid (0.335 g, 84%): 198-199 °C; $\nu_{max}/cm^{-1}$ (KBr) 3386, 2924, 1816, 1740, 1699, 1531, 1261; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.23 [2H, quin, *J* 7.5, C(4″)-$H_2$], 1.51 [2H, quin, *J* 7.5, C(3″)-$H_2$], 1.72 [2H, quin, *J* 7.2, C(5″)-$H_2$], 2.17 [2H, t, *J* 7.3, C(2″)-$H_2$], 4.24 [2H, t, *J* 7.0, C(6″)-$H_2$], 6.67 [1H, t, *J* 7.5, C(6)-H], 6.76 [1H, t, *J* 7.8, C(6′)-H], 6.77 [1H, d, *J* 7.5, C(7)-H], 6.93 [1H, d, *J* 8.0, C(7′)-H], 7.04 [1H, t, *J* 8.0, C(5)-H], 7.09 [1H, t, *J* 7.8, C(5′)-H], 7.43 [1H, d, *J* 8.1, C(4)-H], 7.53 [1H, d, *J* 8.3, C(4′)-H], 7.84 [1H, s, C(2′)-H], 7.89 [1H, s, C(2)-H], 11.92 (1H, bs, NH), 11.99 (1H, bs, COOH); $\delta_c$ (75 MHz, DMSO-$d_6$) 24.0 ($CH_2$), 25.6 ($CH_2$), 29.3 ($CH_2$), 33.5 ($CH_2$), 45.7 ($CH_2$), 104.2 (C, aromatic C), 104.9 (C, aromatic C), 110.5 (CH, aromatic CH), 112.1

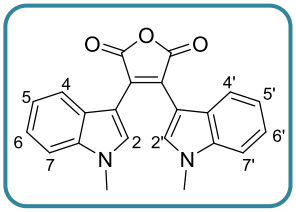
(CH, aromatic CH), 119.8 (CH, aromatic CH), 120.0 (CH, aromatic CH), 121.2 (CH, aromatic CH), 121.4 (CH, aromatic CH), 122.07 (CH, aromatic CH), 122.12 (CH, aromatic CH), 124.7 (C, aromatic C), 125.5 (C, aromatic C), 127.4 (C, aromatic C), 128.2 (C, aromatic C), 130.6 (CH, aromatic CH), 133.0 (CH, aromatic CH), 135.9 (C, aromatic C), 136.1 (C, aromatic C), 166.4 (C=O), 166.5 (C=O), 174.3 (COOH); m/z ($ESI^-$) 441.2 [$(M-H)^-$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{26}H_{23}N_2O_5)^+$ 443.1607. Found 443.1611.

**6,7,8,9,10,11-Hexahydro-5,21:12,17-dimethenodibenzo[*i*,*o*]furo[3,4-*l*][1,8] diazacyclohexadecine-18,20-dione 272**



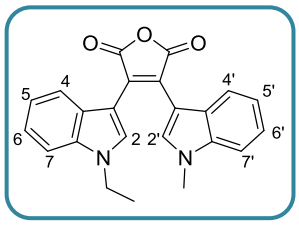

To a solution of 3,4-di(1*H*-indol-3-yl)furan-2,5-dione **255** (1.004 g, 3.06 mmol) in anhydrous DMF (150 mL) at 0 °C under a nitrogen atmosphere was added sodium hydride (60 wt. % oil dispersion, 0.270 g, 6.73 mmol) and the mixture was stirred for 40 minutes at this temperature. 1,6-Dibromohexane (0.50 mL, 0.79 g, 3.2 mmol) was then added to the dark blue mixture and the reaction was allowed to warm slowly to room temperature and stirred for 16 hours. At this point, the reaction mixture was poured into water (400 mL) and extracted with ethyl acetate (5 × 50 mL). The organic layer was washed with 1 M aqueous HCl (3 × 50 mL), followed by water (2 × 50 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced. The crude residue was purified using column chromatography on silica gel with 50:50 DCM/hexane to give desired compound as a reddish/purple solid (0.364 g, 29%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3431, 2927, 1809, 1747, 1626, 1528, 1186, 735; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.09 [4H, bs, C(8, 9)-$H_2$], 1.82 [4H, bs, C(7, 10)-$H_2$], 4.16-4.23 [4H, m, C(6, 11)-$H_2$], 7.15-7.28 [4H, m, C(2, 3, 14, 15)-H], 7.55 [2H, d, *J* 7.6, C(4, 13)-H], 7.57 [2H, s, C(22, 23)-H], 7.87 [2H, dd, *J* 7.1, 1.2, C(1, 16)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 22.5 (2 × $CH_2$), 27.2 (2 × $CH_2$), 44.2 (2 × $CH_2$), 101.9 (2C, 2 × aromatic C), 110.7 (2CH, 2 × aromatic CH), 120.7 (2CH, 2 × aromatic CH), 121.3 (2CH, 2 × aromatic CH), 122.0 (2CH, 2 × aromatic CH), 126.4 (2C, 2 × aromatic C), 132.2 (2CH, 2 × aromatic CH), 132.5 (2C, 2 × aromatic C), 135.4 (2C, 2 × aromatic C), 165.5 (2 × C=O); m/z ($ESI^+$) 411.2 [$(M+H)^+$ 40%].

**3,4-Bis(1-methyl-1*H*-indol-3-yl)furan-2,5-dione 147**[11]



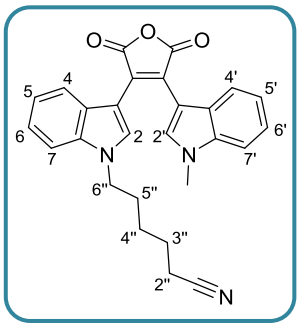
To a solution of 3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **265** (0.301 g, 0.879 mmol) in anhydrous DMF (15 mL) under nitrogen was added sodium hydride (60 wt. % oil dispersion, 0.042 g, 1.14 mmol) and the mixture was allowed to stir for 40 minutes. Iodomethane (0.08 mL, 0.18 g, 1.3 mmol) was then added and the reaction was stirred for a further 16 hours. Water (30 mL) was then added and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were then washed with 1 M aqueous HCl (2 × 20 mL), followed by water (20 mL) and brine (20 mL). The product was recrystallised from the ethyl acetate solution to give desired product as a red solid (0.162 g). Excess solvent was removed from filtrate and the residue was purified using column chromatography on silica gel with 20% ethyl acetate/hexane to give additional product (0.087 g, combined yield 0.249 g, 80%): m.p. 177-178 °C; $\nu_{max}/cm^{-1}$ (KBr) 2924, 2855, 1815, 1740, 1610, 1517, 1226, 735; $\delta_H$ (300 MHz, DMSO-$d_6$) 3.88 (6H, s, 2 × $CH_3$), 6.74 [2H, ddd, *J* 7.8, 7.0, 0.7, C(6, 6′)-H], 6.82 [2H, d, *J* 7.8, C(7, 7′)-H], 7.11 [2H, ddd, *J* 8.2, 7.0, 1.2, C(5, 5′)-H], 7.49 [2H, d, *J* 8.2, C(4, 4′)-H], 7.92 [2H, s, C(2, 2′)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 33.0 (2 × $CH_3$), 104.0 (2C, 2 × aromatic C), 110.5 (2CH, 2 × aromatic CH), 120.0 (2CH, 2 × aromatic CH), 121.3 (2CH, 2 × aromatic CH), 122.2 (2CH, 2 × aromatic CH), 125.4 (2C, 2 × aromatic C), 127.3 (2C, 2 × aromatic C), 134.2 (2CH, 2 × aromatic CH), 136.7 (2C, 2 × aromatic C), 166.4 (2 × C=O); m/z ($ESI^+$) 357.1 [$(M+H)^+$ 100%].

**3-(1-Ethyl-1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione 273**



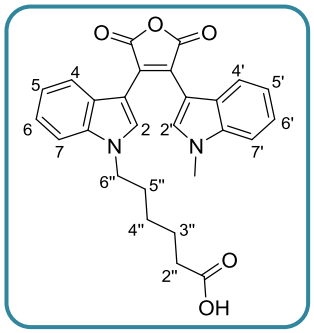

To a solution of 3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **265** (0.262 g, 0.765 mmol) in anhydrous DMF (15 mL) under nitrogen was added sodium hydride (60 wt. % oil dispersion, 0.037 g, 1.07 mmol) and the mixture was allowed to stir for 40 minutes. Iodoethane (0.09 mL, 0.17 g, 1.1 mmol) was then added and the reaction was stirred for a further 16 hours. Water (30 mL) was then added and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were then washed with 1 M aqueous HCl (2 × 20 mL), followed by water (20 mL) and brine (20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was purified using column chromatography on silica gel with 20% ethyl acetate/hexane to give desired product as a red solid (0.204 g, 72%): m.p. 214-216 °C; $\nu_{max}/cm^{-1}$ (KBr) 3420, 3133, 1816, 1758, 1626, 1525, 1219, 737; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.31 (3H, t, *J* 7.2, $CH_2C\underline{H}_3$), 3.89 (3H, s, $CH_3$), 4.26 (2H, q, *J* 7.2, $C\underline{H}_2CH_3$), 6.67-6.74 [2H, m, C(6′, 7′)-H], 6.81 [1H, t, *J* 7.9, C(6)-H], 7.04 [1H, d, *J* 8.0, C(7)-H], 7.08-7.16 [2H, m, (5, 5′)-H], 7.50 [1H, d, *J* 8.4, C(4′)-H], 7.54 [1H, d, *J* 8.4, C(4)-H], 7.81 [1H, s, C(2)-H], 7.97 [1H, s, C(2′)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 15.1 ($CH_2\underline{C}H_3$), 33.0 ($CH_3$), 40.8 ($\underline{C}H_2CH_3$), 103.9 (C, aromatic C), 104.2 (C, aromatic C), 110.4 (CH, aromatic CH), 110.5 (CH, aromatic CH), 120.05 (CH, aromatic CH), 120.08 (CH, aromatic CH), 121.5 (CH, aromatic CH), 121.6 (CH, aromatic CH), 122.2 (2CH, 2 × aromatic CH), 124.9 (C, aromatic C), 125.7 (C, aromatic C), 127.1 (C, aromatic C), 127.7 (C, aromatic C), 132.5 (CH, aromatic CH), 134.4 (CH, aromatic CH), 135.6 (C, aromatic C), 136.8 (C, aromatic C), 166.3 (C=O), 166.5 (C=O); m/z ($ESI^+$) 371.1 $[(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{23}H_{19}N_2O_3)^+$ 371.1396. Found 371.1389.

**6-{3-[4-(1-Methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanenitrile 274**



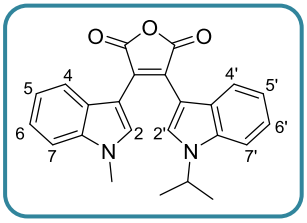

To a solution of 3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **265** (0.505 g, 1.48 mmol) in anhydrous DMF (20 mL) under nitrogen was added sodium hydride (60 wt. % oil dispersion, 0.070 g, 1.77 mmol) and the mixture was stirred for 40 minutes. 6-Bromohexane nitrile (0.30 mL, 0.38 g, 2.2 mmol) was then added and the reaction was stirred for a further 16 hours. Water (40 mL) was then added and the mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were then washed with 1 M aqueous HCl (2 × 30 mL), followed by water (30 mL) and brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was purified using column chromatography on silica gel with 30% ethyl acetate/hexane to give desired product as a red solid (0.258 g, 40%): m.p. 78-79 °C; $\nu_{max}/cm^{-1}$ (KBr) 3411, 2936, 2244, 1816, 1750, 1529, 1254, 1100, 741; $\delta_H$ (400 MHz, DMSO-$d_6$) 1.27-1.35 [2H, m, C(4″)-$H_2$], 1.56 [2H, quin, *J* 7.4, C(3″)-$H_2$], 1.73 [2H, quin, *J* 7.4, C(5″)-$H_2$], 2.44 [2H, t, *J* 6.9, C(2″)-$H_2$], 3.89 (3H, s, $CH_3$), 4.24 [2H, t, *J* 6.9, C(6″)-$H_2$], 6.69-6.72 [2H, m, C(6′, 7′)-H], 6.79 [1H, t, *J* 7.6, C(6)-H], 7.01 [1H, d, *J* 8.15, C(7)-H], 7.07-7.13 [2H, m, (5, 5′)-H], 7.49 [1H, d, *J* 8.15, C(4′)-H], 7.54 [1H, d, 8.49, C(4)-H], 7.82 [1H, s, C(2)-H], 7.97 [1H, s, C(2′)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 16.0 ($CH_2$), 24.3 ($CH_2$), 25.1 ($CH_2$), 28.7 ($CH_2$), 33.0 ($CH_3$), 45.6 ($CH_2$), 103.9 (C, aromatic C), 104.3 (C, aromatic C), 110.50 (CH, aromatic CH), 110.54 (CH, aromatic CH), 120.0 (CH, aromatic CH), 120.1 (CH, aromatic CH), 120.5 (CN), 121.4 (CH, aromatic CH), 121.5 (CH, aromatic CH), 122.1 (CH, aromatic CH), 122.2 (CH, aromatic CH), 125.0 (C, aromatic C), 125.7 (C, aromatic C), 127.0 (C, aromatic C), 127.9 (C, aromatic C), 132.9 (CH, aromatic CH), 134.3 (CH, aromatic CH), 135.9 (C, aromatic C), 136.7 (C, aromatic C), 166.3 (C=O), 166.5 (C=O); m/z ($ESI^+$) 438.2 [$(M+H)^+$ 12%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{27}H_{24}N_3O_3)^+$ 438.1818. Found 438.1802.

**6-{3-[4-(1-Methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanoic acid 279**



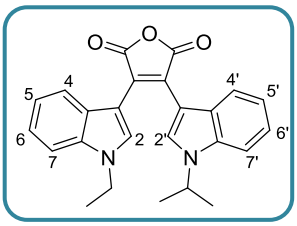

6-{3-[4-(1-Methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydro furan-3-yl]-1*H*-indol-1-yl}hexanenitrile **274** (0.103 g, 0.235 mmol) was dissolved in a mixture of methanol (8 mL) and 10% aqueous potassium hydroxide solution (8 mL). The solution was heated to reflux for 16 hours before being allowed to cool and the solvent evaporated. The residue was dissolved in water (10 mL) and acidified to pH 2 using 20% aqueous HCl, and was then extracted with ethyl acetate (10 mL × 2). The organic layer was washed with water (10 mL × 2) followed by brine (10 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give desired compound as a red solid (0.082 g, 76%): m.p. 95-97 °C; $\nu_{max}/cm^{-1}$ (KBr) 3050, 2937, 1816, 1751, 1705, 1611, 1528, 1255, 740; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.18-1.29 [2H, m, C(4″)-$H_2$], 1.51 [2H, quin, *J* 7.4, C(3″)-$H_2$], 1.71 [2H, quin, *J* 7.3, C(5″)-$H_2$], 2.17 [2H, t, *J* 7.4, C(2″)-$H_2$], 3.90 (3H, s, $CH_3$), 4.23 [2H, t, *J* 6.7, C(6″)-$H_2$], 6.68 [2H, m, C(6′, 7′)-H], 6.78 [1H, t, *J* 7.6, C(6)-H], 7.00 [1H, d, *J* 8.0, C(7)-H], 7.06-7.14 [2H, m, (5, 5′)-H], 7.48 [1H, d, *J* 8.3, C(4′)-H], 7.53 [1H, d, *J* 8.3, C(4)-H], 7.81 [1H, s, C(2)-H], 7.99 [1H, s, C(2′)-H], 11.99 (COOH); $\delta_c$ (75 MHz, DMSO-$d_6$) 24.1 ($CH_2$), 25.9 ($CH_2$), 29.3 ($CH_2$), 33.0 ($CH_3$), 33.8 ($CH_2$), 45.7 ($CH_2$), 103.9 (C, aromatic C), 104.2 (C, aromatic C), 110.48 (CH, aromatic CH), 110.52 (CH, aromatic CH), 120.0 (2CH, 2 × aromatic CH), 121.4 (2CH, 2 × aromatic CH), 122.1 (2CH, 2 × aromatic CH), 125.0 (C, aromatic C), 125.7 (C, aromatic C), 127.0 (C, aromatic C), 127.8 (C, aromatic C), 132.9 (CH, aromatic CH), 134.3 (CH, aromatic CH), 135.9 (C, aromatic C), 136.7 (C, aromatic C), 166.3 (C=O), 166.5 (C=O), 174.5 (COOH); m/z ($ESI^+$) 457.3 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{27}H_{25}N_2O_5)^+$ 457.1763. Found 457.1755.

**3-(1-Isopropyl-1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione 275**



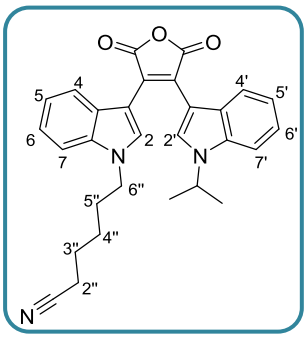

To a solution of 3-(1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione **266** (0.302 g, 0.815 mmol) in anhydrous DMF (15 mL) under nitrogen was added sodium hydride (60 wt. % oil dispersion, 0.037 g, 1.47 mmol) and the mixture was stirred for 40 minutes. Iodomethane (0.06 mL, 0.14 g, 0.97 mmol) was then added and the reaction was allowed to stir for a further 16 hours. Water (30 mL) was then added and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were then washed with 1 M aqueous HCl (2 × 20 mL), followed by water (20 mL) and brine (20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was purified using column chromatography on silica gel with 20% ethyl acetate/hexane to give desired product as a red solid (0.140 g, 46%): m.p. 164-167 °C; $\nu_{max}/cm^{-1}$ (KBr) 3118, 3048, 2970, 1812, 1745, 1627, 1529, 1247, 739; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.37 [6H, d, *J* 6.7, 2 × $CH_3$], 3.90 (3H, s, $CH_3$), 4.81 [1H, sept, *J* 6.6, C<u>H</u>$(CH_3)_2$], 6.60 [1H, d, *J* 8.0, C(7)-H], 6.72 [1H, t, *J* 8.0, C(6)-H], 6.87 [1H, t, *J* 7.8, C(6′)-H], 7.09-7.19 [3H, m, C(5, 5′, 7′)-H], 7.50 [1H, d, *J* 8.4, C(4)-H], 7.59 [1H, d, *J* 8.4, C(4′)-H], 7.73 [1H, s, C(2′)-H], 8.01 [1H, s, C(2)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 22.0 (2 × $CH_3$), 33.0 [<u>C</u>H$(CH_3)_2$], 47.2 ($CH_3$), 103. 9 (C, aromatic C), 104.5 (C, aromatic C), 110.5 (CH, aromatic CH), 110.6 (CH, aromatic CH), 120.1 (CH, aromatic CH), 120.2 (CH, aromatic CH), 121.5 (CH, aromatic CH), 121.6 (CH, aromatic CH), 122.15 (CH, aromatic CH), 122.18 (CH, aromatic CH), 124.5 (C, aromatic C), 125.7 (C, aromatic C), 126.9 (C, aromatic C), 128.1 (C, aromatic C), 129.1 (CH, aromatic CH), 134.65 (CH, aromatic CH), 134.69 (C, aromatic C), 136.9 (C, aromatic C), 166.2 (C=O), 166.5 (C=O); m/z ($ESI^+$) 385.2 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{24}H_{21}N_2O_3)^+$ 385.1552. Found 385.1540.

**3-(1-Ethyl-1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione 276**



To a solution of 3-(1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione **266** (0.167 g, 0.451 mmol) in anhydrous DMF (10 mL) under nitrogen was added sodium hydride (60 wt. % oil dispersion, 0.019 g, 0.811 mmol) and the mixture was stirred for 40 minutes. Iodoethane (0.06 mL, 0.12 g, 0.69 mmol) was then added and the reaction was allowed to stir for a further 16 hours. Water (20 mL) was then added and the mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were then washed with 1 M aqueous HCl (2 × 15 mL), followed by water (15 mL) and brine (15 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was purified using column chromatography on silica gel with 20% ethyl acetate/hexane to give desired product as an orange solid (0.091 g, 51%): m.p. 165-167 °C; $\nu_{max}/cm^{-1}$ (KBr) 2978, 1817, 1746, 1524, 1211, 741; $\delta_H$ (400 MHz, DMSO-$d_6$) 1.34 (3H, t, *J* 7.1, $CH_2C\underline{H_3}$) 1.40 [6H, d, *J* 6.7, 2 × $CH_3$], 4.30 (2H, q, *J* 7.2, $C\underline{H_2}CH_3$), 4.82 [1H, sept, *J* 6.6 $C\underline{H}(CH_3)_2$], 6.75-6.86 [3H, m, C(6, 6′, 7)-H), 7.04 [1H, d, *J* 7.7, C(7′)-H] 7.10-7.16 [2H, m, C(5, 5′)-H), 7.56 [1H, d, *J* 8.1, C(4)-H], 7.60 [1H, d, *J* 8.3, C(4′)-H], 7.80 [1H, s, C(2′)-H], 7.93 [1H, s, C(2)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 15.1 ($CH_3$), 22.1 (2 × $CH_3$), 40.9 ($CH_2$), 47.2 (CH), 104.1 (C, aromatic C), 104.4 (C, aromatic C), 110.5 (CH, aromatic CH), 110.7 (CH, aromatic CH), 120.1 (CH, aromatic CH), 120.2 (CH, aromatic CH), 121.65 (CH, aromatic CH), 121.73 (CH, aromatic CH), 122.1 (CH, aromatic CH), 122.2 (CH, aromatic CH), 125.0 (C, aromatic C), 125.3 (C, aromatic C), 127.4 (C, aromatic C), 127.9 (C, aromatic C), 129.3 (CH, aromatic CH), 132.9 (CH, aromatic CH), 135.5 (C, aromatic C), 135.8 (C, aromatic C), 166.3 (C=O), 166.4 (C=O); m/z ($ESI^+$) 399.2 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{25}H_{23}N_2O_3)^+$ 399.1709. Found 399.1706.

**6-{3-[4-(1-Isopropyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanenitrile 277**

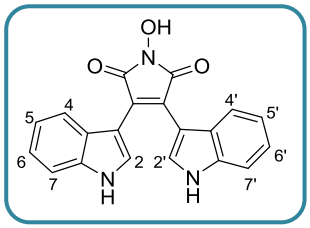



To a solution of 3-(1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione **266** (0.303 g, 0.818 mmol) in anhydrous DMF (15 mL) under nitrogen was added sodium hydride (60 wt. % oil dispersion, 0.049 g, 2.025 mmol) and the mixture was stirred for 40 minutes. 6-Bromohexane nitrile (0.17 mL, 0.23 g, 1.28 mmol) was then added and the reaction was allowed to stir for a further 16 hours. Water (20 mL) was then added and the mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were then washed with 1 M aqueous HCl (2 × 15 mL), followed by water (15 mL) and brine (15 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was purified using column chromatography on silica gel with 30% ethyl acetate/hexane to give desired product as an orange solid (0.127 g, 33%): m.p. 186-189 °C; $\nu_{max}/cm^{-1}$ (KBr) 2937, 2247, 1830, 1754, 1529, 1257, 748; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.34 [2H, quin, *J* 7.4, C(4″)-$H_2$] 1.40 (6H, d, *J* 6.6, 2 × $CH_3$), 1.58 [2H, quin, *J* 7.5, C(3″)-$H_2$], 1.78 [2H, quin, *J* 7.5, C(5″)-$H_2$], 2.45 [2H, t, *J* 7.0, C(2″)-$H_2$], 4.29 [2H, t, *J* 7.0, C(6″)-$H_2$], 4.82 [1H, sept, *J* 6.6, C<u>H</u>$(CH_3)_2$], 6.73-6.84 [3H, m, C(6, 6′, 7)-H], 7.05 [1H, d, *J* 8.0, C(7′)-H] 7.10-7.15 [2H, m, C(5, 5′)-H], 7.56 [1H, d, *J* 8.3, C(4)-H], 7.59 [1H, d, *J* 8.3, C(4′)-H], 7.80 [1H, s, *J* 8.4, C(2′)-H], 7.94 [s, 1H, C(2)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 16.0 ($CH_2$), 22.1 (2 × $CH_3$), 24.3 ($CH_2$), 25.2 ($CH_2$), 28.7 ($CH_2$), 45.6 ($CH_2$), 47.2 [<u>C</u>H$(CH_3)_2$], 104.1 (C, aromatic C), 104.4 (C, aromatic C), 110.6 (2CH, 2 × aromatic CH), 120.1 (CH, aromatic CH), 120.2 (CH, aromatic CH), 120.5 (CN), 121.59 (CH, aromatic CH), 121.62 (CH, aromatic CH), 122.1 (CH, aromatic CH), 122.2 (CH, aromatic CH), 124.9 (C, aromatic C), 125.6 (C, aromatic C), 127.5 (C, aromatic C), 127.8 (C, aromatic C), 129.3 (CH, aromatic CH), 133.4 (CH, aromatic CH), 135.5 (C, aromatic C), 136.1 (C, aromatic C), 166.3 (C=O), 166.4 (C=O); m/z ($ESI^+$) 466.2 [$(M+H)^+$ 20%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{29}H_{28}N_3O_3)^+$ 466.2131. Found 466.2121.

### 7.2.3 Synthesis of hydroxymaleimide analogues

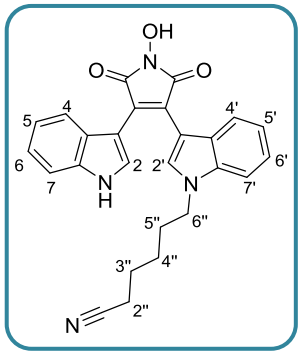
**General procedure (i):** To a solution of the maleic anhydride (1 eq.) in anhydrous DMF (30 mL/mmol) was added hydroxylamine hydrochloride (5 eq.) followed by triethylamine (5 eq.). The mixture was then heated to 70 °C and stirred at this temperature for 24 hours. After cooling, water (100 mL/mmol) was added and the mixture was extracted with ethyl acetate (3 × 40 mL/mmol). The organic layer was washed with 1 M aqueous HCl (2 × 50 mL/mmol), followed by water (50 mL/mmol) and brine (50 mL/mmol) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated to give a dark red solid.

**1-Hydroxy-3,4-di(1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione 280**[12]



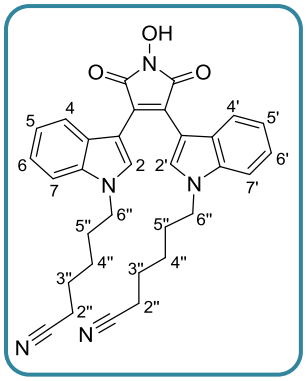

Following general procedure (i) starting from 3,4-di(1*H*-indol-3-yl)furan-2,5-dione **255** (0.505 g, 1.54 mmol) in anhydrous DMF (50 mL) with hydroxylamine hydrochloride (0.529 g, 7.69 mmol) and triethylamine (1.06 mL, 0.77 g, 7.69 mmol) gave desired product as a dark red solid (0.446 g, 87%): m.p. 171-173 °C; (Lit. 160 °C)[12]; $\nu_{max}/cm^{-1}$ (KBr) 3397, 2837, 1767, 1705, 1524, 1414, 1118; $\delta_H$ (300 MHz, DMSO-$d_6$) 6.65 [2H, t, *J* 7.5, C(6, 6′)-H], 6.79 [2H, d, *J* 8.0, C(7, 7′)-H], 6.99 [2H, t, *J* 7.6, C(5, 5′)-H], 7.39 [2H, d, *J* 8.1, C(4, 4′)-H], 7.77 [2H, d, *J* 2.7, C(2, 2′)-H], 10.41 (1H, s, N-OH), 11.71 (2H, d, *J* 2.0, N(1, 1′)-H); m/z ($ESI^-$) 342.4 [$(M-H)^-$ 100%].

**6-{3-[1-Hydroxy-4-(1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-1*H*-indol-1-yl}hexanenitrile 281**



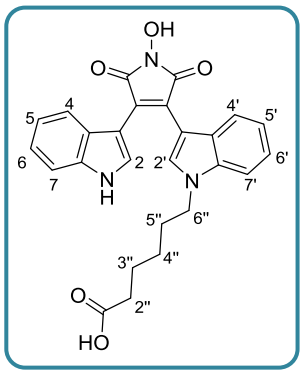

Following general procedure (i) starting from 6-{3-[4-(1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanenitrile **270** (0.150 g, 0.354 mmol) in anhydrous DMF (10 mL) with hydroxylamine hydrochloride (0.123 g, 1.77 mmol) and triethylamine (0.25 mL, 0.18 g, 1.8 mmol) gave desired product as a dark red solid (0.134 g, 86%): m.p. 142-143 °C; $\nu_{max}/cm^{-1}$ (KBr) 3377, 2933, 2247, 1770, 1706, 1527, 1121, 735; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.25-1.36 [2H, m, C(4″)-$H_2$], 1.56 [2H, quin, *J* 7.3, C(3″)-$H_2$], 1.73 [2H, quin, *J* 7.1, C(5″)-$H_2$], 2.44 [2H, t, *J* 7.0, C(2″)-$H_2$], 4.23 [2H, t, *J* 6.7, C(6″)-$H_2$], 6.62 [1H, t, *J* 7.3, C(6)-H], 6.67-6.74 [2H, m, C(6′, 7)-H], 6.87 [1H, d, *J* 8.0, C(7′)-H], 6.99 [1H, t, *J* 7.8, C(5)-H], 7.05 [1H, t, *J* 7.9, C(5′)-H], 7.39 [1H, d, *J* 8.1, C(4)-H], 7.49 [1H, d, *J* 8.2, C(4′)-H], 7.77 [1H, s, C(2′)-H], 7.82 [1H, d, *J* 1.8, C(2)-H], 10.48 (1H, bs, N-OH), 11.76 (1H, bs, NH); $\delta_c$ (75 MHz, DMSO-$d_6$) 16.0 ($CH_2$), 24.3 ($CH_2$), 25.1 ($CH_2$), 28.8 ($CH_2$), 45.4 ($CH_2$), 104.7 (C, aromatic C), 105.3 (C, aromatic C), 110.3 (CH, aromatic CH), 111.9 (CH, aromatic CH), 119.4 (CH, aromatic CH), 119.6 (CH, aromatic CH), 120.6 (CN), 120.9 (CH, aromatic CH), 121.1 (CH, aromatic CH), 121.7 (CH, aromatic CH), 121.8 (CH, aromatic CH), 123.7 (C, aromatic C), 124.6 (C, aromatic C), 125.0 (C, aromatic C), 125.8 (C, aromatic C), 129.5 (CH, aromatic CH), 132.1 (CH, aromatic CH), 135.7 (C, aromatic C), 136.0 (C, aromatic C), 168.39 (C=O), 168.43 (C=O); m/z ($ESI^+$) 439.2 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{26}H_{23}N_4O_3)^+$ 439.1770. Found 439.1763.

**6,6'-[3,3'-(1-Hydroxy-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile 282**



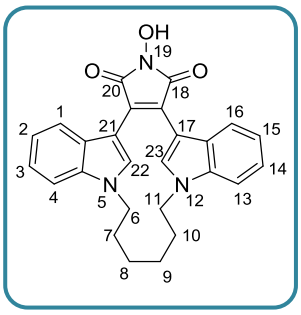

Following general procedure (i) starting from 6,6'-[3,3'-(2,5-dioxo-2,5-dihydrofuran-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile **271** (0.084g, 0.162 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.056 g, 0.809 mmol) and triethylamine (0.11 mL, 0.08 g, 0.81 mmol) gave desired product as a dark red solid (0.076 g, 87%): m.p. 83-84 °C; $\nu_{max}/cm^{-1}$ (KBr) 3257, 2936, 2244, 1771, 1713, 1530, 1392, 1159, 743; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.25-1.38 [4H, m, 2 × C(4′′)-$H_2$], 1.57 [4H, quin, *J* 7.4, 2 × C(3′′)-$H_2$], 1.75 [4H, quin, *J* 7.2, 2 × C(5′′)-$H_2$], 2.45 [4H, t, *J* 7.1, 2 × C(2′′)-$H_2$], 4.25 [4H, t, *J* 6.9, × C(6′′)-$H_2$], 6.68 [2H, t, *J* 7.4, C(6, 6′)-H], 6.80 [2H, d, *J* 7.7, C(7, 7′)-H], 7.05 [2H, t, *J* 7.6, C(5, 5′)-H], 7.50 [2H, d, *J* 8.3, C(4, 4′)-H], 7.82 [2H, s, C(2, 2′)-H], 10.43 (N-OH); $\delta_c$ (75 MHz, DMSO-$d_6$) 16.0 (2 × $CH_2$), 24.3 (2 × $CH_2$), 25.2 (2 × $CH_2$), 28.7 (2 × $CH_2$), 45.4 (2 × $CH_2$), 104.7 (2C, 2 × aromatic C), 110.3 (2CH, 2 × aromatic CH), 119.6 (2CH, 2 × aromatic CH), 120.5 (2 × CN), 121.2 (2CH, 2 × aromatic CH), 121.8 (2CH, 2 × aromatic CH), 124.0 (2C, 2 × aromatic C), 125.6 (2C, 2 × aromatic C), 132.1 (2CH, 2 × aromatic CH), 135.8 (2C, 2 × aromatic C), 168.3 (2 × C=O); m/z ($ESI^+$) 534.3 [$(M+H)^+$ 40%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{32}H_{32}N_5O_3)^+$ 534.2505. Found 534.2496.

**6-{3-[1-Hydroxy-4-(1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-1*H*-indol-1-yl}hexanoic acid 283**



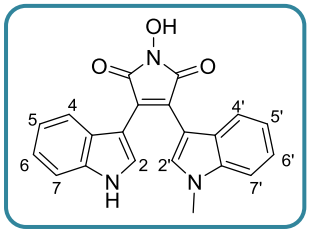

Following general procedure (i) starting from 6-{3-[4-(1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanoic acid **278** (0.120 g, 0.271 mmol) in anhydrous DMF (10 mL) with hydroxylamine hydrochloride (0.094 g, 1.36 mmol) and triethylamine (0.19 mL, 0.14 g, 1.4 mmol) gave desired product as a dark red solid (0.107 g, 86%): m.p. 133-135 °C; $\nu_{max}/cm^{-1}$ (KBr) 3372, 2935, 1768, 1703, 1612, 1527, 1391, 1097, 735; $\delta_H$ (400 MHz, DMSO-$d_6$) 1.18-1.27 [2H, m, C(4″)-$H_2$], 1.51 [2H, quin, *J* 7.5, C(3″)-$H_2$], 1.71 [2H, quin, *J* 7.3, C(5″)-$H_2$], 2.18 [2H, t, *J* 7.4, C(2″)-H], 4.22 [2H, t, *J* 6.9, C(6″)-H], 6.60 [1H, t, *J* 7.6, C(6)-H], 6.67-6.73 [2H, m, C(6′, 7)-H], 6.87 [1H, d, *J* 8.2, C(7′)-H], 6.98 [1H, t, *J* 7.6, C(5)-H], 7.04 [1H, t, *J* 7.7, C(5′)-H], 7.38 [1H, d, *J* 8.2, C(4)-H], 7.48 [1H, d, *J* 8.3, C(4′)-H], 7.77 [1H, s, C(2′)-H], 7.82 [1H, d, *J* 2.9, C(2)-H], 10.45 (1H, s, NOH), 11.75 (1H, bd, *J* 2.4, NH), 12.02 (1H, bs, COOH); $\delta_c$ (75 MHz, DMSO-$d_6$) 24.0 ($CH_2$), 25.6 ($CH_2$), 29.3 ($CH_2$), 33.5 ($CH_2$), 45.6 ($CH_2$), 104.7 (C, aromatic C), 105.4 (C, aromatic C), 110.2 (CH, aromatic CH), 111.8 (CH, aromatic CH), 119.4 (CH, aromatic CH), 119.6 (CH, aromatic CH), 120.9 (CH, aromatic CH), 121.1 (CH, aromatic CH), 121.7 (CH, aromatic CH), 121.8 (CH, aromatic CH), 123.8 (C, aromatic C), 124.6 (C, aromatic C), 125.0 (C, aromatic C), 125.9 (C, aromatic C), 129.5 (CH, aromatic CH), 132.0 (CH, aromatic CH), 135.8 (C, aromatic C), 136.0 (C, aromatic C), 168.39 (C=O), 168.44 (C=O), 174.3 (COOH); m/z ($ESI^+$) 458.2 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{26}H_{24}N_3O_5)^+$ 458.1716. Found 458.1700.

**19-Hydroxy-6,7,8,9,10,11-hexahydro-5,21:12,17-dimetheno-18*H*-dibenzo[*i*,*o*] pyrrolo[3,4-*l*][1,8]diazacyclohexadecine-18,20(19*H*)-dione 284**



Following general procedure (i) starting from 6,7,8,9,10,11-hexahydro-5,21:12,17-dimethenodibenzo[*i*,*o*]furo[3,4-*l*][1,8] diazacyclohexadecine-18,20-dione **272** (0.151 g, 0.368 mmol) in anhydrous DMF (10 mL) with hydroxylamine hydrochloride (0.127 g, 1.84 mmol) and triethylamine (0.26 mL, 0.19 g, 1.8 mmol) gave desired product as a purple solid (0.129 g, 82%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3373, 2924, 1768, 1712, 1619, 1534, 1470, 1390, 735; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.06 [4H, bs, C(8, 9)-$H_2$], 1.80 [4H, bs, C(7, 10)-$H_2$], 4.12-4.22 [4H, m, C(6, 11)-$H_2$], 7.14 [2H, ddd, *J* 7.9, 7.2, 1.0, C(2, 15)-H], 7.21 [2H, ddd, *J* 8.2, 7.1, 1.2, C(3, 14)-H], 7.39 [2H, s, C(22, 23)-H], 7.51 [2H, d, *J* 7.8, C(4, 13)-H], 7.81 [2H, dd, *J* 7.1, 0.9, C(1, 16)-H], 10.47 (N-O<u>H</u>); $\delta_c$ (75 MHz, DMSO-$d_6$) 22.7 (2 × $CH_2$), 27.3 (2 × $CH_2$), 44.2 (2 × $CH_2$), 102.5 (2C, 2 × aromatic C), 110.5 (2CH, 2 × aromatic CH), 120.2 (2CH, 2 × aromatic CH), 121.4 (2CH, 2 × aromatic CH), 121.7 (2CH, 2 × aromatic CH), 126.8 (2C, 2 × aromatic C), 128.6 (2C, 2 × aromatic C), 131.9 (2CH, 2 × aromatic CH), 135.2 (2C, 2 × aromatic C), 167.7 (2 × C=O); m/z ($ESI^+$) 426.2 [$(M+H)^+$ 10%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{26}H_{24}N_3O_3)^+$ 426.1818. Found 426.1827.

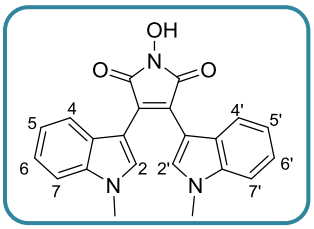
**1-Hydroxy-3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione 285**



Following general procedure (i) starting from 3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **265** (0.104 g, 0.304 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.101 g, 1.52 mmol) and triethylamine (0.20 mL, 0.15 g, 1.5 mmol) gave desired product as a dark red solid (83%): m.p. 137-139 °C; $\nu_{max}/cm^{-1}$ (KBr) 3332, 2930, 1767, 1706, 1612, 1529, 1460, 1023; $\delta_H$ (300 MHz, DMSO-$d_6$) 3.87 (3H, s, $CH_3$), 6.61-6.74 [3H, m, C(6, 6′, 7′)-H], 6.83 [1H, d, *J* 8.0, C(7)-H], 6.99 [1H, t, *J* 7.5, C(5)-H], 7.04 [1H, t, *J* 7.5, C(5′)-H], 7.39 [1H, d, *J* 8.0, C(4)-H], 7.44 [1H, d, *J* 8.1, C(4′)-H], 7.77 [1H, d, *J* 7.8, C(2)-H], 7.87 [1H, s, C(2′)-H], 10.45 (1H, s, N-OH), 11.74 (1H, bs, NH); $\delta_c$ (75 MHz, DMSO-$d_6$) 32.9 ($CH_3$), 104.5 (C, aromatic C), 105.5 (C, aromatic C), 110.2 (CH, aromatic CH), 111.9 (CH, aromatic

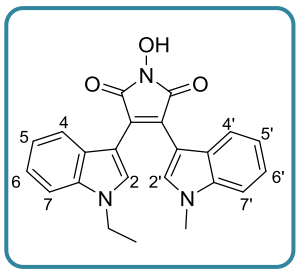
CH), 119.4 (CH, aromatic CH), 119.7 (CH, aromatic CH), 120.8 (CH, aromatic CH), 121.0 (CH, aromatic CH), 121.7 (CH, aromatic CH), 121.8 (CH, aromatic CH), 123.9 (C, aromatic C), 124.0 (C, aromatic C), 125.4 (C, aromatic C), 125.6 (C, aromatic C), 129.3 (CH, aromatic CH), 133.2 (CH, aromatic CH), 135.9 (C, aromatic C), 136.5 (C, aromatic C), 168.5 (2 × C=O); m/z (ESI$^-$) 356.2 [(M-H)$^-$ 50%]; HRMS (ESI$^-$): Exact mass calculated for $(C_{21}H_{14}N_3O_3)^-$ 356.1035. Found 356.1025.

**1-Hydroxy-3,4-bis(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione 286**




Following general procedure (i) starting from 3,4-bis(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **147** (0.092 g, 0.258 mmol) in anhydrous DMF (10 mL) with hydroxylamine hydrochloride (0.090 g, 1.29 mmol) and triethylamine (0.18 mL, 0.13 g, 1.3 mmol) gave desired product as a dark red solid (0.081 g, 84%): m.p. >300 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3224, 1764, 1694, 1610, 1519, 1367, 1097; $\delta_H$ (300 MHz, DMSO-$d_6$) 3.86 (6H, s, 2 × $CH_3$), 6.67 [2H, t, *J* 7.4, C(6, 6′)-H], 6.76 [2H, d, *J* 7.9, C(7, 7′)-H], 7.05 [2H, t, *J* 7.4, C(5, 5′)-H], 7.44 [2H, d, *J* 8.2, C(4, 4′)-H], 7.84 (2H, s, C(2, 2′)-H], 10.44 (1 H, s, N-OH); $\delta_c$ (75 MHz, DMSO-$d_6$) 32.9 (2 × $CH_3$), 104.6 (2C, 2 × aromatic C), 110.2 (2CH, 2 × aromatic CH), 119.6 (2CH, 2 × aromatic CH), 121.1 (2CH, 2 × aromatic CH), 121.8 (2CH, 2 × aromatic CH), 123.7 (2C, 2 × aromatic C), 125.7 (2C, 2 × aromatic C), 133.2 (2CH, 2 × aromatic CH), 136.5 (2C, 2 × aromatic C), 168.4 (2 × C=O); m/z (ESI$^+$) 372.1 [(M+H)$^+$ 100%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{22}H_{18}N_3O_3)^+$ 372.1348. Found 372.1348.

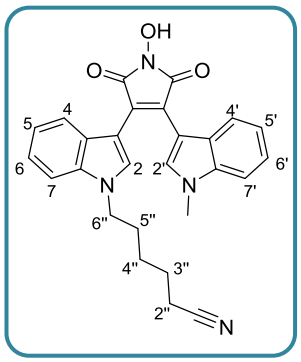
**3-(1-Ethyl-1*H*-indol-3-yl)-1-hydroxy-4-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione 287**




Following general procedure (i) starting from 3-(1-ethyl-1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **273** (0.103 g, 0.278 mmol) in anhydrous DMF (10 mL) with hydroxylamine hydrochloride (0.094 g, 1.39 mmol) and triethylamine (0.2 mL, 0.14 g, 1.4 mmol) gave desired product as a dark red solid (0.072 g, 69%): m.p. 236-238 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3239, 2932, 1763, 1699, 1523, 1219, 739; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.30 (3H, t, *J* 7.6, $CH_2C\underline{H}_3$), 3.87 (3H,

s, $CH_3$), 4.24 (2H, q, *J* 7.1 $C\underline{H}_2CH_3$), 6.62-6.69 [2H, m, C(6′, 7′)-H], 6.75 [1H, t, *J* 7.6, C(6)-H], 6.96 [1H, d, *J* 8.15, C(7)-H], 7.03-7.11 [2H, m, (5, 5′)-H], 7.45 [1H, d, *J* 8.15, C(4′)-H], 7.50 [1H, d, 8.49, C(4)-H], 7.74 [1H, s, C(2)-H], 7.90 [1H, s, C(2′)-H], 10.47 (N-OH); $\delta_c$ (75 MHz, DMSO-$d_6$) 15.2 ($CH_2C\underline{H}_3$), 32.9 ($CH_3$), 40.7 ($C\underline{H}_2CH_3$), 104.4 (C, aromatic C), 104.8 (C, aromatic C), 110.18 (CH, aromatic CH), 110.23 (CH, aromatic CH), 119.65 (CH, aromatic CH), 119.67 (CH, aromatic CH), 121.2 (CH, aromatic CH), 121.3 (CH, aromatic CH), 121.8 (2CH, 2 × aromatic CH), 123.4 (C, aromatic C), 124.1 (C, aromatic C), 125.2 (C, aromatic C), 126.0 (C, aromatic C), 131.6 (CH, aromatic CH), 133.4 (CH, aromatic CH), 135.4 (C, aromatic C), 136.6 (C, aromatic C), 168.37 (C=O), 168.41 (C=O); m/z ($ESI^+$) 386.2 $[(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{23}H_{20}N_3O_3)^+$ 386.1505. Found 386.1509.

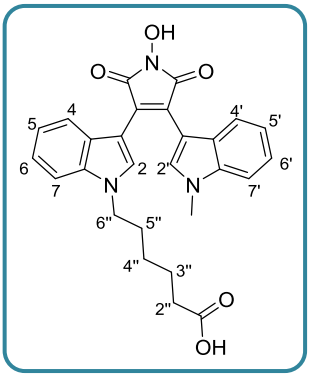
**6-{3-[1-Hydroxy-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-1*H*-indol-1-yl}hexanenitrile 288**




Following general procedure (i) starting from 6-(3-(4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl)-1*H*-indol-1-yl)hexanenitrile **274** (0.103 g, 0.235 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.079 g, 1.18 mmol) and triethylamine (0.16 mL, 0.12 g, 1.2 mmol) gave desired product as a dark red solid (0.087 g, 82%): m.p. 123-125 °C; $\nu_{max}/cm^{-1}$ (KBr) 3229, 2935, 2244, 1769, 1708, 1529, 1099, 742; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.22-1.39 [2H, m, C(4″)-$H_2$], 1.57 [2H, quin, *J* 7.27, C(3″)-$H_2$], 1.73 [2H, quin, *J* 7.04, C(5″)-$H_2$], 2.45 [2H, t, *J* 7.2, C(2″)-$H_2$], 3.88 (3H, s, $CH_3$), 4.23 [2H, t, *J* 6.9, C(6″)-$H_2$], 6.62-6.68 [2H, m, C(6′, 7′)-H], 6.73 [1H, t, *J* 7.7, C(6)-H], 6.94 [1H, d, *J* 7.8, C(7)-H], 7.01-7.10 [2H, m, (5, 5′)-H], 7.45 [1H, d, *J* 8.0, C(4′)-H], 7.50 [1H, d, *J* 8.2, C(4)-H], 7.75 [1H, s, C(2)-H], 7.90 [1H, s, C(2′)-H], 10.43 (1H, s, N-OH); $\delta_c$ (75 MHz, DMSO-$d_6$) 16.0 ($CH_2$), 24.3 ($CH_2$), 25.2 ($CH_2$), 28.8 ($CH_2$), 32.9 ($CH_3$), 45.9 ($CH_2$), 104.4 (C, aromatic C), 104.9 (C, aromatic C), 110.2 (CH, aromatic CH), 110.3 (CH, aromatic CH), 119.6 (2 × CH, 2 aromatic CH), 120.6 (CN), 121.1 (CH, aromatic CH), 121.2 (CH, aromatic CH), 121.77 (CH, aromatic CH), 121.81 (CH, aromatic CH), 123.5 (C, aromatic C), 124.2 (C, aromatic C), 125.3 (C, aromatic C), 126.0 (C, aromatic C), 132.0 (CH, aromatic CH), 133.3 (CH, aromatic CH), 135.7 (C, aromatic C), 136.6 (C, aromatic C), 168.3 (C=O), 168.4 (C=O); m/z ($ESI^-$) 451.3 [(M-

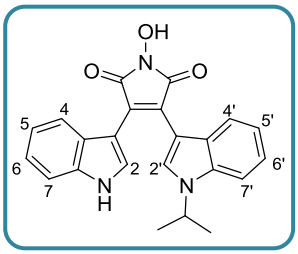
H)$^-$ 50%]; HRMS (ESI$^-$): Exact mass calculated for $(C_{27}H_{23}N_4O_3)^-$ 451.1770. Found 451.1783.

**6-{3-[1-Hydroxy-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-1*H*-indol-1-yl}hexanoic acid 289**



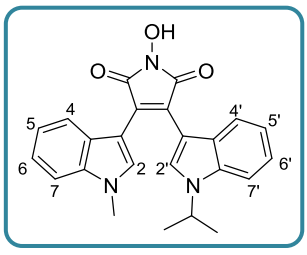

Following general procedure (i) starting from 6-{3-[4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H* -indol-1-yl}hexanoic acid **279** (0.101 g, 0.221 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.076 g, 1.11 mmol) and triethylamine (0.15 mL, 0.11 g, 1.1 mmol) gave desired compound as a dark red solid (0.085 g, 79%): m.p. 196-197 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3114, 2935, 1710, 1609, 1529, 1098, 741; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.16-1.29 [2H, m, C(4″)-H$_2$], 1.51 [2H, quin, *J* 7.4, C(3″)-H$_2$], 1.70 [2H, quin, *J* 7.1, C(5″)-H$_2$], 2.17 [2H, t, 7.3, C(2″)-H$_2$], 3.87 (3H, s, CH$_3$), 4.21 [2H, t, *J* 6.7, C(6″)-H$_2$], 6.58-6.66 [2H, m, C(6′, 7′)-H], 6.72 [1H, t, *J* 7.4, C(6)-H], 6.93 [1H, d, *J* 8.0, C(7)-H], 7.00-7.09 [2H, m, (5, 5′)-H], 7.43 [1H, d, *J* 8.3, C(4′)-H], 7.48 [1H, d, *J* 8.3, C(4)-H], 7.73 [1H, s, C(2)-H], 7.90 [1H, s, C(2′)-H], 10.43 (1H, bs, NH), 11.99 (1H, bs, COOH); $\delta_c$ (75 MHz, DMSO-$d_6$) 24.0 (CH$_2$), 25.6 (CH$_2$), 29.3 (CH$_2$), 32.9 (CH$_3$), 33.5 (CH$_2$), 45.6 (CH$_2$), 104.4 (C, aromatic C), 104.8 (C, aromatic C), 110.17 (CH, aromatic CH), 110.24 (CH, aromatic CH), 119.57 (CH, aromatic CH), 119.59 (CH, aromatic CH), 121.10 (CH, aromatic CH), 121.12 (CH, aromatic CH), 121.8 (2CH, 2 × aromatic CH), 123.5 (C, aromatic C), 124.3 (C, aromatic C), 125.3 (C, aromatic C), 126.0 (C, aromatic C), 132.0 (CH, aromatic CH), 133.3 (CH, aromatic CH), 135.7 (C, aromatic C), 136.6 (C, aromatic C), 168.4 (C=O), 168.4 (C=O), 174.3 (COOH); m/z (ESI$^+$) 472.2 [(M+H)$^+$ 100%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{27}H_{26}N_3O_5)^+$ 472.1872. Found 472.1864.

**1-Hydroxy-3-(1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione 290**



Following general procedure (i) starting from 3-(1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione **266** (0.120 g, 0.323 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.11 g, 1.62 mmol) and triethylamine (0.23 mL, 0.17 g, 1.6 mmol) gave desired product as a dark red solid (0.097 g, 77%): m.p. 106-109 °C; $\nu_{max}/cm^{-1}$ (KBr) 3118, 3135, 2970, 1812, 1745, 1627, 1529, 1247; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.38 (6H, d, *J* 6.6, 2 × $CH_3$), 4.78 [1H, sept, *J* 6.7 C<u>H</u>$(CH_3)_2$], 6.63 (2H, m, C(6, 7)-H], 6.78 [1H, t, *J* 7.2, C(6′)-H], 6.96-7.10 [3H, m, C(5, 5′, 7′)-H], 7.39 [1H, d, *J* 8.1, C(4)-H], 7.53 [1H, d, *J* 8.3, C(4′)-H], 7.69 [1H, s, C(2′)-H], 7.83 [1H, d, *J* 2.8, C(2)-H], 10.42 (1H, s, N-OH), 11.74 (1H, s, N-H); $\delta_c$ (75 MHz, DMSO-$d_6$) 22.1 [CH(<u>C</u>$H_3)_2$], 47.0 [<u>C</u>H$(CH_3)_2$], 105.0 (C, aromatic C), 105.2 (C, aromatic C), 110.3 (CH, aromatic CH), 111.9 (CH, aromatic CH), 119.5 (CH, aromatic CH), 119.8 (CH, aromatic CH), 121.1 (CH, aromatic CH), 121.3 (CH, aromatic CH), 121.71 (CH, aromatic CH), 121.73 (CH, aromatic CH), 123.6 (C, aromatic C), 124.6 (C, aromatic C), 124.9 (C, aromatic C), 125.9 (C, aromatic C), 128.2 (CH, aromatic CH), 129.8 (CH, aromatic CH), 135.3 (C, aromatic C), 136.1 (C, aromatic C), 168.37 (C=O), 168.44 (C=O); m/z (ESI$^+$) 386.4 [$(M+H)^+$ 100%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{23}H_{20}N_3O_3)^+$ 386.1505. Found 386.1495.

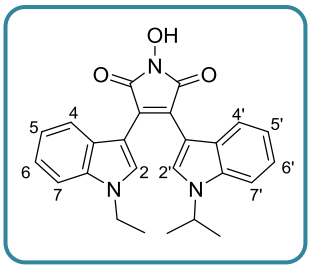
**1-Hydroxy-3-(1-isopropyl-1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione 291**



Following general procedure (i) starting from 3-(1-isopropyl-1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2, 5-dione **275** (0.099 g, 0.258 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.089 g, 1.29 mmol) and triethylamine (0.18 mL, 0.13 g, 1.29 mmol) gave desired product as a dark red solid (0.096 g, 93%): m.p. 84-86 °C; $\nu_{max}/cm^{-1}$ (KBr) 3118, 2930, 1712, 1654, 1463, 1220, 741; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.41 (6H, d, *J* 6.6, 2 × $CH_3$), 3.93 (3H, s, $CH_3$), 4.78 [1H, sept, *J* 6.6, C<u>H</u>$(CH_3)_2$], 6.52 [1H, d, *J* 7.7, C(7)-H] 6.63 [1H, t, *J* 7.2, C(6)-H], 6.80 [1H, t, *J* 7.3, C(6′)-H], 7.03-7.11 [3H, m, C(5, 5′, 7)-H], 7.45

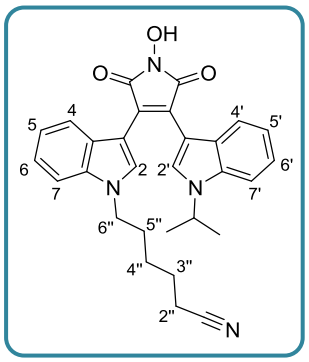
[1H, d, *J* 8.2, C(4)-H], 7.54 [1H, d, *J* 8.5, C(4′)-H], 7.67 [1H, s, C(2′)-H], 7.93 [1H, s, C(2)-H], 10.48 (1H, s, N-OH); $\delta_c$ (75 MHz, DMSO-$d_6$) 22.1 (2 × $CH_3$), 32.9 [$\underline{C}H(CH_3)_2$], 47.0 ($CH_3$), 104.3 (C, aromatic C), 105.1 (C, aromatic C), 110.2 (CH, aromatic CH), 110.3 (CH, aromatic CH), 119.7 (CH, aromatic CH), 119.8 (CH, aromatic CH), 121.3 (2CH, 2 × aromatic CH), 121.75 (CH, aromatic CH), 121.77 (CH, aromatic CH), 123.4 (C, aromatic C), 124.6 (C, aromatic C), 124.9 (C, aromatic C), 126.0 (C, aromatic C), 128.2 (CH, aromatic CH), 133.6 (CH, aromatic CH), 135.3 (C, aromatic C), 136.7 (C, aromatic C), 168.3 (C=O), 168.4 (C=O); m/z ($ESI^+$) 400.2 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{24}H_{22}N_3O_3)^+$ 400.1661. Found 400.1651.

**3-(1-Ethyl-1*H*-indol-3-yl)-1-hydroxy-4-(1-isopropyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione 292**




Following general procedure (i) starting from 3-(1-ethyl-1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)furan-2,5-dione **276** (0.068 g, 0.171 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.059 g, 0.853 mmol) and triethylamine (0.12 mL, 0.87 g, 0.85 mmol) gave desired product as a dark red solid (0.056 g, 80%): m.p. 83-85 °C; $\nu_{max}/cm^{-1}$ (KBr) 3125, 2975, 1769, 1713, 1527, 1214, 741; $\delta_H$ (300 MHz, DMSO-$d_6$,) 1.34 (3H, t, *J* 7.2, $CH_2C\underline{H_3}$) 1.39 (6H, d, *J* 6.6, 2 × $CH_3$), 4.27 (2H, q, *J* 7.2, $C\underline{H_2}CH_3$), 4.79 [1H, sept, *J* 6.6 $C\underline{H}(CH_3)_2$], 6.66-6.72 [2H, m, C(6, 7)-H], 6.76 [1H, t, *J* 7.6, C(6′)-H], 6.95 [1H, d, *J* 7.8, C(7′)-H], 7.04-7.11 [2H, m, C(5, 5′)-H], 7.50 [1H, d, *J* 8.3, C(4)-H], 7.55 [1H, d, *J* 8.4, C(4′)-H], 7.74 [1H, s, C(2′)-H], 7.85 [1H, s, C(2)-H], 10.47 (1H, s, N-OH), $\delta_c$ (75 MHz, DMSO-$d_6$) 15.2 ($CH_3$), 22.1 (2 × $CH_3$), 40.7 ($CH_2$), 47.0 (CH), 104.6 (C, aromatic C), 105.0 (C, aromatic C), 110.2 (CH, aromatic CH), 110.4 (CH, aromatic CH), 119.7 (CH, aromatic CH), 119.8 (CH, aromatic CH), 121.38 (CH, aromatic CH), 121.44 (CH, aromatic CH), 121.7 (CH, aromatic CH), 121.8 (CH, aromatic CH), 123.9 (C, aromatic C), 124.3 (C, aromatic C), 125.3 (C, aromatic C), 125.7 (C, aromatic C), 128.4 (CH, aromatic CH), 131.9 (CH, aromatic CH), 135.4 (C, aromatic C), 135.6 (C, aromatic C), 168.3 (C=O), 168.4 (C=O); m/z ($ESI^+$) 414.2 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{25}H_{24}N_3O_3)^+$ 414.1818. Found 414.1814.

**6-{3-[1-Hydroxy-4-(1-isopropyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-1*H*-indol-1-yl}hexanenitrile 293**

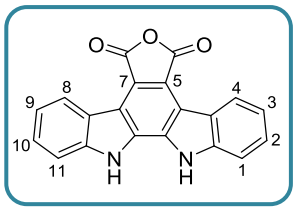



Following general procedure (i) starting from 6-{3-[4-(1-isopropyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl]-1*H*-indol-1-yl}hexanenitrile **277** (0.104 g, 0.223 mmol) in anhydrous DMF (8 mL) with hydroxylamine hydrochloride (0.077 g, 1.12 mmol) and triethylamine (0.16 mL, 1.2 g, 1.1 mmol) gave desired product as a dark red solid (0.083 g, 77%): m.p. 80-82 °C; $\nu_{max}/cm^{-1}$ (KBr) 3230, 2929, 2244, 1789, 1713, 1528, 1216, 742; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.34 [2H, m, C(4″)-H], 1.39 (6H, d, *J* 6.6, 2 × $CH_3$), 1.57 [2H, quin, *J* 7.5, C(3″)-H], 1.76 [2H, quin, *J* 7.2, C(5″)-H], 2.45 [2H, t, *J* 7.1, C(2″)-H], 4.26 [2H, t, *J* 6.9, C(6″)-H], 4.79 [1H, sept, *J* 6.7, C<u>H</u>$(CH_3)_2$], 6.64-6.77 [3H, m, C(6, 6′, 7)-H], 6.96 [1H, d, *J* 8.0, C(7′)-H], 7.03-7.10 [2H, m, C(5, 5′)-H], 7.49-7.55 [2H, m, C(4, 4′)-H], 7.74 [1H, s, C(2′)-H], 7.85 [1H, s, C(2)-H], 10.47 (1H, s, N-OH); $\delta_c$ (75 MHz, DMSO-$d_6$) 16.0 ($CH_2$), 22.1 (2 × $CH_3$), 24.3 ($CH_2$), 25.2 ($CH_2$), 28.8 ($CH_2$), 45.4 ($CH_2$), 47.0 (CH), 104.6 (C, aromatic C), 105.0 (C, aromatic C), 110.3 (2CH, 2 × aromatic CH), 119.6 (CH, aromatic CH), 119.7 (CH, aromatic CH), 120.5 (CN), 121.3 (2CH, 2 × aromatic CH), 121.7 (CH, aromatic CH), 121.8 (CH, aromatic CH), 124.0 (C, aromatic C), 124.3 (C, aromatic C), 125.3 (C, aromatic C), 125.7 (C, aromatic C), 128.3 (CH, aromatic CH), 132.3 (CH, aromatic CH), 135.4 (C, aromatic C), 135.9 (C, aromatic C), 168.31 (C=O), 168.33 (C=O); m/z ($ESI^+$) 481.2 $[(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{29}H_{29}N_4O_3)^+$ 481.2240. Found 481.2225.

## 7.3 Synthesis of indolo[2,3-*a*]carbazoles

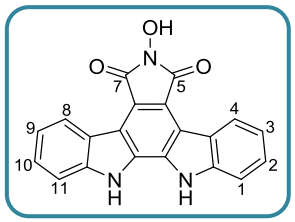
### 7.3.1 Synthesis of unsubstituted ICZs

**Furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione 76**[13]



3,4-Di(1*H*-indol-3-yl)furan-2,5-dione **255** (1.001 g, 30.5 mmol) was dissolved in acetonitrile (1 L) giving a bright red solution which was irradiated using a standard high pressure mercury lamp, with a catalytic amount of iodine in the presence of air for 24 hours. At this point, a yellow precipitate had formed and the suspension was allowed to cool to room temperature. The precipitate was collected using vacuum filtration and washed with saturated aqueous sodium thiosulfate (10 mL), followed by water (10 mL) and allowed to dry to give desired product as a yellow solid (0.723 g, 72%): m.p. >300 °C; (Lit. >300 °C)[13]; $\nu_{max}/cm^{-1}$ (KBr); 3408, 1817, 1752, 1405, 1258, 738; $\delta_H$ (300 MHz, DMSO-$d_6$) 7.33 [2H, t, *J* 7.6, C(3, 9)-H], 7.55 [2H, ddd, *J* 8.3, 7.2, 1.2, C(2, 10)-H], 7.78 [2H, d, *J* 8.2, C(1, 11)-H], 8.69 [2H, d, *J* 8.0, C(4, 8)-H], 11.91 [2H, bs, N(12, 13)-H]; m/z (ESI$^-$) 325.2 [$(M-H)^-$ 50%].

**6-Hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 296**[12]

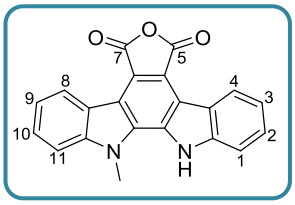


To a solution of furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **76** (0.434 g, 1.33 mmol) in anhydrous DMF (50 mL) was added hydroxylamine hydrochloride (0.464 g, 6.65 mmol) followed by triethylamine (0.93 mL, 0.67 g, 6.7 mmol). The reaction mixture was then heated to 70 °C and stirred at this temperature for 24 hours. After cooling, water (50 mL) was added and the precipitate collected using vacuum filtration and was washed with 1 M aqueous HCl (10 mL) and water (10 mL) before being allowed to dry to give the desired product as a red solid (0.418 g, 92%): m.p. >300 °C; (Lit. >315 °C)[12]; $\nu_{max}/cm^{-1}$ (KBr) 3399, 3209, 1738, 1695, 1563, 1403, 1063, 748; $\delta_H$ (400 MHz, DMSO-$d_6$) 7.38 [2H, t, *J* 7.5, C(3, 9)-H], 7.58 [2H, t, *J* 7.6,

C(2, 10)-H], 7.83 [2H, d, *J* 8.2, C(1, 11)-H], 8.98 [2H, d, *J* 8.0, C(4, 8)-H], 10.58 (1H, s, N-OH), 11.77 [2H, s, N(12, 13)-H]; m/z (ESI$^-$) 340.2 [(M-H)$^-$ 100%].

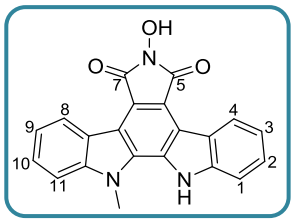
### 7.3.2 Incorporation of methyl and allyl substituents

**12-Methylfuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione 297**



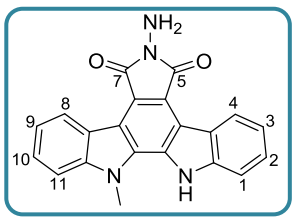

3-(1*H*-Indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)furan-2,5-dione **265** (0.250 g, 0.735 mmol) was dissolved in acetonitrile (250 mL) and irradiated using a standard high pressure mercury lamp, with a catalytic amount of iodine in the presence of air for 16 hours. At this point a yellow precipitate had formed and the suspension was allowed to cool to room temperature. The precipitate was collected using vacuum filtration and washed with saturated sodium thiosulfate, followed by water and allowed to dry to give desired product as a yellow solid (0.103 g, 41%): m.p. >300 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3391, 1820, 1730, 1399, 1341, 1251, 738; $\delta_H$ (600 MHz, DMSO-$d_6$) 4.23 (3H, s, $CH_3$), 7.29 [1H, t, *J* 7.5, C(3 or 9)-H], 7.33 [1H, t, *J* 7.5, C(3 or 9)-H], 7.52 [1H, ddd, *J* 8.0, 7.1, 0.9, C(2 or 10)-H], 7.59 [1H, ddd, *J* 8.1, 7.1, 0.9, C(2 or 10)-H], 7.70-7.73 [2H, m, C(1, 11)-H], 8.68 [1H, d, *J* 8.3, C(4 or 8)-H], 8.70 [1H, d, *J* 8.6, C(4 or 8)-H], 12.14 [1H, bs, N(13)-H]; $\delta_c$ (150 MHz, DMSO-$d_6$, 37 °C) 31.9 ($CH_3$), 110.0 (CH, aromatic CH), 112.1 (CH, aromatic CH), 115.3 (C, aromatic C), 116.5 (C, aromatic C), 117.1 (C, aromatic C), 117.3 (C, aromatic C), 120.2 (2C, 2 × aromatic C), 120.6 (CH, aromatic CH), 120.7 (CH, aromatic CH), 123.2 (CH, aromatic CH), 123.4 (CH, aromatic CH), 127.2 (2CH, 2 × aromatic CH), 129.2 (C, aromatic C), 130.9 (C, aromatic C), 141.0 (C, aromatic C), 141.3 (C, aromatic C), 164.45 (C=O), 164.49 (C=O); m/z (ESI$^-$) 399.1 [(M-H)$^-$ 40%]; HRMS (ESI$^-$): Exact mass calculated for $(C_{21}H_{11}N_2O_3)^-$ 339.0770. Found 339.0775.

**6-Hydroxy-12-methyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 298**




To a solution of 12-methylfuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **297** (0.093 g, 0.273 mmol) in anhydrous DMF (10 mL) was added hydroxylamine hydrochloride (0.095 g, 1.37 mmol) and triethylamine (0.20 mL, 0.15 g, 1.4 mmol). The reaction mixture was then heated to 80 °C and stirred at this temperature for 24 hours. After cooling, water (20 mL) was added and the precipitate collected using vacuum filtration and was washed with 1 M aqueous HCl (5 mL) followed by water (5 mL) before being allowed to dry to give gave desired product as an orange solid (0.084 g, 87%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3333, 2840, 1744, 1693, 1567, 1397, 1108, 733; $\delta_H$ (300 MHz, DMSO-$d_6$) 4.39 (3H, s, $CH_3$), 7.35 [2H, t, *J* 7.5, C(3, 9)-H], 7.56 [1H, t, *J* 7.6, C(2 or 10)-H], 7.60 [1H, t, *J* 7.7, C(2 or 10)-H], 7.77 [1H, d, *J* 8.1, C(1 or 11)-H], 7.78 [1H, d, *J* 8.3, C(1 or 11)-H], 9.02 [2H, d, *J* 8.0, C(4, 8)-H], 10.43 (1H, bs, N-OH) 12.07 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 31.9 ($CH_3$), 109.7 (CH, aromatic CH), 112.0 (CH, aromatic CH), 115.0 (C, aromatic C), 115.3 (C, aromatic C), 115.5 (C, aromatic C), 116.7 (C, aromatic C), 120.1 (CH, aromatic CH), 120.2 (CH, aromatic CH), 120.59 (C, aromatic C), 120.63 (C, aromatic C), 124.0 (CH, aromatic CH), 124.2 (CH, aromatic CH), 126.87 (CH, aromatic CH), 126.91 (CH, aromatic CH), 128.5 (C, aromatic C), 130.3 (C, aromatic C), 141.1 (C, aromatic C), 141.5 (C, aromatic C), 166.28 (C=O), 166.32 (C=O); m/z (ESI$^-$) 354.3 [$(M-H)^-$ 100%]; HRMS (ESI$^-$): Exact mass calculated for $(C_{21}H_{12}N_3O_3)^-$ 354.0879. Found 354.0889.

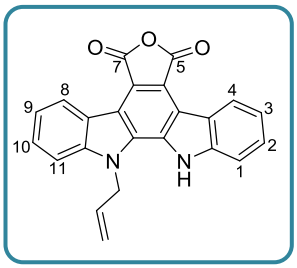
**6-Amino-12-methyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 299**




To a stirred suspension of 12-methylfuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **297** (0.102 g, 0.300 mmol) in THF (20 mL) was added hydrazine hydrate (0.10 mL, 0.10 g, 1.4 mmol) and the mixture was heated to reflux and stirred at this temperature for 16 hours. After cooling the precipitate was collected by vacuum filtration, washed with water and allowed to dry to give an orange solid (0.070 g, 67%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3375, 1687, 1568, 1398, 739; $\delta_H$ (300 MHz, DMSO-$d_6$)

4.31 (3H, s, $CH_3$), 4.83 (2H, s, $NH_2$), 7.32 [2H, t, *J* 7.5, C(3, 9)-H], 7.50-7.63 [2H, m, C(2, 10)-H], 7.72 [1H, d, *J* 8.4, C(1 or 11)-H], 7.75 [1H, d, *J* 8.2, C(1 or 11)-H], 9.02 [1H, d, *J* 7.8, C(4 or 8)-H], 9.03 [1H, d, *J* 7.8, C(4 or 8)-H], 11.53 [1H, bs, N(13)-H]; $\delta_c$ (150 MHz, DMSO-$d_6$) 31.9 ($CH_3$), 109.6 (CH, aromatic CH), 111.9 (CH, aromatic CH), 115.3 (C, aromatic C), 116.52 (C, aromatic C), 116.54 (C, aromatic C), 116.8 (C, aromatic C), 120.0 (CH, aromatic CH), 120.1 (CH, aromatic CH), 120.68 (C, aromatic C), 120.74 (C, aromatic C), 124.1 (CH, aromatic CH), 124.2 (CH, aromatic CH), 126.7 (CH, aromatic CH), 126.8 (CH, aromatic CH), 128.3 (C, aromatic C), 130.1 (C, aromatic C), 141.0 (C, aromatic C), 141.4 (C, aromatic C), 168.6 (C=O), 168.7 (C=O); m/z ($ESI^-$) 353.3 [$(M-H)^-$ 60%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{21}H_{15}N_4O_2)^+$ 355.1195. Found 355.1187.

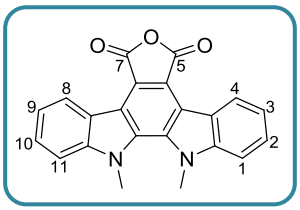
**12-Allyl-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione 300**



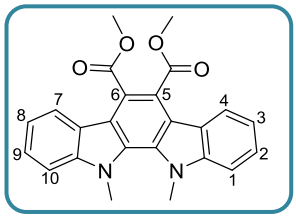

3-(1-Allyl-1*H*-indol-3-yl)-4-(1*H*-indol-3-yl)furan-2,5-dione **269** (0.707 g, 1.92 mmol) was dissolved in acetonitrile (700 mL) and irradiated using a standard high pressure mercury lamp with a catalytic amount of iodine in the presence of air for 24 hours. The yellow precipitate was collected using vacuum filtration and washed with saturated aqueous sodium thiosulfate, followed by water and allowed to dry to give a yellow solid (0.360 g) which analysis showed to be furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione (57% recovery). The filtrate was concentrated to roughly 20 mL and the resulting precipitate was collected using vacuum filtration and washed with saturated aqueous sodium thiosulfate, followed by water to give a yellow solid which the crude NMR showed to be impure desired product (0.072 g): $\delta_H$ (300 MHz, DMSO-$d_6$) 4.67 [1H, d, *J* 17.1, CH=C$\underline{H}_2$ (trans)], 5.05 [1H, d, *J* 10.3, CH=C$\underline{H}_2$ (cis)], 5.56 (2H, bs, $CH_2$), 6.11-6.24 (1H, m, C$\underline{H}$=$CH_2$), 7.32-7.45 [2H, m, C(3, 9)-H], 7.55-7.67 [2H, m, C(2, 10)-H], 7.79 [2H, d, *J* 8.3, C(1, 11)-H], 8.83 [1H, d, *J* 8.0, C(4 or 8)-H], 8.88 [1H, d, *J* 7.9, C(4 or 8)-H], 12.12 [1H, s, N(13)-H]; m/z ($ESI^-$) 365.3 [$(M-H)^-$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{23}H_{15}N_2O_3)^+$ 367.1083. Found 367.1065.

**12,13-Dimethylfuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione 301**

Method A



To a stirred suspension of furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **76** (0.504 g, 1.54 mmol) in anhydrous DMF (20 mL) in a flame-dried flask under nitrogen was added sodium hydride (60 wt. % oil dispersion, 0.180 g, 4.63 mmol). After stirring for 40 minutes, iodomethane (0.29 mL, 0.66 g, 4.6 mmol) was added to the dark blue solution and the mixture was stirred for 16 hours resulting in a pale yellow solution. Due to the presence of starting material in TLC analysis a further portion of sodium hydride (60 wt. % oil dispersion, 0.183 g, 4.63 mmol) was added followed after 40 minutes by iodomethane (0.29 mL, 0.66 g, 4.6 mmol) and the mixture was stirred for a further 6 hours. The reaction mixture was then poured into water (50 mL) and the off-white precipitate was collected by vacuum filtration, washed with 1 M aqueous HCl (5 mL) followed by water (2 × 2 mL) and allowed to dry to give an off-white solid which on analysis was identified as dimethyl 11,12-dimethyl-11,12-dihydroindolo[2,3-*a*]carbazole-5,6-dicarboxylate **188** (0.507 g, 1.3 mmol, 82%): m.p. 224-226 °C; (Lit. 213-214 °C)[14]; $\nu_{max}/cm^{-1}$ (KBr) 2952, 2852, 1713, 1549, 1480, 1261, 1070, 733; $\delta_H$ (300 MHz, DMSO-$d_6$) 4.03 (6H, s, 2 × $OCH_3$), 4.24 (6H, s, 2 × $NCH_3$), 7.33 [2H, ddd, *J* 8.1, 7.1, 1.0, C(3, 8)-H], 7.60 [2H, ddd, *J* 8.3, 7.1, 1.1, C(2, 9)-H], 7.78 [2H, d, *J* 8.3, C(1, 10)-H], 8.06 [2H, d, *J* 7.8, C(4, 7)-H]; m/z ($ESI^+$) 402.2 [$(M+H)^+$ 20%].



Method B

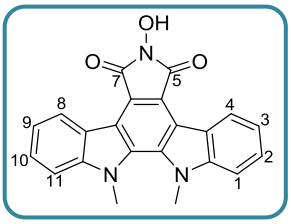
A stirred suspension of furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **76** (1.004 g, 3.08 mmol) in anhydrous DMF (30 mL) in a flame-dried flask under nitrogen was cooled to -12 °C using an ice/salt bath. Sodium hydride (60 wt. % oil dispersion, 0.260 g, 6.47 mmol) was then added and the mixture was stirred for 45 minutes. At this point, iodomethane (0.42 mL, 0.96 g, 6.5 mmol) was added dropwise and the mixture was allowed to slowly warm to room temperature and stirred for 16 hours. The reaction mixture was poured into 1 M aqueous HCl (60 mL) and the precipitate collected using vacuum filtration, washed with water and allowed to dry. The crude product was recrystallised from DMSO to give desired product as a bright yellow solid (0.775 g, 71%): m.p. > 300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3058, 2934, 1817, 1747, 1566, 1250, 745; $\delta_H$

(600 MHz, DMSO-$d_6$) 4.34 (6H, s, 2 × $CH_3$), 7.48 [2H, t, *J* 7.1, C(3, 9)-H], 7.72 [2H, ddd, *J* 8.2, 7.1, 1.1, C(2, 10)-H], 7.88 [2H, d, *J* 8.3, C(1, 11)-H], 8.95 [2H, d, *J* 7.9, C(4, 8)-H]; $\delta_c$ (150 MHz, DMSO-$d_6$) 37.1 (2 × $CH_3$), 111.4 (2CH, 2 × aromatic CH), 117.9 (2C, 2 × aromatic C), 118.1 (2C, 2 × aromatic C), 120.8 (2C, 2 × aromatic C), 121.4 (2CH, 2 × aromatic CH), 123.5 (2CH, 2 × aromatic CH), 128.0 (2CH, 2 × aromatic CH), 132.7 (2C, 2 × aromatic C), 143.8 (2C, 2 × aromatic C), 164.5 (2 × C=O); m/z ($ESI^+$) 354.1 [$(M)^+$ 20%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{22}H_{14}N_2O_3)^+$ 354.1004. Found 354.1003.

Method C

To a stirred suspension of dimethyl 11,12-dimethyl-11,12-dihydroindolo[2,3-*a*]carbazole-5,6-dicarboxylate **188** (0.479 g, 1.20 mmol) in methanol (45 mL) was added 50% aqueous sodium hydroxide (5 mL) and the mixture was heated to reflux for 24 hours. The reaction mixture was then allowed to cool and the solvent was evaporated under reduced pressure. The residue was suspended in water (20 mL) and the mixture was acidified to pH 2 with 10% aqueous HCl. The product was then collected using vacuum filtration and washed with water (10 mL) before being allowed to dry. The off-white solid was then dissolved in acetic anhydride (10 mL) and heated to reflux for 4 hours. After cooling, the solvent was evaporated under reduced pressure and the residue suspended in saturated aqueous sodium bicarbonate solution (20 mL). The product was collected by vacuum filtration, washed with water (5 mL) and allowed to dry yielding desired compound as a bright yellow powder (0.340 g, 80%). Analysis was identical to that reported above.

**6-Hydroxy-12,13-dimethyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 305**

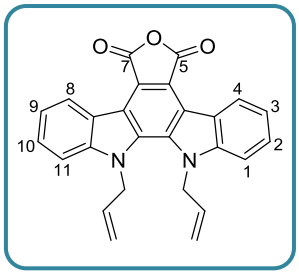



To a stirred suspension of 12,13-dimethylfuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **301** (0.320 g, 0.903 mmol) in anhydrous DMF (25 mL) was added hydroxylamine hydrochloride (0.311 g, 4.52 mmol) and triethylamine (0.60 mL, 0.44 g, 4.5 mmol) and the mixture was heated to 80 °C for 24 hours. At this point the orange solution was allowed to cool before being poured into 1 M aqueous HCl (40 mL) and the precipitate collected by vacuum filtration and washed with water (10 mL) to

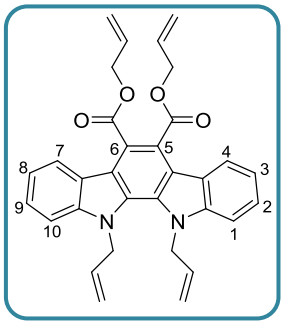
give desired product as an orange solid (0.302 g, 91%); m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3194, 2926, 1752, 1692, 1470, 1007, 747; $\delta_H$ (400 MHz, DMSO-$d_6$) 4.26 (6H, s, 2 × $CH_3$), 7.35 [2H, t, *J* 7.6, C(3, 9)-H], 7.62 [2H, ddd, *J* 8.2, 7.2, 1.1, C(2, 10)-H], 7.76 [2H, d, *J* 8.3, C(1, 11)-H], 9.03 [2H, d, *J* 7.8, C(4, 8)-H], 10.65 (1H, bs, N-OH); $\delta_c$ (150 MHz, DMSO-$d_6$) 36.9 (2 × $CH_3$) 110.9 (2CH, 2 × aromatic CH), 115.5 (2C, 2 × aromatic C), 118.2 (2C, 2 × aromatic C), 120.8 (2CH, 2 × aromatic CH), 121.2 (2 C, 2 × aromatic C), 124.2 (2CH, 2 × aromatic CH), 127.5 (2CH, 2 × aromatic CH), 132.0 (2C, 2 × aromatic C), 144.1 (2C, 2 × aromatic C), 166.0 (2 × C=O); m/z ($ESI^+$) 370.1 [$(M+H)^+$ 5%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{22}H_{16}N_3O_3)^+$ 370.1192. Found 370.1180.

**12,13-Diallyl-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione 304**

Method A



To a stirred suspension of furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **76** (0.198 g, 0.607 mmol) in anhydrous DMF (20 mL) in a flame-dried flask at room temperature under nitrogen was added sodium hydride (60 wt. %, 0.054 g, 1.27 mmol) carefully and the mixture was stirred at this temperature for 40 minutes. Allyl bromide (0.12 mL, 0.17 g, 1.4 mmol) was then added and the mixture was stirred for 16 hours. At this point TLC analysis showed that some starting material still remained and so a second portion of sodium hydride was added (60 wt. %, 0.030 g, 0.750 mmol) followed by a second portion of allyl bromide (0.12 mL, 0.17 g, 1.4 mmol) and the mixture was stirred for a further 16 hours. The reaction mixture was then poured cautiously into water (60 mL) and the precipitate collected by vacuum filtration. The solid was washed with 1 M aqueous HCl (10 mL), followed by water (10 mL) before being allowed to dry to give a pale yellow solid which analysis showed to be diallyl 11,12-diallyl-11,12-dihydroindolo[2,3-*a*]carbazole-5,6-dicarboxylate **303** (0.228 g, 74%): m.p. 179-181 °C; $\nu_{max}/cm^{-1}$ (KBr) 3079, 1820, 1722, 1334, 1237, 1195, 937, 741; $\delta_H$ (300 MHz, DMSO-$d_6$) 4.98 [4H, dt, *J* 5.7, 1.3, 2 × $OCH_2$], 5.10 [4H, bs, 2 × $NCH_2$], 5.25 [2H, dd, *J* 17.2, 1.2, 2 × $OCH_2CH=CH_2$ (trans)], 5.35 [4H, dd, *J* 10.4, 1.6, 4 × $CH=CH_2$ (cis)], 5.47 [2H, dq, *J* 17.2, 1.6, 2 × $NCH_2CH=CH_2$ (trans)], 6.03-6.18 [4H, m, 4 × $CH=CH_2$],

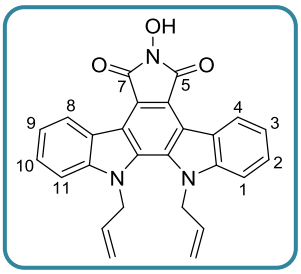


7.32 [2H, ddd, *J* 8.1, 7.1, 1.0, C(3, 8)-H], 7.55 [2H, ddd, *J* 8.3, 7.1, 1.1, C(2, 9)-H], 7.64 [2H, d, *J* 8.2, C(1, 10)-H], 8.07 [2H, d, *J* 7.9, C(4, 7)-H]; $\delta_c$ (150 MHz, DMSO-$d_6$) 50.7 (2 × $CH_2$), 66.7 (2 × $CH_2$), 112.8 (2CH, 2 × aromatic CH), 117.6 (2$CH_2$, 2 × CH=$\underline{C}H_2$), 119.4 (2$CH_2$, 2 × CH=$\underline{C}H_2$), 119.5 (2C, 2 × aromatic C), 119.7 (2C, 2 × aromatic C), 121.5 (2CH, 2 × aromatic CH), 122.0 (2CH, 2 × aromatic CH), 123.0 (2C, 2 × aromatic C), 127.1 (2CH, 2 × aromatic CH), 130.6 (2C, 2 × aromatic C), 132.5 (2CH, 2 × $\underline{C}H=CH_2$), 133.9 (2CH, 2 × $\underline{C}H=CH_2$), 144.3 (2C, 2 × aromatic C), 168.0 (2 × C=O); m/z ($ESI^+$) 505.2 [$(M+H)^+$ 5%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{32}H_{29}N_2O_4)^+$ 505.2127. Found 505.2137.

Method B

A stirred suspension of furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **76** (0.201 g, 0.616 mmol) in anhydrous DMF (20 mL) in a flame-dried flask under nitrogen was cooled to -12 °C using an ice/salt bath. Sodium hydride (60 wt. % oil dispersion, 0.054 g, 1.27 mmol) was then added and the mixture was stirred for 45 minutes. At this point, allyl bromide (0.11 mL, 0.15 g, 1.3 mmol) was added and the mixture was allowed to slowly warm to room temperature and stirred for 16 hours. The reaction mixture was poured into 1 M aqueous HCl (50 mL) and the precipitate collected using vacuum filtration and washed with water (5 mL) and allowed to dry. The crude product was recrystallised from DMSO to give desired product as a bright yellow solid (0.176 g, 72%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3426, 3014, 1819, 1750, 1569, 1335, 1216, 747; $\delta_H$ (600 MHz, DMSO-$d_6$) 5.22 (4H, bt, *J* 2.0, 2 × N$\underline{C}H_2$), 5.27 [2H, dd, *J* 17.3, 1.1, 2 × $CH=CH_2$ (trans)], 5.40 [2H, dd, *J* 10.8, 1.1, 2 × $CH=CH_2$ (cis)], 6.19 [2H, ddt, *J* 17.3, 10.7, 4.0, 2 × C$\underline{H}$=$CH_2$], 7.52 [2H, dt, *J* 7.4, 1.0, C(3, 9)-H], 7.69 [2H, dt, *J* 7.6, 1.1, C(2, 10)-H], 7.73 [2H, d, *J* 8.2, C(1, 11)-H], 9.02 [2H, d, *J* 7.9, C(4, 8)-H]; $\delta_c$ (150 MHz, DMSO-$d_6$) 51.1 (2 × $CH_2$), 113.0 (2CH, 2 × aromatic CH), 117.8 (2 × CH=$\underline{C}H_2$), 119.7 (2C, 2 × aromatic C), 120.0 (2C, 2 × aromatic C), 122.2 (2C, 2 × aromatic C), 122.5 (2CH, 2 × aromatic CH), 124.2 (2CH, 2 × aromatic CH), 128.7 (2CH, 2 × aromatic CH), 133.6 (2 × $\underline{C}H=CH_2$, and 2C, 2 × aromatic C), 144.6 (2C, 2 × aromatic C), 164.8 (2 × C=O); m/z ($ESI^+$) 406.1 [$(M)^+$ 5%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{26}H_{18}N_2O_3)^+$ 406.1317. Found 406.1320.

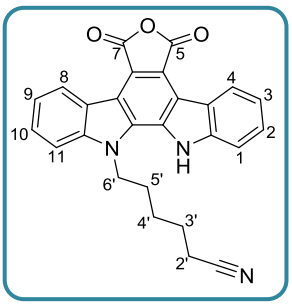
**12,13-Diallyl-6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 306**




To a stirred solution of 12,13-diallyl-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione **304** (0.150 g, 0.369 mmol) in anhydrous DMF (15 mL) was added hydroxylamine hydrochloride (0.128 g, 1.85 mmol) and triethylamine (0.26 mL, 0.19 g, 1.9 mmol) and the resulting mixture was stirred at 80 °C for 24 hours. The orange solution was then allowed to cool and poured into 1 M aqueous HCl (60 mL) and the precipitate collected using vacuum filtration. The solid was suspended in a mixture of water (5 mL) and methanol (5 mL) and allowed to stir for 2 hours before the solid was collected using vacuum filtration and allowed to dry. This gave desired product as an orange solid (0.141 g, 90%): m.p. 248-250 °C; $\nu_{max}/cm^{-1}$ (KBr) 3294, 3082, 1757, 1693, 1442, 1330, 1113, 750; $\delta_H$ (300 MHz, DMSO-$d_6$) 5.06 (4H, bs, 2 × NC$\underline{H}_2$), 5.25 (2H, bd, *J* 17.8, CH=C$\underline{H}_2$ trans), 5.37 (2H, bd, *J* 11.3, CH=C$\underline{H}_2$ cis), 6.14 (ddt, *J* 17.3, 10.7, 3.8, C$\underline{H}$=CH$_2$), 7.38 [2H, ddd, *J* 8.0, 5.0, 3.1, C(3, 9)-H], 7.55-7.62 [4H, m, C(1, 2, 10, 11)-H], 9.09 [2H, d, *J* 7.9, C(4, 8)-H], 10.67 (N-OH); $\delta_c$ (75 MHz, DMSO-$d_6$) 51.0 (2 × $\underline{C}$H$_2$CH=CH$_2$), 112.5 (2CH, 2 × aromatic CH), 116.5 (2C, 2 × aromatic C), 117.7 (2 × CH=$\underline{C}$H$_2$), 120.0 (2C, 2 × aromatic C), 121.9 (2CH, 2 × aromatic CH), 122.6 (2C, 2 × aromatic C), 124.9 (2CH, 2 × aromatic CH), 128.2 (2CH, 2 × aromatic CH), 132.8 (2C, 2 × aromatic C), 133.8 (2 × $\underline{C}$H=CH$_2$), 144.8 (2C, 2 × aromatic C), 166.2 (2 × C=O); m/z (ESI$^+$) 422.2 [(M+H)$^+$ 95%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{26}H_{20}N_3O_3)^+$ 422.1505. Found 422.1511.

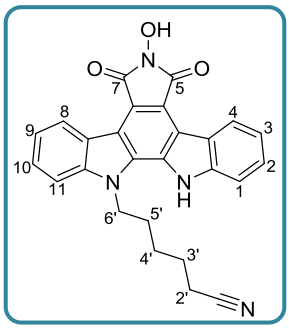
### 7.3.3 Synthesis and derivatisation of 6-[5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl]hexanenitrile

**6-[5,7-Dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl]hexanenitrile 307**



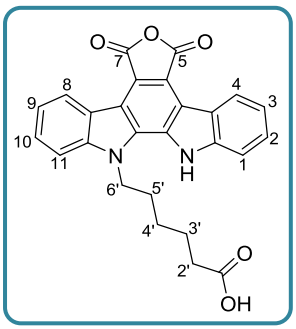

Furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **76** (0.403 g, 1.24 mmol) was suspended in anhydrous DMF (20 mL) in a flame-dried flask under nitrogen and at 0 °C. To this was added sodium hydride (60 wt. % oil dispersion, 0.074 g, 1.85 mmol) resulting in a dark purple solution which was stirred at this temperature for 30 minutes. At this point, 6-bromohexane nitrile (0.27 mL, 0.35 g, 2.0 mmol) was added, followed by sodium iodide (0.278 g, 1.85 mmol) and the mixture was allowed to warm to room temperature and stirred for 16 hours. Water was then added cautiously to quench the reaction and the reaction mixture was poured into 1 M aqueous HCl (50 mL) and stirred for 2 hours. The resulting precipitate was collected using vacuum filtration, washed with copious amounts of water and allowed to dry to give a bright yellow solid (0.327 g, 65%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3392, 2240, 1808, 1743, 1335, 1252, 747; $\delta_H$ (600 MHz, DMSO-$d_6$) 1.35-1.42 [2H, m, C(4′)-$H_2$], 1.50 [2H, quin, *J* 7.3, C(3′)-$H_2$] , 1.83 [2H, quin, *J* 7.0, C(5′)-$H_2$], 2.36 [2H, t, *J* 7.0, C(2′)-$H_2$], 4.88 [2H, t, *J* 6.9, C(6′)-$H_2$], 7.34 [1H, t, *J* 7.5, C(3 or 9)-H], 7.36 [1H, t, *J* 7.6, C(3 or 9)-H], 7.59 [1H, t, *J* 7.7, C(2 or 10)-H], 7.62 [1H, t, *J* 7.7, C(2 or 10)-H], 7.82 [1H, d, *J* 8.1, C(1 or 11)-H], 7.82 [1H, d, *J* 8.2, C(1 or 11)-H], 8.77 [1H, d, *J* 8.0, C(4 or 8)-H], 8.80 [1H, d, *J* 7.9, C(4 or 8)-H], 12.15 [1H, s, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 16.0 ($CH_2$), 24.5 ($CH_2$), 25.1 ($CH_2$), 29.7 ($CH_2$), 43.9 ($CH_2$), 110.4 (CH, aromatic CH), 112.4 (CH, aromatic CH), 116.0 (C, aromatic C),116.9 (C, aromatic C), 117.3 (C, aromatic C), 117.5 (C, aromatic C), 120.3 (C, aromatic C), 120.4 (C, aromatic C), 120.5 (CN), 120.79 (CH, aromatic CH), 120.84 (CH, aromatic CH), 123.3 (CH, aromatic CH), 123.5 (CH, aromatic CH), 127.35 (CH, aromatic CH), 127.39 (CH, aromatic CH), 128.8 (C, aromatic C), 130.1 (C, aromatic C), 140.9 (C, aromatic C), 141.0 (C, aromatic C), 164.5 (C=O), 164.9 (C=O); m/z ($ESI^+$) 422.2 $[(M+H)^+$ 10%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{26}H_{20}N_3O_3)^+$ 422.1505. Found 422.1493.

**6-[6-Hydroxy-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12(13*H*)-yl]hexanenitrile 308**



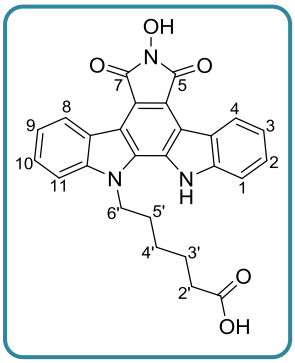

To a stirred solution of 6-[5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl]hexanenitrile **307** (0.203 g, 0.482 mmol) in pyridine (20 mL) was added hydroxylammonium hydrochloride (0.164 mg, 2.41 mmol). The mixture was heated to reflux for 24 hours before being allowed to cool to room temperature. The orange solution was poured carefully into 1 M aqueous HCl (50 mL) before being extracted with 10% MeOH/DCM (3 × 30 mL). The organic layer was washed with 1 M aqueous HCl (50 mL), followed by water (2 × 50 mL), before being dried with anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was purified using column chromatography on silica gel with 2% MeOH/DCM to give an orange solid (0.056 g, 27%): m.p. 266-267 °C; $\nu_{max}/cm^{-1}$ (KBr) 3278, 2933, 2243, 1757, 1699, 1406, 1334, 747; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.36-1.59 [4H, m, C(3′, 4′)-$H_2$], 1.86 [2H, quin, *J* 7.2, C(5′)-$H_2$], 2.38 [2H, t, *J* 6.9, C(2′)-$H_2$], 4.97 [2H, t, *J* 6.9, C(6′)-$H_2$], 7.39 [2H, t, *J* 7.2, C(3, 9)-H], 7.57-7.67 [2H, m, C(2, 10)-H], 7.82 [1H, d, *J* 8.2, C(1 or 11)-H], 7.86 [1H, d, *J* 8.3, C(1 or 11)-H], 9.08 [1H, d, *J* 7.4, C(4 or 8)-H], 9.10 [1H, d, *J* 7.3, C(4 or 8)-H], 10.63 (1H, bs, N-O<u>H</u>), 11.97 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 16.0 ($CH_2$), 24.9 ($CH_2$), 25.2 ($CH_2$), 29.6 ($CH_2$), 43.8 ($CH_2$), 110.2 (CH, aromatic CH), 112.1 (CH, aromatic CH), 115.3 (C, aromatic C), 115.5 (C, aromatic C), 116.2 (C, aromatic C), 117.1 (C, aromatic C), 120.4 (2 × CH, aromatic CH), 120.5 (CN), 120.8 (C, aromatic C), 120.9 (C, aromatic C), 124.1 (CH, aromatic CH), 124.4 (CH, aromatic CH), 127.10 (CH, aromatic CH), 127.14 (CH, aromatic CH), 128.3 (C, aromatic C), 129.6 (C, aromatic C), 141.2 (C, aromatic C), 141.3 (C, aromatic C), 166.3 (2 × C=O); m/z ($ESI^+$) 437.2 [$(M+H)^+$ 30%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{26}H_{21}N_4O_3)^+$ 437.1614. Found 437.1626.

**6-(5,7-Dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl)hexanoic acid 309**



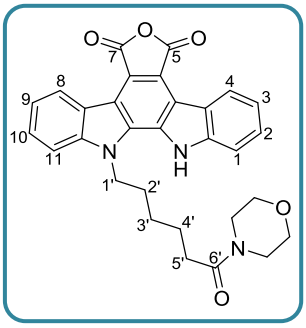
To a stirred suspension of 6-(5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl)hexanenitrile **307** (0.211 g, 0.501 mmol) in a mixture of methanol (20 mL) and water (5 mL) was added potassium hydroxide (85 wt. %, 0.320 g, 5.01 mmol) and the mixture was heated to reflux for 60 hours until TLC analysis showed complete consumption of starting material. After cooling, the solvent was evaporated under reduced pressure and 10% aqueous HCl was added to the residue and the suspension was stirred for 10 minutes before the yellow solid was collected using vacuum filtration, washed with water (5 mL) and allowed to dry. The crude product was recrystallised from acetone to give desired product as a yellow solid (0.172 g, 77%): m.p. 289-291 °C; $\nu_{max}/cm^{-1}$ (KBr) 3388, 2933, 1816, 1749, 1337, 1250, 744; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.26-1.37 [2H, m, C(4′)-$H_2$], 1.46 [2H, quin, *J* 7.2, C(3′)-$H_2$], 1.83 [2H, quin, *J* 7.3, C(5′)-$H_2$], 2.08 [2H, t, *J* 7.1, C(2′)-$H_2$], 4.91 [2H, t, *J* 6.7, C(6′)-$H_2$], 7.38 [1H, t, *J* 7.0, C(3 or 9)-H], 7.40 [1H, t, *J* 7.0, C(3 or 9)-H], 7.57-7.68 [2H, m, C(2, 10)-H], 7.84 [2H, d, *J* 8.1, C(1, 11)-H], 8.83 [1H, d, *J* 9.1, C(4 or 8)-H], 8.86 [1H, d, *J* 8.7, C(4 or 8)-H], 12.18 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 24.2 ($CH_2$), 25.4 ($CH_2$), 30.0 ($CH_2$), 33.5 ($CH_2$), 43.9 ($CH_2$), 110.3 (CH, aromatic CH), 112.3 (CH, aromatic CH), 115.9 (C, aromatic C), 116.8 (C, aromatic C), 117.2 (C, aromatic C), 117.4 (C, aromatic C), 120.3 (C, aromatic C), 120.4 (C, aromatic C), 120.67 (CH, aromatic CH), 120.70 (CH, aromatic CH), 123.2 (CH, aromatic CH), 123.5 (CH, aromatic CH), 127.2 (CH, aromatic CH), 127.3 (CH, aromatic CH), 128.7 (C, aromatic C), 130.0 (C, aromatic C), 140.9 (2C, 2 × aromatic C), 164.36 (C=O), 164.43 (C=O), 174.3 (COOH); m/z ($ESI^-$) 439.2 [$(M-H)^-$ 70%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{26}H_{21}N_2O_5)^+$ 441.1450. Found 441.1457.

**6-[6-Hydroxy-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12(13*H*)-yl]hexanoic acid 310**



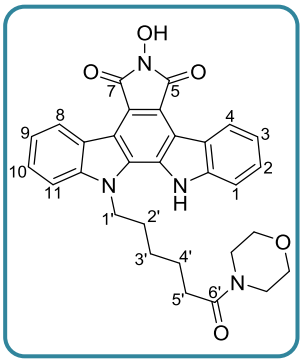

To a stirred solution of 6-[5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl]hexanoic acid **309** (0.120 g, 0.272 mmol) in anhydrous DMF (15 mL) was added hydroxylamine hydrochloride (0.095 g, 1.36 mmol) and triethylamine (0.20 mL, 0.15 g, 1.4 mmol) and the mixture was heated to 80 °C for 24 hours. After cooling, the reaction mixture was poured into 1 M aqueous HCl (40 mL) and extracted using 10% methanol/DCM (3 × 30 mL). The organic layer was washed with 1 M aqueous HCl (40 mL) followed by water (3 × 30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. A red precipitate formed during extraction was collected using vacuum filtration and found to be desired product (0.021 g). The extraction residue was purified using column chromatography on silica gel with 10% methanol/DCM with acetic acid (4 drops per 100 mL) to give desired product as a red solid (0.039 g, combined yield 0.060 g, 49%): m.p. 225-227 °C; $\nu_{max}/cm^{-1}$ (KBr) 3378, 2938, 1749, 1715, 1405, 1015, 735; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.28-1.40 [2H, m, C(4′)-$H_2$], 1.48 [2H, quin, *J* 7.1, C(3′)-$H_2$], 1.84 [2H, quin, *J* 6.9, C(5′)-$H_2$], 2.09 [2H, t, *J* 7.0, C(2′)-$H_2$], 4.94 [2H, t, *J* 6.6, C(6′)-$H_2$], 7.37 [1H, t, *J* 7.5, C(3 or 9)-H], 7.38 [1H, t, *J* 7.6, C(3 or 9)-H], 7.55-7.66 [2H, m, C(2, 10)-H], 7.82 [2H, d, *J* 8.2, C(1, 11)-H], 9.06 [1H, d, *J* 8.0, C(4 or 8)-H], 9.09 [1H, d, *J* 7.9, C(4 or 8)-H], 10.97 (1H, bs, N-OH), 11.99 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 24.3 ($CH_2$), 25.5 ($CH_2$), 30.0 ($CH_2$), 33.6 ($CH_2$), 43.9 ($CH_2$), 110.1 (CH, aromatic CH), 112.2 (CH, aromatic CH), 115.3 (C, aromatic C), 115.5 (C, aromatic C), 116.1 (C, aromatic C), 117.1 (C, aromatic C), 120.4 (CH, aromatic CH), 120.7 (CH, aromatic CH), 120.9 (2C, 2 × aromatic C), 124.1 (CH, aromatic CH), 124.4 (CH, aromatic CH), 127.1 (2CH, 2 × aromatic CH), 128.3 (C, aromatic C), 129.6 (C, aromatic C), 141.2 (C, aromatic C), 141.3 (C, aromatic C), 166.3 (2C, 2 × C=O), 174.4 (COOH); m/z ($ESI^-$) 454.1 [$(M-H)^-$ 20%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{26}H_{22}N_3O_5)^+$ 456.1559. Found 456.1548.

**12-(6-Morpholino-6-oxohexyl)furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione 311**



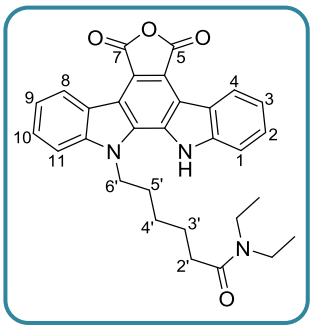
To a stirred suspension of 6-(5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl)hexanoic acid **309** (0.122 g, 0.277 mmol) in DCM (25 mL) in a flame-dried flask under nitrogen was added oxalyl chloride (0.04 mL, 0.06 g, 0.42 mmol) followed by a drop of anhydrous DMF. The mixture was allowed to stir for 2 hours resulting in a pale yellow solution before the solvent and excess oxalyl chloride were removed under reduced pressure. Morpholine (3 mL) was then added slowly to the residue while stirring and the reaction mixture was stirred for a further 2 hours. At this point, water (20 mL) was added and the mixture was acidified to pH 2 using 10% HCl. The crude product was extracted into 10% methanol/DCM (2 × 15 mL) and the organic layer was washed with water (3 × 20 mL), followed by brine (20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was washed with hot acetone (15 mL) and allowed to dry to give desired product as a bright yellow solid (0.082 g, 60%): m.p. 259-261 °C; $\nu_{max}/cm^{-1}$ (KBr) 3288, 2859, 1817, 1762, 1624, 1337, 1247, 739; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.19-1.32 [2H, m, C(3′)-$H_2$], 1.43 [2H, quin, *J* 7.2, C(4′)-$H_2$], 1.85 [2H, quin, *J* 7.0, C(2′)-$H_2$], 2.12 [2H, t, *J* 7.4, C(5′)-$H_2$], 3.18-3.46 [8H, $H_2O$, m, N(C$\underline{H_2}$C$\underline{H_2}$)$_2$O], 4.92 [2H, t, *J* 6.5, C(1′)-$H_2$], 7.37 [1H, t, *J* 7.1, C(3 or 9)-H], 7.40 [1H, *J* 7.1, C(3 or 9)-H], 7.61 [1H, t, *J* 8.2, C(2 or 10)-H], 7.64 [1H, t, *J* 8.3, C(2 or 10)-H], 7.84 [2H, d, *J* 8.1, C(1, 11)-H], 8.83 [1H, d, *J* 8.8, C(4 or 8)-H], 8.86 [1H, d, *J* 8.6, C(4 or 8)-H], 12.16 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 25.0 ($CH_2$), 26.1 ($CH_2$), 30.6 ($CH_2$), 32.1 ($CH_2$), 41.8 [one of (N$CH_2$)$_2$], 44.5 ($CH_2$), 45.7 [one of (N$CH_2$)$_2$], 66.5 (2 × O$CH_2$), 110.8 (CH, aromatic CH), 112.8 (CH, aromatic CH), 116.3 (C, aromatic C), 117.3 (C, aromatic C), 117.6 (C, aromatic C), 117.8 (C, aromatic C), 120.7 (C, aromatic C), 120.9 (C, aromatic C), 121.2 (2CH, 2 × aromatic CH), 123.7 (CH, aromatic CH), 124.0 (CH, aromatic CH), 127.7 (CH, aromatic CH), 127.8 (CH, aromatic CH), 129.2 (C, aromatic C), 130.5 (C, aromatic C), 141.38 (C, aromatic C), 141.40 (C, aromatic C), 164.8 (C=O), 164.9 (C=O), 171.1 ($CH_2\underline{C}$=O); m/z (ESI$^-$) 508.2 [$(M-H)^-$ 40%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{30}H_{28}N_3O_5)^+$ 510.2029. Found 510.2018.

**6-Hydroxy-12-(6-morpholino-6-oxohexyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 314**



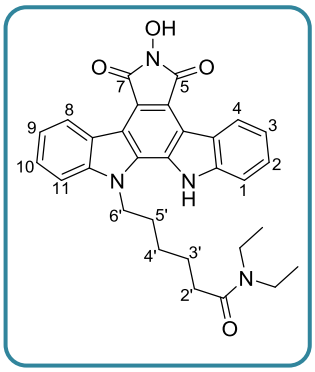

To a stirred solution of 12-(6-morpholino-6-oxohexyl)furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **311** (0.092 g, 0.181 mmol) in anhydrous DMF (10 mL) was added hydroxylamine hydrochloride (0.064 g, 0.903 mmol) and triethylamine (0.13 mL, 0.09 g, 0.92 mmol) and the mixture was heated to 80 °C for 24 hours. After cooling, the reaction mixture was poured into 1 M aqueous HCl (20 mL) and extracted using 10% methanol/DCM (3 × 15 mL). The organic layer was washed with 1 M aqueous HCl (20 mL) followed by water (3 × 15 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was purified using column chromatography on silica gel with 1% MeOH/DCM to give desired product as a red solid (0.054 g, 57%): m.p. 164-166 °C; $\nu_{max}/cm^{-1}$ (KBr) 3381, 2929, 1759, 1709, 1618, 1336, 1116, 733; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.20-1.33 [2H, m, C(3′)-$H_2$], 1.43 [2H, quin, *J* 7.1, C(4′)-$H_2$], 1.86 [2H, quin, *J* 7.0, C(2′)-$H_2$], 2.12 [2H, t, *J* 7.1, C(5′)-$H_2$], 3.19-3.46 [8H, $H_2O$, m, N(C$\underline{H}_2$C$\underline{H}_2$)$_2$O], 4.96 [2H, t, *J* 6.1, C(1′)-$H_2$], 7.39 [2H, t, *J* 7.2, C(3, 9)-H], 7.60 [1H, t, *J* 8.0, C(2 or 10)-H], 7.63 [1H, t, *J* 8.1, C(2 or 10)-H], 7.82 [1H, d, *J* 8.3, C(1 or 11)-H], 7.85 [1H, d, *J* 8.4, C(1 or 11)-H], 9.07 [1H, d, *J* 7.5, C(4 or 8)-H], 9.10 [1H, d, *J* 7.4, C(4 or 8)-H], 10.65 (1H, bs, N-OH), 11.99 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 24.6 ($CH_2$), 25.6 ($CH_2$), 30.1 ($CH_2$), 31.6 ($CH_2$), 41.3 [one of $(NCH_2)_2$], 44.0 ($CH_2$), 45.3 [one of $(NCH_2)_2$], 66.0 (2 × $OCH_2$), 110.2 (CH, aromatic CH), 112.1 (CH, aromatic CH), 115.3 (C, aromatic C), 115.5 (C, aromatic C), 116.1 (C, aromatic C), 117.1 (C, aromatic C), 120.4 (2CH, 2 × aromatic CH), 120.8 (C, aromatic C), 120.9 (C, aromatic C), 124.1 (CH, aromatic CH), 124.4 (CH, aromatic CH), 127.07 (CH, aromatic CH), 127.14 (CH, aromatic CH), 128.3 (C, aromatic C), 129.7 (C, aromatic C), 141.2 (C, aromatic C), 141.3 (C, aromatic C), 166.3 (2C, 2 × C=O), 170.6 ($CH_2\underline{C}$=O); m/z (ESI$^-$) 523.2 [$(M-H)^-$ 100%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{30}H_{29}N_4O_5)^+$ 525.2138. Found 525.2150.

**6-(5,7-Dioxo-7,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*)-yl)-*N*,*N*-diethylhexanamide 312**



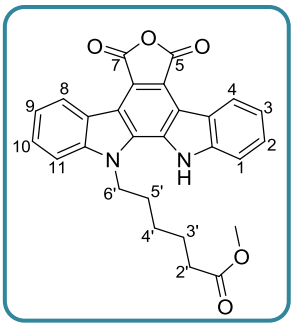
To a stirred suspension of 6-(5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl)hexanoic acid **309** (0.121 g, 0.275 mmol) in DCM (25 mL) in a flame-dried flask under nitrogen was added oxalyl chloride (0.04 mL, 0.06 g, 0.41 mmol) followed by a drop of anhydrous DMF. The mixture was allowed to stir for 2 hours resulting in a pale yellow solution before the solvent and excess oxalyl chloride were removed under reduced pressure. Diethylamine (3 mL) was then added and the mixture was stirred for 2 hours before DCM (10 mL) was added. The resulting solution was stirred for a further 1 hour before excess solvent was removed under reduced pressure. The residue was acidified to pH 2 and stirred for 30 minutes before the yellow solid was collected using vacuum filtration, washed with water and allowed to dry. Washes with hot acetone (2 × 10 mL) gave desired product as a yellow solid (0.098 g, 73%): m.p. 113-114 °C; $\nu_{max}/cm^{-1}$ (KBr) 3280, 2933, 1818, 1752, 1616, 1249, 742; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.83 (3H, t, *J* 6.9, one of $CH_2C\underline{H}_3$), 0.84 (3H, t, *J* 6.9, one of $CH_2C\underline{H}_3$), 1.17-1.29 [2H, m, C(4′)-$H_2$], 1.43 [2H, quin, *J* 7.2, C(3′)-$H_2$], 1.84 [2H, quin, *J* 7.5, C(5′)-$H_2$], 2.06 [2H, t, *J* 7.0, C(2′)-$H_2$], 3.00-3.15 [4H, m, 2 × $C\underline{H}_2CH_3$), 4.91 [2H, t, *J* 6.3, C(6′)-$H_2$], 7.34-7.43 [2H, m, C(3, 9)-H], 7.61 [1H, t, *J* 7.9, C(2 or 10)-H], 7.63 [1H, t, *J* 8.0, C(2 or 10)-H], 7.83 [1H, d, *J* 8.1, C(1 or 11)-H], 7.84 [1H, d, *J* 8.4, C(1 or 11)-H], 8.82 [1H, d, *J* 8.6, C(4 or 8)-H], 8.85 [1H, d, *J* 8.5, C(4 or 8)-H], 12.18 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 13.4 ($CH_3$), 14.6 ($CH_3$), 25.2 ($CH_2$), 26.0 ($CH_2$), 30.6 ($CH_2$), 32.1 ($CH_2$), 39.7 ($CH_2$), 41.6 ($CH_2$), 44.4 ($CH_2$), 110.8 (CH, aromatic CH), 112.8 (CH, aromatic CH), 116.3 (C, aromatic C), 117.3 (C, aromatic C), 117.6 (C, aromatic C), 117.8 (C, aromatic C), 120.7 (C, aromatic C), 120.9 (C, aromatic C), 121.2 (2CH, 2 × aromatic CH), 123.7 (CH, aromatic CH), 124.0 (CH, aromatic CH), 127.7 (CH, aromatic CH), 127.8 (CH, aromatic CH), 129.2 (C, aromatic C), 130.5 (C, aromatic C), 141.3 (C, aromatic C), 141.4 (C, aromatic C), 164.85 (C=O), 164.91 (C=O), 171.1 ($CH_2\underline{C}$=O); m/z ($ESI^+$) 496.2 $[(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{30}H_{30}N_3O_4)^+$ 496.2236. Found 496.2234.

**_N_,_N_-Diethyl-6-(6-hydroxy-5,7-dioxo-5,6,7,13-tetrahydro-12_H_-indolo[2,3-_a_]pyrrolo[3,4-_c_]carbazol-12-yl)hexanamide 315**



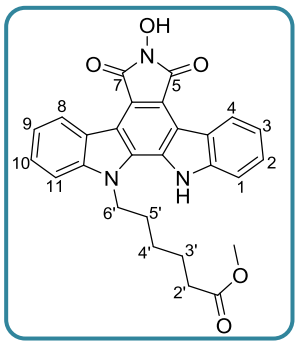

To a stirred solution of 6-(5,7-dioxo-7,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*)-yl)-*N*,*N*-diethylhexanamide **312** (0.077 g, 0.155 mmol) in anhydrous DMF (8 mL) was added hydroxylamine hydrochloride (0.054 g, 0.777 mmol) and triethylamine (0.11 mL, 0.08 g, 0.78 mmol) and the mixture was heated to 80 °C for 24 hours. After cooling, the reaction mixture was poured into 1 M aqueous HCl (20 mL) and stirred for 30 minutes before the orange solid was collected using vacuum filtration. The solid was washed with water (10 mL), followed by methanol (5 mL) to give a yellow/orange solid (0.063 g, 76%): m.p. 239-240 °C; $\nu_{max}/cm^{-1}$ (KBr) 3353, 2934, 1748, 1701, 1623, 1461, 1335, 745; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.83 (3H, t, *J* 7.0, one of $CH_2C\underline{H}_3$), 0.85 (3H, t, *J* 7.0, one of $CH_2C\underline{H}_3$), 1.21-1.33 [2H, m, C(4′)-$H_2$], 1.46 [2H, quin, *J* 7.3, C(3′)-$H_2$], 1.85 [2H, quin, *J* 7.0, C(5′)-$H_2$], 2.07 [2H, t, *J* 7.1, C(2′)-$H_2$], 3.04 (2H, q, *J* 6.9, one of $C\underline{H}_2CH_3$), 3.11 (2H, q, *J* 6.9, one of $C\underline{H}_2CH_3$), 4.96 [2H, t, *J* 6.5, C(6′)-$H_2$], 7.35-7.43 [2H, m, C(3, 9)-H], 7.56-7.67 [2H, m, C(2, 10)-H], 7.81 [1H, d, *J* 7.4, C(1 or 11)-H], 7.84 [1H, d, *J* 7.8, C(1 or 11)-H], 9.08 [1H, d, *J* 7.9, C(4 or 8)-H], 9.1 [1H, d, *J* 7.7, C(4 or 8)-H], 10.60 (1H, bs, N-OH), 11.96 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 13.4 ($CH_3$), 14.6 ($CH_3$), 25.2 ($CH_2$), 26.1 ($CH_2$), 30.6 ($CH_2$), 32.1 ($CH_2$), 39.7 ($CH_2$), 41.6 ($CH_2$), 44.5 ($CH_2$), 110.7 (CH, aromatic CH), 112.6 (CH, aromatic CH), 115.8 (C, aromatic C), 116.0 (C, aromatic C), 116.6 (C, aromatic C), 117.6 (C, aromatic C), 120.87 (CH, aromatic CH), 120.89 (CH, aromatic CH), 121.2 (C, aromatic C), 121.4 (C, aromatic C), 124.6 (CH, aromatic CH), 124.9 (CH, aromatic CH), 127.5 (CH, aromatic CH), 127.6 (CH, aromatic CH), 128.8 (C, aromatic C), 130.2 (C, aromatic C), 141.7 (C, aromatic C), 141.8 (C, aromatic C), 166.8 (2C, 2 × C=O), 171.2 ($CH_2\underline{C}$=O); m/z ($ESI^+$) 511.2 [$(M+H)^+$ 70%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{30}H_{31}N_4O_4)^+$ 511.2345. Found 511.2321.

**Methyl 6-(5,7-dioxo-7,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*)-yl)hexanoate 313**



To a stirred suspension of 6-(5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl)hexanoic acid **309** (0.122 g, 0.277 mmol) in DCM (25 mL) in a flame-dried flask under nitrogen was added oxalyl chloride (0.04 mL, 0.06 g, 0.41 mmol) followed by a drop of anhydrous DMF. The mixture was allowed to stir for 2 hours resulting in a pale yellow solution before the solvent and excess oxalyl chloride were removed under reduced pressure. Methanol (3mL) was added slowly to the residue while stirring and the reaction mixture was stirred for a further 2 hours under nitrogen. The yellow precipitate was then collected by vacuum filtration and washed with hot acetone (10 mL) to give desired product as a yellow solid (0.092 g, 75%): m.p. 263-265 °C; $\nu_{max}/cm^{-1}$ (KBr) 3377, 2949, 1819, 1750, 1704, 1406, 1340, 1253, 737; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.23-1.34 [2H, m, C(4′)-$H_2$], 1.46 [2H, quin, *J* 7.3, C(3′)-$H_2$], 1.82 [2H, quin, *J* 7.3, C(5′)-$H_2$], 2.15 [2H, t, *J* 7.2, C(2′)-$H_2$], 3.45 (3H, s, $CH_3$), 4.90 [2H, t, *J* 6.7, C(6′)-$H_2$], 7.34-7.43 [2H, m, C(3 and 9)-H], 7.61 [1H, t, *J* 8.0, C(2)-H], 7.63 [1H, t, *J* 8.0, C(10)-H], 7.83 [2H, d, *J* 8.2, C(1, 11)-H], 8.81 [1H, d, *J* 8.5, C(4)-H], 8.84 [1H, d, *J* 8.4, C(8)-H], 12.17 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 24.7 ($CH_2$), 25.8 ($CH_2$), 30.4 ($CH_2$), 33.5 ($CH_2$), 44.4 ($CH_2$), 51.5 ($CH_3$), 110.8 (CH, aromatic CH), 112.8 (CH, aromatic CH), 116.3 (C, aromatic C), 117.3 (C, aromatic C), 117.7 (C, aromatic C), 117.9 (C, aromatic C), 120.7 (C, aromatic C), 120.9 (C, aromatic C), 121.2 (2CH, 2 × aromatic CH), 123.7 (CH, aromatic CH), 124.0 (CH, aromatic CH), 127.7 (CH, aromatic CH), 127.8 (CH, aromatic CH), 129.2 (C, aromatic C), 130.5 (C, aromatic C), 141.35 (C, aromatic C), 141.39 (C, aromatic C), 164.86 (C=O), 164.92 (C=O), 173.6 ($CH_2\underline{C}$=O); m/z (ESI$^-$) 453.2 [$(M-H)^-$ 40%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{27}H_{22}N_2O_5)^+$ 454.1529. Found 454.1522.

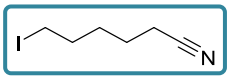
**Methyl 6-(6-hydroxy-5,7-dioxo-5,6,7,13-tetrahydro-12*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12-yl)hexanoate 316**




To a stirred solution of methyl 6-(5,7-dioxo-7,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*)-yl)hexanoate **313** (0.070 g, 0.154 mmol) in anhydrous DMF (8 mL) was added hydroxylamine hydrochloride (0.054 g, 0.770 mmol) and triethylamine (0.11 mL, 0.08 g, 0.77 mmol) and the mixture was heated to 80 °C for 24 hours. After cooling, the reaction mixture was poured into 1 M aqueous HCl (20 mL) and extracted using 10% methanol/DCM (3 × 15 mL). The organic layer was washed with 1 M aqueous HCl (20 mL) followed by water (3 × 15 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give a red solid (crude yield 0.056 g). The crude product was purified using column chromatography on silica gel with 1% MeOH/DCM to give a red solid (0.020 g, 28%): m.p. 243-244 °C; $\nu_{max}/cm^{-1}$ (KBr) 3402, 2944, 1750, 1704, 1405, 1334, 741; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.26-1.38 [2H, m, C(4′)-$H_2$], 1.49 [2H, quin, *J* 7.3, C(3′)-$H_2$], 1.84 [2H, quin, *J* 7.2, C(5′)-$H_2$], 2.17 [2H, t, *J* 7.3, C(2′)-$H_2$], 3.46 (3H, s, $CH_3$), 4.95 [2H, t, *J* 6.8, C(6′)-$H_2$], 7.38-7.42 [2H, m, C(3, 9)-H], 7.60 [1H, ddd, *J* 8.2, 7.1, 1.1, C(2)-H], 7.64 [1H, ddd, *J* 8.2, 7.1, 1.1, C(10)-H], 7.82 [1H, d, *J* 7.3, C(1 or 11)-H], 7.84 [1H, d, *J* 7.8, C(1 or 11)-H], 9.08 [1H, d, *J* 7.8, C(4)-H], 9.11 [1H, d, *J* 7.7, C(8)-H], 10.59 (1H, bs, N-OH), 11.95 [1H, bs, N(13)-H]; $\delta_c$ (150 MHz, DMSO-$d_6$) 24.7 ($CH_2$), 25.8 ($CH_2$), 30.4 ($CH_2$), 33.5 ($CH_2$), 44.4 ($CH_2$), 51.5 ($CH_3$), 110.7 (CH, aromatic CH), 112.7 (CH, aromatic CH), 115.8 (C, aromatic C), 116.0 (C, aromatic C), 116.7 (C, aromatic C), 117.6 (C, aromatic C), 121.0 (2CH, 2 × aromatic CH), 121.2 (C, aromatic C), 121.3 (C, aromatic C), 124.6 (CH, aromatic CH), 124.9 (CH, aromatic CH), 127.6 (CH, aromatic CH), 127.7 (CH, aromatic CH), 128.8 (C, aromatic C), 130.1 (C, aromatic C), 141.7 (C, aromatic C), 141.8 (C, aromatic C), 166.8 (2 × C=O), 173.6 ($CH_2\underline{C}$=O); m/z ($ESI^-$) 468.2 [$(M-H)^-$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{27}H_{24}N_3O_5)^+$ 470.1716. Found 470.1693.

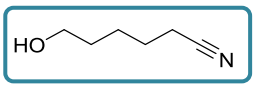
### 7.3.4 Incorporation of second *N*-substituent

**6-Iodohexanenitrile 319**[15]



Sodium iodide (1.201 g, 8.01 mmol) was suspended in a solution of 6-bromohexane nitrile **318** (0.70 mL, 0.93 g, 5.3 mmol) in acetone (40 mL) and the mixture was heated to reflux for 16 hours. The mixture was then cooled, filtered and the filtrate concentrated to dryness under reduced pressure. The residue was dissolved in DCM (40 mL) and the organic layer washed with saturated aqueous sodium thiosulfate (30 mL), followed by water (3 × 30 mL), before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give desired product as a clear oil which was used without further purification (0.989 g, 84%): $\nu_{max}/cm^{-1}$ (KBr) 2937, 2865, 2246, 1459, 1426, 1206, 726, 503; $\delta_H$ (400 MHz, $CDCl_3$) 1.54-1.63 [2H, m, C(4)-$H_2$], 1.70 [2H, quin, *J* 7.2, C(3)-$H_2$], 1.87 [2H, quin, *J* 7.0, C(5)-$H_2$], 2.38 [2H, t, *J* 7.0, C(2)-$H_2$], 3.20 [2H, t, *J* 6.9, C(6)-$H_2$].

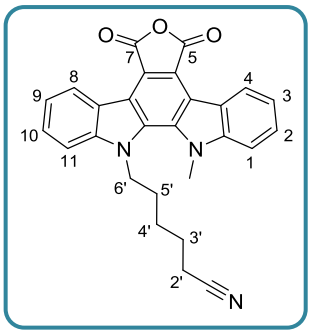
**6-Hydroxyhexanenitrile 320**[16]



To a solution of 6-bromohexane nitrile **318** (1.00 mL, 1.32 g, 7.50 mmol) in THF (20 mL) was added 10% aqueous sodium hydroxide (5 mL). The biphasic mixture was stirred for 2 hours before the layers were separated and the aqueous layer was extracted with 10% MeOH/DCM (3 × 15 mL). The organic layer was washed with 1 M aqueous HCl (20 mL), followed by water (2 × 20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give a colourless oil which was used without further purification (0.760 g, 89%): $\nu_{max}/cm^{-1}$ (NaCl) 3536, 2941, 2868, 2246, 1461, 1428, 1271, 1242, 731; $\delta_H$ (300 MHz, $CDCl_3$) 1.56-1.76 [4H, m, C(3, 4)-$H_2$], 1.91 [2H, quin, *J* 6.9, C(5)-$H_2$], 2.37 [2H, t, *J* 6.9, C(2)-$H_2$], 3.42 [2H, t, *J* 6.6, C(6)-$H_2$]; m/z ($ESI^+$) 114.1 [$(M+H)^+$ 5%].

**6-(13-Methyl-5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl)hexanenitrile 317**

Method A



To a stirred suspension of 12-methylfuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **297** (0.104 g, 0.306 mmol) in anhydrous DMF (10 mL) under nitrogen was added sodium hydride (60 wt. % oil suspension, 0.015 g, 0.367 mmol) and the dark purple solution was stirred for 30 minutes. A solution of 6-iodohexanenitrile **319** (0.089 g, 0.40 mmol) in anhydrous DMF (1 mL) was then added and the mixture was stirred for a further 16 hours. Water (30 mL) was then added and the resulting precipitate was collected using vacuum filtration and washed with 1 M aqueous HCl (5 mL), followed by water (10 mL) and allowed to dry to give a yellow solid. NMR analysis showed only starting material to be present.

Method B

A suspension of 12-methylfuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **297** (0.102 g, 0.306 mmol), triphenylphosphine (0.234 g, 0.892 mmol) and 6-hydroxyhexanenitrile **320** (0.999 g, 0.883 mmol) in dry THF (10 mL) was cooled to -78 °C under a nitrogen atmosphere. Diisopropyl azodicarboxylate (0.175 g, 0.865 mmol) was then added at this temperature and the mixture was allowed to warm to room temperature over 16 hours. The reaction mixture was poured into 0.1 M HCl (10 mL) before the yellow suspension was collected by vacuum filtration, washed with water (5 mL) and allowed to dry. NMR analysis showed only starting material to be present.

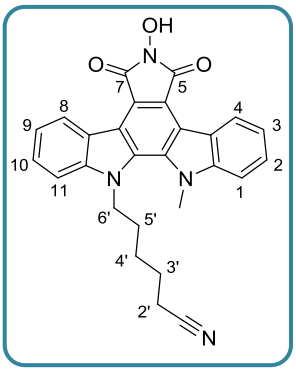
Method C

To a stirred suspension of 6-[5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl]hexanenitrile **307** (0.201 g, 0.477 mmol) in anhydrous DMF (15 mL) under nitrogen was added sodium hydride (0.021 g, 0.525 mmol) and the dark purple solution was stirred for 30 minutes. Iodomethane (0.04 mL, 0.09 g, 0.57 mmol) was then added and the reaction mixture was stirred for a further 20 hours before pouring into water (40 mL). The yellow precipitate was collected using vacuum filtration, washed with 1 M aqueous HCl (10 mL), followed by water (5 mL) and allowed to dry. The crude product was purified using column chromatography on silica gel with DCM

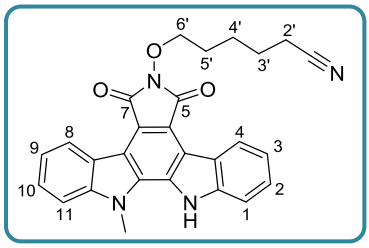
as eluent to give a yellow solid (0.067 g, 33%): m.p. 208-209 °C; $\nu_{max}/cm^{-1}$ (KBr) 3431, 2944, 2243, 1819, 1752, 1335, 1252, 742; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.02-1.14 [2H, m, C(4″)-$H_2$], 1.39 [2H, quin, *J* 7.4, C(3″)-$H_2$], 1.68 [2H, quin, *J* 7.6, C(5″)-$H_2$], 2.28 [2H, t, *J* 7.1, C(2″)-$H_2$], 4.25 (3H, s, $CH_3$), 4.79 [2H, t, *J* 7.5, C(6″)-$H_2$], 7.43 [1H, t, *J* 7.7, C(9)-H], 7.46 [1H, t, *J* 7.8, C(3)-H], 7.67 [1H, ddd, *J* 8.3, 7.2, 1.2, C(10)-H], 7.70 [1H, ddd, *J* 8.3, 7.1, 1.1, C(2)-H], 7.83 [1H, d, *J* 8.3, C(1)-H], 7.95 [1H, d, *J* 8.3, C(11)-H], 8.88 [1H, d, *J* 7.9, C(4)-H], 8.89 [1H, d, *J* 7.8, C(8)-H]; $\delta_c$ (150 MHz, DMSO-$d_6$) 16.3 ($CH_2$), 24.6 ($CH_2$), 25.4 ($CH_2$), 28.1 ($CH_2$), 37.1 ($CH_3$), 48.5 ($CH_2$), 112.0 (CH, aromatic CH), 112.9 (CH, aromatic CH), 118.2 (C, aromatic C), 118.7 (C, aromatic C), 118.9 (C, aromatic C), 119.6 (C, aromatic C), 120.9 (CN), 121.4 (C, aromatic C), 122.1 (2 CH, 2 × aromatic CH), 122.2 (C, aromatic C), 124.0 (CH, aromatic CH), 124.2 (CH, aromatic CH), 128.3 (CH, aromatic CH), 128.5 (CH, aromatic CH), 132.8 (C, aromatic C), 133.8 (C, aromatic C), 143.8 (C, aromatic C), 144.7 (C, aromatic C), 164.7 (2 × C=O); m/z ($ESI^+$) 436.2 [$(M+H)^+$ 10%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{27}H_{22}N_3O_3)^+$ 436.1661. Found 436.1649.

**6-[6-Hydroxy-13-methyl-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12(13*H*)-yl]hexanenitrile 322**

Method A




To a solution of 6-hydroxy-12-methyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **308** (0.090 g, 0.25 mmol) in anhydrous DMF (15 mL) under a nitrogen atmosphere was added sodium hydride (0.033 g, 0.75 mmol) and the mixture was allowed to stir for 30 minutes. At this point 6-iodohexanenitrile (0.082 g, 0.36 mmol) was added and the mixture was allowed to stir for 16 hours. Water (5 mL) was then added cautiously to quench the reaction before the reaction mixture was poured into 1 M aqueous HCl (25 mL). The precipitate was collected by vacuum filtration and washed with water (2 × 5 mL) before being allowed to dry to give a yellow/orange solid which analysis showed to be 6-[(12-methyl-5,7-dioxo-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-6(7*H*,12*H*,13*H*)-yl)oxy]hexanenitrile **321** (0.090 g, 80%): m.p. 240-243 °C; $\nu_{max}/cm^{-1}$ (KBr) 3576, 3384, 2924, 2246, 1755, 1708, 1569, 1397, 1334, 1107, 733; $\delta_H$



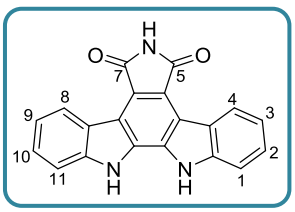
(300 MHz, DMSO-$d_6$) 1.60-1.76 [4H, m, C(3′, 4′)-$H_2$], 1.82 [2H, quin, *J* 6.5, C(5′)-$H_2$], 2.59 [2H, t, *J* 6.6, C(2′)-$H_2$], 4.27 [2H, t, *J* 6.1, C(6′)-$H_2$], 4.32 (3H, s, $CH_3$), 7.28-7.38 [2H, m, C(3, 9)-H], 7.54 [1H, t, *J* 7.5, C(2 or 10)-H], 7.59 [1H, t, *J* 7.5, C(2 or 10)-H], 7.73 [1H, d, *J* 8.3, C(1 or 11)-H], 7.74 [1H, d, *J* 7.8, C(1 or 11)-H], 8.95 [2H, d, *J* 7.9, C(4, 8)-H], 12.02 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 16.6 ($CH_2$), 25.0 ($CH_2$), 25.1 ($CH_2$), 27.5 ($CH_2$), 32.3 ($CH_3$), 77.7 ($CH_2$), 110.0 (CH, aromatic CH), 112.4 (CH, aromatic CH), 115.1 (C, aromatic C), 115.4 (C, aromatic C), 115.9 (C, aromatic C), 117.1 (C, aromatic C), 120.6 (CH, aromatic CH), 120.6 (CH, aromatic CH), 121.95 (C, aromatic C), 120.98 (C, aromatic C), 121.2 (CN), 124.4 (CH, aromatic CH), 124.6 (CH, aromatic CH), 127.3 (CH, aromatic CH), 127.4 (CH, aromatic CH), 129.0 (C, aromatic C), 130.7 (C, aromatic C), 141.5 (C, aromatic C), 141.8 (C, aromatic C), 165.9 (2 × C=O); m/z ($ES^+$) 451.2 [$(M+H)^+$ 20%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{27}H_{23}N_4O_3)^+$ 451.1770. Found 451.1761.

Method B

To a suspension of 6-[13-methyl-5,7-dioxofuro[3,4-*c*]indolo[2,3-*a*]carbazol-12(5*H*,7*H*,13*H*)-yl]hexanenitrile **317** (0.110 g, 0.25 mmol) in anhydrous DMF (10 mL) was added hydroxylamine hydrochloride (0.088 g, 1.3 mmol) and triethylamine (0.18 mL, 0.13 g, 1.3 mmol) and the resulting mixture was heated to 80 °C and stirred for 24 hours at this temperature. At this point, TLC analysis showed incomplete consumption of starting material and so further portions of both hydroxylamine hydrochloride (0.089 g, 1.3 mmol) and triethylamine (0.18 mL, 0.13 g, 1.3 mmol) were added and the temperature was increased to 120 °C for a further 48 hours until TLC analysis showed no starting material remained. The reaction mixture was then cooled to room temperature and poured into 1 M aqueous HCl (20 mL). The precipitate was collected by vacuum filtration and washed with water (2 × 5 mL) before being allowed to dry. The crude product was purified using column chromatography on silica gel with 2% MeOH/DCM as eluent to give an orange solid (0.071 g, 63%): m.p. 225-227 °C; $\nu_{max}/cm^{-1}$ (KBr) 3226, 2936, 2247, 1758, 1705, 1453, 1332, 1007, 752; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.00-1.12 [2H, m, C(4′)-$H_2$], 1.37 [2H, quin, *J* 7.4, C(3′)-$H_2$], 1.63 [2H, quin, *J* 7.4, C(5′)-$H_2$], 2.27 [2H, t, *J* 7.1, C(2′)-$H_2$], 4.23 (3H, s, $CH_3$), 4.78 [2H, t, *J* 7.4, C(6′)-$H_2$], 7.38-7.47 [2H, m, C(3, 9)-H], 7.65 [1H, ddd, *J* 8.3, 7.0, 1.2, C(10)-H], 7.67

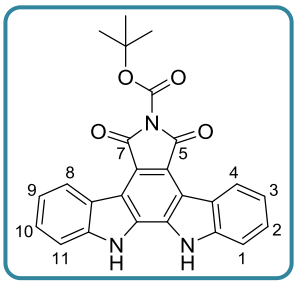
[1H, ddd, *J* 8.3, 7.2, 1.2, C(2)-H], 7.79 [1H, d, *J* 8.2, C(1)-H], 7.91 [1H, d, *J* 8.3, C(11)-H], 9.09 [1H, d, *J* 7.9, C(4 )-H], 9.12 [1H, d, *J* 7.8, C(8)-H], 10.71 (N-OH); $\delta_c$ (75 MHz, DMSO-$d_6$) 16.3 ($CH_2$), 24.6 ($CH_2$), 25.4 ($CH_2$), 27.9 ($CH_2$), 37.1 ($CH_3$), 48.4 ($CH_2$), 111.7 (CH, aromatic CH), 112.7 (CH, aromatic CH), 116.1 (C, aromatic C), 116.6 (C, aromatic C), 119.2 (C, aromatic C), 119.8 (C, aromatic C), 120.8 (CN), 121.6 (2CH, 2 × aromatic CH), 122.0 (C, aromatic C), 122.8 (C, aromatic C), 124.8 (CH, aromatic CH), 125.0 (CH, aromatic CH), 127.9 (CH, aromatic CH), 128.2 (CH, aromatic CH), 132.4 (C, aromatic C), 133.5 (C, aromatic C), 144.1 (C, aromatic C), 145.3 (C, aromatic C), 166.3 (C=O), 166.4 (C=O); m/z ($ESI^+$) 451.2 [$(M+H)^+$ 20%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{27}H_{23}N_4O_3)^+$ 451.1770. Found 451.1767.

**12,13-Dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 58**[13]

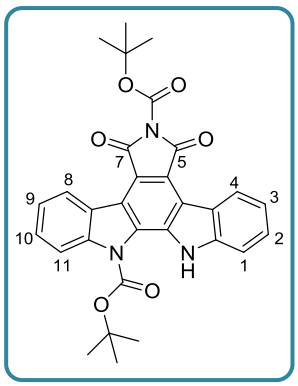


To a solution of furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **76** (0.933 g, 2.86 mmol) in anhydrous DMF (15 mL) was added 1,1,1,3,3,3-hexamethyldisilazane (6.0 mL, 4.6 g, 28.6 mmol), followed by methanol (0.58 mL, 0.45 g, 14.3 mmol). The reaction mixture was then stirred for 16 hours before pouring into water (40 mL). The precipitate was collected by vacuum filtration and allowed to dry. The solid was then suspended in methanol and stirred for 2 hours before being collected by vacuum filtration and allowed to dry to give an orange solid (0.849 g, 91%): m.p. > 300 °C; (Lit. >300 °C)[13]; $\nu_{max}/cm^{-1}$ (KBr) 3583, 3364, 3226, 1719, 1681, 1567, 1405, 1332, 748; $\delta_H$ (300 MHz, DMSO-$d_6$) 7.34 [2H, t, *J* 7.6, C(3, 9)-H], 7.54 [2H, ddd, *J* 8.2, 7.2, 1.2, C(2, 10)-H], 7.80 [2H, d, *J* 8.1, C(1, 11)-H], 8.99 [2H, d, *J* 7.84, C(4, 8)-H], 10.96 (1H, bs, C=ONHC=O), 11.72 [2H, bs, N(12, 13)-H]; m/z ($ESI^+$) 324.2 [$(M+H)^+$ 100%].

**_tert_-Butyl 5,7-dioxo-5,7,12,13-tetrahydro-6*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-6-carboxylate 329**

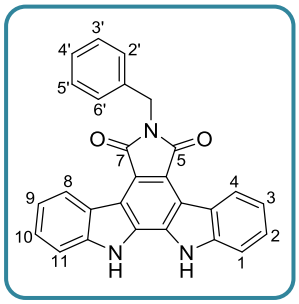


To a stirred suspension of 12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **58** (0.503 g, 1.54 mmol) in acetonitrile (50 mL) was added di-*tert*-butyl dicarbonate (0.42 mL, 0.40 g, 1.8 mmol) followed by DMAP (0.019 g, 0.15 mmol) and the reaction mixture was stirred at room temperature for 2 hours. At this point, TLC analysis showed incomplete consumption of starting material and so a further 1 equivalent of di-*tert*-butyl dicarbonate (0.34 mL, 0.33 g, 1.5 mmol) was added and stirring was continued for a further 1 hour. The solvent was removed under reduced pressure and the yellow residue suspended in a mixture of water (20 mL) and methanol (20 mL). The solid was collected by vacuum filtration and allowed to dry to give a yellow solid found on analysis to be di-*tert*-butyl 5,7-dioxo-7,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-



*c*]carbazole-6, 12-dicarboxylate **330**[17] (0.615 g, 78%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3358, 3209, 1702, 1573, 1321, 1146, 749; $\delta_H$ (300 MHz, $CDCl_3$) 1.74 [9H, s, $C(CH_3)_3$], 1.84 [9H, s, $C(CH_3)_3$], 7.36 [1H, ddd, *J* 8.1, 5.0, 2.8, C(3)-H], 7.42-7.59 [4H, m, C(1, 2, 9, 10)-H], 8.09 [1H, d, *J* 8.2, C(11)-H], 9.19 [1H, d, *J* 8.1, C(4)-H], 9.37 [1H, d, *J* 7.9, C(8)-H], 11.31 (1H, bs, NH); $\delta_c$ (75 MHz, $CDCl_3$) 28.1 (3 × $CH_3$), 28.3 (3 × $CH_3$), 84.7 [$\underline{C}(CH_3)_3$], 87.0 [$\underline{C}(CH_3)_3$], 111.2 (CH, aromatic CH), 115.9 (CH, aromatic CH), 116.6 (C, aromatic C), 119.6 (C, aromatic C), 120.9 (C, aromatic C), 121.07 (C, aromatic C), 121.10 (CH, aromatic CH), 122.6 (C, aromatic C), 124.2 (CH, aromatic CH), 124.3 (C, aromatic C), 125.77 (CH, aromatic CH), 125.84 (CH, aromatic CH), 127.30 (C, aromatic C), 128.1 (CH, aromatic CH), 128.3 (CH, aromatic CH), 131.4 (C, aromatic C), 138.3 (C, aromatic C), 139.6 (C, aromatic C), 147.3 (C=O), 151.9 (C=O), 164.9 (C=O), 165.1 (C=O).

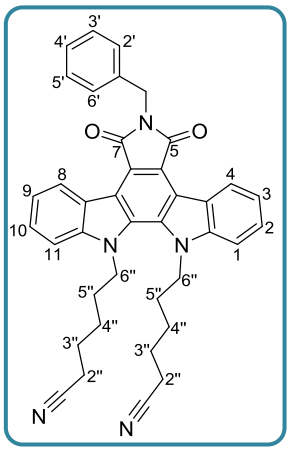
**6-Benzyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 166**[18]



To a suspension of furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **76** (0.150 g, 0.460 mmol) in anhydrous DMF (8 mL) was added benzylamine (0.25 mL, 0.25 g, 2.3 mmol) and the mixture was heated to 100 °C for 16 hours. After cooling, the precipitate was collected by vacuum filtration, washed with 1 M aqueous HCl (3 × 5 mL), followed by water (5 mL) and allowed to dry to give an orange solid (0.146 g, 76%): m.p. > 300 °C; (Lit 308-310 °C)[17]; $\nu_{max}/cm^{-1}$ (KBr) 3528, 3372, 1742, 1674, 1651, 1559, 1380, 1245, 742; $\delta_H$ (300 MHz, DMSO-$d_6$) 4.90 (2H, s, $CH_2$), 7.24-7.31 [1H, m, C(4′)-H], 7.32-7.39 [4H, m, C(3, 9, 3′, 5′)-H], 7.41-7.46 [2H, m, C(2′, 6′)-H], 7.56 [2H, ddd, *J* 8.2, 7.2, 1.1, C(2, 10)-H], 7.81 [2H, d, *J* 8.2, C(1, 11)-H], 8.99 [2H, d, *J* 7.9, C(4, 8)-H], 11.76 [2H, bs, N(12,13)-H]; m/z ($ESI^+$) 414.3 [$(M+H)^+$ 60%].

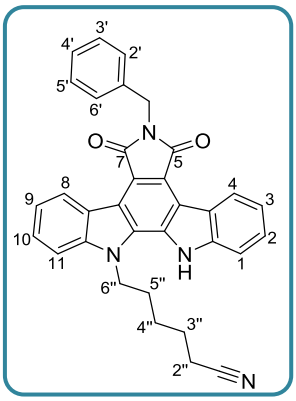
**6,6'-(6-Benzyl-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-12,13-diyl)dihexanenitrile 332**

Method A



6-Benzyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **166** (0.553 g, 1.33 mmol) was suspended in anhydrous DMF (20 mL) in a flame-dried flask under nitrogen and at 0 °C. To this was added sodium hydride (60 wt. % oil suspension, 0.117 g, 2.93 mmol) resulting in a dark blue suspension which was stirred at this temperature for 30 minutes. At this point, a solution of 6-iodohexane nitrile (0.690 g, 3.09 mmol) in anhydrous DMF (2 mL) was added and the mixture was allowed to warm to room temperature and stirred for 24 hours. A small amount of water was then added cautiously to quench the reaction before the reaction mixture was poured into water (60 mL) and extracted using 10% MeOH/DCM (3 × 30 mL). The organic layer was washed with water (4 × 30 mL), followed by brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered, and the solvent evaporated

under reduced pressure to give a yellow solid found on analysis to be mono alkylated 6-(6-benzyl-5,7-dioxo-5,6,7,13-tetrahydro-12*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbaz ol-12-yl)hexanenitrile **331** (0.562 g, 85%): $\nu_{max}/cm^{-1}$ (KBr) 3391, 2933, 2252, 1743, 1692, 1573, 1403, 1338, 743; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.35-1.45 [2H, m, C(4″)-$H_2$], 1.51 [2H, quin, *J* 7.2, C(3″)-$H_2$], 1.85 [2H, quin, *J* 6.9, C(5″)-$H_2$], 2.36 [2H, t, *J* 6.9, C(2″)-$H_2$], 4.87 (2H, s, $CH_2$), 4.93 [2H, t, *J* 6.7, C(6″)-$H_2$], 7.23-7.46 [7H, m, C(3, 9, 2′, 3′, 4′, 5′, 6′)-H], 7.59 [1H, t, *J* 7.4, C(2 or 10)-H], 7.62 [1H, t, *J* 7.6, C(2 or 10)-H], 7.82 [2H, d, *J* 8.3, C(1, 11)-H], 9.08 [1H, d, *J* 7.8, C(4 or 8)-H], 9.10 [1H, d, *J* 7.8, C(4 or 8)-H], 11.94 [1H, s, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 16.5 ($CH_2$), 25.1 ($CH_2$), 25.6 ($CH_2$), 30.1 ($CH_2$), 41.1 ($CH_2$), 44.2 ($CH_2$), 110.5 (CH, aromatic CH), 112.6 (CH, aromatic CH), 116.6 (C, aromatic C), 117.5 (C, aromatic C), 118.8 (C, aromatic C), 118.9 (C, aromatic C), 120.8 (2CH, 2 × aromatic CH), 121.0 (CN), 121.3 (C, aromatic C), 121.4 (C, aromatic C), 124.7 (CH, aromatic CH), 124.9 (CH, aromatic CH), 127.4 (CH, aromatic CH), 127.5 (CH, aromatic CH), 127.7 (CH, aromatic CH), 127.9 (2CH, 2 × aromatic CH), 128.7 (C, aromatic C), 129.1 (2CH, 2 × aromatic CH), 129.9 (C, aromatic C), 138.0 (C, aromatic C), 141.58 (C, aromatic C), 141.62 (C, aromatic C), 169.7 (2 × C=O); m/z ($ESI^-$) 509.2 [$(M-H)^-$ 40%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{33}H_{27}N_4O_2)^+$ 511.2134. Found 511.2137.

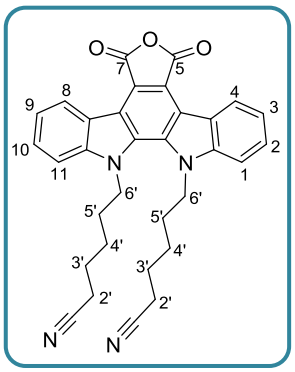



Method B

To a suspension of 6-benzyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **166** (0.179, 0.431 mmol) in anhydrous DMF (20 mL) under nitrogen was added caesium carbonate (0.350 g, 1.07 mmol) and the mixture was stirred for 40 minutes slowly becoming a dark purple solution. To this was added a solution of 6-iodohexanenitrile (0.243 g, 1.09 mmol) in anhydrous DMF (2 mL) and the reaction mixture was allowed to stir for 60 hours until TLC analysis showed the reaction was complete. Water (50 mL) was then added and the aqueous layer extracted with 10% MeOH/DCM (2 × 30 mL). The organic layer was washed with 1 M aqueous HCl (2 × 40 mL) followed by water (40 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was purified using column chromatography on silica gel with DCM to give desired product as an orange solid (0.228 g, 87%): m.p. 207-208 °C; $\nu_{max}/cm^{-1}$ (KBr) 3423,

2936, 2244, 1748, 1693, 1574, 1332, 748; $\delta_H$ (300 MHz, $CDCl_3$) 0.93-1.06 [4H, m, 2 × C(4″)-$H_2$], 1.35 [4H, quin, *J* 7.4, 2 × C(3″)-$H_2$], 1.42 [4H, quin, *J* 7.4, 2 × C(5″)-$H_2$], 2.02 [4H, t, *J* 7.0, 2 × C(2″)-$H_2$], 4.63 [4H, t, *J* 7.3, 2 × C(6″)-$H_2$], 5.00 (2H, s, $CH_2$), 7.22-7.29 [1H, m, C(4′)-H], 7.30-7.37 [2H, m, C(3′, 5′)-H], 7.43 [2H, ddd, 8.0, 5.6, 2.3, C(3, 9)-H], 7.54-7.64 [6H, m, C(1, 2, 10, 11, 2′, 6′)-H], 9.27 [2H, d, *J* 7.9, C(4, 8)-H]; $\delta_c$ (75 MHz, $CDCl_3$) 16.8 (2 × $CH_2$), 24.7 (2 × $CH_2$), 25.5 (2 × $CH_2$), 27.2 (2 × $CH_2$), 41.6 ($CH_2$ benzyl), 48.5 (2 × $CH_2$), 112.0 (2CH, 2 × aromatic CH), 119.2 (2C, 2 × aromatic C), 120.6 (2C, 2 × aromatic C), 121.4 (2C, 2 × aromatic C), 122.1 (2CH, 2 × aromatic CH), 124.5 (4C, 2 × aromatic C, 2 × CN), 126.1 (2CH, 2 × aromatic CH), 127.6 (CH, aromatic CH), 127.7 (2CH, 2 × aromatic CH), 128.6 (2CH, 2 × aromatic CH), 128.7 (2CH, 2 × aromatic CH), 133.6 (2C, 2 × aromatic C), 137.3 (C, aromatic C), 144.5 (2C, 2 × aromatic C), 169.4 (2 × C=O); m/z (ESI$^+$) 606.3 [$(M+H)^+$ 15%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{39}H_{36}N_5O_2)^+$ 606.2869. Found 606.2874.

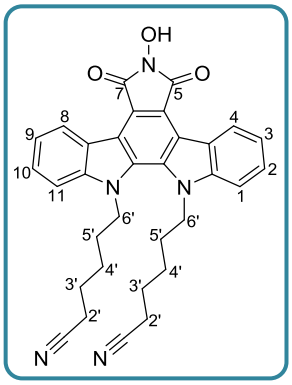
**6,6'-(5,7-Dioxo-5,7-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-12,13-diyl)dihexanenitrile 333**




To a stirred suspension of 6,6'-(6-benzyl-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-12,13-diyl)dihexane nitrile **332** (0.190 g, 0.314 mmol) in a mixture of ethanol (20 mL) and water (5 mL) was added potassium hydroxide (85 wt. %, 0.197 g, 2.98 mmol) and the mixture was heated to reflux for 24 hours. After cooling, the solvent was evaporated under reduced pressure and the residue acidified with 10% aqueous HCl. The aqueous layer was extracted with 10% MeOH/DCM (2 × 30 mL) and the organic layer was then washed with water (3 × 30 mL), dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was purified using column chromatography on silica gel with 2% MeOH/DCM to give desired product as a yellow solid (0.090 g, 56%): m.p. 123-124 °C; $\nu_{max}/cm^{-1}$ (KBr) 3435, 2941, 2254, 1820, 1751, 1473, 1336, 746; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.89-1.02 [4H, m, 2 × C(4′)-$H_2$], 1.31 [4H, quin, *J* 7.4, 2 × C(3′)-$H_2$], 1.55 [4H, quin, *J* 7.4, 2 × C(5′)-$H_2$], 2.22 [4H, t, *J* 7.1, 2 × C(2′)-$H_2$], 4.80 [4H, t, *J* 7.2, 2 × C(6′)-$H_2$], 7.50 [2H, t, *J* 7.5, C(3, 9)-H], 7.71 [2H, t, *J* 7.8, C(2, 10)-H], 8.00 [2H, d, *J* 8.3, C(1, 11)-H], 8.96 [2H, d, *J* 7.8, C(4, 8)-H]; $\delta_c$ (150 MHz, DMSO-$d_6$) 16.3 (2 ×

$CH_2$), 24.4 (2 × $CH_2$), 25.3 (2 × $CH_2$), 27.8 (2 × $CH_2$), 48.5 (2 × $CH_2$), 113.6 (2CH, 2 × aromatic CH), 119.2 (2C, 2 × aromatic C), 120.2 (2C, 2 × aromatic C), 120.8 (2 × CN), 122.4 (2CH, 2 × aromatic CH), 122.8 (2C, 2 × aromatic C), 124.4 (2CH, 2 × aromatic CH), 128.5 (2CH, 2 × aromatic CH), 134.0 (2C, 2 × aromatic C), 144.2 (2C, 2 × aromatic C), 164.8 (2 × C=O); m/z ($ESI^+$) 539.2 [$(M+Na)^+$ 10%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{32}H_{28}N_4O_3Na)^+$ 539.2059. Found 539.2054.

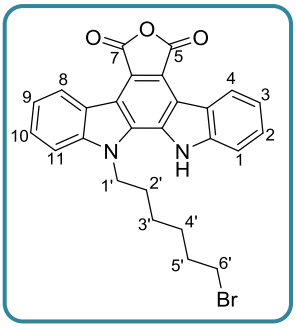
**6,6'-(6-Hydroxy-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-12,13-diyl)dihexanenitrile 334**




To a stirred suspension of 6,6'-(5,7-dioxo-5,7-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-12,13-diyl)dihexanenitrile **333** (0.110 g, 0.207 mmol) in anhydrous DMF (8 mL) was added hydroxylamine hydrochloride (0.074 g, 1.03 mmol) and triethylamine (0.15 mL, 0.11 g, 1.0 mmol) and the mixture was heated to 80 °C for 24 hours. After cooling, the reaction mixture was poured into 1 M aqueous HCl (20 mL) and the precipitate collected by vacuum filtration. The orange solid was suspended in a mixture of 1 M aqueous HCl (10 mL) and methanol (10 mL) and stirred for 2 hours before the solid was collected using vacuum filtration, washed with water (5 mL) and allowed to dry to give desired product as an orange solid (0.083 g, 73%): m.p. 199-201 °C; $\nu_{max}/cm^{-1}$ (KBr) 3321, 2935, 2245, 1761, 1708, 1447, 1332, 1113, 750; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.89-1.02 [4H, m, 2 × C(4′)-$H_2$], 1.31 [4H, quin, *J* 7.3, 2 × C(3′)-$H_2$], 1.49 [4H, quin, *J* 7.3, 2 × C(5′)-$H_2$], 2.22 [4H, t, *J* 7.0, 2 × C(2′)-$H_2$], 4.73 [4H, t, *J* 7.1, 2 × C(6′)-$H_2$], 7.44 [2H, t, *J* 7.5, C(3, 9)-H], 7.66 [2H, t, *J* 7.7, C(2, 10)-H], 7.92 [2H, d, *J* 8.3, C(1, 11)-H], 9.11 [2H, d, *J* 7.8, C(4, 8)-H], 10.70 (1H, bs, N-OH); $\delta_c$ (150 MHz, DMSO-$d_6$) 16.3 (2 × $CH_2$), 24.5 (2 × $CH_2$), 25.4 (2 × $CH_2$), 27.6 (2 × $CH_2$), 48.4 (2 × $CH_2$), 113.3 (2CH, 2 × aromatic CH), 116.8 (2C, 2 × aromatic C), 120.3 (2C, 2 × aromatic C), 120.8 (2 × CN), 122.0 (2CH, 2 × aromatic CH), 123.4 (2C, 2 × aromatic C), 125.2 (2CH, 2 × aromatic CH), 128.1 (2CH, 2 × aromatic CH), 133.4 (2C, 2 × aromatic C), 144.6 (2C, 2 × aromatic C), 166.4 (2C, 2 × C=O); m/z ($ESI^+$) 532.2 [$(M+H)^+$ 20%]. HRMS ($ESI^+$): Exact mass calculated for $(C_{32}H_{30}N_5O_3)^+$ 532.2349. Found 532.2343.

### 7.3.5 Synthesis and derivatisation of 12-(6-bromohexyl)-6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione

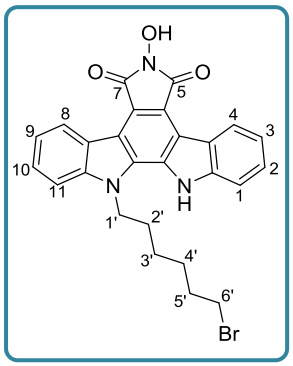
**12-(6-Bromohexyl)-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione 335**



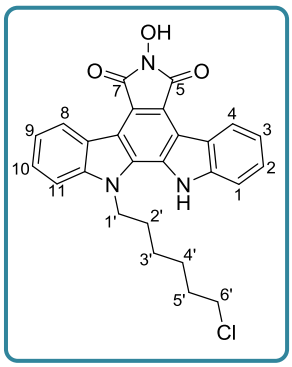

Furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **76** (0.101 g, 0.309 mmol) was suspended in anhydrous DMF (10 mL) in a flame-dried flask and the mixture was cooled to 0 °C under nitrogen. Sodium hydride (60 wt. % oil suspension, 0.027 g, 0.67 mmol) was then added carefully and the mixture was stirred at this temperature for 1 hour resulting in a dark blue solution. A solution of 1,6 dibromohexane (0.24 mL, 0.38 g, 1.5 mmol) in anhydrous DMF (40 mL) was prepared in a separate flame-dried flask and to this was added the dark blue solution, dropwise over 90 minutes at room temperature. The resulting dark purple solution was stirred for a further 16 hours. The reaction mixture was then poured into water (400 mL) and extracted with 10% MeOH/DCM (4 × 50 mL). The organic layer was washed with water (4 × 50 mL), followed by brine (50 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The sticky residue was triturated with methanol (10 mL) and the product collected by vacuum filtration giving a bright yellow solid (0.097 g, 65%): m.p. > 300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3364, 2931, 1818, 1738, 1336, 1251, 746; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.26 [4H, m, C(3′, 4′)-$H_2$], 1.64 [2H, quin, *J* 6.7, C(5′)-$H_2$], 1.76-1.89 [2H, m, C(2′)-$H_2$], 3.39 [2H, t, *J* 6.6, C(6′)-$H_2$], 4.90 [2H, t, *J* 6.7, C(1′)-$H_2$], 7.37 [1H, t, *J* 7.6, C(3 or 9)-H], 7.38 [1H, t, *J* 7.5, C(3 or 9)-H], 7.60 [1H, t, *J* 8.0, C(2 or 10)-H], 7.63 [ 1H, t, *J* 7.7, C(2 or 10)-H], 7.84 [2H, d, *J* 8.3, C(1, 11)-H], 8.81 [1H, d, *J* 8.2, C(4 or 8)-H], 8.84 [1H, d, *J* 8.2, C(4 or 8)-H], 12.13 [1H, bs, N(13)-H]; $\delta_c$ (150 MHz, DMSO-$d_6$) 25.4 ($CH_2$), 27.7 ($CH_2$), 30.6 ($CH_2$), 32.5 ($CH_2$), 35.4 ($CH_2$), 44.5 ($CH_2$), 111.0 (CH, aromatic CH), 112.9 (CH, aromatic CH), 116.5 (C, aromatic C), 117.4 (C, aromatic C), 117.9 (C, aromatic C), 118.2 (C, aromatic C), 120.9 (C, aromatic C), 121.0 (C, aromatic C), 121.4 (CH, aromatic CH), 121.4 (CH, aromatic CH), 123.8 (CH, aromatic CH), 124.1 (CH, aromatic CH), 127.87 (CH, aromatic CH), 127.93 (CH, aromatic CH), 129.4 (C, aromatic C), 130.7 (C, aromatic C), 141.5 (C, aromatic C), 141.6 (C, aromatic C),

165.0 (C=O), 165.1 (C=O); m/z (ESI$^+$) 488.1 [(M)$^+$ 80%]; HRMS (ESI$^+$): Exact mass calculated for ($C_{26}H_{21}N_2O_3{}^{79}Br)^+$ 488.0736. Found 488.0745.

**12-(6-Bromohexyl)-6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 387**



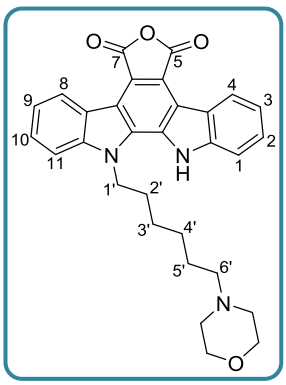

To a solution of 12-(6-bromohexyl)-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione **335** (0.898 g, 1.84 mmol) in anhydrous DMF (40 mL) was added hydroxylamine hydrochloride (0.637 g, 9.18 mmol) and triethylamine (1.30 mL, 0.95 g, 9.2 mmol) and the resulting mixture was stirred at 80 °C for 2 hours. The orange solution was then allowed to cool and poured into water (200 mL) which was extracted using 10% MeOH/DCM (3 × 50 mL). The organic layer was washed with water (4 × 50 mL) followed by brine (50 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was purified using column chromatography on silica gel using ethyl acetate as eluent to give a red solid which was identified on analysis to be 12-(6-chlorohexyl)-6-hydroxy-12,13-




dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **336** (0.327 g, 40%): m.p. 298-300 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3421, 2933, 1755, 1695, 1403, 1332, 1112, 748; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.28-1.37 [4H, m, C(3′, 4′)-H$_2$], 1.57 [2H, quin, *J* 6.5, C(5′)-H$_2$], 1.78-1.90 [2H, m, C(2′)-H$_2$], 3.50 [2H, t, *J* 6.6, C(6′)-H$_2$], 4.95 [2H, t, *J* 6.7, C(1′)-H$_2$], 7.38 [2H, t, *J* 7.6, C(3, 9)-H], 7.60 [1H, t, *J* 8.0, C(2 or 10)-H], 7.62 [1H, t, *J* 8.0, C(2 or 10)-H], 7.82 [1H, d, *J* 8.1, C(1 or 11)-H], 7.84 [1H, d, *J* 8.3, C(1 or 11)-H], 9.07 [1H, d, *J* 7.2, C(4 or 8)-H], 9.10 [1H, d, *J* 7.3, C(4 or 8)-H], 10.63 (1H, bs, N-O<u>H</u>), 11.95 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 25.6 ($CH_2$), 26.5 ($CH_2$), 30.6 ($CH_2$), 32.3 ($CH_2$), 44.4 ($CH_2$), 45.6 ($CH_2$), 110.6 (CH, aromatic CH), 112.6 (CH, aromatic CH), 115.8 (C, aromatic C), 116.0 (C, aromatic C), 116.6 (C, aromatic C), 117.6 (C, aromatic C), 120.89 (CH, aromatic CH), 120.91 (CH, aromatic CH), 121.2 (C, aromatic C), 121.3 (C, aromatic C), 124.6 (CH, aromatic CH), 124.9 (CH, aromatic CH), 127.56 (CH, aromatic CH), 127.64 (CH, aromatic CH), 128.8 (C, aromatic C), 130.1 (C, aromatic C), 141.7 (C, aromatic C), 141.8 (C, aromatic C), 166.8 (2 × C=O); m/z (ESI$^+$) 460.1 [(M+H)$^+$

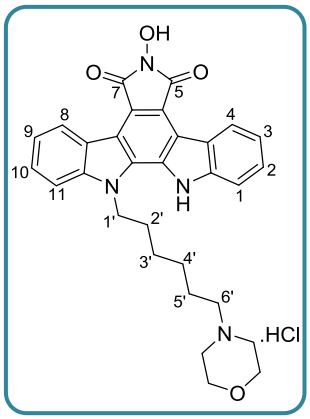
60%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{26}H_{23}N_3O_3{}^{35}Cl)^+$ 460.1428. Found 460.1423.

**12-(6-Morpholinohexyl)furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione 337**



To a stirred suspension of 12-(6-bromohexyl)-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione **335** (0.201 g, 0.411 mmol) in absolute ethanol (20 mL) was added morpholine (0.35 mL, 0.35 g, 4.1 mmol) and the mixture was heated to reflux for 24 hours. After cooling, the solvent was evaporated under reduced pressure and the residue was dissolved in 10% MeOH/DCM (30 mL). The organic layer was washed with 1 M aqueous HCl (30 mL), followed by saturated aqueous sodium bicarbonate (30 mL) and then water (2 × 30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was recrystallised from acetone to give a yellow solid (0.110 g, 55%): m.p. 259-261 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3308, 2933, 1819, 1752, 1405, 1338, 1252, 1116, 742; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.14-1.29 [6H, m, C(3′, 4′, 5′)-H$_2$], 1.84 [2H, quin, *J* 7.3, C(2′)-H$_2$], 2.04 [2H, t, *J* 6.7, C(6′)-H$_2$], 2.14 [4H, t, *J* 4.4, N(C<u>H</u>$_2$CH$_2$)$_2$O], 3.43 [4H, t, *J* 4.6, N(CH$_2$C<u>H</u>$_2$)$_2$O], 4.94 [2H, t, *J* 6.6, C(1′)-H$_2$], 7.40 [1H, t, *J* 7.5, C(3 or 9)-H], 7.41 [1H, t, *J* 7.4, C(3 or 9)-H], 7.62 [1H, t, *J* 8.0, C(2 or 10)-H], 7.65 [1H, t, *J* 8.0, C(2 or 10)-H], 7.85 [1H, d, *J* 8.2, C(1 or 11)-H], 7.86 [1H, d, *J* 8.3, C(1 or 11)-H], 8.86 [1H, d, *J* 8.2, C(4 or 8)-H], 8.89 [1H, d, *J* 8.3, C(4 or 8)-H], 12.18 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 26.0 (CH$_2$), 26.1 (CH$_2$), 27.0 (CH$_2$), 30.6 (CH$_2$), 44.5 (CH$_2$), 53.7 (2 × CH$_2$), 58.5 (CH$_2$), 66.5 (2 × CH$_2$), 110.6 (CH, aromatic CH), 112.8 (CH, aromatic CH), 116.4 (C, aromatic C), 117.4 (C, aromatic C), 117.8 (C, aromatic C), 118.0 (C, aromatic C), 120.8 (C, aromatic C), 120.9 (C, aromatic C), 121.2 (2CH, 2 × aromatic CH), 123.7 (CH, aromatic CH), 124.0 (CH, aromatic CH), 127.7 (CH, aromatic CH), 127.8 (CH, aromatic CH), 129.3 (C, aromatic C), 130.7 (C, aromatic C), 141.4 (C, aromatic C), 141.5 (C, aromatic C), 164.9 (C=O), 165.0 (C=O); m/z (ESI$^+$) 496.2 [(M+H)$^+$ 30 %]; HRMS (ESI$^+$): Exact mass calculated for $(C_{30}H_{30}N_3O_4)^+$ 496.2236. Found 496.2214.

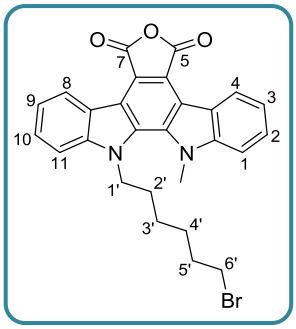
**4-[6-(6-hydroxy-5,7-dioxo-5,6,7,13-tetrahydro-12*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12-yl)hexyl]morpholin-4-ium chloride 338**



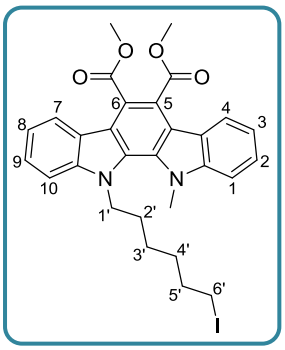

To a solution of 12-(6-morpholinohexyl)furo[3,4-*c*]indolo[2,3-*a*]carbazole-5,7(12*H*,13*H*)-dione **337** (0.093 g, 0.19 mmol) in anhydrous DMF (10 mL) was added hydroxylamine hydrochloride (0.065 g, 0.94 mmol) and triethylamine (0.13 mL, 0.09 g, 0.94 mmol) and the resulting mixture was stirred at 80 °C for 24 hours. After cooling, the reaction mixture was poured into 1M aqueous HCl (20 mL) and the precipitate collected by vacuum filtration, washed with water (5 mL) and allowed to dry to give desired product as an orange solid (0.056 g, 54%): m.p. 226-228 °C; $\nu_{max}/cm^{-1}$ (KBr) 3383, 3244, 2931, 1757, 1705, 1571, 1401, 1336, 1108, 749, 732; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.17-1.42 [4H, m, C(3′, 4′)-$H_2$], 1.46-1.59 [2H, m, C(5′)-$H_2$], 1.86 [2H, quin, *J* 7.3, C(2′)-$H_2$], 2.83-2.96 [4H, m, C(6′)-$H_2$, one of each of $O(CH_2C\underline{H}_2)_2N$], 3.28 [2H, d, *J* 12.2, one of each of $O(CH_2C\underline{H}_2)_2N$], 3.69 [2H, t, *J* 12.1, one of each of $O(C\underline{H}_2CH_2)_2N$], 3.87 [2H, dd, *J* 12.5, 2.6, one of each of $O(C\underline{H}_2CH_2)_2N$], 5.01 [2H, t, *J* 6.6, C(1′)-H], 7.39 [1H, t, *J* 7.6, C(3 or 9)-H], 7.41 [1H, t, *J* 7.6, C(3 or 9)-H], 7.60 [1H, ddd, *J* 8.3, 7.2, 1.2, C(2 or 10)-H], 7.64 [1H, ddd, *J* 8.3, 7.2, 1.2, C(2 or 10)-H], 7.87 [1H, d, *J* 8.3, C(1 or 11)-H], 7.88 [1H, d, *J* 8.2, C(1 or 11)-H], 9.08 [1H, d, *J* 8.3, C(4 or 8)-H], 9.11 [1H, d, *J* 8.7, C(4 or 8)-H], 10.52 [1H, bs, NH (salt)], 10.61 (1H, bs, N-OH), 12.16 [1H, bs, N(13)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 22.9 ($CH_2$), 25.9 ($CH_2$), 26.3 ($CH_2$), 30.5 ($CH_2$), 44.5 ($CH_2$), 51.3 (2 × $CH_2$), 56.2 ($CH_2$), 63.6 (2 × $CH_2$), 110.7 (CH, aromatic CH), 112.8 (CH, aromatic CH), 115.8 (C, aromatic C), 115.9 (C, aromatic C), 116.6 (C, aromatic C), 117.5 (C, aromatic C), 120.9 (2CH, 2 × aromatic CH), 121.2 (C, aromatic C), 121.4 (C, aromatic C), 124.6 (CH, aromatic CH), 124.9 (CH, aromatic CH), 127.6 (2CH, 2 × aromatic CH), 128.8 (C, aromatic C), 130.1 (C, aromatic C), 141.75 (C, aromatic C), 141.78 (C, aromatic C), 166.8 (2 × C=O); m/z ($ESI^+$) 511.2 $[(M+H)^+$ 80%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{30}H_{31}N_4O_4)^+$ 511.2345. Found 511.2334.

**12-(6-Bromohexyl)-13-methyl-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione 340**

Method A



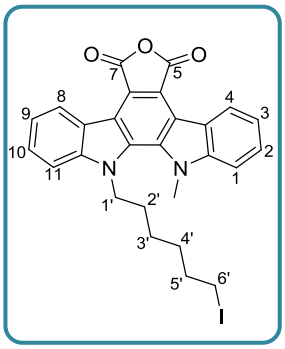
To a stirred suspension of 12-(6-bromohexyl)-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione **335** (0.637 g, 1.30 mmol) in anhydrous DMF (30 mL) in a flame-dried flask under nitrogen was added sodium hydride (60 wt. % oil dispersion, 0.120 g, 3.00 mmol) carefully, and the purple mixture was stirred for 40 minutes. Iodomethane (0.19 mL, 0.44 g, 3.1 mmol) was then added and the mixture was stirred for a further 16 hours. At this point TLC analysis showed that some starting material remained and so a further portion of sodium hydride (60 wt. % oil dispersion, 0.061 g, 1.53 mmol) was added followed by a further portion of iodomethane (0.10 mL, 0.23 g, 1.6 mmol) and the mixture was stirred for a further 16 hours. The reaction mixture was then cautiously poured into water (70 mL) and the precipitate collected using vacuum filtration. The solid was washed with copious amounts of water and allowed to dry. The crude residue was purified using column chromatography on silica gel using DCM to give a pale yellow solid which on analysis was shown to be dimethyl 11-(6-iodohexyl)-12-methyl-11,12-dihydroindolo[2,3-*a*]carbazole-5,6-dicarboxylate **339** (0.442 g, 57%): m.p. 134-136 °C; $\nu_{max}/cm^{-1}$ (KBr) 3431, 2945, 1720, 1333, 1256, 1068, 739; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.81-0.93 [2H, m, C(3′)-$H_2$], 1.11 [2H, quin, *J* 7.3, C(4′)-$H_2$], 1.39-1.58 [4H, m, C(2′, 5′)-$H_2$], 3.00 [2H, t, *J* 6.9, C(6′)-$H_2$], 4.03 (6H, s, 2 × $OCH_3$), 4.16 (3H, s, $NCH_3$), 4.74 [2H, t, *J* 7.0, C(1′)-$H_2$], 7.28-7.36 [2H, m, C(3, 8)-H], 7.57 [1H, t, *J* 7.7, C(9)-H], 7.59 [1H, t, *J* 7.5, C(2)-H], 7.76 [1H, d, *J* 8.3, C(1)-H], 7.85 [1H, d, *J* 8.3, C(10)-H], 8.05 [1H, d, *J* 8.0, C(4 or 7)-H], 8.06 [1H, d, *J* 7.9, C(4 or 7)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 8.8 ($CH_2$), 25.0 ($CH_2$), 28.0 ($CH_2$), 29.5 ($CH_2$), 33.0 ($CH_2$), 36.9 ($CH_3$), 48.0 ($CH_2$), 53.3 (2 × $CH_3$), 112.0 (CH, aromatic CH), 113.0 (CH, aromatic CH), 119.0 (C, aromatic C), 119.4 (C, aromatic C), 119.57 (C, aromatic C), 119.62 (C, aromatic C), 121.3 (2CH, 2 × aromatic CH), 121.9 (CH, aromatic CH), 122.2 (CH, aromatic CH), 122.6 (C, aromatic C), 123.4 (C, aromatic C), 126.8 (CH, aromatic CH), 127.1 (CH, aromatic CH), 130.4 (C, aromatic C), 131.4 (C, aromatic C), 143.6 (C, aromatic C), 144.9 (C, aromatic C), 168.8 (2 ×



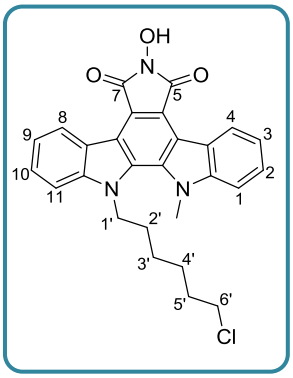
C=O); m/z (ESI$^+$) 597.1 [(M+H)$^+$ 20%]; HRMS (ESI$^+$): Exact mass calculated for ($C_{29}H_{30}N_2O_4{}^{127}I$)$^+$ 597.1250. Found 597.1245.

Method B

To a stirred suspension of 12-(6-bromohexyl)-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione **335** (0.350 g, 0.715 mmol) in anhydrous DMF (30 mL) in a flame-dried flask under nitrogen was added sodium hydride (60 wt. % oil suspension, 0.034 g, 0.85 mmol) and the mixture was stirred for 40 minutes. Iodomethane (0.14 mL, 0.32 g, 2.3 mmol) was then added and the mixture was stirred for a further 48 hours. The reaction mixture was then cautiously poured into water (70 mL) and the precipitate collected using vacuum filtration. The solid was washed with copious amounts of water and allowed to dry. The crude product was recrystallised from acetone to give a yellow solid which on analysis was found to be 12-(6-iodohexyl)-13-methyl-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione (0.203 g, 52%): m.p. 186-187 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 2932, 1819, 1753, 1480, 1334, 1250, 1203, 741; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.86-0.98 [2H, quin, *J* 7.4, C(3′)-$H_2$], 1.08-1.19 [2H, quin, *J* 7.4, C(4′)-$H_2$], 1.47 [2H, quin, *J* 7.2, C(5′)-$H_2$], 1.65 [2H, quin, *J* 7.3, C(2′)-$H_2$], 3.02 [2H, t, *J* 6.9, C(6′)-$H_2$], 4.26 (3H, s, $CH_3$), 4.81 [2H, t, *J* 7.3, C(1′)-$H_2$], 7.42-7.51 [2H, m, C(3, 9)-H], 7.68 [1H, ddd, *J* 8.4, 7.2, 1.2, C(10) -H], 7.72 [1H, ddd, *J* 8.3, 7.1, 1.2, C(2)-H], 7.85 [1H, d, *J* 8.3, C(1)-H], 7.95 [1H, d, *J* 8.3, C(11)-H], 8.91 [1H, d, *J* 7.7, C(4)-H], 8.93 [1H, d, *J* 7.5, C(8)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 8.9 ($CH_2$), 24.9 ($CH_2$), 28.5 ($CH_2$), 29.6 ($CH_2$), 32.9 ($CH_2$), 37.0 ($CH_3$), 48.6 ($CH_2$), 111.8 (CH, aromatic CH), 112.8 (CH, aromatic CH), 118.1 (C, aromatic C), 118.5 (C, aromatic C), 118.9 (C, aromatic C), 119.4 (C, aromatic C), 121.4 (C, aromatic C), 121.95 (CH, aromatic CH), 121.97 (CH, aromatic CH), 122.1 (C, aromatic C), 123.9 (CH, aromatic CH), 124.2 (CH, aromatic CH), 128.2 (CH, aromatic CH), 128.5 (CH, aromatic CH), 132.8 (C, aromatic C), 133.7 (C, aromatic C), 143.7 (C, aromatic C), 144.6 (C, aromatic C), 164.5 (2 × C=O); m/z (ESI$^+$) 551.1 [(M+H)$^+$ 20%]; HRMS (ESI$^+$): Exact mass calculated for ($C_{27}H_{24}N_2O_3{}^{127}I$)$^+$ 551.0832. Found 551.0818.

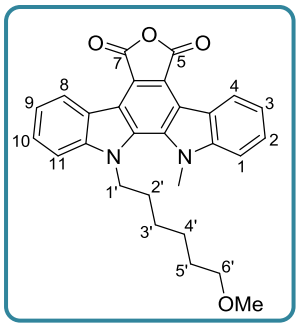


**12-(6-Chlorohexyl)-6-hydroxy-13-methyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 341**



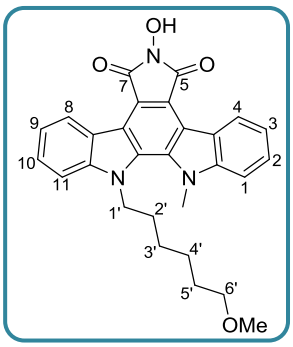

To a solution of 12-(6-iodohexyl)-13-methyl-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione **340** (0.120 g, 0.218 mmol) in anhydrous DMF (12 mL) was added hydroxylamine hydrochloride (0.083 g, 1.19 mmol) and triethylamine (0.17 mL, 0.12 g, 1.2 mmol) and the resulting mixture was stirred at 80 °C for 2 hours. After cooling, the reaction mixture was poured into 1M aqueous HCl (20 mL) and the precipitate collected by vacuum filtration, washed with water (5 mL) and allowed to dry. The crude residue was purified using column chromatography on silica gel with 1% MeOH/DCM to give desired product as an orange solid (0.076 g, 74%): m.p. 123-125 °C; $\nu_{max}/cm^{-1}$ (KBr) 3199, 2934, 1762, 1702, 1450, 1331, 1006, 750; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.91 [2H, quin, *J* 7.5, C(3′)-$H_2$], 1.15 [2H, quin, *J* 7.5, C(4′)-$H_2$], 1.40 [2H, quin, *J* 7.0, C(5′)-$H_2$], 1.60 [2H, quin, *J* 7.2, C(2′)-$H_2$], 3.36 [2H, t, *J* 6.6, C(6′)-$H_2$], 4.19 (3H, s, $CH_3$), 4.75 [2H, t, *J* 6.9, C(1′)-$H_2$], 7.38 [1H, t, *J* 7.5, C(9)-H], 7.40 [1H, t, *J* 7.6, C(3)-H], 7.62 [1H, t, *J* 8.4, C(10)-H], 7.65 [1H, t, *J* 8.2, C(2)-H], 7.77 [1H, d, *J* 8.2, C(1)-H], 7.86 [1H, d, *J* 8.3, C(11)-H], 9.06 [1H, d, *J* 7.5, C(4)-H], 9.09 [1H, d, *J* 7.5, C(8)-H], 10.70 (1H, bs, OH); $\delta_c$ (75 MHz, DMSO-$d_6$) 25.4 ($CH_2$), 26.0 ($CH_2$), 28.3 ($CH_2$), 32.1 ($CH_2$), 37.2 ($CH_3$), 45.4 ($CH_2$), 48.4 ($CH_2$), 111.8 (CH, aromatic CH), 112.7 (CH, aromatic CH), 116.2 (C, aromatic C), 116.6 (C, aromatic C), 119.2 (C, aromatic C), 119.8 (C, aromatic C), 121.6 (2CH, 2 × aromatic CH), 122.1 (C, aromatic C), 122.9 (C, aromatic C), 124.8 (CH, aromatic CH), 125.0 (CH, aromatic CH), 127.9 (CH, aromatic CH), 128.2 (CH, aromatic CH), 132.6 (C, aromatic C), 133.6 (C, aromatic C), 144.2 (C, aromatic C), 145.3 (C, aromatic C), 166.35 (C=O), 166.40 (C=O); m/z ($ESI^+$) 474.2 [$(M+H)^+$ 50%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{27}H_{25}N_3O_3{}^{35}Cl)^+$ 474.1584. Found 474.1570.

**12-(6-Methoxyhexyl)-13-methyl-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione 342**




To a stirred suspension of 11-(6-iodohexyl)-12-methyl-11,12-dihydroindolo[2,3-*a*]carbazole-5,6-dicarboxylate **339** (0.411 g, 0.689 mmol) in a mixture of methanol (8 mL) and water (2 mL) was added potassium hydroxide (85 wt. %, 0.306 g, 4.64 mmol) and the mixture was heated to reflux for 24 hours. After cooling, the solvent was evaporated under reduced pressure and the residue suspended in 1 M aqueous HCl (20 mL). The solid was collected using vacuum filtration and dried overnight at 50 °C. The pale yellow solid was suspended in acetic anhydride (10 mL) and heated to reflux for 2 hours. After cooling, the solvent was evaporated under reduced pressure and the residue was washed with saturated sodium bicarbonate solution (30 mL). The crude product was extracted with 10% MeOH/DCM (2 × 20 mL) and the organic layer was then washed with saturated sodium bicarbonate solution (20 mL), followed by water (3 × 20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was purified using column chromatography on silica gel with DCM to give desired product as a bright yellow solid (0.153 g, 49%): m.p. 168-169 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 2932, 1818, 1753, 1334, 1251, 741; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.90 [2H, quin, *J* 7.4, C(4′)-$H_2$], 1.03-1.14 [2H, m, C(3′)-$H_2$], 1.21 [2H, quin, *J* 6.6, C(5′)-$H_2$], 1.65 [2H, quin, *J* 7.3, C(2′)-$H_2$], 3.04 [2H, t, *J* 6.6, C(6′)-$H_2$], 3.06 (3H, s, OC$\underline{H}_3$), 4.21 (3H, s, $NCH_3$), 4.74 [2H, t, *J* 7.4, C(1′)-$H_2$], 7.40 [1H, t, *J* 7.6, C(3 or 9)-H], 7.44 [1H, t, *J* 7.6, C(3 or 9)-H], 7.65 [1H, ddd, *J* 8.4, 7.2, 1.2, C(2 or 10)-H], 7.69 [1H, ddd, *J* 8.3, 7.1, 1.1, C(2 or 10)-H], 7.82 [1H, d, *J* 8.3, C(11)-H], 7.90 [1H, d, *J* 8.4, C(1)-H], 8.85 [2H, d, *J* 7.9, C(4, 8)-H]; $\delta_c$ (150 MHz, DMSO-$d_6$) 25.3 ($CH_2$), 25.8 ($CH_2$), 28.6 ($CH_2$), 29.1 ($CH_2$), 37.1 ($CH_3$), 48.5 ($CH_2$), 58.1 ($CH_3$), 72.0 ($CH_2$), 112.0 (CH, aromatic CH), 112.9 (CH, aromatic CH), 118.2 (C, aromatic C), 118.6 (C, aromatic C), 118.9 (C, aromatic C), 119.5 (C, aromatic C), 121.4 (C, aromatic C), 122.0 (CH, aromatic CH), 122.1 (CH, aromatic CH), 122.2 (C, aromatic C), 124.0 (CH, aromatic CH), 124.2 (CH, aromatic CH), 128.3 (CH, aromatic CH), 128.5 (CH, aromatic CH), 132.9 (C, aromatic C), 133.8 (C, aromatic C), 143.7 (C, aromatic C), 144.7 (C, aromatic C), 164.7 (2 × C=O); m/z (ESI$^-$) 453.2 [(M-H)$^-$ 50%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{28}H_{27}N_2O_4)^+$ 455.1971. Found 455.1966.

**6-Hydroxy-12-(6-methoxyhexyl)-13-methyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 343**

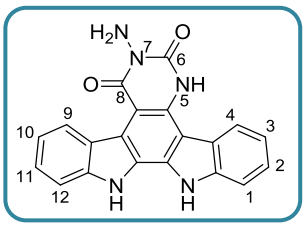



To a solution of 12-(6-methoxyhexyl)-13-methyl-12,13-dihydrofuro[3,4-*c*]indolo[2,3-*a*]carbazole-5,7-dione **342** (0.110 g, 0.242 mmol) in anhydrous DMF (10 mL) was added hydroxylamine hydrochloride (0.079 g, 1.21 mmol) and triethylamine (0.16 mL, 0.12 g, 1.1 mmol) and the resulting mixture was stirred at 80 °C for 24 hours. The orange solution was then allowed to cool and poured into 1 M aqueous HCl (60 mL) and the precipitate collected using vacuum filtration. The solid was suspended in a mixture of water (5 mL) and methanol (5 mL) and allowed to stir for 2 hours before the solid was collected using vacuum filtration and allowed to dry. This gave desired product as an orange solid (0.083 g, 73%): m.p. 180-182 °C; $\nu_{max}/cm^{-1}$ (KBr) 2934, 2836, 1760, 1709, 1473, 1333, 1104, 727; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.85 [2H, quin, *J* 7.3, C(4′)-$H_2$], 0.98-1.10 [2H, m, C(3′)-$H_2$], 1.16 [2H, quin, *J* 6.7, C(5′)-$H_2$], 1.60 [2H, quin, *J* 7.1, C(2′)-$H_2$], 2.98 [2H, t, *J* 6.3, C(6′)-$H_2$], 3.03 (3H, s, OC$\underline{H}_3$), 4.21 (3H, s, $NCH_3$), 4.78 [2H, t, *J* 7.0, C(1′)-$H_2$], 7.41 [1H, t, *J* 7.2, C(3 or 9)-H], 7.43 [1H, t, *J* 7.1, C(3 or 9)-H], 7.64 [1H, t, *J* 8.1, C(10)-H], 7.67 [1H, t, *J* 7.8, C(2)-H], 7.79 [1H, d, *J* 8.2, C(1 or 11)-H], 7.88 [1H, d, *J* 8.2, C(1 or 11)-H], 9.08 [1H, d, *J* 8.0, C(4)-H], 9.11 [1H, d, *J* 8.1, C(8)-H], 10.73 (N-OH); $\delta_c$ (150 MHz, DMSO-$d_6$) 25.3 ($CH_2$), 25.8 ($CH_2$), 28.4 ($CH_2$), 29.0 ($CH_2$), 37.2 ($CH_3$), 48.4 ($CH_2$), 58.1 ($CH_3$), 71.1 ($CH_2$), 111.9 (CH, aromatic CH), 112.9 (CH, aromatic CH), 116.2 (C, aromatic C), 116.6 (C, aromatic C), 119.3 (C, aromatic C), 119.8 (C, aromatic C), 121.67 (CH, aromatic CH), 121.71 (CH, aromatic CH), 122.1 (C, aromatic C), 122.9 (C, aromatic C), 124.8 (CH, aromatic CH), 125.1 (CH, aromatic CH), 128.0 (CH, aromatic CH), 128.3 (CH, aromatic CH), 132.7 (C, aromatic C), 133.7 (C, aromatic C), 144.2 (C, aromatic C), 145.4 (C, aromatic C), 166.39 (C=O), 166.44 (C=O); m/z ($ESI^+$) 470.2 [$(M+H)^+$ 50%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{28}H_{28}N_3O_4)^+$ 470.2080. Found 470.2065.

## 7.4 Lossen Rearrangement

**7-Amino-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 345**

Method A



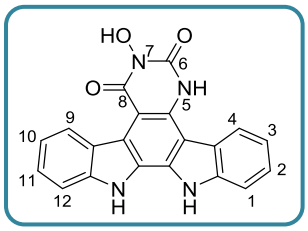
To a stirred suspension of 6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **296** (0.152 g, 0.455 mmol) in pyridine (15 mL) was added DMAP (0.062 g, 0.490 mmol) and *p*-toluenesulfonyl chloride (0.096 g, 0.490 mmol). The solution was stirred for 2 hours at room temperature before hydrazine hydrate (0.07 mL, 0.07 g, 2.3 mmol) was added and the mixture was heated to 80 °C for 16 hours. After cooling, the solvent was evaporated under reduced pressure. The residue was suspended in ethanol (10 mL) and the precipitate collected by vacuum filtration. Washes with ethanol (2 × 2 mL) gave the product as a grey solid (0.111 g, 69%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3274, 1702, 1651, 1575, 1443, 1389, 740; $\delta_H$ (300 MHz, DMSO-$d_6$) 5.73 (2H, bs, $NH_2$), 7.22 [1H, t, *J* 7.4, C(10)-H], 7.34 [1H, t, *J* 7.5, C(3)-H], 7.44 [1H, t, *J* 7.8, C(11)-H], 7.50 [1H, t, *J* 7.8, C(2)-H], 7.72 [1H, d, *J* 8.0, C(12)-H], 7.79 [1H, d, *J* 8.0, C(1)-H], 8.69 [1H, d, *J* 8.0, C(4)-H], 9.57 [1H, d, *J* 8.2, C(9)-H], 10.53 [1H, bs, N(5)-H], 11.45 (1H, s, NH), 11.82 (1H, s, NH); $\delta_c$ (75 MHz, DMSO-$d_6$) 101.8 (C, aromatic C), 106.7 (C, aromatic C), 111.9 (CH, aromatic CH), 112.3 (CH, aromatic CH), 116.4 (C, aromatic C), 119.2 (CH, aromatic CH), 120.7 (CH, aromatic CH), 122.1 (C, aromatic C), 122.4 (CH, aromatic CH), 123.3 (C, aromatic C), 123.5 (C, aromatic C), 125.5 (CH, aromatic CH), 125.6 (CH, aromatic CH), 127.4 (CH, aromatic CH), 130.8 (C, aromatic C), 131.4 (C, aromatic C), 139.4 (C, aromatic C), 140.2 (C, aromatic C), 148.9 (C=O), 160.6 (C=O); m/z ($ESI^-$) 354.3 [$(M-H)^-$ 20%]; HRMS ($ESI^-$): Exact mass calculated for $(C_{20}H_{12}N_5O_2)^-$ 354.0991. Found 354.0982.

Method B

To a stirred suspension of 6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **296** (0.100 g, 0.293 mmol) in pyridine (10 mL) was added DMAP (0.038 g, 0.322 mmol) and *p*-toluenesulfonyl chloride (0.059 g, 0.322 mmol) and the resulting solution was stirred for 2 hours at room temperature. Water (20 mL)

was then added and the yellow precipitate was collected using vacuum filtration and washed with water (2 × 2 mL) to give tosylate ester as a yellow solid (0.120 g, 82%) which was used without further purification or full characterisation: $\nu_{max}/cm^{-1}$ (KBr) 3398, 1772, 1732, 1406, 1327, 1178, 744, 566; $\delta_H$ (300 MHz, DMSO-$d_6$) 2.48 (3H, s, $CH_3$), 7.37 [2H, t, *J* 7.7, C(3, 9)-H], 7.55-7.62 [4H, m, C(2, 10, 3′, 5′)-H], 7.84 [2H, d, *J* 8.2, C(1, 11)-H], 8.03 [2H, d, *J* 8.4, C(2′, 6′)-H], 8.75 [2H, d, *J* 8.0, C(4, 8)-H], 11.91 [2H, s, N(12, 13)-H]. Tosylate ester (0.102 g, 0.21 mmol) was then suspended in ethanol (10 mL) and hydrazine hydrate (0.02 mL, 0.02 g, 0.62 mmol) was added. The reaction mixture was heated to reflux for 16 hours before cooling. Water (20 mL) was then added and the red precipitate collected using vacuum filtration. Analysis showed the resulting red solid to be hydroxymaleimide starting material.

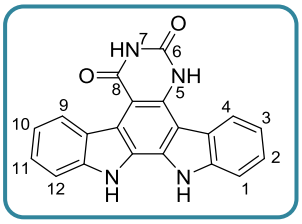
**7-Hydroxy-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 346**




To a stirred suspension of 6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **296** (0.154 g, 0.451 mmol) in pyridine (15 mL) was added DMAP (0.063 g, 0.496 mmol) and *p*-toluenesulfonyl chloride (0.097 g, 0.496 mmol). The solution was stirred for 2 hours at this temperature before hydroxylammonium hydrochloride (0.160 g, 2.26 mmol) was added and the mixture was heated to 80 °C. After 16 hours the mixture was cooled and the solvent evaporated under reduced pressure. The residue was suspended in ethanol (10 mL) and the precipitate collected by vacuum filtration. Washes with ethanol gave the desired product as a light brown solid (0.116 g, 71%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3305, 1699, 1648, 1577, 1371, 1184, 725; $\delta_H$ (300 MHz, DMSO-$d_6$) 7.22 [1H, t, *J* 7.7, C(10)-H], 7.35 [1H, t, *J* 7.3, C(3)-H], 7.45 [1H, t, *J* 8.0, C(11)-H], 7.51 [1H, t, *J* 7.9, C(2)-H], 7.74 [1H, d, *J* 8.0, C(12)-H], 7.81 [1H, d, *J* 8.0, C(1)-H], 8.67 [1H, d, *J* 8.1, C(4)-H], 9.52 [1H, d, *J* 8.2, C(9)-H], 10.49 [1H, s, N(5)-H], 10.59 (1H, bs, O-H), 11.47 (1H, s, NH), 11.84 (1H, s, NH); $\delta_c$ (75 MHz, DMSO-$d_6$) 102.4 (C, aromatic C), 106.8 (C, aromatic C), 111.9 (CH, aromatic CH), 112.3 (CH, aromatic CH), 116.6 (C, aromatic C), 119.3 (CH, aromatic CH), 120.7 (CH, aromatic CH), 122.1 (C, aromatic C), 122.4 (CH, aromatic CH), 123.3 (C, aromatic C), 123.4 (C, aromatic C), 125.6 (CH, aromatic CH), 125.7 (CH, aromatic CH), 127.3 (CH, aromatic CH), 130.9 (C, aromatic C), 131.5 (C,

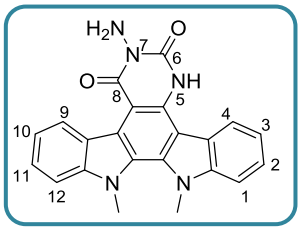
aromatic C), 139.4 (C, aromatic C), 140.2 (C, aromatic C), 149.4 (C=O), 160.9 (C=O); m/z (ESI$^-$) 355.3 [(M-H)$^-$ 90%]; HRMS (ESI$^-$): Exact mass calculated for $(C_{20}H_{11}N_4O_3)^-$ 355.0831. Found 355.0822.

**13,14-Dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 348**



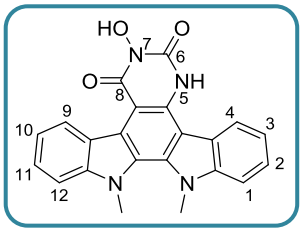
7-Amino-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbaz ole-6,8(5*H*,7*H*)-dione **345** (0.150 g, 0.421 mmol) was suspended in a mixture of acetic acid (5 mL) and water (5 mL). Sodium nitrite (0.150 g, 2.11 mmol) was added and the mixture was stirred at 50 °C for 2 hours. Further portions of sodium nitrite (0.150 g, 2.11 mmol) were added at 2 hours and 4 hours and the mixture was then stirred for 16 hours. Water was then added and the precipitate collected by filtration and allowed to dry. The off-white solid was then suspended in DMSO (5 mL) and heated to 80 °C for 1 hour resulting in a clear, brown solution. After cooling, water (15 mL) was added and the precipitate collected using vacuum filtration. Washes with water (2 × 2 mL) resulted in an off-white solid (0.122 g, 85%): m.p. >300 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3250, 3052, 1696, 1652, 1576, 1444, 1388, 1325, 743; $\delta_H$ (300 MHz, DMSO-$d_6$) 7.20 [1H, ddd, *J* 8.2, 7.0, 1.1, C(10)-H], 7.33 [1H, t, *J* 7.6, C(3)-H], 7.43 [1H, ddd, *J* 8.2, 7.0, 1.2, C(11)-H], 7.50 [1H, ddd, *J* 8.0, 7.3, 0.9, C(2)-H], 7.71 [1H, d, *J* 8.0, C(12)-H], 7.80 [1H, d, *J* 7.9, C(1)-H], 8.67 [ 1H, d, *J* 8.0, C(4)-H], 9.50 [1H, d, *J* 8.2, C(9)-H], 10.09 [1H, bs, N(5)-H], 11.30 [1H, bs, N(7)-H], 11.40 (1H, s, NH), 11.81 (1H, s, NH); $\delta_c$ (150 MHz, DMSO-$d_6$) 102.5 (C, aromatic C), 106.9 (C, aromatic C), 111.8 (CH, aromatic CH), 112.3 (CH, aromatic CH), 116.9 (C, aromatic C), 119.1 (CH, aromatic CH), 120.7 (CH, aromatic CH), 122.2 (C, aromatic C), 122.4 (CH, aromatic CH), 123.1 (C, aromatic C), 123.5 (C, aromatic C), 125.5 (CH, aromatic CH), 125.6 (CH, aromatic CH), 127.6 (CH, aromatic CH), 131.0 (C, aromatic C), 134.2 (C, aromatic C), 139.4 (C, aromatic C), 140.2 (C, aromatic C), 151.1 (C=O), 164.2 (C=O); m/z (ESI$^+$) 341.1 [(M+H)$^+$ 100%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{20}H_{13}N_4O_2)^+$ 341.1039. Found 341.1025.

**7-Amino-13,14-dimethyl-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 349**



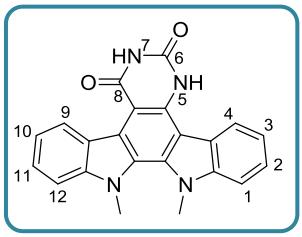
To a stirred suspension of 6-hydroxy-12,13-dimethyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **305** (0.151 g, 0.409 mmol) in pyridine (15 mL) was added DMAP (0.055 g, 0.450 mmol) and *p*-toluenesulfonyl chloride (0.085 g, 0.450 mmol). The solution was stirred for 2 hours at room temperature before hydrazine hydrate (0.10 mL, 0.10 g, 2.4 mmol) was added and the mixture was heated to 80 °C for 16 hours. After cooling, the solvent was evaporated under reduced pressure. The residue was suspended in ethanol (10 mL) and the precipitate collected by vacuum filtration. Washes with ethanol (2 × 2 mL) gave the desired product as a cream solid (0.122 g, 87%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3365, 2918, 1709, 1651, 1442, 1088, 734; $\delta_H$ (300 MHz, DMSO-$d_6$) 4.13 (3H, s, $CH_3$), 4.23 (3H, s, $CH_3$), 5.70 (2H, bs, $NH_2$), 7.29 [1H, ddd, *J* 8.0, 7.1, 1.0, C(10)-H], 7.42 [1H, t, *J* 7.2, C(3)-H], 7.55 [1H, ddd, *J* 8.1, 7.0, 1.0, C(11)-H], 7.60 [1H, t, *J* 7.7, C(2)-H], 7.70 [1H, d, *J* 8.1, C(12)-H], 7.78 [1H, d, *J* 8.2, C(1)-H], 8.66 [1H, d, *J* 8.0, C(4)-H], 9.59 [1H, d, *J* 8.0, C(9)-H], 10.63 (1H, s, NH); $\delta_c$ (150 MHz, DMSO-$d_6$) 36.7 ($CH_3$), 37.5 ($CH_3$), 102.2 (C, aromatic C), 109.1 (C, aromatic C), 111.3 (CH, aromatic CH), 111.4 (CH, aromatic CH), 119.5 (C, aromatic C), 120.1 (CH, aromatic CH), 121.4 (CH, aromatic CH), 121.9 (C, aromatic C), 122.1 (C, aromatic C), 122.3 (CH, aromatic CH), 123.9 (C, aromatic C), 126.0 (CH, aromatic CH), 126.3 (CH, aromatic CH), 126.9 (C, aromatic C), 127.5 (CH, aromatic CH), 133.8 (C, aromatic C), 143.2 (C, aromatic C), 145.1 (C, aromatic C), 149.0 (C=O), 160.1 (C=O); m/z ($ESI^+$) 384.1 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{22}H_{18}N_5O_2)^+$ 384.1461. Found 384.1464.

**7-Hydroxy-13,14-dimethyl-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 350**



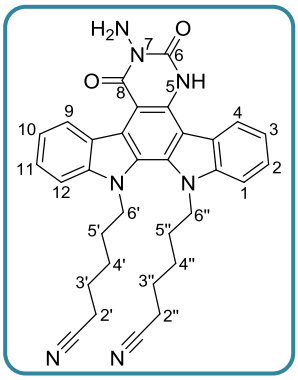

To a stirred suspension of 6-hydroxy-12,13-dimethyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione **305** (0.204 g, 0.552 mmol) in pyridine (15 mL) was added DMAP (0.079 g, 0.662 mmol) and *p*-toluenesulfonyl chloride (0.123 g, 0.662 mmol). The solution was stirred for 2 hours at this temperature before hydroxylammonium hydrochloride (0.188 g, 2.76 mmol) was added and the mixture was heated to 80 °C for 16 hours. After cooling, the solvent was evaporated under reduced pressure. The residue was suspended in ethanol (10 mL) and the precipitate collected by vacuum filtration. Washes with ethanol (2 × 2 mL) gave the desired product as a light brown solid (0.119 g, 57%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr) 3391, 2917, 1712, 1664, 1596, 1456, 1325, 739; $\delta_H$ (300 MHz, DMSO-$d_6$) 4.14 (3H, s, $CH_3$), 4.24 (3H, s, $CH_3$), 7.29 [1H, t, *J* 7.4, C(10)-H], 7.43 [1H, t, *J* 7.4, C(3)-H], 7.55 [1H, t, *J* 7.2, C(11)-H], 7.60 [1H, t, *J* 7.5, C(2)-H], 7.71 [1H, d, *J* 8.2, C(12)-H], 7.79 [1H, d, *J* 8.2, C(1)-H], 8.64 [1H, d, *J* 7.9, C(4)-H], 9.54 [1H, d, *J* 8.2, C(9)-H], 10.59 (1H, bs, OH), 10.60 (1H, s, NH); $\delta_c$ (150 MHz, DMSO-$d_6$) 36.7 ($CH_3$), 37.5 ($CH_3$), 102.8 (C, aromatic C), 109.1 (C, aromatic C), 111.3 (CH, aromatic CH), 111.4 (CH, aromatic CH), 119.7 (C, aromatic C), 120.1 (CH, aromatic CH), 121.4 (CH, aromatic CH), 122.1 (2C, 2 × aromatic C), 122.3 (CH, aromatic CH), 123.8 (C, aromatic C), 126.1 (CH, aromatic CH), 126.4 (CH, aromatic CH), 126.9 (C, aromatic C), 127.4 (CH, aromatic CH), 133.8 (C, aromatic C), 143.2 (C, aromatic C), 145.1 (C, aromatic C), 149.4 (C=O), 160.5 (C=O); m/z ($ESI^+$) 385.1 [$(M+H)^+$ 1%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{22}H_{17}N_4O_3)^+$ 385.1301. Found 385.1314.

**13,14-Dimethyl-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 352**



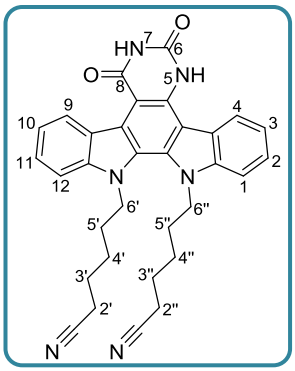
7-Amino-13,14-dimethyl-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione **349** (0.102 g, 0.266 mmol) was suspended in a mixture of acetic acid (6 mL) and water (6 mL). Sodium nitrite (0.090 g, 1.33 mmol) was added and the mixture was stirred at 50 °C for 2 hours. Further portions of sodium nitrite (0.090 g, 1.33 mmol) were added at 2 hours and 4 hours and the mixture was then stirred for 16 hours at this temperature. Water (20 mL) was then added and the precipitate collected by filtration and allowed to dry. The off-white solid was then suspended in DMSO (5 mL) and heated to 80 °C for 1 hour resulting in a clear, brown solution. After cooling, water (20 mL) was added and the precipitate collected using vacuum filtration. Washes with water (2 × 2 mL) gave desired product as an off-white solid (83 mg, 86%): m.p. >300 °C; $\nu_{max}/cm^{-1}$ (KBr); 3462, 3162, 3046, 2827, 1690, 1618, 1447, 1321, 738; $\delta_H$ (300 MHz, DMSO-$d_6$) 4.11 (3H, s, $CH_3$), 4.21 (3H, s, $CH_3$), 7.26 [1H, t, *J* 7.5, C(10)-H], 7.40 [1H, t, *J* 7.4, C(3)-H], 7.53 [1H, t, *J* 7.7, C(11)-H], 7.58 [1H, t, *J* 7.9, C(2)-H], 7.67 [1H, d, *J* 8.1, C(12)-H, 7.76 [1H, d, *J* 8.1, C(1)-H], 8.62 [1H, d, *J* 7.8, C(4)-H], 9.53 [1H, d, *J* 8.3, C(9)-H], 10.16 [1H, bs, N(5)-H], 11.34 [1H, bs, N(7)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 36.7 ($CH_3$), 37.5 ($CH_3$), 102.9 (C, aromatic C), 109.1 (C, aromatic C), 111.2 (CH, aromatic CH), 111.3 (CH, aromatic CH), 119.99 (CH, aromatic CH), 120.03 (C, aromatic C), 121.4 (CH, aromatic CH), 122.1 (C, aromatic C), 122.2 (CH, aromatic CH), 123.9 (C, aromatic C), 126.0 (CH, aromatic CH), 126.4 (CH, aromatic CH), 126.7 (C, aromatic C), 127.6 (CH, aromatic CH), 134.0 (C, aromatic C), 134.7 (C, aromatic C), 143.2 (C, aromatic C), 145.2 (C, aromatic C), 151.1 (C=O), 163.7 (C=O); m/z ($ESI^+$) 369.1 [$(M+H)^+$ 10%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{22}H_{17}N_4O_2)^+$ 369.1352. Found 369.1346.

**6,6'-(7-Amino-6,8-dioxo-5,6,7,8-tetrahydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-13,14-diyl)dihexanenitrile 351**




To a stirred suspension of 6,6'-(6-hydroxy-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-12,13-diyl)di hexanenitrile **334** (0.130 g, 0.245 mmol) in pyridine (13 mL) was added DMAP (0.033 g, 0.269 mmol) and *p*-toluenesulfonyl chloride (0.051 g, 0.269 mmol). The solution was stirred for 2 hours at room temperature before hydrazine hydrate (0.04 mL, 0.04 g, 1.2 mmol) was added and the mixture was heated to 80 °C for 16 hours. After cooling, the solvent was evaporated under reduced pressure. The residue was suspended in methanol (10 mL) and the precipitate collected by vacuum filtration. Washes with methanol (2 × 2 mL) gave the desired product as an off-white solid (0.080 g, 61%): m.p. 135-137 °C; $\nu_{max}/cm^{-1}$ (KBr) 3330, 2937, 2243, 1713, 1648, 1594, 1432, 1336, 749; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.79-0.98 [4H, m, C(4′, 4″)-$H_2$], 1.16-1.33 [6H, m, C(3′, 3″, 5′)-$H_2$], 1.42 [2H, quin, *J* 7.2, C(5″)-$H_2$], 2.16 [2H, t, *J* 6.9, C(2′ or 2″)-$H_2$], 2.17 [2H, t, *J* 6.9, C(2′ or 2″)-$H_2$], 4.64 [2H, t, *J* 6.9, C(6′)-$H_2$], 4.74 [2H, t, *J* 7.0, C(6″)-$H_2$], 5.71 (2H, bs, $NH_2$), 7.30 [1H, t, *J* 7.7, C(10)-H], 7.42 [1H, t, *J* 7.5, C(3)-H], 7.53 [1H, t, *J* 7.3, C(11)-H], 7.58 [1H, t, *J* 7.5, C(2)-H], 7.83 [1H, d, *J* 8.2, C(12)-H], 7.89 [1H, d, *J* 8.2, C(1)-H], 8.69 [1H, d, *J* 7.9, C(4)-H], 9.60 [1H, d, *J* 8.2, C(9)-H], 10.66 (1H, bs, NH); $\delta_c$ (75 MHz, DMSO-$d_6$) 16.2 [2 × $CH_2$], 24.4 [$CH_2$], 24.5 [$CH_2$], 25.4 [$CH_2$], 25.5 [$CH_2$], 26.8 [$CH_2$], 27.6 [$CH_2$], 47.7 [$CH_2$], 48.4 [$CH_2$], 102.9 (C, aromatic C), 110.1 (C, aromatic C), 113.0 (CH, aromatic CH), 113.1 (CH, aromatic CH), 120.6 (CH, aromatic CH), 120.7 (2 × CN), 121.4 (C, aromatic C), 121.7 (CH, aromatic CH), 122.7 (CH, aromatic CH), 123.5 (C, aromatic C), 125.8 (C, aromatic C), 126.0 (CH, aromatic CH), 126.3 (CH, aromatic CH), 127.3 (C, aromatic C), 127.9 (CH, aromatic CH), 132.2 (C, aromatic C), 134.5 (C, aromatic C), 142.5 (C, aromatic C), 144.7 (C, aromatic C), 148.9 (C=O), 160.1 (C=O); m/z ($ESI^+$) 546.2 [$(M+H)^+$ 20%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{32}H_{32}N_7O_2)^+$ 546.2617. Found 546.2600.

**6,6'-(6,8-Dioxo-5,6,7,8-tetrahydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-13,14-diyl)dihexanenitrile 353**



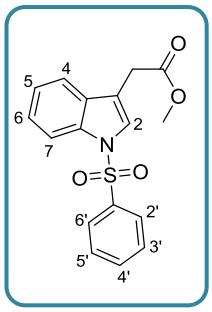
6,6'-(7-Amino-6,8-dioxo-5,6,7,8-tetrahydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-13,14-diyl)dihexanenitrile **351** (0.070 g, 0.13 mmol) was suspended in a mixture of acetic acid (3 mL) and water (3 mL). Sodium nitrite (0.044 g, 0.64 mmol) was added and the mixture was stirred at 50 °C for 2 hours. Further portions of sodium nitrite (0.044 g, 0.64 mmol) were added at 2 hours and 4 hours and the mixture was then stirred for 16 hours at this temperature. Water (15 mL) was then added and the precipitate collected by filtration and allowed to dry. The light brown solid was then suspended in DMSO (3 mL) and heated to 80 °C for 1 hour resulting in a clear, brown solution. After cooling, water (10 mL) was added and the aqueous layer was extracted with 20% MeOH/DCM (15 mL). The organic layer was washed once with water (10 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. Methanol (4 mL) was added to the residue and the mixture was stirred for 30 minutes before the precipitate was collected using vacuum filtration to give an off-white solid (0.042 g, 61%): m.p. 124-126 °C; $\nu_{max}/cm^{-1}$ (KBr) 3419, 3190, 2939, 2244, 1706, 1594, 1460, 1369, 750; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.82-0.99 [4H, m, C(4′, 4″)-$H_2$], 1.15-1.34 [6H, m, C(3′, 3″, 5′)-$H_2$], 1.42 [2H, quin, *J* 6.7, C(5″)-$H_2$], 2.18 [2H, t, *J* 6.8, C(either 2′ or 2″)-$H_2$], 2.19 [2H, t, *J* 6.8, C(either 2′ or 2″)-$H_2$], 4.63 [2H, t, *J* 7.0, C(6′)-$H_2$], 4.74 [2H, t, *J* 7.1, C(6″)-$H_2$], 7.27 [1H, t, *J* 7.6, C(10)-H], 7.41 [1H, t, *J* 7.6, C(3)-H], 7.52 [1H, t, *J* 7.9, C(11)-H], 7.58 [1H, t, *J* 7.9, C(2)-H], 7.82 [1H, d, *J* 8.2, C(12)-H], 7.89 [1H, d, *J* 8.2, C(1)-H], 8.65 [1H, d, *J* 8.0, C(4)-H], 9.53 [1H, d, *J* 8.1, C(9)-H], 10.20 (1H, bs, NH), 11.36 (1H, bs, C=ONHC=O); $\delta_c$ (125 MHz, DMSO-$d_6$) 16.2 ($CH_2$), 16.3 ($CH_2$), 24.46 ($CH_2$), 24.49 ($CH_2$), 25.4 ($CH_2$), 25.6 ($CH_2$), 26.8 ($CH_2$), 27.5 ($CH_2$), 47.7 ($CH_2$), 48.4 ($CH_2$), 103.7 (C, aromatic C), 110.2 (C, aromatic C), 113.0 (CH, aromatic CH), 113.1 (CH, aromatic CH), 120.6 (CH, aromatic CH), 120.8 (2 × CN), 121.75 (CH, aromatic CH), 121.84 (C, aromatic C), 122.7 (CH, aromatic CH), 123.6 (C, aromatic C), 125.8 (C, aromatic C), 125.9 (CH, aromatic CH), 126.4 (CH, aromatic CH), 127.1 (C, aromatic C), 128.0 (CH, aromatic CH), 134.7 (C, aromatic C), 135.0 (C, aromatic C), 142.5 (C, aromatic C), 144.9 (C, aromatic C), 151.1 (C=O), 163.7 (C=O);

m/z (ESI$^+$) 531.2 [(M+H)$^+$ 40%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{32}H_{31}N_6O_2)^+$ 531.2508. Found 531.2495.

## 7.5 Synthesis and derivatisation of novel methyl 2,3-di(1*H*-indol-3-yl)-3-oxopropanoates

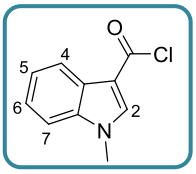
### 7.5.1 Synthesis of 13-methyl-5,6,12,13-tetrahydro-7*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-7-one

**Methyl 2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]acetate 355**[19]



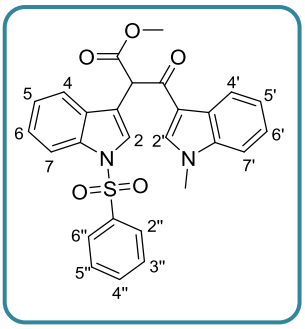

To a suspension of 2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]acetic acid **256** (13.846 g, 43.9 mmol) in distilled methanol (200 mL) was added dropwise thionyl chloride (4.78 mL, 7.84 g, 65.9 mmol) and the mixture was heated to reflux for 16 hours. After cooling, the solvent and excess thionyl chloride were evaporated under reduced pressure. Saturated aqueous sodium bicarbonate solution (60 mL) was added to the residue which was then extracted was ethyl acetate (2 × 40 mL). The organic layer was washed with saturated aqueous sodium bicarbonate (40 mL) followed by water (2 × 40 mL), before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was purified using column chromatography on silica gel with 20% ethyl acetate/ hexane gave desired product as a pale yellow solid (11.859 g, 82%): m.p 64-65 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3150, 2949, 1742, 1447, 1366, 1180, 755, 724; $\delta_H$ (300 MHz, $CDCl_3$) 3.69 (2H, d, *J* 1.1, $CH_2$), 3.70 (3H, s, $CH_3$), 7.25 [1H, ddd, *J* 8.0, 7.0, 1.1, C(6)-H], 7.33 [1H, ddd, *J* 8.4, 7.3, 1.4, C(5)-H], 7.39-7.46 [2H, m, C(3′, 5′)-H], 7.47-7.55 [2H, m, C(7, 4′)-H], 7.57 [1H, s, C(2)-H], 7.85-7.90 [2H, m, C(2′, 6′)-H], 7.98 [1H, d, *J* 8.1, C(4)-H]; m/z (ESI$^+$) 330.1 [(M+H)$^+$ 15%].

**1-Methyl-1*H*-indole-3-carbonyl chloride 176**[20]



To a suspension of 1-methyl-1*H*-indole-3-carboxylic acid **361** (1.910 g, 10.9 mmol) in DCM (100 mL) at 0 °C under nitrogen was added dropwise oxalyl chloride (0.93 mL, 1.38 g, 21.8 mmol) and the mixture was stirred for 16 hours at room temperature eventually becoming a light brown solution. The solvent and excess oxalyl chloride were removed under reduced pressure to give desired compound as an off-white solid which was used without further purification: $\nu_{max}/cm^{-1}$ (KBr) 1746, 1526, 1358, 1197, 1031, 792, 750.

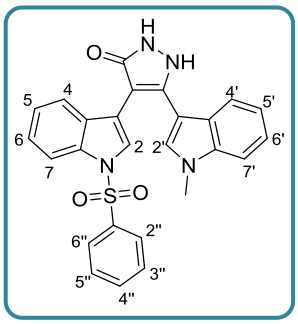
**Methyl 3-(1-methyl-1*H*-indol-3-yl)-3-oxo-2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]propanoate 357**



A stirred solution of diisopropylamine (5.90 mL, 4.26 g, 42.1 mmol) in THF (200 mL) in a flame-dried three necked flask under nitrogen was cooled to -60 °C. *n*-Butyllithium (2.5 M, 16 mL, 40 mmol) was then added slowly and the mixture was stirred for 30 minutes at this temperature. The mixture was then cooled to -78 °C before a solution of methyl 2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]acetate **355** (6.004 g, 18.2 mmol) in THF (30 mL) was added slowly and then mixture was stirred for 1 hour at this temperature. A suspension of 1-methyl-1*H*-indole-3-carbonyl chloride **176** (21.8 mmol) in THF (50 mL) was then added slowly and the mixture was allowed to warm to room temperature and stirred for 16 hours. At this point, water (5 mL) was added to quench the reaction and the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (200 mL) which was washed with saturated aqueous sodium bicarbonate solution (100 mL), water (3 × 100 mL) and then brine (100 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was purified using column chromatography on silica gel using 40% ethyl acetate/ hexane followed by recrystallisation from ethyl acetate to give desired product as a white solid (3.985 g, 45%): m.p. 169-171 °C; $\nu_{max}/cm^{-1}$ (KBr) 1748, 1634, 1525, 1369, 1176, 1130, 752, 728; $\delta_H$ (300 MHz, $CDCl_3$) 3.74 (3H, s, $CH_3$), 3.75 (3H, s, $CH_3$), 5.54 (1H, d, *J* 0.7, CH), 7.19-7.39 [7H, m, C(5, 5′, 6, 6′, 7′, 3″, 5″)-H], 7.43-7.50 [1H, m, C(4″)-H], 7.60 [1H, d, *J* 7.0, C(7)-H], 7.78 [1H, s, C(2)-H], 7.83-7.87 [2H, m, C(2″,

6″)-H], 7.89 [1H, d, *J* 0.5, C(2′)-H], 7.95 [1H, d, *J* 7.5, C(4)-H], 8.35-8.41 [1H, m, C(4′)-H]; $\delta_c$ (75 MHz, $CDCl_3$) 33.7 (N-$CH_3$), 52.9 (O-$CH_3$), 53.7 (CH), 109.8 (CH, aromatic CH), 113.7 (CH, aromatic CH), 114.8 (C, aromatic C), 116.0 (C, aromatic C), 119.8 (CH, aromatic CH), 122.8 (CH, aromatic CH), 123.2 (CH, aromatic CH), 123.5 (CH, aromatic CH), 123.9 (CH, aromatic CH), 125.0 (CH, aromatic CH), 125.9 (CH, aromatic CH), 126.7 (C, aromatic C), 126.8 (2CH, 2 × aromatic CH), 129.3 (2CH, 2 × aromatic CH), 130.0 (C, aromatic C), 133.8 (CH, aromatic CH), 134.9 (C, aromatic C), 136.2 (CH, aromatic CH), 137.4, (C, aromatic C), 138.2 (C, aromatic C), 169.0 (C=O), 186.0 (C=O); m/z ($ESI^+$) 487.3 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{27}H_{23}N_2O_5S)^+$ 487.1328. Found 487.1327.

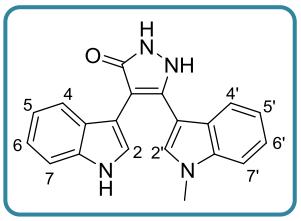
**5-(1-Methyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]-1*H*-pyrazol-3(2*H*)-one 358**




To a stirred suspension of methyl 3-(1-methyl-1*H*-indol-3-yl)-3-oxo-2-[1-(phenylsulfonyl)-1*H*-indol-3-yl]propanoate **357** (0.102 g, 0.210 mmol) in ethanol (20 mL) was added camphoric acid (0.040 g, 0.210 mmol) and hydrazine hydrate (0.013 mL, 0.013 g, 0.21 mmol). The mixture was heated to reflux for 2 hours before an excess of hydrazine hydrate (0.026 mL, 0.026 g, 0.42 mmol) was added and the mixture was heated at reflux for a further 24 hours. The mixture was then cooled and the solvent evaporated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL) and washed with saturated aqueous sodium bicarbonate (30 mL), followed by water (2 × 30 mL) and brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude residue was purified using column chromatography on silica gel with ethyl acetate as eluent to give desired product as a white solid (0.044 g, 45%): m.p. 257-259 °C; $\nu_{max}/cm^{-1}$ (KBr) 3353, 2598, 1637, 1520, 1358, 1177, 1131, 742, 593; $\delta_H$ (300 MHz, DMSO-$d_6$) 3.76 (3H, s, $CH_3$), 6.72 [1H, t, *J* 7.5, C(6′)-H], 7.03 [1H, t, *J* 7.7, C(6)-H], 7.07-7.14 [2H, m, C(5′, 7′)-H], 7.20-7.28 [2H, m, C(5, 7)-H], 7.41 [1H, s, C(2′)-H], 7.43 [1H, d, *J* 8.7, C(4′)-H], 7.50-7.58 [2H, m, C(3″, 5″)-H], 7.53 [1H, s, C(2)-H], 7.65-7.72 [1H, m, C(4″)-H], 7.81-7.86 [2H, m, C(2″, 6″)-H], 7.88 [1H, d, *J* 8.3, C(4)-H], 10.24 (1H, bs, NH), 11.75 (1H, bs, NH); $\delta_c$ (75 MHz, DMSO-$d_6$) 33.1 ($CH_3$), 94.3 (C, aromatic C), 105.1 (C, aromatic C), 110.4 (CH, aromatic CH), 113.4

(CH, aromatic CH), 115.9 (C, aromatic C), 119.9 (CH, aromatic CH), 120.0 (CH, aromatic CH), 122.0 (CH, aromatic CH), 122.1 (CH, aromatic CH), 123.4 (CH, aromatic CH), 124.2 (CH, aromatic CH), 125.0 (CH, aromatic CH), 126.0 (C, aromatic C), 127.01 (2CH, 2 × aromatic CH), 129.1 (CH, aromatic CH), 130.2 (2CH, 2 × aromatic CH), 130.8 (C, aromatic C), 134.6 (C, aromatic C), 134.9 (CH, aromatic CH), 136.7 (C, aromatic C), 136.8 (C, aromatic C), 137.5 (C, aromatic C), 160.0 (C=O); m/z (ESI$^-$) 467.2 [(M-H)$^-$ 100%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{26}H_{21}N_4O_3S)^+$ 469.1334. Found 469.1322.

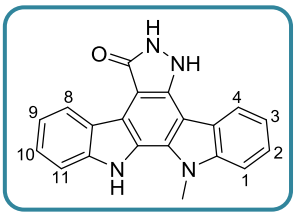
**4-(1*H*-Indol-3-yl)-5-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrazol-3(2*H*)-one 359**



To a suspension of 5-(1-methyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]-1*H*-pyrazol-3(2*H*)-one **358** (0.706 g, 1.51 mmol) in a mixture of methanol (40 mL) and water (10 mL) was added potassium hydroxide (85 wt.%, 0.366 g, 6.03 mmol) and the mixture was heated to reflux for 60 hours until reaction was complete. After cooling, the solvent was evaporated under reduced pressure. The residue was then dissolved in water (40 mL) and acidified to pH 2 using 20% aqueous HCl before being extracted with ethyl acetate (2 × 30 mL). The organic layer was washed with water (3 × 30 mL) followed by brine (30 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was purified using column chromatography on silica gel with 2% MeOH/DCM to give desired product as a light brown solid (0.142 g, 28%): m.p. 192-194 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3401, 3051, 2685, 1704, 1614, 1566, 1248, 739; $\delta_H$ (300 MHz, DMSO-$d_6$) 3.64 (3H, s, $CH_3$), 6.72 [1H, ddd, *J* 7.9, 7.1, 0.9, C(6)-H], 6.79 [1H, ddd, *J* 7.9, 7.1, 0.9, C(6′)-H], 6.92 [1H, ddd, *J* 8.1, 7.0, 1.0, C(5)-H], 6.99-7.06 [2H, m, C(2, 5′)-H], 7.16 [1H, d, *J* 7.9, C(7)-H], 7.19-7.26 [2H, m, C(4, 7′)-H], 7.23 [1H, s, C(2′)-H], 7.32 [1H, d, *J* 8.2, C(4′)-H], 9.91 (1H, bs, NH), 10.82 [1H, s, N(1)-H], 11.18 (1H, bs, NH); $\delta_c$ (75 MHz, DMSO-$d_6$) 33.0 ($CH_3$), 96.9 (C, aromatic C), 106.2 (C, aromatic C), 107.2 (C, aromatic C), 110.2 (CH, aromatic CH), 111.6 (CH, aromatic CH), 118.5 (CH, aromatic CH), 119.7 (CH, aromatic CH), 120.6 (CH, aromatic CH), 120.7 (CH, aromatic CH), 121.1 (CH, aromatic CH), 121.9 (CH, aromatic CH), 124.4 (CH, aromatic CH), 126.2 (C, aromatic C), 127.5 (C, aromatic C), 128.8 (CH, aromatic CH), 136.1 (C, aromatic C), 136.4 (C, aromatic C), 136.8 (C, aromatic C), 160.2 (C=O);

m/z (ESI$^+$) 329.3 [(M+H)$^+$ 20%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{20}H_{17}N_4O)^+$ 329.1402. Found 329.1416.

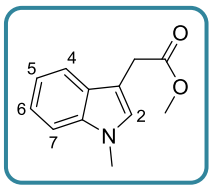
**13-Methyl-5,6,12,13-tetrahydro-7*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-7-one 360**




5-(1-Methyl-1*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]-1*H*-pyrazol-3(2*H*)-one (0.120 g, 0.256 mmol) **358** was dissolved in methanol (100 mL) and irradiated using a standard high pressure mercury lamp in the presence of air for 2 hours. The solvent was then evaporated under reduced pressure and the residue dissolved in 10% MeOH/DCM (30 mL). The organic layer was washed with water (3 × 20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was recrystallised from MeOH/DCM to give desired product as a light brown solid (0.021 g, 25%): m.p. >300 °C; $\nu_{max}$/cm$^{-1}$ (KBr) 3407, 3267, 3052, 1618, 1602, 1328, 741; $\delta_H$ (300 MHz, DMSO-$d_6$) 4.43 (3H, s, $CH_3$), 7.20 [1H, t, *J* 7.5, C(9)-H], 7.32 [1H, t, *J* 7.5, C(3)-H], 7.37 [1H, t, *J* 7.6, C(10)-H], 7.47 [1H, t, *J* 7.7, C(2)-H], 7.68 [1H, d, *J* 8.1, C(11)-H], 7.76 [1H, d, *J* 8.2, C(1)-H], 8.46 [1H, d, *J* 7.7, C(4)-H], 8.94 [1H, d, *J* 7.9, C(8)-H], 10.78 (1H, bs, NH), 11.51 [1H, s, N(12)-H], 11.66 (1H, bs, NH); $\delta_c$ (150 MHz, DMSO-$d_6$) 32.5 ($CH_3$), 101.0 (C, aromatic C), 104.3 (C, aromatic C), 109.9 (CH, aromatic CH), 112.0 (CH, aromatic CH), 114.0 (C, aromatic C), 119.2 (CH, aromatic CH), 120.2 (CH, aromatic CH), 121.3 (CH, aromatic CH), 121.6 (C, aromatic C), 121.8 (C, aromatic C), 123.1 (C, aromatic C), 123.9 (CH, aromatic CH), 124.2 (CH, aromatic CH), 124.5 (CH, aromatic CH), 129.5 (C, aromatic C), 136.0 (C, aromatic C), 139.6 (C, aromatic C), 139.7 (C, aromatic C), 159.2 (C=O); m/z (ESI$^+$) 327.1 [(M+H)$^+$ 100%]; HRMS (ESI$^+$): Exact mass calculated for $(C_{20}H_{15}N_4O)^+$ 327.1246. Found 327.1234.

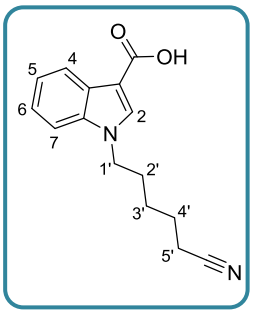
### 7.5.2 Synthesis of 6-[6-amino-13-methyl-8-oxo-8,13-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazol-14(7*H*)-yl]hexanenitrile

**Methyl 2-(1-methyl-1*H*-indol-3-yl)acetate 177**[20]



To a solution of indole-3-acetic acid **254** (5.001 g, 28.5 mmol) in anhydrous DMF (50 mL) was added potassium carbonate (5.903 g, 42.8 mmol) and dimethyl carbonate (8.40 mL, 7.64 g, 100 mmol) and the mixture was heated to 130 °C and stirred for 16 hours at this temperature. After cooling to room temperature, water (100 mL) was added and the crude product was extracted into ethyl acetate (60 mL). The organic layer was then washed with water (3 × 50 mL) and brine (50 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was purified using column chromatography with 10% ethyl acetate/ hexane to give the desired product as a light brown oil which was used without further purification (4.563 g, 79%): $\nu_{max}/cm^{-1}$ (KBr) 2950, 1737, 1475, 1333, 1163, 742; $\delta_H$ (300 MHz, $CDCl_3$) 3.69 (3H, s, $CH_3$), 3.75 (3H, s, $CH_3$), 3.77 (2H, d, *J* 0.7, $CH_2$), 7.03 [1H, s, C(2)-H], 7.12 [1H, ddd, *J* 8.0, 6.8, 1.3, C(5)-H], 7.23 [1H, ddd, 8.0, 6.8, 1.2, C(6)-H], 7.29 [1H, dt, *J* 8.0, 1.0, C(7)-H], 7.59 [1H, dt, *J* 7.9, 1.0, C(4)-H]; m/z ($ESI^+$) 204.4 [$(M+H)^+$ 20%].

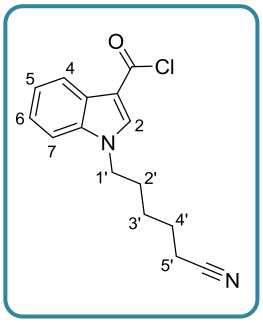
**1-(5-Cyanopentyl)-1*H*-indole-3-carboxylic acid 362**



To a solution of indole-3-carboxylic acid **361** (3.002 g, 18.6 mmol) in anhydrous DMF (50 mL) at 0 °C under nitrogen was added sodium hydride (1.561 g, 39.0 mmol) cautiously. The mixture was stirred at this temperature for 30 minutes before a solution of 6-bromohexane nitrile **318** (2.70 mL, 3.58 g, 20.4 mmol) in anhydrous DMF (10 mL) was added. The mixture was stirred for a further 16 hours before water was added cautiously to quench the reaction. The solution was then poured into water (120 mL) and acidified to pH 2 with 10% HCl. The aqueous mixture then extracted with ethyl acetate (3 × 50 mL) and the organic layer was washed with water (60 mL × 3) followed by brine (60 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure to give

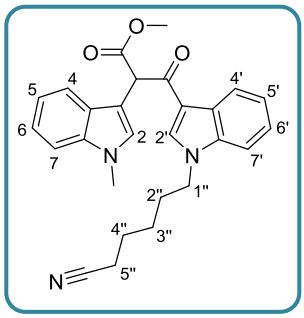
desired product as a white solid (4.442 g, 93%): m.p. 107-108 °C; $\nu_{max}/cm^{-1}$ (KBr) 3047, 2947, 2692, 2248, 1647, 1523, 1193, 757; $\delta_H$ (300 MHz, DMSO-$d_6$) 1.28-1.40 [2H, m, C(3′)-$H_2$], 1.58 [2H, quin, *J* 7.3, C(4′)-$H_2$], 1.81 [2H, quin, *J* 7.4, C(2′)-$H_2$], 2.46 [2H, t, *J* 7.1, C(5′)-$H_2$], 4.25 [2H, t, *J* 7.0, C(1′)-$H_2$], 7.16-7.27 [2H, m, C(5, 6)-H], 7.58 [1H, d, *J* 7.5, C(7)-H], 8.03 [1H, dd, *J* 6.8, 1.4, C(4)-H], 8.08 [1H, s, C(2)-H], 11.92 (1H, bs, COOH); $\delta_c$ (75 MHz, DMSO-$d_6$) 16.5 ($CH_2$), 24.8 ($CH_2$), 25.8 ($CH_2$), 29.2 ($CH_2$), 46.2 ($CH_2$), 106.9 (C, aromatic C), 111.2 (CH, aromatic CH), 121.1 (CN), 121.3 (CH, aromatic CH), 121.7 (CH, aromatic CH), 122.6 (CH, aromatic CH), 127.0 (C, aromatic C), 135.7 (CH, aromatic CH), 136.7 (C, aromatic C), 166.1 (C=O); m/z ($ESI^-$) 255.4 $[(M-H)^-$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{15}H_{17}N_2O_2)^+$ 257.1290. Found 257.1278.

**1-(5-Cyanopentyl)-1*H*-indole-3-carbonyl chloride 363**




To a solution of 1-(5-cyanopentyl)-1*H*-indole-3-carboxylic acid **362** (3.572 g, 13.9 mmol) in DCM (120 mL) at 0 °C under nitrogen was added oxalyl chloride (2.40 mL, 3.55 g, 27.9 mmol) dropwise. The mixture was then allowed to warm to room temperature and stirred for a further 16 hours. Excess oxalyl chloride and solvent were then removed under reduced pressure to give the acyl chloride as a brown oil which was used directly in the next reaction: $\nu_{max}/cm^{-1}$ ($CDCl_3$) 2943, 2245, 1736, 1525, 1370, 800, 749.

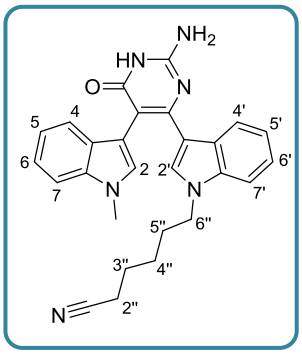
**Methyl 3-[1-(5-cyanopentyl)-1*H*-indol-3-yl]-2-(1-methyl-1*H*-indol-3-yl)-3-oxopropanoate 364**




A stirred solution of diisopropylamine (3.80 mL, 2.74 g, 27.1 mmol) in THF (150 mL) in a flame-dried three necked flask under nitrogen was cooled to -60 °C. *n*-Butyllithium (2.5 M, 10.2 mL, 25.5 mmol) was then added slowly and the mixture was stirred for 30 minutes at this temperature. The mixture was then cooled to -78 °C before a solution of methyl 2-(1-methyl-1*H*-indol-3-yl)acetate **177** (2.363 g, 11.6 mmol) in THF (20 mL) was added slowly and then mixture was stirred for 1 hour at this temperature. A suspension of 1-(5-

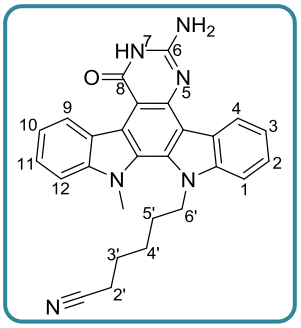
cyanopentyl)-1*H*-indole-3-carbonyl chloride **363** (3.821 g, 13.9 mmol) in THF (30 mL) was then added while maintaining the temperature and then the mixture was allowed to warm to room temperature overnight while stirring. At this point, water (5 mL) was added to quench the reaction and the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (150 mL) and washed with saturated aqueous sodium bicarbonate solution (70 mL), water (3 × 70 mL) and then brine (70 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was purified using column chromatography on silica gel using DCM to give desired product as a pale yellow foamy solid (1.098 g, 21%): m.p. 68-70 °C; $\nu_{max}/cm^{-1}$ (KBr) 2946, 2244, 1747, 1642, 1526, 1383, 1152, 745; $\delta_H$ (300 MHz, $CDCl_3$) 1.23-1.36 [2H, m, C(3″)-$H_2$], 1.55 [2H, quin, *J* 7.3, C(4″)-$H_2$], 1.80 [2H, quin, *J* 7.5, C(2″)-$H_2$], 2.19 [2H, t, *J* 7.0, C(5″)-$H_2$], 3.74 (3H, s, $CH_3$), 3.77 (3H, s, $CH_3$), 4.07 [2H, t, *J* 7.0, C(1″)-$H_2$], 5.71 (1H, s, CH), 7.15 [1H, ddd, *J* 8.0, 6.9, 1.3, C(5′)-H], 7.23 [1H, ddd, *J* 8.1, 6.9, 1.2, C(6′)-H], 7.27-7.32 [4H, m, C(5, 6, 7, 7′)-H], 7.33 [1H, s, C(2)-H], 7.69 [1H, d, *J* 7.8, C(4)-H], 7.80 [1H, s, C(2′)-H], 8.41-8.47 [1H, m, C(4′)-H]; $\delta_c$ (75 MHz, $CDCl_3$) 17.0 ($CH_2$), 24.8 ($CH_2$), 25.8 ($CH_2$), 28.9 ($CH_2$), 33.0 ($CH_3$), 46.8 ($CH_2$), 52.7 ($CH_3$), 53.2 (CH), 107.5 (C, aromatic C), 109.6 (CH, aromatic CH), 109.7 (CH, aromatic CH), 115.04 (C, aromatic C), 118.2 (CH, aromatic CH), 119.4 (CN), 119.6 (CH, aromatic CH), 121.8 (CH, aromatic CH), 122.98 (CH, aromatic CH), 123.00 (CH, aromatic CH), 123.7 (CH, aromatic CH), 127.0 (C, aromatic C), 127.1 (C, aromatic C), 129.0 (CH, aromatic CH), 135.0 (CH, aromatic CH), 136.5 (C, aromatic C), 136.7 (C, aromatic C), 170.3 (C=O), 188.2 (C=O); m/z ($ESI^-$) 440.3 [$(M-H)^-$ 40%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{27}H_{28}N_3O_3)^+$ 442.2131. Found 442.2121.

**6-{3-[2-Amino-5-(1-methyl-1*H*-indol-3-yl)-6-oxo-1,6-dihydropyrimidin-4-yl]-1*H*-indol-1-yl}hexanenitrile 365**




Sodium metal (0.622 g, 27.0 mmol) was added in small portions to distilled methanol (12 mL) in a flame-dried flask under nitrogen and stirred until fully dissolved. Guanidine carbonate (2.451 g, 13.6 mmol) was then added and the reaction mixture was stirred for 30 minutes. A solution of methyl 3-[1-(5-cyanopentyl)-1*H*-indol-3-yl]-2-(1-methyl-1*H*-indol-3-yl)-3-oxopropanoate **364** (0.605 g, 1.37 mmol) in distilled methanol (3 mL) was then added and the reaction mixture was heated to reflux for 48 hours. After cooling, the solvent was evaporated under reduced pressure. The residue was dissolved in water (20 mL), acidified to pH 7 using 10% aqueous HCl and the resulting precipitate was collected using vacuum filtration. The crude product was purified using column chromatography on silica gel with a gradient of 2-4% MeOH/DCM to give a pale yellow solid (0.245 g, 40%): m.p. 169-170 °C; $\nu_{max}/cm^{-1}$ (KBr) 3317, 3049, 2933, 2243, 1635, 1517, 1461, 1390, 1225, 740; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.73-0.86 [2H, m, C(4″)-$H_2$], 1.14 [2H, quin, *J* 6.8, C(5″)-$H_2$], 1.29 [2H, quin, *J* 7.3, C(3″)-$H_2$], 2.30 [2H, t, *J* 7.2, C(2″)-$H_2$], 3.77 [2H, t, *J* 6.0, C(6″)-$H_2$], 3.82 (3H, s, $CH_3$), 6.45 (2H, bs, $NH_2$), 6.58 [1H, s, C(2′)-H], 6.75 [1H, t, *J* 7.3, C(6)-H], 6.88 [1H, d, *J* 7.8, C(7)-H], 6.99-7.12 [3H, m, C(5, 5′, 6′)-H], 7.24 [1H, s, C(2)-H], 7.32 [1H, d, *J* 8.0, C(7′)-H], 7.41 [1H, d, *J* 8.2, C(4)-H], 8.45 [1H, d, *J* 7.5, C(4′)-H], 10.85 (1H, bs, NH); $\delta_c$ (75 MHz, DMSO-$d_6$) 16.3 ($CH_2$), 24.7 ($CH_2$), 25.2 ($CH_2$), 28.8 ($CH_2$), 32.9 ($CH_3$), 45.2 ($CH_2$), 103.2 (C, aromatic C), 109.5 (C, aromatic C), 109.7 (CH, aromatic CH), 109.9 (CH, aromatic CH), 113.4 (C, aromatic C), 118.8 (CH, aromatic CH), 120.0 (CH, aromatic CH), 120.2 (CH, aromatic CH), 121.01 (CH, aromatic CH), 121.04 (CN), 121.7 (CH, aromatic CH), 123.8 (CH, aromatic CH), 127.4 (C, aromatic C), 127.6 (C, aromatic C), 130.4 (CH, aromatic CH), 131.7 (CH, aromatic CH), 135.9 (C, aromatic C), 137.1 (C, aromatic C), 154.0 (C, aromatic C), 159.0 (C, aromatic C), 163.7 (C=O); m/z ($ESI^+$) 451.3 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{27}H_{27}N_6O)^+$ 451.2246. Found 451.2252.

**6-[6-Amino-13-methyl-8-oxo-8,13-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazol-14(7*H*)-yl]hexanenitrile 366**

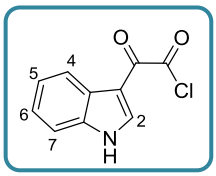



6-{3-[2-Amino-5-(1-methyl-1*H*-indol-3-yl)-6-oxo-1,6-dihydro pyrimidin-4-yl]-1*H*-indol-1-yl}hexanenitrile **365** (0.100 g, 0.222 mmol) was dissolved in a mixture of acetonitrile (60 mL) and methanol (40 mL) along with a catalytic amount of iodine and irradiated using a standard high pressure mercury lamp in the presence of air for 16 hours. The solvent was then evaporated under reduced pressure and the residue dissolved in 10% MeOH/DCM (30 mL). The organic layer was washed with saturated aqueous sodium thiosulfate (20 mL), followed by water (3 × 20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The crude product was purified using column chromatography on silica gel with 2% MeOH/DCM to give desired product as a light brown solid (0.032 g, 32%): m.p. 181-183 °C; $\nu_{max}/cm^{-1}$ (KBr) 3369, 2927, 2244, 1638, 1600, 1439, 1324, 748; $\delta_H$ (300 MHz, DMSO-$d_6$) 0.97-1.10 [2H, m, C(4′)-$H_2$], 1.36 [2H, quin, *J* 7.3, C(3′)-$H_2$], 1.59 [2H, quin, *J* 7.2, C(5′)-$H_2$], 2.25 [2H, t, *J* 7.1, C(2′)-$H_2$], 4.06 (3H, s, $CH_3$), 4.74 [2H, t, *J* 7.1, C(6′)-$H_2$], 6.42 (2H, s, $NH_2$), 7.24 [1H, t, *J* 7.5, C(10)-H], 7.32 [1H, t, *J* 7.4, C(3)-H], 7.48 [2H, t, *J* 7.6, C(2, 11)-H], 7.64 [1H, d, *J* 8.1, C(12)-H], 7.80 [1H, d, *J* 8.2, C(1)-H], 9.09 [1H, d, *J* 7.6, C(4)-H], 9.66 [1H, d, *J* 8.2, C(9)-H], 10.95 [1H, s, N(7)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 16.3 ($CH_2$), 24.6 ($CH_2$), 25.6 ($CH_2$), 28.0 ($CH_2$), 37.2 ($CH_3$), 47.7 ($CH_2$), 105.9 (C, aromatic C), 111.2 (CH, aromatic CH), 112.0 (CH, aromatic CH), 116.6 (C, aromatic C), 119.8 (CH, aromatic CH), 120.3 (C, aromatic C), 120.8 (CN), 121.0 (CH, aromatic CH), 124.4 (CH, aromatic CH), 125.0 (CH, aromatic CH), 125.2 (C, aromatic C), 125.7 (CH, aromatic CH), 126.3 (C, aromatic C), 127.6 (C, aromatic C), 127.7 (CH, aromatic CH), 133.5 (C, aromatic C), 142.5 (C, aromatic C), 145.5 (C, aromatic C), 147.7 (C, aromatic C), 152.0 (C, aromatic C), 162.5 (C=O); m/z ($ESI^+$) 449.3 [$(M+H)^+$ 50%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{27}H_{25}N_6O)^+$ 449.2090. Found 449.2087.

## 7.6 Routes towards 3,3'-(1*H*-imidazole-4,5-diyl)bis(1*H*-indole)s

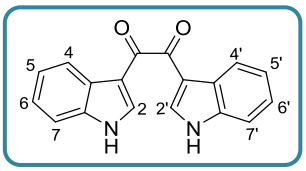
### 7.6.1 Synthesis of 3,3'-(1*H*-imidazole-4,5-diyl)bis(1*H*-indole)

**2-(1*H*-Indol-3-yl)-2-oxoacetyl chloride 368**[4]



To a solution of indole **251** (1.006 g, 8.59 mmol) in diethyl ether (20 mL) was added oxalyl chloride (1.10 mL, 1.63 g, 12.8 mmol) dropwise over 10 minutes. The yellow suspension was then stirred for a further 30 minutes. The product was collected using vacuum filtration and washed with cool diethyl ether (5 mL) to give yellow crystals (1.587 g, 94%): m.p. 133-135 °C; (Lit. 135 °C)[4]; $\nu_{max}/cm^{-1}$ (KBr) 3218, 1783, 1620, 1429, 1232, 982, 778, 727, 661; m/z ($ESI^+$) 190.2 $[(acid+H)^+$ 100%].

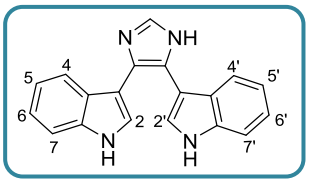
**1,2-Bis(1*H*-indol-3-yl)ethane-1,2-dione 367**[21]



*Note: this reaction was carried out using a 3-necked flask fitted with an overhead stirrer.*

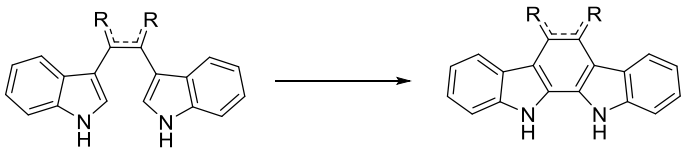
2-(1*H*-Indol-3-yl)-2-oxoacetyl chloride **368** (16.123 g, 78.0 mmol) was added portionwise to a suspension of aluminium chloride (45.307 g, 340 mmol) in a mixture of 1,2 dichloroethane (100 mL) and heptane (50 mL) over 5 minutes with stirring at room temperature. A solution of indole **251** (13.576 g, 116 mmol) in 1,2 dichloroethane (100 mL) was added dropwise under vigorous stirring to the dark brown mixture and then stirred for a further 16 hours. The reaction mixture was poured onto ice water (400 mL) and extracted with ethyl acetate (3 × 300 mL). The combined extracts were washed with water (2 × 300 mL) before being, dried over magnesium sulfate, filtered and evaporated under reduced pressure. The brown residue was triturated with ether (200 mL) and the product was collected by vacuum filtration as a brown powder (13.383 g, 60%): m.p. 269-270 °C; (Lit. 267-269 °C)[21]; $\nu_{max}/cm^{-1}$ (KBr) 3305, 1604, 1511, 1422, 1240, 1114, 778, 732; $\delta_H$ (300 MHz, DMSO-$d_6$) 7.26-7.32 [4H, m, C(5, 5', 6, 6')-H], 7.51-7.58 [2H, m, C(7, 7')-H], 8.28 [2H, d, *J* 3.2, C(2, 2')-H], 8.25-8.31 [2H, m, C(4, 4')-H], 12.26 [2H, s, N(1, 1′)-H]; m/z ($ESI^+$) 289.1 $[(M+H)^+$ 100%].

**3,3'-(1*H*-Imidazole-4,5-diyl)bis(1*H*-indole) 369**



To a suspension of 1,2-bis(1*H*-indol-3-yl)ethane-1,2-dione **367** (4.009 g, 13.9 mmol) and ammonium acetate (10.467 g, 139 mmol) in acetic acid (120 mL) was added formaldehyde dimethyl acetal (1.16 mL, 1.00 g, 13.9 mmol) and the mixture was stirred at reflux for 48 hours. The reaction mixture was then allowed to cool and concentrated under reduced pressure. The residue was washed with saturated aqueous sodium bicarbonate (100 mL) and extracted with 10% MeOH/DCM (3 × 100 mL). The organic layer was dried over magnesium sulfate, filtered and evaporated under reduced pressure. The crude product was purified using column chromatography on alumina with 4% MeOH/DCM to give desired product as a dark brown solid (1.396 g, 34%): m.p. 113-114 °C; $\nu_{max}/cm^{-1}$ (KBr) 3386, 3052, 1559, 1455, 742; $\delta_H$ (300 MHz, DMSO-$d_6$) 6.92 [2H, ddd, *J* 8.0, 7.1, 0.9, C(5, 5′)-H], 7.08 [2H, ddd, *J* 8.1, 7.1, 1.1, C(6, 6′)-H], 7.31 [2H, d, *J* 2.4, C(2, 2′)-H], 7.39 [2H, d, *J* 8.1, C(7, 7′)-H], 7.55 [2H, d, *J* 7.9, C(4, 4′)-H], 8.02 [1H, s, N-CH-N], 11.17 [2H, s, N(1, 1′)-H]; $\delta_c$ (75 MHz, DMSO-$d_6$) 107.3 (2C, 2 × aromatic C), 111.5 (2CH, 2 × aromatic CH), 118.8 (2CH, 2 × aromatic CH), 120.3 (2CH, 2 × aromatic CH), 121.2 (2CH, 2 × aromatic CH), 123.9 (2CH, 2 × aromatic CH), 125.4 (2C, 2 × aromatic C), 125.9 (2C, 2 × aromatic C), 134.11 (CH, aromatic CH), 135.9 (2C, 2 × aromatic C); m/z ($ESI^+$) 299.1 [$(M+H)^+$ 100%]; HRMS ($ESI^+$): Exact mass calculated for $(C_{19}H_{15}N_4)^+$ 299.1297. Found 299.1304.

### 7.6.2 Derivatives of 3,3'-(1*H*-imidazole-4,5-diyl)bis(1*H*-indole)




| Reaction No. | Compound | Oxidant | Stoichiometry | Conditions | Product |
|---|---|---|---|---|---|
| 1 | Imidazole | hν/$I_2$ | 0.1 eq. | $CH_3CN$/MeOH (1:1) reflux | complex mixture |
| 2 | Imidazole | hν | - | $CH_3CN$/MeOH (1:1) reflux | complex mixture |
| 3 | Imidazole | Δ | - | $CH_3CN$/MeOH (1:1) reflux | starting material |
| 4 | Imidazole | $Pd(OAc)_2$ | 1 eq. | AcOH, 120 °C | complex mixture |
| 5 | Imidazole | $MnO_2$ | 10 eq. | Acetone, r.t. | complex mixture |
| 6 | Imidazole | DDQ | 1.2 eq. | TFA, reflux | complex mixture |
| 7 | Dione | hν/$I_2$ | 0.1 eq. | $CH_3CN$, reflux | starting material |
| 8 | Dione | $FeCl_3$ | 3 eq. | DCM, r.t. | starting material |

**Reactions 1, 2 and 7:** A solution of either imidazole **369** or dione **367** (0.100 g) with or without a catalytic amount of iodine was irradiated using a standard high pressure mercury lamp in the presence of air for 16 hours. The solvent was then evaporated under reduced pressure and the residue dissolved in 10% MeOH/DCM (30 mL). The organic layer was washed with saturated aqueous sodium thiosulfate (20 mL), followed by water (3 × 20 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure.

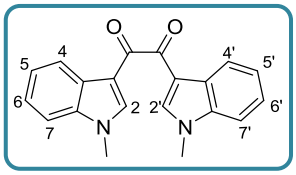
**Reaction 3:** A solution of imidazole **369** (0.100 g) in acetonitrile/methanol was heated to reflux for 16 hours before the solvent was evaporated under reduced pressure.

**Reactions 4, 5 and 8:** A solution of imidazole **369** or dione **367** (0.100 g) with the required oxidant was stirred using the conditions given above. After cooling, the reaction mixture was filtered through a pad of Celite® and washed with further portions of solvent (3 × 10 mL). The solvent was removed under reduced pressure.

**Reaction 6:** Imidazole **369** (0.100 g, 0.33 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.092 g, 0.40 mmol) were suspended in trifluoroacetic acid (10 mL) and the reaction was heated to reflux for 24 hours. After cooling, the solvent was removed under reduced pressure and the residue washed with saturated aqueous sodium bicarbonate solution and extracted into 10% MeOH/DCM. The organic layer was washed with saturated aqueous sodium bicarbonate solution, followed by brine before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure.

**Purification:** As HRMS indicated some formation of desired indolocarbazole product, crude reaction mixtures from reactions 1, 2, 4, 5 and 6 were subjected to careful column chromatography on silica gel with a gradient of 2-5% MeOH/DCM. Unfortunately, no pure samples were obtained. Column chromatography on alumina with a gradient of 2-4% MeOH/DCM was also unsuccessful due to multiple products co-eluting.

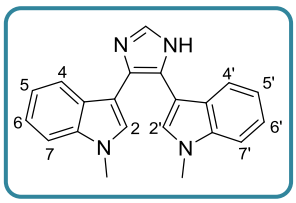
**1,2-Bis(1-methyl-1*H*-indol-3-yl)ethane-1,2-dione 372**[5]




To a solution of 1,2-bis(1*H*-indol-3-yl)ethane-1,2-dione **367** (1.005 g, 3.47 mmol) in anhydrous DMF (35 mL) under nitrogen was added sodium hydride (60 wt. % oil suspension, 0.606 g, 13.9 mmol) and the reaction mixture was allowed to stir for 30 minutes. Iodomethane (0.90 mL, 2.05 g, 14.6 mmol) was then added and the reaction was stirred for a further 16 hours. Water (60 mL) was then added and the product was extracted into ethyl acetate (3 × 50 mL). The organic layer was washed with 1 M aqueous HCl (2 × 50 mL), followed by water (50 mL) before being dried over anhydrous magnesium

sulfate, filtered and the solvent evaporated under reduced pressure. The crude material was purified using column chromatography on silica gel with 20% ethyl acetate/hexane to give desired product as a brown solid (0.911 g, 83%): m.p. 265-267 °C; (Lit. 268-269 °C)[5]; $\nu_{max}/cm^{-1}$ (KBr) 2931, 1623, 1607, 1525, 1373, 1077, 738; $\delta_H$ (300 MHz, DMSO-$d_6$) 3.89 [6H, s, N-$CH_3$], 7.31-7.40 [4H, m, C(5, 5', 6, 6')-H], 7.59-7.64 [2H, m, C(7, 7')-H], 8.27-8.32 [4H, m, C(2, 2', 4, 4')-H]; m/z ($ESI^+$) 317.1 [$(M+H)^+$ 90%].

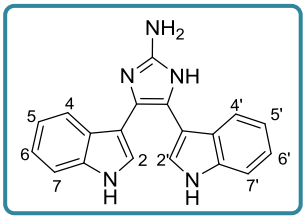
**3,3'-(1*H*-Imidazole-4,5-diyl)bis(1-methyl-1*H*-indole) 373**



Formaldehyde dimethyl acetal (0.17 mL, 0.15 g, 1.9 mmol) was added to a suspension of 1,2-bis(1-methyl-1*H*-indol-3-yl)ethane-1,2-dione **372** (0.601 g, 1.9 mmol) and ammonium acetate (1.467 g, 19 mmol) in acetic acid (20 mL) and the mixture was stirred at reflux for 48 hours. The reaction mixture was then concentrated under reduced pressure. The residue washed with saturated sodium bicarbonate solution (10 mL) and extracted with 10% MeOH/DCM (3 × 10 mL). The organic layer was then dried over magnesium sulfate, filtered and evaporated under reduced pressure to give a dark brown residue which on analysis was shown to be starting material.

### 7.6.3 Alternative Routes

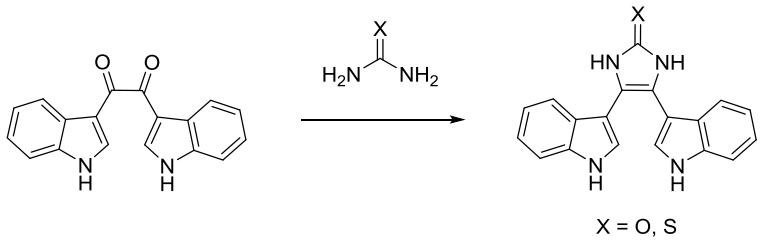
**4,5-Di(1*H*-indol-3-yl)-1*H*-imidazol-2-amine 374**



Guanidine carbonate (2.00 g, 11.1 mmol) was suspended in a solution of sodium hydroxide (0.9641 g, 22.2 mmol) in methanol (25 mL) and the mixture was stirred for 1 hour before being filtered. The filtrate was evaporated to dryness under reduced pressure. The residue was then dissolved in ethanol (17 mL) and filtered. The ethanol was then evaporated under reduced pressure to give guanidine as a semi crystalline material. Guanidine (0.5361 g, 9.0 mmol) was then added to a solution of 1,2-bis(1*H*-indol-3-yl)ethane-1,2-dione **367** (2.441 g, 8.5 mmol) in methanol (15 mL) and the mixture was refluxed for 48 hours. The precipitated solid was then collected using vacuum filtration and washed with methanol. The light brown solid (0.21 g, 0.61

mmol) was then suspended in methanol (10 mL) and 10% Pd/C (0.036 g) was added. The suspension was agitated under a hydrogen atmosphere at 10 psi for 24 hours. The reaction mixture was filtered through a pad of Celite® and washed with a further portion of methanol (20 mL). The filtrate was then evaporated to dryness under reduced pressure. Analysis using $^{1}$H NMR and mass spectrometry showed no product had formed.

Attempted condensation with ureas




| Reaction No. (X) | Stoichiometry (eq. of urea) | Additive | Conditions | Product |
|---|---|---|---|---|
| **1** (O) | 1.3 | CSA (1 eq.) | Ethanol reflux 24 hours | starting material |
| **2** (O) | 5 | *p*-TsOH (1 eq.), | Acetonitrile, reflux, $N_2$, 4 Å mol. sieves, 24 hours | starting material |
| **3** (O) | 1.5 | $Zn(OTf)_2$, (0.2 eq.) | Acetonitrile, reflux, $N_2$, 24 hours | starting material |
| **4** (O) | 5 | - | Pyridine, reflux, 24 hours | starting material |
| **5** (S) | 5 | TFA (cat.) | THF, reflux, $N_2$, 24 hours | starting material |
| **6** (S) | 5 | NaOMe (10 eq.) | Methanol, reflux, $N_2$, 24 hours | starting material |

**General Procedure:** A mixture of 1,2-bis(1*H*-indol-3-yl)ethane-1,2-dione **367** (0.100 g, 0.35 mmol), either urea or thiourea, additive and appropriate solvent (10 mL) were

reacted under the conditions given above. After cooling, the solvent was evaporated under reduced pressure and the residue dissolved in ethyl acetate (20 mL). The organic layer was washed with water (3 × 10 mL) before being dried over anhydrous magnesium sulfate, filtered and the solvent evaporated under reduced pressure. In all cases $^{1}$H NMR analysis showed only starting material to be present.

## 7.7 Protocol for Biological Evaluation

### 7.7.1 NCI-60 Cell Screen Experimental Methodology

Cell lines are initially grown in RPMI 1640 culture medium containing 10% heat-inactivated fetal bovine serum under standard culture conditions (37 °C, 5% $CO_2$, 95% air and 100% humidity).[22] Prior to the addition of the candidate compound, the cells are dispensed into 96-well microtiter plates in 100 μL of culture medium at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines before being incubated under standard culture conditions for 24 hours. Two plates of each cell line are then fixed *in situ* with trichloroacetic acid (TCA) to represent a measurement of the cell population for each cell line at the time of drug addition (Tz).

Candidate compounds are dissolved in DMSO at 400-fold the desired final test concentration and stored frozen until required. This stock solution can then be diluted to the necessary concentrations – single dose screening is carried out at 10 μM and five dose screening at five different concentrations (100 μM, 10 μM, 1 μM, 100 nM and 10 nM).

Following the addition of 100 μL of the drug solution to the appropriate wells and the plates are incubated for a further 48 hours under standard culture conditions. The assay is terminated by the addition of 50 μL of cold TCA (50% TCA for adherent cells and 80% TCA for settled suspension cells), which fixes the cells *in situ*, and incubation for 60 minutes at 4 °C. The supernatant is discarded and the plates are washed with water before staining with sulforhodamine B solution (100 μL) at 4% (w/v) in 1% acetic acid is added to each well, and the plates are incubated at room temperature for 10 minutes. The absorbance is read on an automated plate reader at a wavelength of 515 nm, and using the absorbance measurements for time zero (Tz), control growth (C), and test growth in the presence of the drug at the particular concentration level (Ti) the percentage growth is calculated. For the single dose assay the results are given as percentage growth. For the five dose assay, the percentage growths at each concentration are used to form a concentration response curve from which the concentration parameters $GI_{50}$ (concentration to inhibit cell growth by 50%) and $LC_{50}$ (concentration required for 50% cell death) are extracted by linear extrapolation. TGI

(total growth inhibition) is read as the x-axis intercept from the five different drug concentrations.

### 7.7.2 Kinase Screen Experimental Methodology

All kinase assays were performed in 96-well plates in appropriate buffer, with either protein or peptide as substrate in the presence of 15μM [γ-$^{33}$P] ATP (3,000 Ci/mmol; 10 mCi/ml) in a final volume of 30 μl using the following method.[23] The reaction mixture was pipetted in four steps in the following order: (i) 20 μL of buffer (see below), (ii) 5 μL of [γ-$^{33}$P] ATP solution (in water), (iii) 5 μL of test compound (in 10% DMSO) and (iv) 10 μL of substrate/ 10 μL of enzyme solution (premixed). The final DMSO concentration was 1% in all assays. The mixtures were incubated at 30 °C for 80 minutes before the reaction was stopped with 50 μL of 2% (v/v) phosphoric acid and the plates were washed with water or 0.9% (w/v) sodium chloride solution. Determination of incorporated $^{33}P_i$ was achieved with a microplate scintillation counter. $IC_{50}$ values were then calculated from the residual activities (in %) obtained for each concentration.

Buffers:

(A) 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 μg/mL heparin.

(B) 60 mM β-glycerophosphate, 30 mM *p*-nitrophenyl-phosphate, 25 mM MOPS (pH 7), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 0.1 mM sodium orthovanadate.

(D) 25 mM MOPS, pH 7.2, 12.5 mM β-glycerolphosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA, 0.25 mM DTT.

(H) MOPS 25 mM pH 7.5, 10 mM $MgCl_2$.

(K) Tris 50 mM pH 7.5, 20 mM $MgCl_2$, 2 mM $MnCl_2$.

(R) 5 mM MOPS pH 7.2, 2.5 mM β-glycerophosphate, 4 mM $MgCl_2$, 2.5 mM $MnCl_2$, 1 mM EGTA, 0.4 mM EDTA, 50 μg/mL BSA, 0.05 mM DTT.

*Hs*RIPK3 (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed in buffer R with 0.1 μg/μL of MBP as substrate.

*Hs*PIM1 (human proto-oncogene, recombinant, expressed in bacteria) was assayed in buffer B with 0.8 μg/μL of histone H1 (Sigma #H5505) as substrate.

*Hs*Haspin-kd (human, kinase domain, amino acids 470 to 798, recombinant, expressed in bacteria) was assayed in buffer H with 0.007 µg/µL of Histone H3 (1-21) peptide (ARTKQTARKSTGGKAPRKQLA) as substrate;

*Hs*CDK2/CyclinA (cyclin-dependent kinase-2, human, kindly provided by Dr. A. Echalier-Glazer, Leicester, UK) was assayed in buffer A (+ 0.15 mg/mL of BSA + 0.23 mg/mL of DTT) with 0.8 µg/µL of histone H1 as substrate.

*Hs*CDK9/CyclinT (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed in buffer A (+ 0.15 mg/mL of BSA + 0.23 mg/mL of DTT) with 0.27 µg/µL of the following peptide: YSPTSPSYSPTSPSYSPTSPSKKKK, as substrate.

*Hs*CDK5/p25 (human, recombinant, expressed in bacteria) was assayed in buffer B, with 0.8 µg/µL of histone H1 as substrate.

*Hs*AuroraB (human, recombinant, expressed by baculovirus in Sf9 insect cells, SignalChem, product #A31-10G) was assayed in buffer D with 0.2 µg/µL of MBP as substrate.

*Ssc*GSK-3α/β (glycogen synthase kinase-3, porcine brain, native, affinity purified) was assayed in buffer A (+ 0.15 mg/mL of BSA + 0.23 mg/mL of DTT), with 0.010 µg/µL of GS-1 peptide, a GSK-3-selective substrate (YRRAAVPPSPSLSRHSSPHQSpE DEEE, "Sp" stands for phosphorylated serine).

*Ssc*CK1δ/ε (casein kinase 1δ/ε, porcine brain, native, affinity purified) was assayed in buffer B, with 0.022 µg/µL of the following peptide: RRKHAAIGSpAYSITA as CK1-specific substrate.

*Rn*DYRK1A-kd (*Rattus norvegicus,* amino acids 1 to 499 including the kinase domain, recombinant, expressed in bacteria, DNA vector kindly provided by Dr. W. Becker, Aachen, Germany) was assayed in buffer A (+ 0.5 mg/mL of BSA + 0.23 mg/mL of DTT) with 0.033 µg/µL of the following peptide: KKISGRLSPIMTEQ as substrate.

*Mm*CLK1 (from *Mus musculus*, recombinant, expressed in bacteria) was assayed in buffer A (+ 0.15 mg/mL of BSA + 0.23 mg/mL of DTT) with 0.027 µg/µL of the following peptide: GRSRSRSRSRSR.

*Pf*GSK-3 (from *Plasmodium falciparum*, recombinant, expressed in bacteria) was assayed in buffer A (+ 0.15 mg/mL of BSA +0.23 mg/mL of DTT) with 0.010 µg/µL GS-1 peptide, a GSK-3-selective substrate (YRRAAVPPSPSLSRHSSPHQSpEDEEE, "Sp" stands for phosphorylated serine).

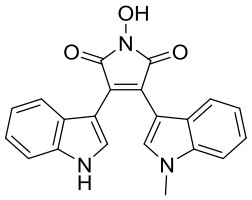
*Ld*TLK (tousled-like kinase, from *Leishmania donovani*, recombinant, expressed in bacteria), was assayed in buffer K with 0.6 µg/µL of casein dephosphorylated from bovine milk (Sigma #C4032).

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# Appendices

**1-Hydroxy-3-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione 285**



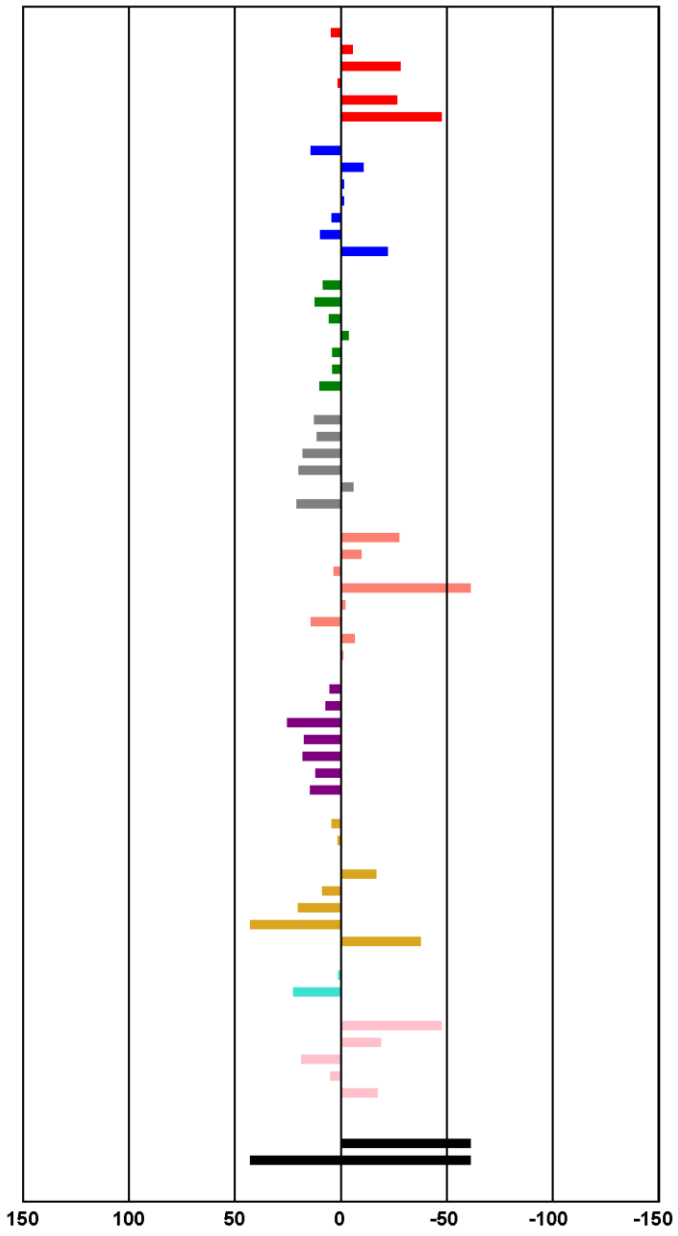

**Developmental Therapeutics Program**

**One Dose Mean Graph**

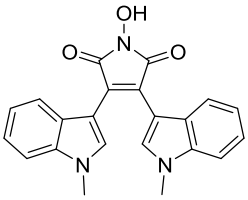
| NSC: D-775309 / 1 | Conc: 1.00E-5 Molar | Test Date: May 20, 2013 |
|---|---|---|
| Experiment ID: 1305OS96 | | Report Date: Jul 12, 2013 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 82.18 |
| HL-60(TB) | 72.85 |
| K-562 | 50.25 |
| MOLT-4 | 78.98 |
| RPMI-8226 | 51.73 |
| SR | 30.86 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 91.86 |
| HOP-62 | 67.86 |
| NCI-H226 | 76.88 |
| NCI-H23 | 76.86 |
| NCI-H322M | 81.88 |
| NCI-H460 | 87.38 |
| NCI-H522 | 56.27 |
| Colon Cancer | |
| COLO 205 | 85.99 |
| HCC-2998 | 89.87 |
| HCT-116 | 83.29 |
| HCT-15 | 74.83 |
| HT29 | 81.53 |
| KM12 | 81.73 |
| SW-620 | 87.70 |
| CNS Cancer | |
| SF-268 | 90.40 |
| SF-295 | 88.84 |
| SF-539 | 95.54 |
| SNB-19 | 97.47 |
| SNB-75 | 72.46 |
| U251 | 98.50 |
| Melanoma | |
| LOX IMVI | 51.02 |
| MALME-3M | 68.69 |
| M14 | 81.04 |
| MDA-MB-435 | 17.09 |
| SK-MEL-2 | 76.42 |
| SK-MEL-28 | 91.98 |
| SK-MEL-5 | 71.97 |
| UACC-62 | 77.37 |
| Ovarian Cancer | |
| IGROV1 | 83.02 |
| OVCAR-3 | 84.71 |
| OVCAR-4 | 102.98 |
| OVCAR-5 | 95.03 |
| OVCAR-8 | 95.64 |
| NCI/ADR-RES | 89.48 |
| SK-OV-3 | 92.08 |
| Renal Cancer | |
| 786-0 | 81.98 |
| A498 | 79.11 |
| ACHN | 77.90 |
| CAKI-1 | 61.52 |
| RXF 393 | 86.35 |
| SN12C | 97.81 |
| TK-10 | 120.39 |
| UO-31 | 40.55 |
| Prostate Cancer | |
| PC-3 | 78.70 |
| DU-145 | 100.22 |
| Breast Cancer | |
| MCF7 | 30.92 |
| MDA-MB-231/ATCC | 59.54 |
| HS 578T | 96.32 |
| BT-549 | 82.70 |
| T-47D | 60.91 |
| Mean | 77.99 |
| Delta | 60.90 |
| Range | 103.30 |

Mean Growth Percent - Growth Percent



## 1-Hydroxy-3,4-bis(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione 286



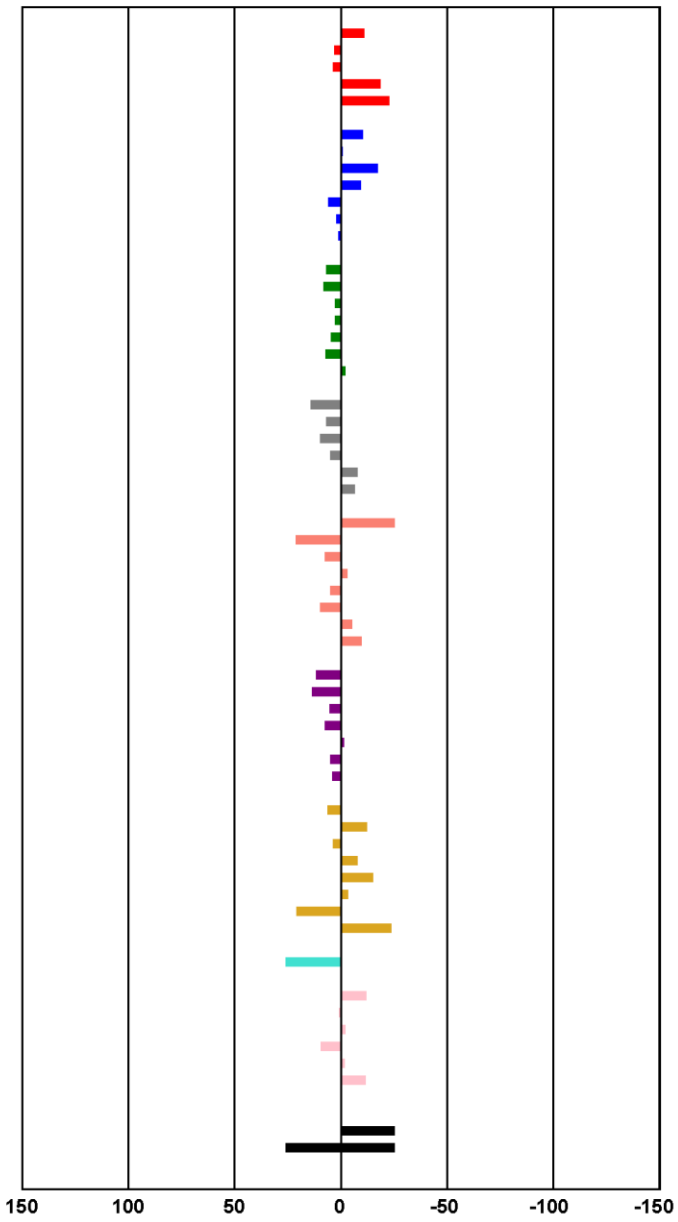

**Developmental Therapeutics Program**

**One Dose Mean Graph**

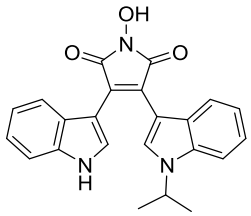
| NSC: D-774887 / 1 | Conc: 1.00E-5 Molar | Test Date: May 06, 2013 |
|---|---|---|
| Experiment ID: 1305OS83 | | Report Date: Jun 21, 2013 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| HL-60(TB) | 87.06 |
| K-562 | 100.40 |
| MOLT-4 | 100.97 |
| RPMI-8226 | 79.32 |
| SR | 75.38 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 87.75 |
| HOP-62 | 97.05 |
| HOP-92 | 80.78 |
| NCI-H226 | 88.73 |
| NCI-H322M | 103.23 |
| NCI-H460 | 99.54 |
| NCI-H522 | 98.44 |
| Colon Cancer | |
| COLO 205 | 104.02 |
| HCC-2998 | 105.52 |
| HCT-116 | 99.90 |
| HCT-15 | 99.93 |
| HT29 | 101.88 |
| KM12 | 104.48 |
| SW-620 | 95.89 |
| CNS Cancer | |
| SF-268 | 111.59 |
| SF-295 | 104.21 |
| SF-539 | 107.12 |
| SNB-19 | 102.17 |
| SNB-75 | 90.33 |
| U251 | 91.39 |
| Melanoma | |
| LOX IMVI | 72.78 |
| MALME-3M | 118.46 |
| M14 | 104.68 |
| MDA-MB-435 | 94.89 |
| SK-MEL-2 | 102.32 |
| SK-MEL-28 | 107.16 |
| SK-MEL-5 | 92.82 |
| UACC-257 | 88.32 |
| Ovarian Cancer | |
| IGROV1 | 109.02 |
| OVCAR-3 | 110.76 |
| OVCAR-4 | 102.68 |
| OVCAR-5 | 104.71 |
| OVCAR-8 | 96.48 |
| NCI/ADR-RES | 102.32 |
| SK-OV-3 | 101.41 |
| Renal Cancer | |
| 786-0 | 103.44 |
| A498 | 85.78 |
| ACHN | 101.04 |
| CAKI-1 | 90.06 |
| RXF 393 | 82.85 |
| SN12C | 94.54 |
| TK-10 | 118.25 |
| UO-31 | 74.19 |
| Prostate Cancer | |
| DU-145 | 123.17 |
| Breast Cancer | |
| MCF7 | 86.17 |
| MDA-MB-231/ATCC | 97.99 |
| HS 578T | 95.85 |
| BT-549 | 106.85 |
| T-47D | 96.37 |
| MDA-MB-468 | 86.44 |
| Mean | 97.62 |
| Delta | 24.84 |
| Range | 50.39 |

Mean Growth Percent - Growth Percent



**1-Hydroxy-3-(1*H*-indol-3-yl)-4-(1-isopropyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione**
**290**

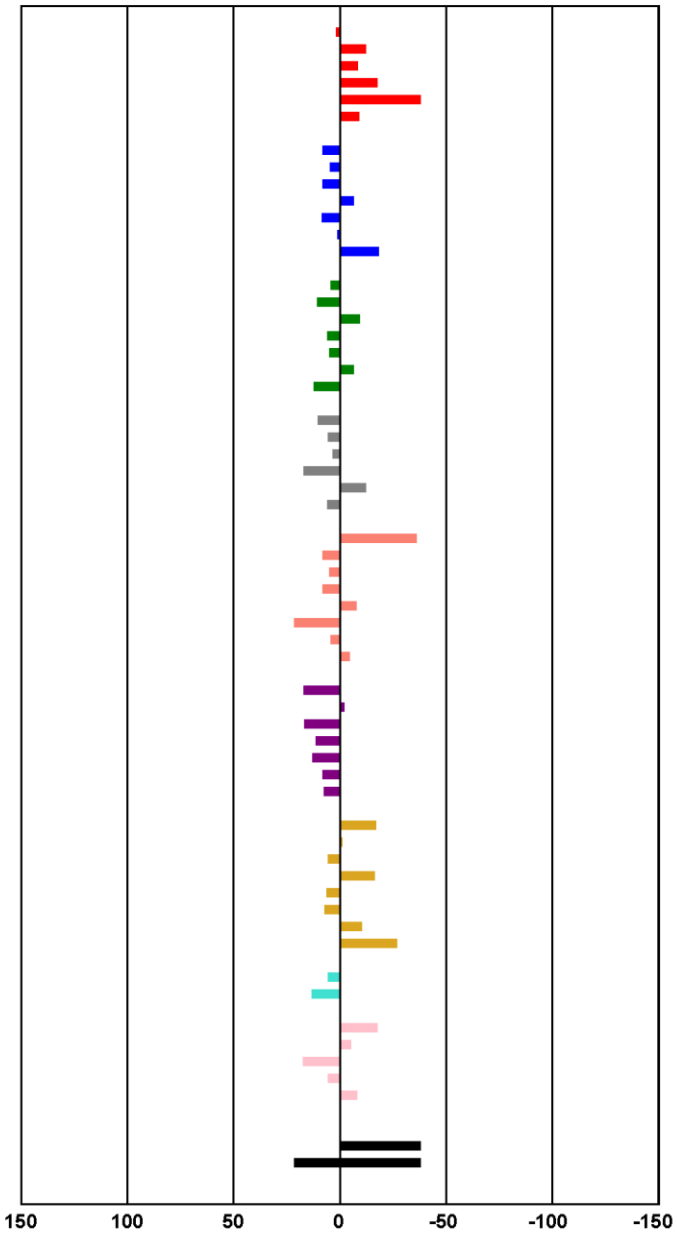



**Developmental Therapeutics Program**

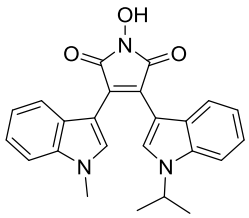
**One Dose Mean Graph**

| NSC: D-775308 / 1 | Conc: 1.00E-5 Molar | Test Date: May 20, 2013 |
|---|---|---|
| Experiment ID: 1305OS96 | | Report Date: Jul 12, 2013 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 95.84 |
| HL-60(TB) | 82.60 |
| K-562 | 86.28 |
| MOLT-4 | 76.99 |
| RPMI-8226 | 56.92 |
| SR | 85.74 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 102.14 |
| HOP-62 | 98.58 |
| NCI-H226 | 102.27 |
| NCI-H23 | 88.20 |
| NCI-H322M | 102.61 |
| NCI-H460 | 95.32 |
| NCI-H522 | 76.41 |
| Colon Cancer | |
| COLO 205 | 98.45 |
| HCC-2998 | 104.76 |
| HCT-116 | 85.44 |
| HCT-15 | 99.89 |
| HT29 | 99.16 |
| KM12 | 88.35 |
| SW-620 | 106.21 |
| CNS Cancer | |
| SF-268 | 104.49 |
| SF-295 | 99.65 |
| SF-539 | 97.33 |
| SNB-19 | 111.21 |
| SNB-75 | 82.61 |
| U251 | 99.97 |
| Melanoma | |
| LOX IMVI | 58.81 |
| MALME-3M | 102.11 |
| M14 | 98.91 |
| MDA-MB-435 | 102.30 |
| SK-MEL-2 | 86.84 |
| SK-MEL-28 | 115.69 |
| SK-MEL-5 | 98.52 |
| UACC-62 | 90.05 |
| Ovarian Cancer | |
| IGROV1 | 111.05 |
| OVCAR-3 | 92.74 |
| OVCAR-4 | 110.72 |
| OVCAR-5 | 105.33 |
| OVCAR-8 | 106.91 |
| NCI/ADR-RES | 102.36 |
| SK-OV-3 | 101.53 |
| Renal Cancer | |
| 786-0 | 77.89 |
| A498 | 93.51 |
| ACHN | 99.59 |
| CAKI-1 | 78.30 |
| RXF 393 | 100.20 |
| SN12C | 101.21 |
| TK-10 | 84.47 |
| UO-31 | 67.94 |
| Prostate Cancer | |
| PC-3 | 99.74 |
| DU-145 | 107.26 |
| Breast Cancer | |
| MCF7 | 77.10 |
| MDA-MB-231/ATCC | 89.55 |
| HS 578T | 111.27 |
| BT-549 | 99.69 |
| T-47D | 86.72 |
| Mean | 94.39 |
| Delta | 37.47 |
| Range | 58.77 |



**1-Hydroxy-3-(1-isopropyl-1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione 291**

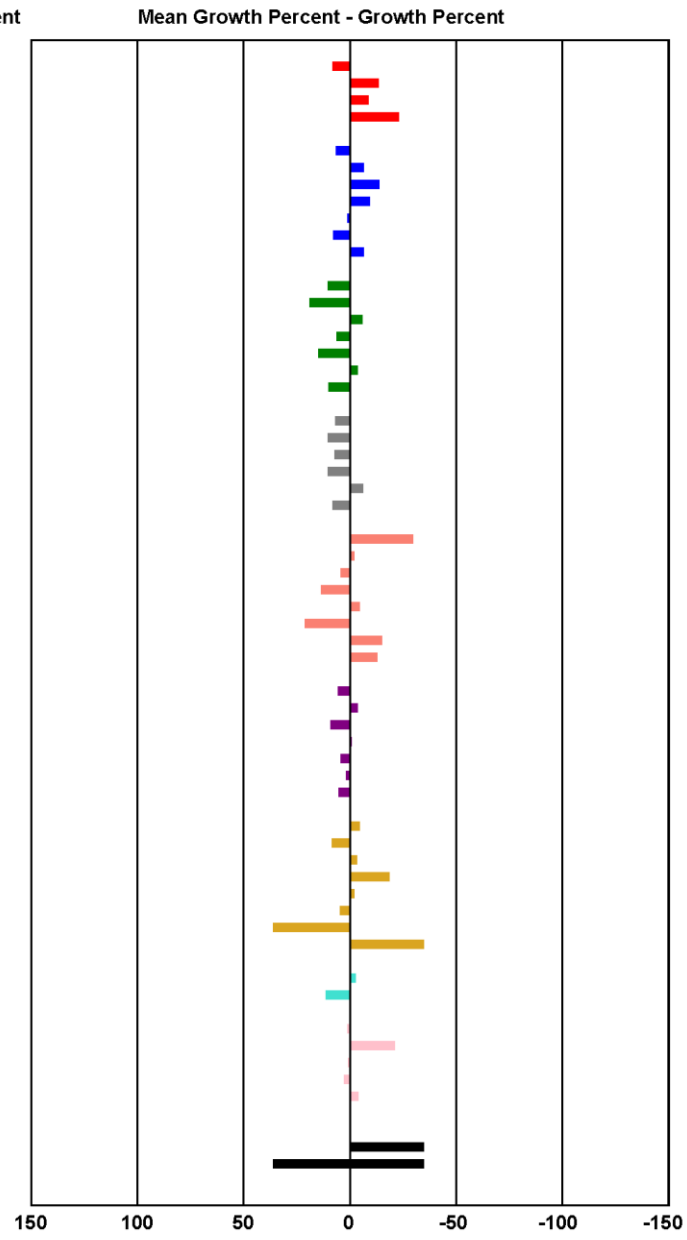



**Developmental Therapeutics Program**

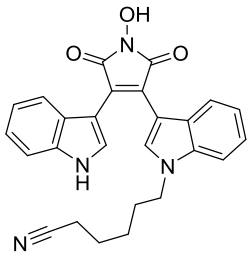
**One Dose Mean Graph**

| NSC: D-775307 / 1 | Conc: 1.00E-5 Molar | Test Date: May 20, 2013 |
|---|---|---|
| Experiment ID: 1305OS96 | | Report Date: Jul 12, 2013 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| HL-60(TB) | 98.66 |
| K-562 | 77.74 |
| MOLT-4 | 82.39 |
| RPMI-8226 | 68.27 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 97.06 |
| HOP-62 | 84.59 |
| NCI-H226 | 77.23 |
| NCI-H23 | 81.76 |
| NCI-H322M | 91.73 |
| NCI-H460 | 98.32 |
| NCI-H522 | 84.69 |
| Colon Cancer | |
| COLO 205 | 100.90 |
| HCC-2998 | 109.60 |
| HCT-116 | 85.38 |
| HCT-15 | 96.89 |
| HT29 | 105.19 |
| KM12 | 87.41 |
| SW-620 | 100.53 |
| CNS Cancer | |
| SF-268 | 97.27 |
| SF-295 | 100.87 |
| SF-539 | 97.62 |
| SNB-19 | 100.97 |
| SNB-75 | 84.93 |
| U251 | 98.78 |
| Melanoma | |
| LOX IMVI | 61.53 |
| MALME-3M | 89.02 |
| M14 | 94.83 |
| MDA-MB-435 | 104.04 |
| SK-MEL-2 | 86.58 |
| SK-MEL-28 | 111.84 |
| SK-MEL-5 | 76.23 |
| UACC-62 | 78.22 |
| Ovarian Cancer | |
| IGROV1 | 96.24 |
| OVCAR-3 | 87.58 |
| OVCAR-4 | 99.63 |
| OVCAR-5 | 90.28 |
| OVCAR-8 | 94.79 |
| NCI/ADR-RES | 92.38 |
| SK-OV-3 | 95.85 |
| Renal Cancer | |
| 786-0 | 86.49 |
| A498 | 99.02 |
| ACHN | 88.00 |
| CAKI-1 | 72.74 |
| RXF 393 | 89.16 |
| SN12C | 95.15 |
| TK-10 | 126.73 |
| UO-31 | 56.43 |
| Prostate Cancer | |
| PC-3 | 88.39 |
| DU-145 | 101.98 |
| Breast Cancer | |
| MCF7 | 91.60 |
| MDA-MB-231/ATCC | 70.11 |
| HS 578T | 91.20 |
| BT-549 | 93.15 |
| T-47D | 87.26 |
| Mean | 90.84 |
| Delta | 34.41 |
| Range | 70.30 |



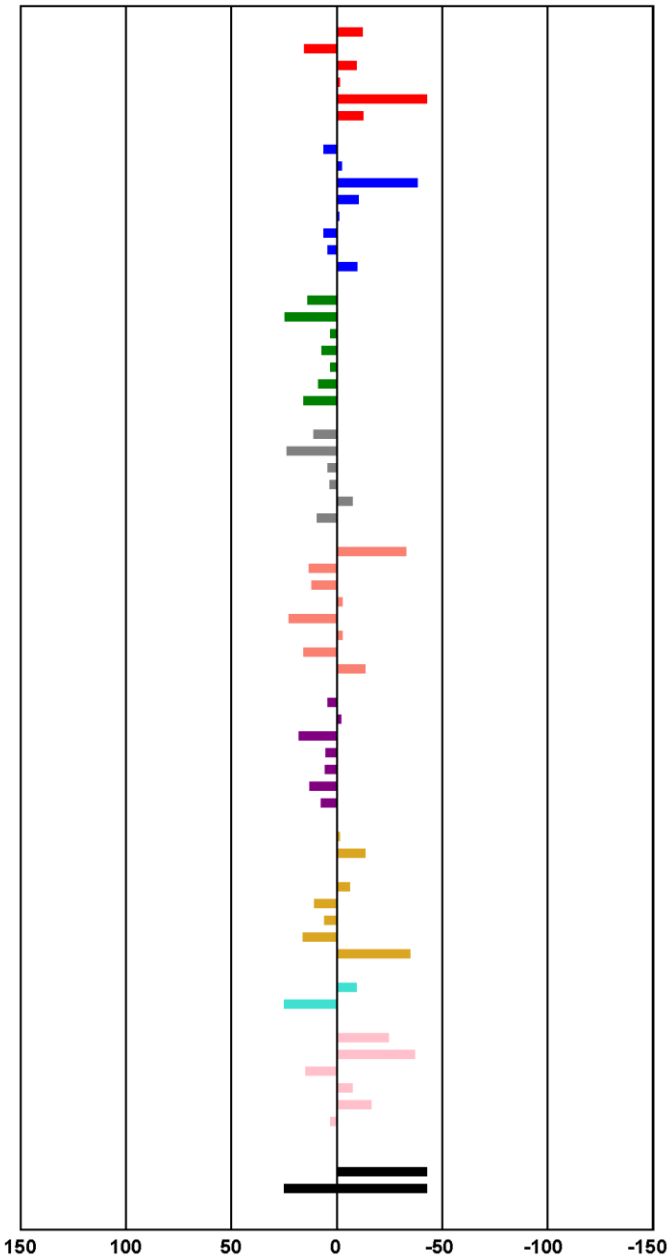
**6-{3-[1-Hydroxy-4-(1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-1*H*-indol-1-yl}hexanenitrile 281**



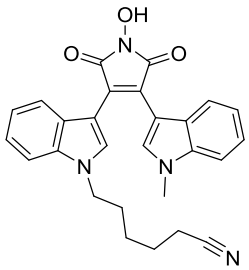

| Developmental Therapeutics Program | NSC: D-776690 / 1 | Conc: 1.00E-5 Molar | Test Date: Jul 22, 2013 |
|---|---|---|---|
| One Dose Mean Graph | Experiment ID: 1307OS38 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 73.84 |
| HL-60(TB) | 101.09 |
| K-562 | 76.96 |
| MOLT-4 | 84.78 |
| RPMI-8226 | 43.47 |
| SR | 73.75 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 91.73 |
| HOP-62 | 83.78 |
| HOP-92 | 47.97 |
| NCI-H226 | 75.83 |
| NCI-H23 | 84.94 |
| NCI-H322M | 91.69 |
| NCI-H460 | 89.87 |
| NCI-H522 | 76.65 |
| Colon Cancer | |
| COLO 205 | 99.41 |
| HCC-2998 | 110.25 |
| HCT-116 | 88.52 |
| HCT-15 | 92.59 |
| HT29 | 88.53 |
| KM12 | 94.19 |
| SW-620 | 101.20 |
| CNS Cancer | |
| SF-268 | 96.64 |
| SF-295 | 109.28 |
| SF-539 | 89.87 |
| SNB-19 | 88.77 |
| SNB-75 | 78.86 |
| U251 | 94.94 |
| Melanoma | |
| LOX IMVI | 53.33 |
| M14 | 98.75 |
| MDA-MB-435 | 97.43 |
| SK-MEL-2 | 83.40 |
| SK-MEL-28 | 108.22 |
| SK-MEL-5 | 83.60 |
| UACC-257 | 101.29 |
| UACC-62 | 72.55 |
| Ovarian Cancer | |
| IGROV1 | 89.68 |
| OVCAR-3 | 84.19 |
| OVCAR-4 | 103.50 |
| OVCAR-5 | 90.87 |
| OVCAR-8 | 91.17 |
| NCI/ADR-RES | 98.40 |
| SK-OV-3 | 93.08 |
| Renal Cancer | |
| 786-0 | 84.68 |
| A498 | 72.72 |
| ACHN | 85.85 |
| CAKI-1 | 80.09 |
| RXF 393 | 96.08 |
| SN12C | 91.28 |
| TK-10 | 101.47 |
| UO-31 | 51.27 |
| Prostate Cancer | |
| PC-3 | 76.79 |
| DU-145 | 110.40 |
| Breast Cancer | |
| MCF7 | 61.41 |
| MDA-MB-231/ATCC | 49.16 |
| HS 578T | 100.37 |
| BT-549 | 78.87 |
| T-47D | 69.81 |
| MDA-MB-468 | 88.62 |
| Mean | 85.82 |
| Delta | 42.35 |
| Range | 66.93 |

Mean Growth Percent - Growth Percent



**6-{3-[1-Hydroxy-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-1*H*-indol-1-yl}hexanenitrile 288**

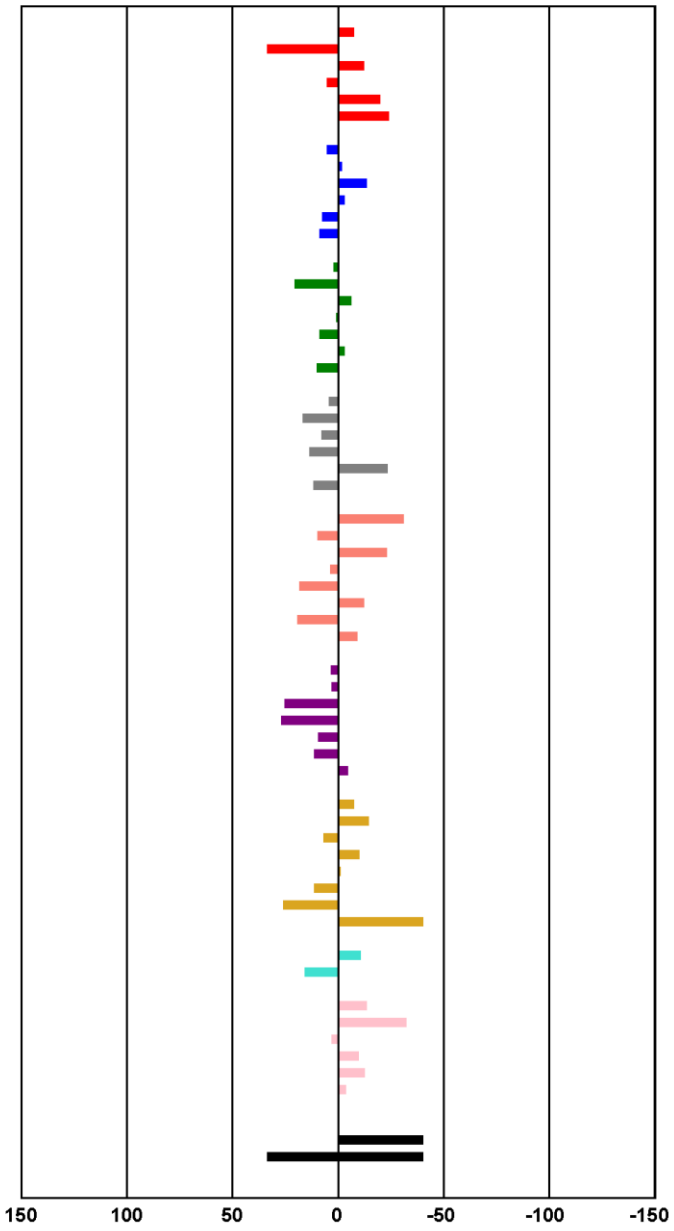



**Developmental Therapeutics Program**

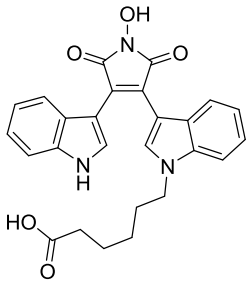
**One Dose Mean Graph**

| NSC: D-776694 / 1 | Conc: 1.00E-5 Molar | Test Date: Jul 22, 2013 |
|---|---|---|
| Experiment ID: 1307OS38 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 83.84 |
| HL-60(TB) | 124.17 |
| K-562 | 78.79 |
| MOLT-4 | 95.69 |
| RPMI-8226 | 71.15 |
| SR | 67.20 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 95.84 |
| HOP-62 | 89.57 |
| NCI-H226 | 77.80 |
| NCI-H23 | 88.11 |
| NCI-H322M | 97.95 |
| NCI-H460 | 99.33 |
| Colon Cancer | |
| COLO 205 | 92.62 |
| HCC-2998 | 110.87 |
| HCT-116 | 85.08 |
| HCT-15 | 91.18 |
| HT29 | 99.10 |
| KM12 | 88.24 |
| SW-620 | 100.55 |
| CNS Cancer | |
| SF-268 | 94.87 |
| SF-295 | 107.24 |
| SF-539 | 98.37 |
| SNB-19 | 104.02 |
| SNB-75 | 67.96 |
| U251 | 102.18 |
| Melanoma | |
| LOX IMVI | 60.16 |
| M14 | 100.17 |
| MDA-MB-435 | 68.10 |
| SK-MEL-2 | 94.13 |
| SK-MEL-28 | 108.72 |
| SK-MEL-5 | 78.85 |
| UACC-257 | 109.66 |
| UACC-62 | 82.00 |
| Ovarian Cancer | |
| IGROV1 | 93.77 |
| OVCAR-3 | 93.61 |
| OVCAR-4 | 115.82 |
| OVCAR-5 | 117.22 |
| OVCAR-8 | 100.05 |
| NCI/ADR-RES | 101.88 |
| SK-OV-3 | 86.50 |
| Renal Cancer | |
| 786-0 | 83.69 |
| A498 | 76.68 |
| ACHN | 97.36 |
| CAKI-1 | 81.32 |
| RXF 393 | 90.05 |
| SN12C | 101.88 |
| TK-10 | 116.50 |
| UO-31 | 50.82 |
| Prostate Cancer | |
| PC-3 | 80.55 |
| DU-145 | 106.12 |
| Breast Cancer | |
| MCF7 | 77.71 |
| MDA-MB-231/ATCC | 58.86 |
| HS 578T | 93.45 |
| BT-549 | 81.36 |
| T-47D | 78.54 |
| MDA-MB-468 | 87.62 |
| Mean | 90.80 |
| Delta | 39.98 |
| Range | 73.35 |



**6-{3-[1-Hydroxy-4-(1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-1*H*-indol-1-yl}hexanoic acid 283**



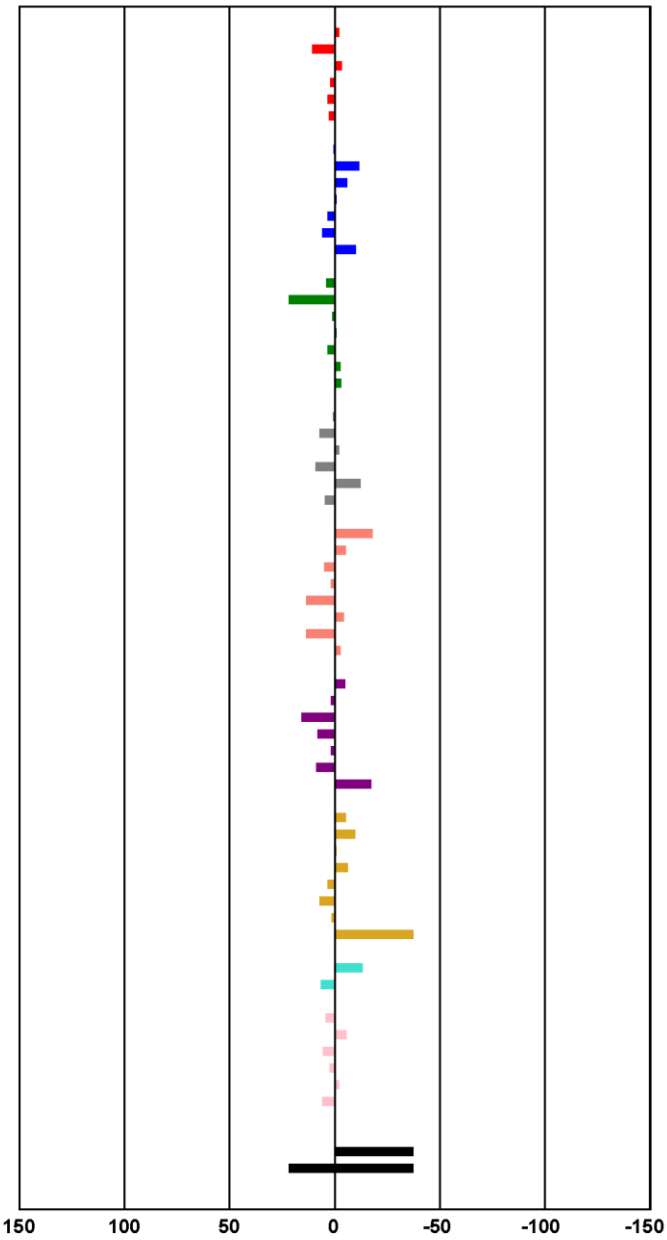

**Developmental Therapeutics Program**

**One Dose Mean Graph**

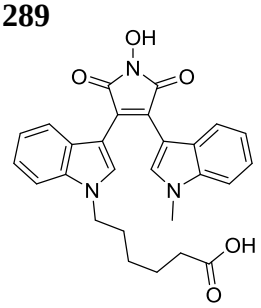
| NSC: D-776692 / 1 | Conc: 1.00E-5 Molar | Test Date: Jul 22, 2013 |
|---|---|---|
| Experiment ID: 1307OS38 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 96.15 |
| HL-60(TB) | 108.19 |
| K-562 | 94.80 |
| MOLT-4 | 99.71 |
| RPMI-8226 | 101.04 |
| SR | 100.25 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 98.16 |
| HOP-62 | 86.79 |
| NCI-H226 | 92.53 |
| NCI-H23 | 97.62 |
| NCI-H322M | 100.89 |
| NCI-H460 | 103.33 |
| NCI-H522 | 88.30 |
| Colon Cancer | |
| COLO 205 | 101.52 |
| HCC-2998 | 119.23 |
| HCT-116 | 98.86 |
| HCT-15 | 97.34 |
| HT29 | 100.93 |
| KM12 | 95.61 |
| SW-620 | 95.32 |
| CNS Cancer | |
| SF-268 | 98.29 |
| SF-295 | 104.72 |
| SF-539 | 96.31 |
| SNB-19 | 106.76 |
| SNB-75 | 85.99 |
| U251 | 102.15 |
| Melanoma | |
| LOX IMVI | 80.41 |
| M14 | 92.93 |
| MDA-MB-435 | 102.39 |
| SK-MEL-2 | 99.41 |
| SK-MEL-28 | 111.10 |
| SK-MEL-5 | 93.90 |
| UACC-257 | 111.19 |
| UACC-62 | 95.66 |
| Ovarian Cancer | |
| IGROV1 | 93.39 |
| OVCAR-3 | 99.26 |
| OVCAR-4 | 113.35 |
| OVCAR-5 | 105.72 |
| OVCAR-8 | 99.41 |
| NCI/ADR-RES | 106.32 |
| SK-OV-3 | 81.04 |
| Renal Cancer | |
| 786-0 | 93.01 |
| A498 | 88.50 |
| ACHN | 97.55 |
| CAKI-1 | 92.19 |
| RXF 393 | 100.98 |
| SN12C | 104.78 |
| TK-10 | 99.02 |
| UO-31 | 61.01 |
| Prostate Cancer | |
| PC-3 | 84.93 |
| DU-145 | 103.97 |
| Breast Cancer | |
| MCF7 | 101.78 |
| MDA-MB-231/ATCC | 92.63 |
| HS 578T | 103.08 |
| BT-549 | 99.92 |
| T-47D | 96.22 |
| MDA-MB-468 | 103.55 |
| Mean | 97.88 |
| Delta | 36.87 |
| Range | 58.22 |

Mean Growth Percent - Growth Percent



**6-{3-[1-Hydroxy-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-1*H*-indol-1-yl}hexanoic acid 289**



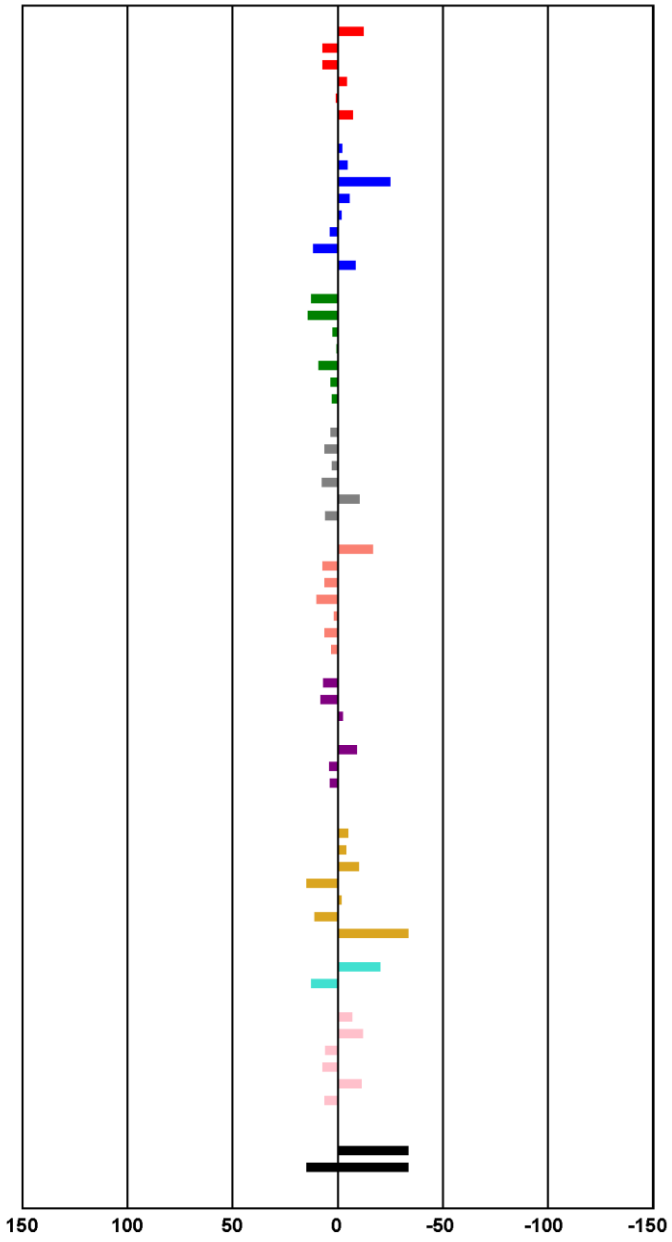

**Developmental Therapeutics Program**

**One Dose Mean Graph**

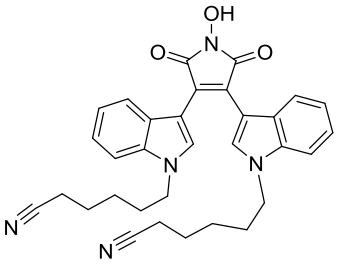
| NSC: D-776691 / 1 | Conc: 1.00E-5 Molar | Test Date: Jul 22, 2013 |
|---|---|---|
| Experiment ID: 1307OS38 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 84.39 |
| HL-60(TB) | 103.10 |
| K-562 | 103.24 |
| MOLT-4 | 92.61 |
| RPMI-8226 | 96.79 |
| SR | 89.64 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 94.60 |
| HOP-62 | 92.02 |
| HOP-92 | 71.82 |
| NCI-H226 | 91.35 |
| NCI-H23 | 95.17 |
| NCI-H322M | 99.75 |
| NCI-H460 | 107.82 |
| NCI-H522 | 88.35 |
| Colon Cancer | |
| COLO 205 | 108.67 |
| HCC-2998 | 110.40 |
| HCT-116 | 98.41 |
| HCT-15 | 96.46 |
| HT29 | 105.09 |
| KM12 | 99.29 |
| SW-620 | 98.94 |
| CNS Cancer | |
| SF-268 | 99.51 |
| SF-295 | 102.27 |
| SF-539 | 98.92 |
| SNB-19 | 103.57 |
| SNB-75 | 86.46 |
| U251 | 102.01 |
| Melanoma | |
| LOX IMVI | 80.05 |
| M14 | 103.28 |
| MDA-MB-435 | 102.29 |
| SK-MEL-28 | 106.25 |
| SK-MEL-5 | 97.82 |
| UACC-257 | 102.24 |
| UACC-62 | 98.97 |
| Ovarian Cancer | |
| IGROV1 | 102.95 |
| OVCAR-3 | 104.34 |
| OVCAR-4 | 94.53 |
| OVCAR-5 | 96.27 |
| OVCAR-8 | 87.74 |
| NCI/ADR-RES | 100.00 |
| SK-OV-3 | 99.75 |
| Renal Cancer | |
| 786-0 | 96.27 |
| A498 | 91.92 |
| ACHN | 92.93 |
| CAKI-1 | 86.78 |
| RXF 393 | 110.77 |
| SN12C | 95.17 |
| TK-10 | 107.06 |
| UO-31 | 63.24 |
| Prostate Cancer | |
| PC-3 | 76.61 |
| DU-145 | 108.65 |
| Breast Cancer | |
| MCF7 | 89.95 |
| MDA-MB-231/ATCC | 84.73 |
| HS 578T | 101.88 |
| BT-549 | 103.21 |
| T-47D | 85.41 |
| MDA-MB-468 | 102.24 |
| Mean | 96.38 |
| Delta | 33.14 |
| Range | 47.53 |

Mean Growth Percent - Growth Percent



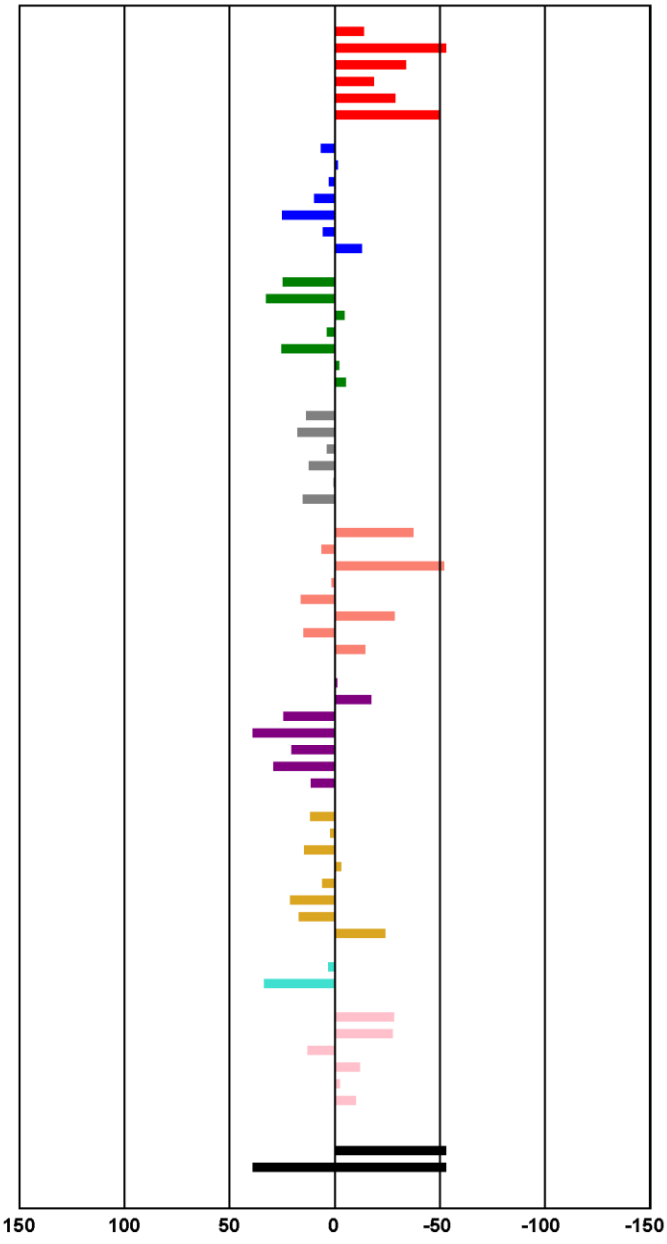
**6,6'-[3,3'-(1-Hydroxy-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile 282**



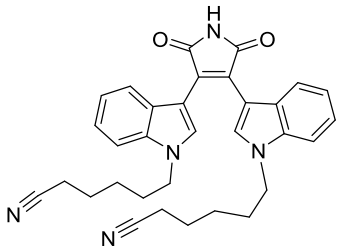

| Developmental Therapeutics Program | NSC: D-776693 / 1 | Conc: 1.00E-5 Molar | Test Date: Jul 22, 2013 |
|---|---|---|---|
| One Dose Mean Graph | Experiment ID: 1307OS38 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 54.19 |
| HL-60(TB) | 15.06 |
| K-562 | 33.87 |
| MOLT-4 | 49.41 |
| RPMI-8226 | 39.13 |
| SR | 17.79 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 73.61 |
| HOP-62 | 66.45 |
| NCI-H226 | 70.04 |
| NCI-H23 | 76.93 |
| NCI-H322M | 92.26 |
| NCI-H460 | 72.68 |
| NCI-H522 | 54.93 |
| Colon Cancer | |
| COLO 205 | 91.72 |
| HCC-2998 | 99.64 |
| HCT-116 | 63.26 |
| HCT-15 | 70.84 |
| HT29 | 92.36 |
| KM12 | 65.71 |
| SW-620 | 62.47 |
| CNS Cancer | |
| SF-268 | 80.59 |
| SF-295 | 84.99 |
| SF-539 | 70.73 |
| SNB-19 | 79.47 |
| SNB-75 | 67.70 |
| U251 | 82.19 |
| Melanoma | |
| LOX IMVI | 30.40 |
| M14 | 73.41 |
| MDA-MB-435 | 15.95 |
| SK-MEL-2 | 68.72 |
| SK-MEL-28 | 83.33 |
| SK-MEL-5 | 39.41 |
| UACC-257 | 82.11 |
| UACC-62 | 53.29 |
| Ovarian Cancer | |
| IGROV1 | 66.84 |
| OVCAR-3 | 50.57 |
| OVCAR-4 | 91.58 |
| OVCAR-5 | 106.29 |
| OVCAR-8 | 87.59 |
| NCI/ADR-RES | 96.29 |
| SK-OV-3 | 78.53 |
| Renal Cancer | |
| 786-0 | 78.74 |
| A498 | 69.16 |
| ACHN | 81.66 |
| CAKI-1 | 64.95 |
| RXF 393 | 73.23 |
| SN12C | 88.25 |
| TK-10 | 84.25 |
| UO-31 | 43.80 |
| Prostate Cancer | |
| PC-3 | 70.31 |
| DU-145 | 100.83 |
| Breast Cancer | |
| MCF7 | 39.87 |
| MDA-MB-231/ATCC | 40.31 |
| HS 578T | 80.04 |
| BT-549 | 56.00 |
| T-47D | 65.55 |
| MDA-MB-468 | 57.99 |
| Mean | 67.50 |
| Delta | 52.44 |
| Range | 91.23 |

Mean Growth Percent - Growth Percent



**6,6'-[3,3'-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile 327**

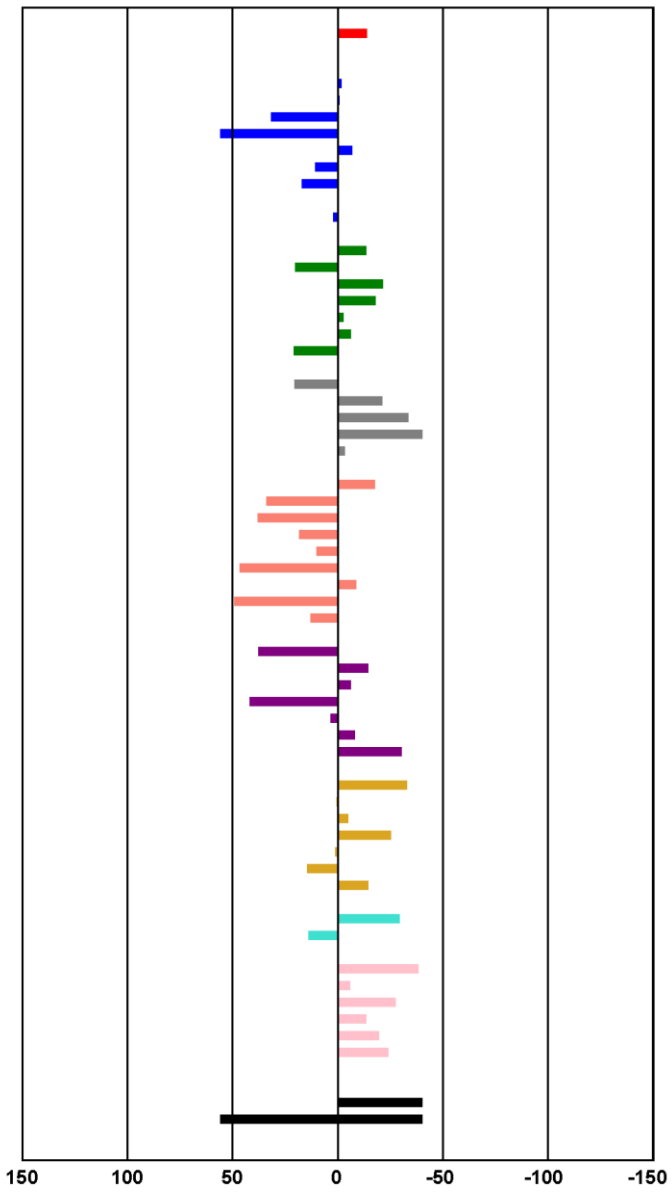



**Developmental Therapeutics Program**

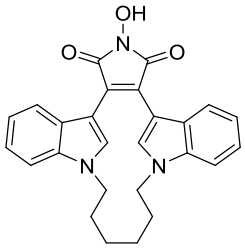
**One Dose Mean Graph**

| | | |
|---|---|---|
| **NSC:** D-781333 / 1 | **Conc:** 1.00E-5 Molar | **Test Date:** Jun 30, 2014 |
| **Experiment ID:** 1406OS12 | | **Report Date:** Jul 25, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 73.46 |
| RPMI-8226 | 86.85 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 85.56 |
| EKVX | 86.46 |
| HOP-62 | 118.17 |
| HOP-92 | 142.48 |
| NCI-H226 | 80.39 |
| NCI-H23 | 97.11 |
| NCI-H322M | 103.47 |
| NCI-H460 | 86.76 |
| NCI-H522 | 88.65 |
| Colon Cancer | |
| COLO 205 | 73.76 |
| HCC-2998 | 106.89 |
| HCT-116 | 65.94 |
| HCT-15 | 69.42 |
| HT29 | 84.40 |
| KM12 | 80.97 |
| SW-620 | 107.55 |
| CNS Cancer | |
| SF-268 | 106.96 |
| SF-295 | 66.22 |
| SF-539 | 53.68 |
| SNB-75 | 47.18 |
| U251 | 83.82 |
| Melanoma | |
| LOX IMVI | 69.49 |
| MALME-3M | 120.60 |
| M14 | 124.53 |
| MDA-MB-435 | 104.84 |
| SK-MEL-2 | 96.46 |
| SK-MEL-28 | 133.02 |
| SK-MEL-5 | 78.67 |
| UACC-257 | 135.59 |
| UACC-62 | 99.31 |
| Ovarian Cancer | |
| IGROV1 | 124.36 |
| OVCAR-3 | 72.65 |
| OVCAR-4 | 81.16 |
| OVCAR-5 | 128.48 |
| OVCAR-8 | 89.88 |
| NCI/ADR-RES | 79.00 |
| SK-OV-3 | 57.03 |
| Renal Cancer | |
| A498 | 54.46 |
| ACHN | 87.04 |
| CAKI-1 | 82.48 |
| RXF 393 | 62.14 |
| SN12C | 87.69 |
| TK-10 | 100.95 |
| UO-31 | 72.65 |
| Prostate Cancer | |
| PC-3 | 57.98 |
| DU-145 | 100.45 |
| Breast Cancer | |
| MCF7 | 48.85 |
| MDA-MB-231/ATCC | 81.38 |
| HS 578T | 59.69 |
| BT-549 | 73.60 |
| T-47D | 67.64 |
| MDA-MB-468 | 63.26 |
| Mean | 86.88 |
| Delta | 39.70 |
| Range | 95.30 |



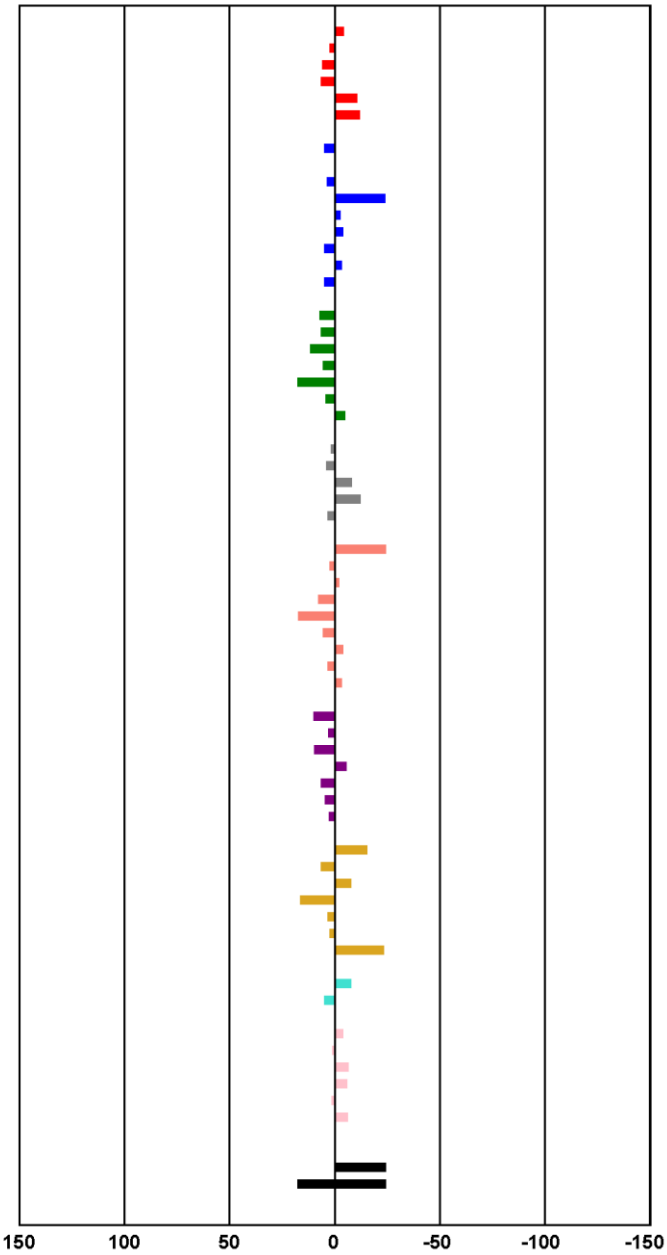
**19-Hydroxy-6,7,8,9,10,11-hexahydro-5,21:12,17-dimetheno-18*H*-dibenzo[*i*,*o*] pyrrolo[3,4-*l*][1,8]diazacyclohexadecine-18,20(19*H*)-dione 284**



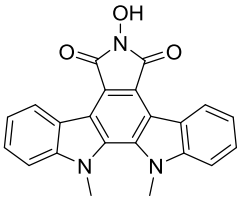

| Developmental Therapeutics Program | NSC: D-781334 / 1 | Conc: 1.00E-5 Molar | Test Date: Jun 30, 2014 |
|---|---|---|---|
| One Dose Mean Graph | Experiment ID: 1406OS12 | | Report Date: Jul 25, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 99.44 |
| HL-60(TB) | 105.36 |
| K-562 | 108.87 |
| MOLT-4 | 109.50 |
| RPMI-8226 | 92.92 |
| SR | 91.89 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 108.01 |
| EKVX | 103.12 |
| HOP-62 | 106.60 |
| HOP-92 | 79.57 |
| NCI-H226 | 100.83 |
| NCI-H23 | 99.66 |
| NCI-H322M | 107.79 |
| NCI-H460 | 100.20 |
| NCI-H522 | 107.90 |
| Colon Cancer | |
| COLO 205 | 110.09 |
| HCC-2998 | 109.50 |
| HCT-116 | 114.69 |
| HCT-15 | 108.42 |
| HT29 | 120.75 |
| KM12 | 107.33 |
| SW-620 | 98.64 |
| CNS Cancer | |
| SF-268 | 104.64 |
| SF-295 | 107.08 |
| SF-539 | 95.69 |
| SNB-75 | 91.36 |
| U251 | 106.23 |
| Melanoma | |
| LOX IMVI | 79.33 |
| MALME-3M | 105.29 |
| M14 | 101.70 |
| MDA-MB-435 | 110.89 |
| SK-MEL-2 | 120.36 |
| SK-MEL-28 | 108.44 |
| SK-MEL-5 | 99.58 |
| UACC-257 | 106.26 |
| UACC-62 | 100.47 |
| Ovarian Cancer | |
| IGROV1 | 113.03 |
| OVCAR-3 | 106.04 |
| OVCAR-4 | 112.80 |
| OVCAR-5 | 98.16 |
| OVCAR-8 | 109.65 |
| NCI/ADR-RES | 107.67 |
| SK-OV-3 | 105.67 |
| Renal Cancer | |
| A498 | 88.34 |
| ACHN | 109.49 |
| CAKI-1 | 95.74 |
| RXF 393 | 119.36 |
| SN12C | 106.20 |
| TK-10 | 105.44 |
| UO-31 | 80.26 |
| Prostate Cancer | |
| PC-3 | 95.78 |
| DU-145 | 107.83 |
| Breast Cancer | |
| MCF7 | 99.57 |
| MDA-MB-231/ATCC | 104.11 |
| HS 578T | 97.24 |
| BT-549 | 97.63 |
| T-47D | 104.46 |
| MDA-MB-468 | 97.47 |
| Mean | 103.28 |
| Delta | 23.95 |
| Range | 41.42 |

Mean Growth Percent - Growth Percent



**6-Hydroxy-12,13-dimethyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 305**

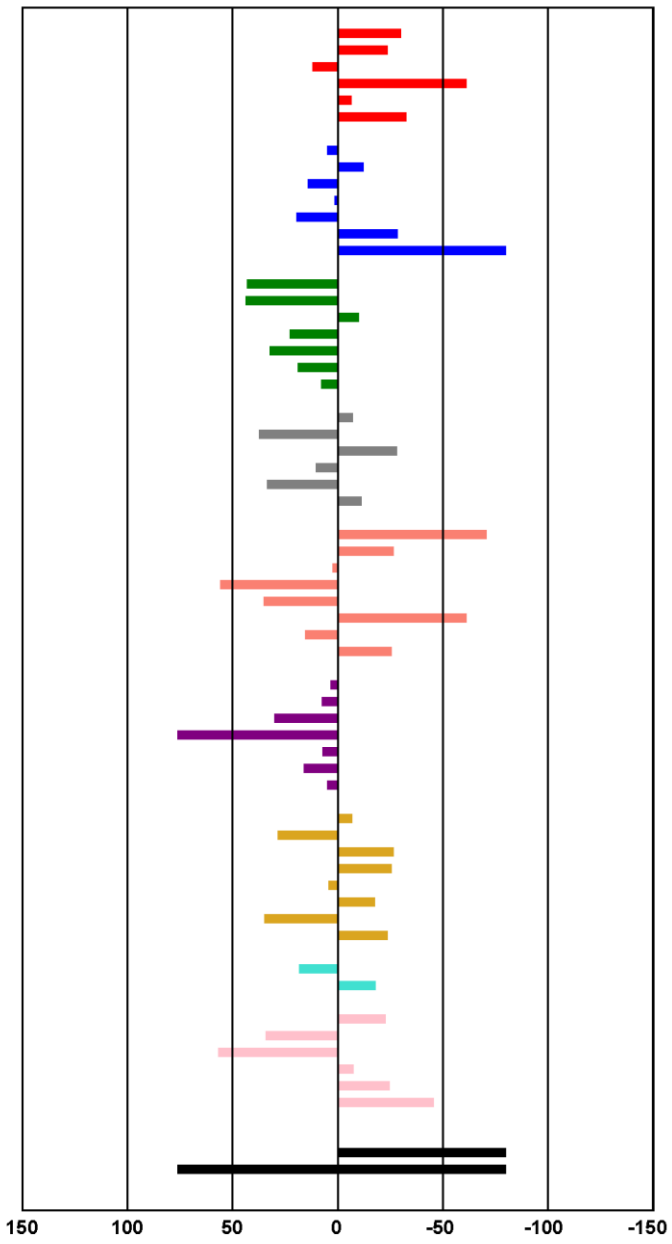



**Developmental Therapeutics Program**

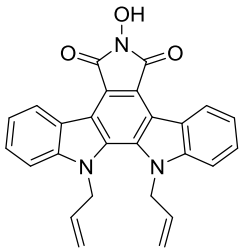
**One Dose Mean Graph**

| NSC: D-776701 / 1 | Conc: 1.00E-5 Molar | Test Date: Jul 22, 2013 |
|---|---|---|
| Experiment ID: 1307OS38 | | Report Date: Oct 29, 2013 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 3.15 |
| HL-60(TB) | 9.78 |
| K-562 | 44.49 |
| MOLT-4 | -28.02 |
| RPMI-8226 | 26.66 |
| SR | 0.60 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 37.47 |
| HOP-62 | 21.18 |
| NCI-H226 | 46.78 |
| NCI-H23 | 34.17 |
| NCI-H322M | 52.21 |
| NCI-H460 | 4.88 |
| NCI-H522 | -46.60 |
| Colon Cancer | |
| COLO 205 | 75.70 |
| HCC-2998 | 76.47 |
| HCT-116 | 23.43 |
| HCT-15 | 55.27 |
| HT29 | 65.03 |
| KM12 | 51.72 |
| SW-620 | 40.49 |
| CNS Cancer | |
| SF-268 | 26.04 |
| SF-295 | 69.96 |
| SF-539 | 5.12 |
| SNB-19 | 42.96 |
| SNB-75 | 66.07 |
| U251 | 22.00 |
| Melanoma | |
| LOX IMVI | -37.35 |
| M14 | 6.91 |
| MDA-MB-435 | 34.97 |
| SK-MEL-2 | 88.47 |
| SK-MEL-28 | 67.89 |
| SK-MEL-5 | -27.77 |
| UACC-257 | 48.07 |
| UACC-62 | 7.72 |
| Ovarian Cancer | |
| IGROV1 | 35.84 |
| OVCAR-3 | 40.24 |
| OVCAR-4 | 62.78 |
| OVCAR-5 | 108.70 |
| OVCAR-8 | 39.88 |
| NCI/ADR-RES | 48.66 |
| SK-OV-3 | 37.62 |
| Renal Cancer | |
| 786-0 | 26.57 |
| A498 | 61.15 |
| ACHN | 6.92 |
| CAKI-1 | 7.62 |
| RXF 393 | 36.89 |
| SN12C | 15.57 |
| TK-10 | 67.49 |
| UO-31 | 9.71 |
| Prostate Cancer | |
| PC-3 | 50.99 |
| DU-145 | 15.47 |
| Breast Cancer | |
| MCF7 | 10.65 |
| MDA-MB-231/ATCC | 66.75 |
| HS 578T | 89.41 |
| BT-549 | 26.00 |
| T-47D | 8.74 |
| MDA-MB-468 | -12.16 |
| Mean | 32.94 |
| Delta | 79.54 |
| Range | 155.30 |



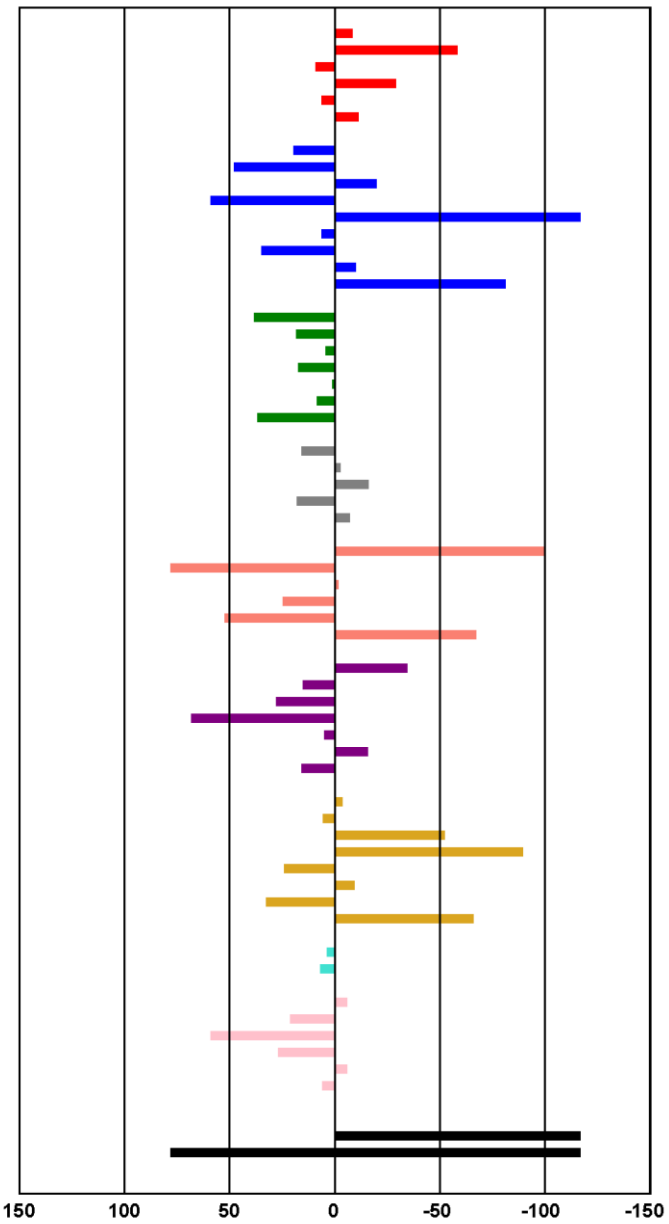
**12,13-Diallyl-6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 306**



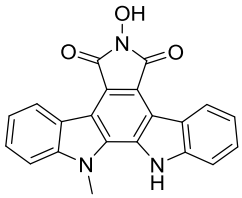

| Developmental Therapeutics Program<br>One Dose Mean Graph | NSC: D-783510 / 1 | Conc: 1.00E-5 Molar | Test Date: Mar 09, 2015 |
|---|---|---|---|
| | Experiment ID: 1503OS44 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 10.22 |
| HL-60(TB) | -39.53 |
| K-562 | 27.28 |
| MOLT-4 | -10.36 |
| RPMI-8226 | 24.39 |
| SR | 7.52 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 37.68 |
| EKVX | 65.99 |
| HOP-62 | -1.11 |
| HOP-92 | 77.24 |
| NCI-H226 | -97.94 |
| NCI-H23 | 24.25 |
| NCI-H322M | 52.78 |
| NCI-H460 | 8.86 |
| NCI-H522 | -62.50 |
| Colon Cancer | |
| COLO 205 | 56.45 |
| HCC-2998 | 36.49 |
| HCT-116 | 22.27 |
| HCT-15 | 35.53 |
| HT29 | 19.18 |
| KM12 | 26.41 |
| SW-620 | 54.92 |
| CNS Cancer | |
| SF-268 | 33.71 |
| SF-295 | 16.20 |
| SF-539 | 2.86 |
| SNB-19 | 36.10 |
| U251 | 11.49 |
| Melanoma | |
| LOX IMVI | -81.21 |
| MALME-3M | 96.12 |
| M14 | 17.18 |
| MDA-MB-435 | 42.67 |
| SK-MEL-2 | 70.41 |
| SK-MEL-5 | -48.49 |
| Ovarian Cancer | |
| IGROV1 | -15.62 |
| OVCAR-3 | 33.17 |
| OVCAR-4 | 45.81 |
| OVCAR-5 | 86.36 |
| OVCAR-8 | 22.94 |
| NCI/ADR-RES | 2.91 |
| SK-OV-3 | 33.90 |
| Renal Cancer | |
| 786-0 | 15.09 |
| A498 | 23.70 |
| ACHN | -33.58 |
| CAKI-1 | -70.64 |
| RXF 393 | 42.22 |
| SN12C | 9.32 |
| TK-10 | 50.79 |
| UO-31 | -47.03 |
| Prostate Cancer | |
| PC-3 | 21.84 |
| DU-145 | 24.85 |
| Breast Cancer | |
| MCF7 | 12.99 |
| MDA-MB-231/ATCC | 39.38 |
| HS 578T | 77.24 |
| BT-549 | 44.95 |
| T-47D | 13.02 |
| MDA-MB-468 | 23.95 |
| Mean | 18.40 |
| Delta | 116.34 |
| Range | 194.06 |

Mean Growth Percent - Growth Percent



**6-Hydroxy-12-methyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 298**

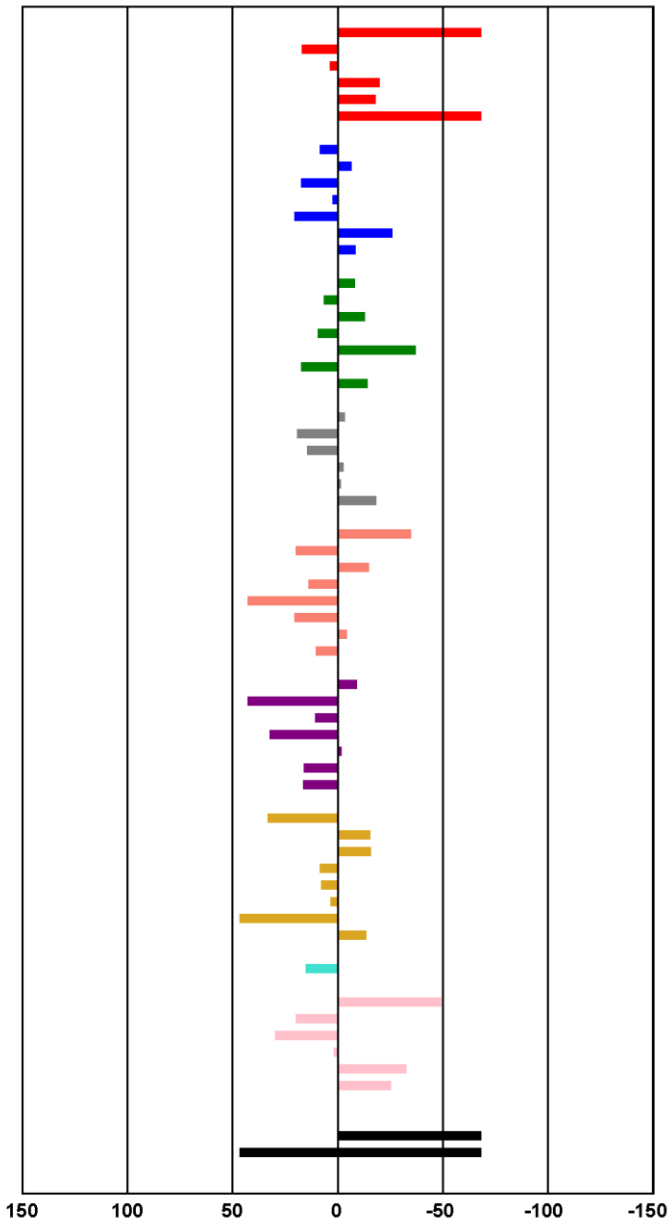



**Developmental Therapeutics Program**

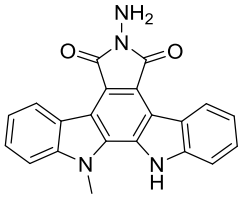
**One Dose Mean Graph**

| NSC: D-774888 / 1 | Conc: 1.00E-5 Molar | Test Date: May 06, 2013 |
|---|---|---|
| Experiment ID: 1305OS83 | | Report Date: Jun 21, 2013 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 8.81 |
| HL-60(TB) | 93.63 |
| K-562 | 80.18 |
| MOLT-4 | 57.26 |
| RPMI-8226 | 59.17 |
| SR | 9.05 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 85.06 |
| HOP-62 | 70.69 |
| HOP-92 | 93.76 |
| NCI-H226 | 78.97 |
| NCI-H322M | 96.94 |
| NCI-H460 | 51.32 |
| NCI-H522 | 68.80 |
| Colon Cancer | |
| COLO 205 | 69.04 |
| HCC-2998 | 83.09 |
| HCT-116 | 64.23 |
| HCT-15 | 85.89 |
| HT29 | 40.16 |
| KM12 | 93.87 |
| SW-620 | 63.09 |
| CNS Cancer | |
| SF-268 | 73.66 |
| SF-295 | 95.83 |
| SF-539 | 90.99 |
| SNB-19 | 74.37 |
| SNB-75 | 75.75 |
| U251 | 59.02 |
| Melanoma | |
| LOX IMVI | 42.47 |
| MALME-3M | 96.26 |
| M14 | 62.46 |
| MDA-MB-435 | 90.29 |
| SK-MEL-2 | 119.34 |
| SK-MEL-28 | 97.15 |
| SK-MEL-5 | 72.90 |
| UACC-257 | 86.74 |
| Ovarian Cancer | |
| IGROV1 | 68.08 |
| OVCAR-3 | 119.16 |
| OVCAR-4 | 87.22 |
| OVCAR-5 | 108.90 |
| OVCAR-8 | 75.44 |
| NCI/ADR-RES | 92.58 |
| SK-OV-3 | 92.87 |
| Renal Cancer | |
| 786-0 | 109.79 |
| A498 | 61.67 |
| ACHN | 61.51 |
| CAKI-1 | 84.88 |
| RXF 393 | 84.38 |
| SN12C | 79.68 |
| TK-10 | 123.05 |
| UO-31 | 63.62 |
| Prostate Cancer | |
| DU-145 | 91.44 |
| Breast Cancer | |
| MCF7 | 28.06 |
| MDA-MB-231/ATCC | 96.22 |
| HS 578T | 106.23 |
| BT-549 | 78.15 |
| T-47D | 44.51 |
| MDA-MB-468 | 51.77 |
| Mean | 76.78 |
| Delta | 67.97 |
| Range | 114.24 |



**6-Amino-12-methyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 299**

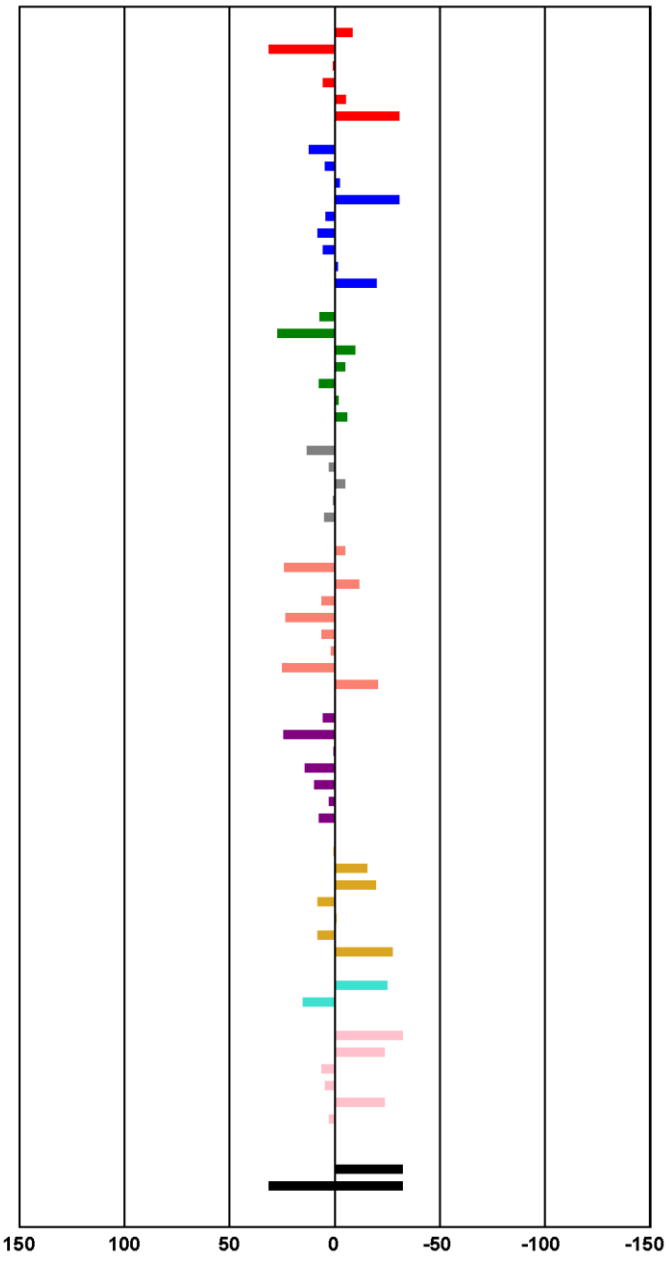



**Developmental Therapeutics Program**

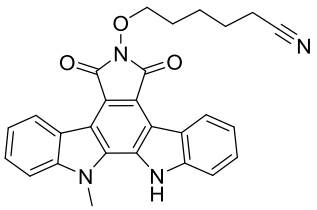
**One Dose Mean Graph**

| NSC: D-781337 / 1 | Conc: 1.00E-5 Molar | Test Date: Jun 30, 2014 |
|---|---|---|
| Experiment ID: 1406OS12 | | Report Date: Jul 25, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 81.13 |
| HL-60(TB) | 120.05 |
| K-562 | 89.56 |
| MOLT-4 | 94.28 |
| RPMI-8226 | 84.09 |
| SR | 58.68 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 100.89 |
| EKVX | 93.37 |
| HOP-62 | 87.03 |
| HOP-92 | 58.74 |
| NCI-H226 | 93.07 |
| NCI-H23 | 96.95 |
| NCI-H322M | 94.23 |
| NCI-H460 | 87.98 |
| NCI-H522 | 69.65 |
| Colon Cancer | |
| COLO 205 | 96.00 |
| HCC-2998 | 116.09 |
| HCT-116 | 79.79 |
| HCT-15 | 84.55 |
| HT29 | 96.26 |
| KM12 | 87.82 |
| SW-620 | 83.42 |
| CNS Cancer | |
| SF-268 | 102.01 |
| SF-295 | 91.40 |
| SF-539 | 84.58 |
| SNB-75 | 89.56 |
| U251 | 93.63 |
| Melanoma | |
| LOX IMVI | 84.39 |
| MALME-3M | 112.69 |
| M14 | 77.93 |
| MDA-MB-435 | 94.92 |
| SK-MEL-2 | 111.97 |
| SK-MEL-28 | 94.92 |
| SK-MEL-5 | 90.38 |
| UACC-257 | 113.80 |
| UACC-62 | 68.89 |
| Ovarian Cancer | |
| IGROV1 | 94.28 |
| OVCAR-3 | 113.20 |
| OVCAR-4 | 89.18 |
| OVCAR-5 | 103.05 |
| OVCAR-8 | 98.51 |
| NCI/ADR-RES | 91.30 |
| SK-OV-3 | 96.36 |
| Renal Cancer | |
| A498 | 89.11 |
| ACHN | 74.02 |
| CAKI-1 | 69.77 |
| RXF 393 | 96.82 |
| SN12C | 88.55 |
| TK-10 | 96.92 |
| UO-31 | 62.01 |
| Prostate Cancer | |
| PC-3 | 64.44 |
| DU-145 | 103.75 |
| Breast Cancer | |
| MCF7 | 57.08 |
| MDA-MB-231/ATCC | 65.65 |
| HS 578T | 95.09 |
| BT-549 | 93.48 |
| T-47D | 65.71 |
| MDA-MB-468 | 91.34 |
| Mean | 89.04 |
| Delta | 31.96 |
| Range | 62.97 |



**6-[(12-Methyl-5,7-dioxo-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-6(7*H*,12*H*,13*H*)-yl)oxy]hexanenitrile 321**

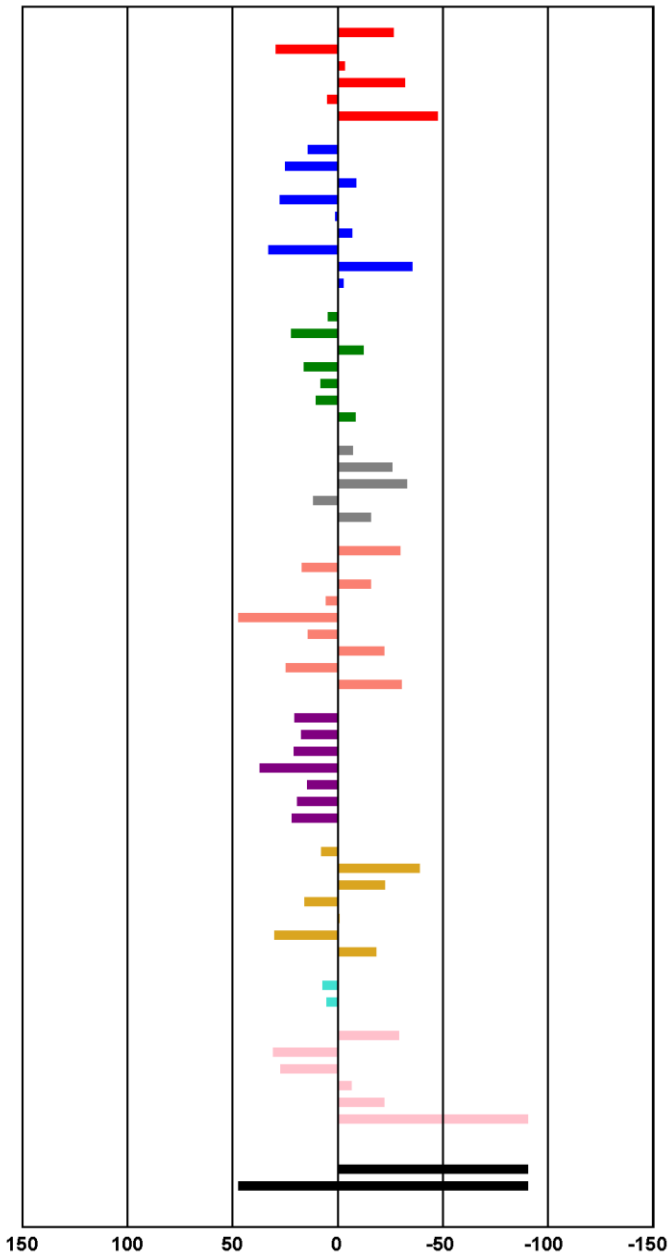


**Developmental Therapeutics Program**

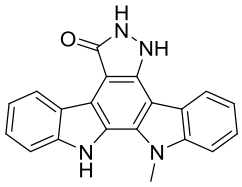
**One Dose Mean Graph**

| NSC: D-781338 / 1 | Conc: 1.00E-5 Molar | Test Date: Jun 30, 2014 |
|---|---|---|
| Experiment ID: 1406OS12 | | Report Date: Jul 25, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 26.42 |
| HL-60(TB) | 81.82 |
| K-562 | 49.65 |
| MOLT-4 | 21.08 |
| RPMI-8226 | 57.22 |
| SR | 5.54 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 66.36 |
| EKVX | 77.32 |
| HOP-62 | 44.34 |
| HOP-92 | 79.97 |
| NCI-H226 | 53.41 |
| NCI-H23 | 46.15 |
| NCI-H322M | 85.15 |
| NCI-H460 | 17.47 |
| NCI-H522 | 50.20 |
| Colon Cancer | |
| COLO 205 | 56.79 |
| HCC-2998 | 74.38 |
| HCT-116 | 40.66 |
| HCT-15 | 68.24 |
| HT29 | 60.29 |
| KM12 | 62.80 |
| SW-620 | 44.71 |
| CNS Cancer | |
| SF-268 | 45.83 |
| SF-295 | 27.11 |
| SF-539 | 20.18 |
| SNB-75 | 63.88 |
| U251 | 37.28 |
| Melanoma | |
| LOX IMVI | 23.39 |
| MALME-3M | 69.17 |
| M14 | 37.18 |
| MDA-MB-435 | 57.93 |
| SK-MEL-2 | 99.67 |
| SK-MEL-28 | 66.52 |
| SK-MEL-5 | 31.00 |
| UACC-257 | 76.81 |
| UACC-62 | 22.62 |
| Ovarian Cancer | |
| IGROV1 | 72.70 |
| OVCAR-3 | 69.56 |
| OVCAR-4 | 73.03 |
| OVCAR-5 | 89.34 |
| OVCAR-8 | 66.72 |
| NCI/ADR-RES | 71.58 |
| SK-OV-3 | 74.01 |
| Renal Cancer | |
| A498 | 59.98 |
| ACHN | 14.19 |
| CAKI-1 | 30.72 |
| RXF 393 | 67.91 |
| SN12C | 52.18 |
| TK-10 | 82.49 |
| UO-31 | 34.80 |
| Prostate Cancer | |
| PC-3 | 59.62 |
| DU-145 | 57.43 |
| Breast Cancer | |
| MCF7 | 23.90 |
| MDA-MB-231/ATCC | 82.85 |
| HS 578T | 79.50 |
| BT-549 | 46.58 |
| T-47D | 30.87 |
| MDA-MB-468 | -37.45 |
| Mean | 52.60 |
| Delta | 90.05 |
| Range | 137.12 |



**13-Methyl-5,6,12,13-tetrahydro-7*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-7-one 360**

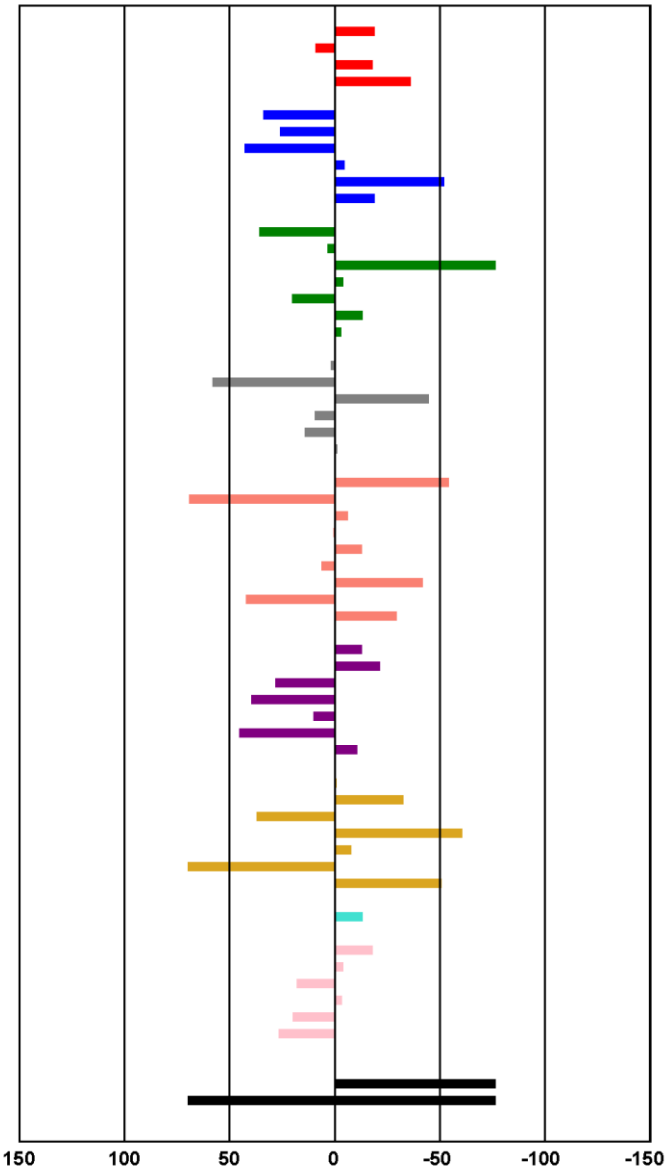


**Developmental Therapeutics Program**

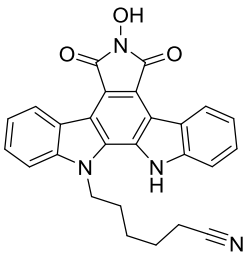
**One Dose Mean Graph**

| NSC: D-785683 / 1 | Conc: 1.00E-5 Molar | Test Date: Jul 27, 2015 |
|---|---|---|
| Experiment ID: 1507OS29 | | Report Date: Aug 14, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| HL-60(TB) | 4.77 |
| K-562 | 32.32 |
| MOLT-4 | 5.90 |
| SR | -12.15 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 56.92 |
| EKVX | 49.04 |
| NCI-H226 | 65.75 |
| NCI-H23 | 19.15 |
| NCI-H460 | -28.21 |
| NCI-H522 | 4.72 |
| Colon Cancer | |
| COLO 205 | 58.98 |
| HCC-2998 | 26.46 |
| HCT-116 | -52.80 |
| HCT-15 | 19.72 |
| HT29 | 43.33 |
| KM12 | 10.74 |
| SW-620 | 20.66 |
| CNS Cancer | |
| SF-268 | 25.02 |
| SF-295 | 81.27 |
| SF-539 | -20.81 |
| SNB-19 | 32.52 |
| SNB-75 | 37.23 |
| U251 | 22.69 |
| Melanoma | |
| LOX IMVI | -30.30 |
| MALME-3M | 92.29 |
| M14 | 17.42 |
| MDA-MB-435 | 23.64 |
| SK-MEL-2 | 10.94 |
| SK-MEL-28 | 29.28 |
| SK-MEL-5 | -18.10 |
| UACC-257 | 65.33 |
| UACC-62 | -5.51 |
| Ovarian Cancer | |
| IGROV1 | 11.04 |
| OVCAR-3 | 2.46 |
| OVCAR-4 | 51.27 |
| OVCAR-5 | 62.63 |
| OVCAR-8 | 33.11 |
| NCI/ADR-RES | 68.45 |
| SK-OV-3 | 13.18 |
| Renal Cancer | |
| 786-0 | 22.95 |
| ACHN | -8.79 |
| CAKI-1 | 60.17 |
| RXF 393 | -36.68 |
| SN12C | 16.13 |
| TK-10 | 92.98 |
| UO-31 | -26.87 |
| Prostate Cancer | |
| DU-145 | 10.53 |
| Breast Cancer | |
| MCF7 | 5.82 |
| MDA-MB-231/ATCC | 19.79 |
| HS 578T | 41.04 |
| BT-549 | 20.49 |
| T-47D | 42.89 |
| MDA-MB-468 | 49.51 |
| Mean | 23.40 |
| Delta | 76.20 |
| Range | 145.78 |



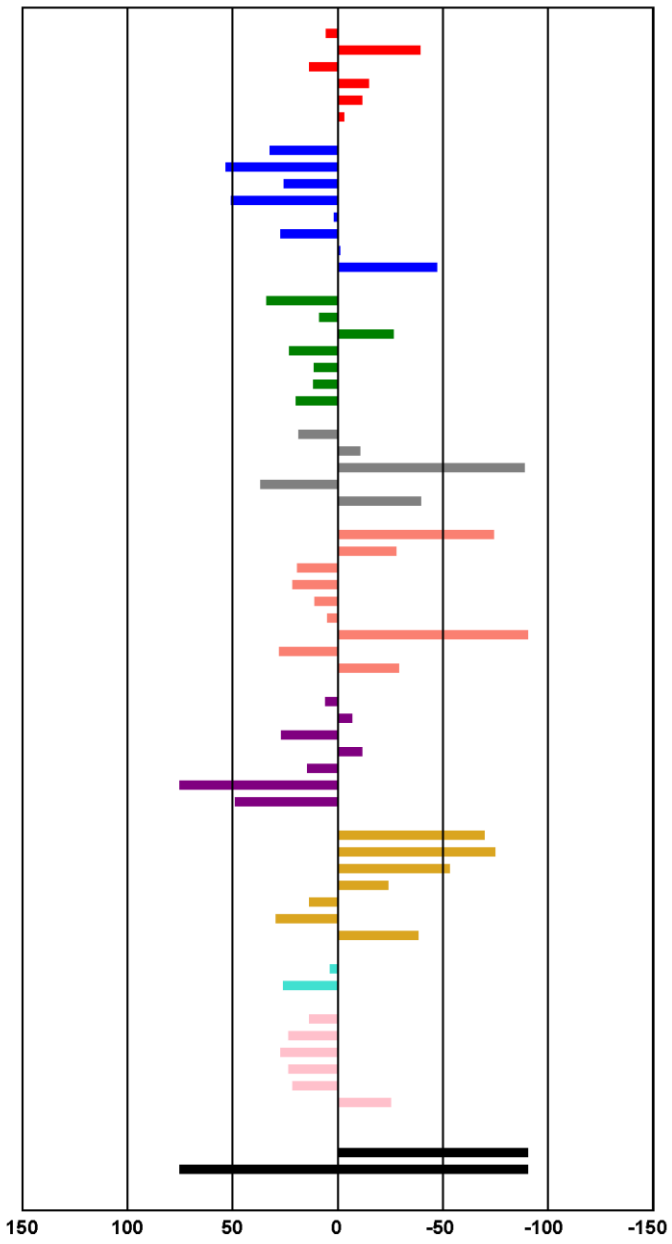
**6-[6-Hydroxy-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12(13*H*)-yl]hexanenitrile 308**



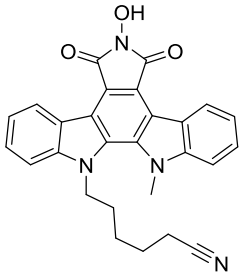

| Developmental Therapeutics Program | NSC: D-781336 / 1 | Conc: 1.00E-5 Molar | Test Date: Jun 30, 2014 |
|---|---|---|---|
| One Dose Mean Graph | Experiment ID: 1406OS12 | | Report Date: Jul 25, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | -1.67 |
| HL-60(TB) | -46.00 |
| K-562 | 6.27 |
| MOLT-4 | -21.30 |
| RPMI-8226 | -18.35 |
| SR | -9.51 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 25.04 |
| EKVX | 46.00 |
| HOP-62 | 18.44 |
| NCI-H226 | 43.33 |
| NCI-H23 | -5.54 |
| NCI-H322M | 19.95 |
| NCI-H460 | -7.73 |
| NCI-H522 | -53.89 |
| Colon Cancer | |
| COLO 205 | 26.69 |
| HCC-2998 | 1.36 |
| HCT-116 | -33.25 |
| HCT-15 | 15.88 |
| HT29 | 3.99 |
| KM12 | 4.16 |
| SW-620 | 12.51 |
| CNS Cancer | |
| SF-268 | 11.33 |
| SF-295 | -17.19 |
| SF-539 | -95.42 |
| SNB-75 | 29.38 |
| U251 | -46.19 |
| Melanoma | |
| LOX IMVI | -80.96 |
| MALME-3M | -34.59 |
| M14 | 11.95 |
| MDA-MB-435 | 14.28 |
| SK-MEL-2 | 3.52 |
| SK-MEL-28 | -2.36 |
| SK-MEL-5 | -97.00 |
| UACC-257 | 20.48 |
| UACC-62 | -35.71 |
| Ovarian Cancer | |
| IGROV1 | -1.36 |
| OVCAR-3 | -13.37 |
| OVCAR-4 | 19.48 |
| OVCAR-5 | -18.30 |
| OVCAR-8 | 7.06 |
| NCI/ADR-RES | 67.88 |
| SK-OV-3 | 41.35 |
| Renal Cancer | |
| A498 | -76.30 |
| ACHN | -81.62 |
| CAKI-1 | -59.91 |
| RXF 393 | -30.66 |
| SN12C | 6.13 |
| TK-10 | 22.04 |
| UO-31 | -44.89 |
| Prostate Cancer | |
| PC-3 | -3.49 |
| DU-145 | 18.54 |
| Breast Cancer | |
| MCF7 | 6.24 |
| MDA-MB-231/ATCC | 16.06 |
| HS 578T | 19.78 |
| BT-549 | 16.13 |
| T-47D | 14.22 |
| MDA-MB-468 | -32.03 |
| Mean | -7.00 |
| Delta | 90.00 |
| Range | 164.88 |

Mean Growth Percent - Growth Percent



**6-[6-Hydroxy-13-methyl-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12(13*H*)-yl]hexanenitrile 322**



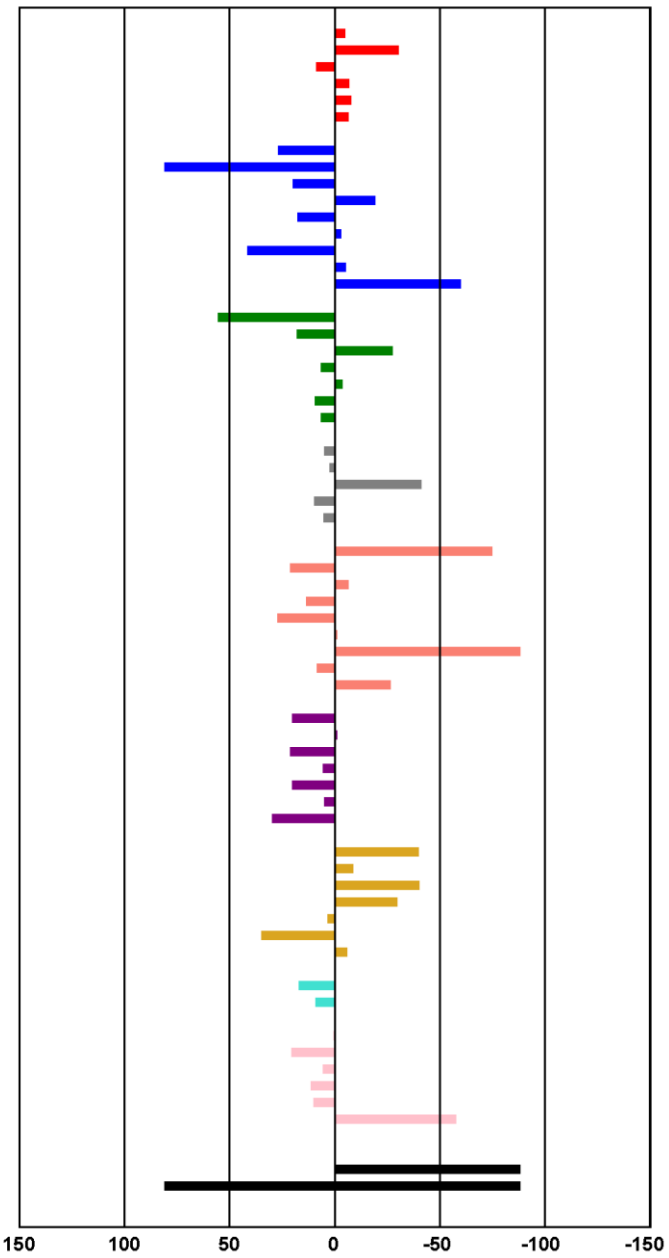

**Developmental Therapeutics Program**

**One Dose Mean Graph**

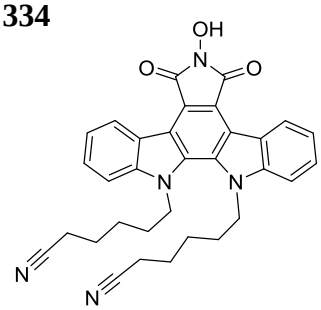
| NSC: D-781335 / 1 | Conc: 1.00E-5 Molar | Test Date: Jun 30, 2014 |
|---|---|---|
| Experiment ID: 1406OS12 | | Report Date: Jul 25, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 3.22 |
| HL-60(TB) | -22.27 |
| K-562 | 16.21 |
| MOLT-4 | 1.13 |
| RPMI-8226 | 0.13 |
| SR | 1.39 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 34.15 |
| EKVX | 88.19 |
| HOP-62 | 27.18 |
| HOP-92 | -11.17 |
| NCI-H226 | 25.11 |
| NCI-H23 | 4.85 |
| NCI-H322M | 48.83 |
| NCI-H460 | 2.84 |
| NCI-H522 | -51.92 |
| Colon Cancer | |
| COLO 205 | 62.77 |
| HCC-2998 | 25.34 |
| HCT-116 | -19.37 |
| HCT-15 | 13.85 |
| HT29 | 4.37 |
| KM12 | 16.59 |
| SW-620 | 14.01 |
| CNS Cancer | |
| SF-268 | 12.19 |
| SF-295 | 9.59 |
| SF-539 | -33.00 |
| SNB-75 | 17.19 |
| U251 | 12.75 |
| Melanoma | |
| LOX IMVI | -66.73 |
| MALME-3M | 28.57 |
| M14 | 1.43 |
| MDA-MB-435 | 20.90 |
| SK-MEL-2 | 34.48 |
| SK-MEL-28 | 6.74 |
| SK-MEL-5 | -80.30 |
| UACC-257 | 15.62 |
| UACC-62 | -18.49 |
| Ovarian Cancer | |
| IGROV1 | 27.58 |
| OVCAR-3 | 6.92 |
| OVCAR-4 | 28.40 |
| OVCAR-5 | 12.77 |
| OVCAR-8 | 27.52 |
| NCI/ADR-RES | 12.21 |
| SK-OV-3 | 37.07 |
| Renal Cancer | |
| A498 | -32.01 |
| ACHN | -0.62 |
| CAKI-1 | -32.31 |
| RXF 393 | -21.78 |
| SN12C | 10.84 |
| TK-10 | 42.07 |
| UO-31 | 2.03 |
| Prostate Cancer | |
| PC-3 | 24.25 |
| DU-145 | 16.34 |
| Breast Cancer | |
| MCF7 | 7.79 |
| MDA-MB-231/ATCC | 27.92 |
| HS 578T | 13.01 |
| BT-549 | 18.51 |
| T-47D | 17.42 |
| MDA-MB-468 | -49.55 |
| Mean | 7.63 |
| Delta | 87.93 |
| Range | 168.49 |

Mean Growth Percent - Growth Percent



**6,6'-(6-Hydroxy-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-12,13-diyl)dihexanenitrile 334**



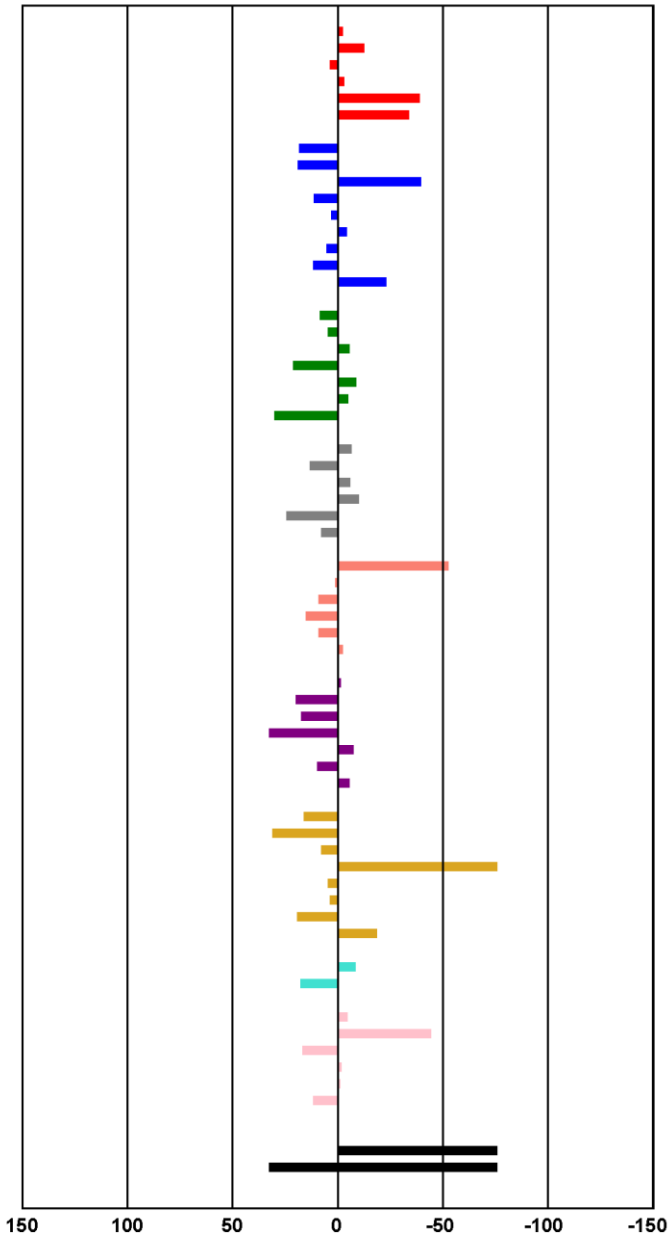

**Developmental Therapeutics Program**

**One Dose Mean Graph**

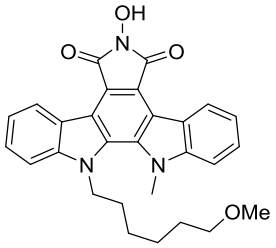
| NSC: D-783507 / 1 | Conc: 1.00E-5 Molar | Test Date: Mar 09, 2015 |
|---|---|---|
| Experiment ID: 1503OS44 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 74.46 |
| HL-60(TB) | 64.28 |
| K-562 | 79.73 |
| MOLT-4 | 73.79 |
| RPMI-8226 | 37.75 |
| SR | 42.89 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 94.52 |
| EKVX | 94.98 |
| HOP-62 | 37.14 |
| HOP-92 | 87.40 |
| NCI-H226 | 79.07 |
| NCI-H23 | 72.49 |
| NCI-H322M | 81.49 |
| NCI-H460 | 87.74 |
| NCI-H522 | 53.72 |
| Colon Cancer | |
| COLO 205 | 84.63 |
| HCC-2998 | 80.80 |
| HCT-116 | 71.32 |
| HCT-15 | 97.30 |
| HT29 | 67.98 |
| KM12 | 71.80 |
| SW-620 | 106.06 |
| CNS Cancer | |
| SF-268 | 70.31 |
| SF-295 | 89.16 |
| SF-539 | 71.04 |
| SNB-19 | 66.65 |
| SNB-75 | 100.32 |
| U251 | 83.82 |
| Melanoma | |
| LOX IMVI | 24.05 |
| MALME-3M | 77.11 |
| M14 | 85.27 |
| MDA-MB-435 | 91.30 |
| SK-MEL-2 | 85.04 |
| SK-MEL-5 | 74.24 |
| Ovarian Cancer | |
| IGROV1 | 75.20 |
| OVCAR-3 | 96.10 |
| OVCAR-4 | 93.43 |
| OVCAR-5 | 108.60 |
| OVCAR-8 | 69.43 |
| NCI/ADR-RES | 85.88 |
| SK-OV-3 | 71.15 |
| Renal Cancer | |
| 786-0 | 92.08 |
| A498 | 107.01 |
| ACHN | 83.85 |
| CAKI-1 | 0.85 |
| RXF 393 | 80.75 |
| SN12C | 79.84 |
| TK-10 | 95.35 |
| UO-31 | 58.22 |
| Prostate Cancer | |
| PC-3 | 68.47 |
| DU-145 | 93.63 |
| Breast Cancer | |
| MCF7 | 72.21 |
| MDA-MB-231/ATCC | 32.31 |
| HS 578T | 92.67 |
| BT-549 | 75.03 |
| T-47D | 75.75 |
| MDA-MB-468 | 87.76 |
| Mean | 76.41 |
| Delta | 75.56 |
| Range | 107.75 |

Mean Growth Percent - Growth Percent



**6-Hydroxy-12-(6-methoxyhexyl)-13-methyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 343**



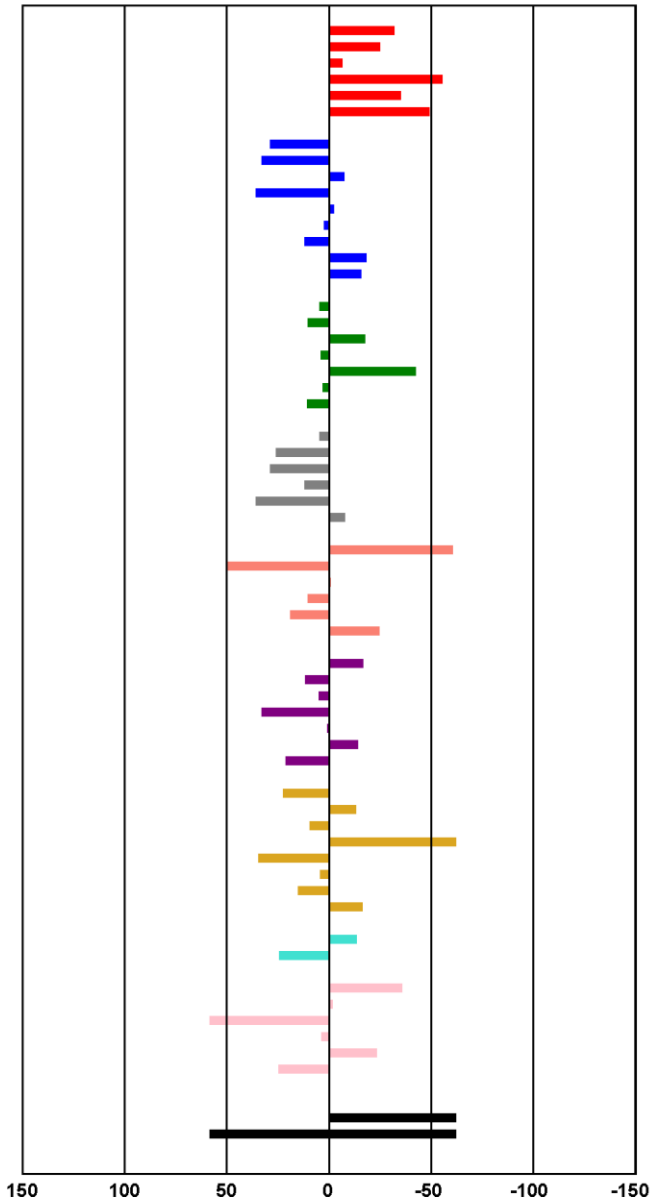

**Developmental Therapeutics Program**

**One Dose Mean Graph**

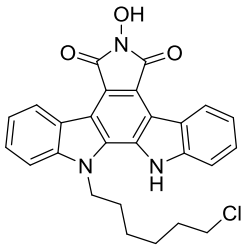
| NSC: D-783508 / 1 | Conc: 1.00E-5 Molar | Test Date: Mar 09, 2015 |
|---|---|---|
| Experiment ID: 1503OS44 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 34.32 |
| HL-60(TB) | 41.21 |
| K-562 | 59.72 |
| MOLT-4 | 10.88 |
| RPMI-8226 | 31.21 |
| SR | 17.05 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 94.47 |
| EKVX | 98.58 |
| HOP-62 | 58.71 |
| HOP-92 | 101.25 |
| NCI-H226 | 63.75 |
| NCI-H23 | 68.02 |
| NCI-H322M | 77.40 |
| NCI-H460 | 48.06 |
| NCI-H522 | 50.43 |
| Colon Cancer | |
| COLO 205 | 70.13 |
| HCC-2998 | 76.00 |
| HCT-116 | 48.56 |
| HCT-15 | 69.48 |
| HT29 | 23.73 |
| KM12 | 68.76 |
| SW-620 | 76.39 |
| CNS Cancer | |
| SF-268 | 70.15 |
| SF-295 | 91.47 |
| SF-539 | 94.31 |
| SNB-19 | 77.51 |
| SNB-75 | 101.33 |
| U251 | 58.37 |
| Melanoma | |
| LOX IMVI | 5.80 |
| MALME-3M | 115.26 |
| M14 | 65.37 |
| MDA-MB-435 | 75.90 |
| SK-MEL-2 | 84.63 |
| SK-MEL-5 | 41.65 |
| Ovarian Cancer | |
| IGROV1 | 49.58 |
| OVCAR-3 | 77.20 |
| OVCAR-4 | 70.47 |
| OVCAR-5 | 98.57 |
| OVCAR-8 | 66.29 |
| NCI/ADR-RES | 52.09 |
| SK-OV-3 | 86.88 |
| Renal Cancer | |
| 786-0 | 88.02 |
| A498 | 52.93 |
| ACHN | 74.87 |
| CAKI-1 | 4.21 |
| RXF 393 | 100.21 |
| SN12C | 70.00 |
| TK-10 | 80.60 |
| UO-31 | 49.98 |
| Prostate Cancer | |
| PC-3 | 52.88 |
| DU-145 | 89.95 |
| Breast Cancer | |
| MCF7 | 30.44 |
| MDA-MB-231/ATCC | 64.46 |
| HS 578T | 123.81 |
| BT-549 | 69.23 |
| T-47D | 42.92 |
| MDA-MB-468 | 90.38 |
| Mean | 65.89 |
| Delta | 61.68 |
| Range | 119.60 |

Mean Growth Percent - Growth Percent



## 12-(6-Chlorohexyl)-6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 336



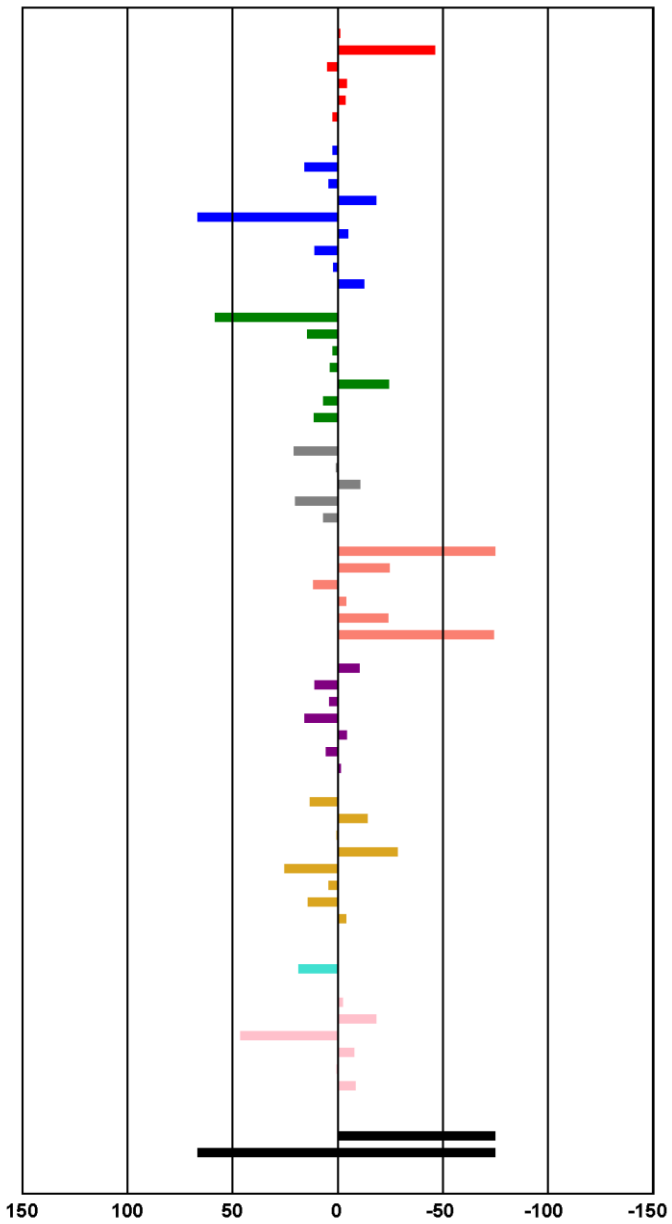

**Developmental Therapeutics Program**

**One Dose Mean Graph**

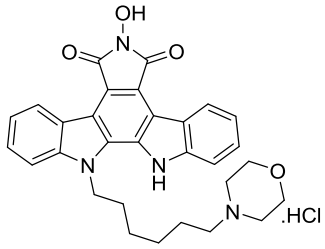
| NSC: D-783509 / 1 | Conc: 1.00E-5 Molar | Test Date: Mar 09, 2015 |
|---|---|---|
| Experiment ID: 1503OS44 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 3.53 |
| HL-60(TB) | -41.74 |
| K-562 | 8.97 |
| MOLT-4 | 0.34 |
| RPMI-8226 | 1.01 |
| SR | 6.48 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 6.36 |
| EKVX | 19.82 |
| HOP-62 | 8.33 |
| HOP-92 | -13.56 |
| NCI-H226 | 70.49 |
| NCI-H23 | -0.17 |
| NCI-H322M | 14.81 |
| NCI-H460 | 5.88 |
| NCI-H522 | -7.94 |
| Colon Cancer | |
| COLO 205 | 62.41 |
| HCC-2998 | 18.35 |
| HCT-116 | 6.41 |
| HCT-15 | 7.75 |
| HT29 | -19.65 |
| KM12 | 10.85 |
| SW-620 | 15.12 |
| CNS Cancer | |
| SF-268 | 24.66 |
| SF-295 | 4.87 |
| SF-539 | -6.00 |
| SNB-19 | 24.22 |
| U251 | 10.87 |
| Melanoma | |
| LOX IMVI | -70.23 |
| MALME-3M | -19.98 |
| M14 | 15.55 |
| MDA-MB-435 | 0.62 |
| SK-MEL-2 | -19.42 |
| SK-MEL-5 | -69.53 |
| Ovarian Cancer | |
| IGROV1 | -5.71 |
| OVCAR-3 | 14.96 |
| OVCAR-4 | 7.84 |
| OVCAR-5 | 19.77 |
| OVCAR-8 | 0.34 |
| NCI/ADR-RES | 9.64 |
| SK-OV-3 | 3.04 |
| Renal Cancer | |
| 786-0 | 17.29 |
| A498 | -9.63 |
| ACHN | 4.33 |
| CAKI-1 | -23.72 |
| RXF 393 | 29.10 |
| SN12C | 8.36 |
| TK-10 | 18.15 |
| UO-31 | 0.63 |
| Prostate Cancer | |
| PC-3 | 4.24 |
| DU-145 | 22.60 |
| Breast Cancer | |
| MCF7 | 2.38 |
| MDA-MB-231/ATCC | -13.76 |
| HS 578T | 50.28 |
| BT-549 | -3.13 |
| T-47D | 4.32 |
| MDA-MB-468 | -3.78 |
| Mean | 4.23 |
| Delta | 74.46 |
| Range | 140.72 |

Mean Growth Percent - Growth Percent



**4-[6-(6-hydroxy-5,7-dioxo-5,6,7,13-tetrahydro-12*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12-yl)hexyl]morpholin-4-ium chloride 338**

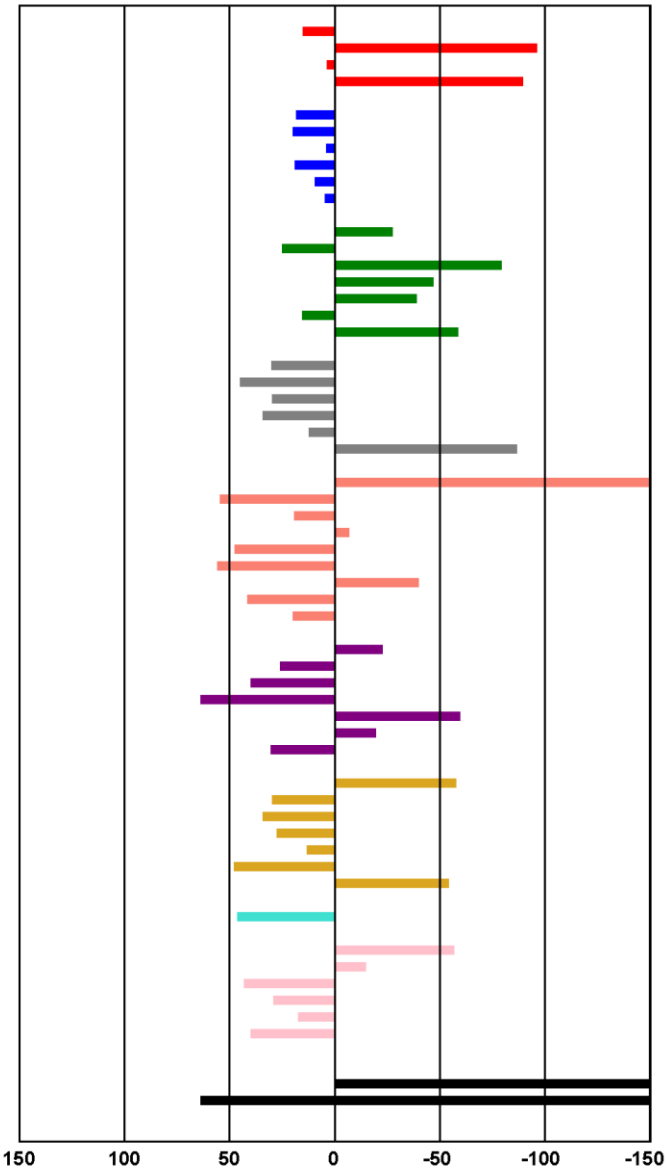



**Developmental Therapeutics Program**

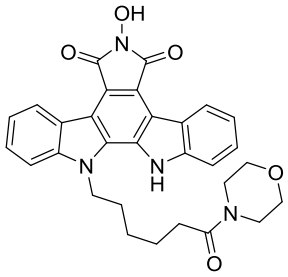
**One Dose Mean Graph**

| NSC: D-785682 / 1 | Conc: 1.00E-5 Molar | Test Date: Jul 27, 2015 |
|---|---|---|
| Experiment ID: 1507OS29 | | Report Date: Aug 14, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| HL-60(TB) | 77.71 |
| K-562 | -32.87 |
| MOLT-4 | 66.26 |
| SR | -26.15 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 81.07 |
| EKVX | 82.44 |
| NCI-H226 | 66.75 |
| NCI-H23 | 81.53 |
| NCI-H460 | 72.17 |
| NCI-H522 | 67.27 |
| Colon Cancer | |
| COLO 205 | 35.96 |
| HCC-2998 | 87.76 |
| HCT-116 | -15.95 |
| HCT-15 | 16.46 |
| HT29 | 24.34 |
| KM12 | 78.01 |
| SW-620 | 4.79 |
| CNS Cancer | |
| SF-268 | 92.87 |
| SF-295 | 107.54 |
| SF-539 | 92.43 |
| SNB-19 | 96.97 |
| SNB-75 | 74.94 |
| U251 | -23.24 |
| Melanoma | |
| LOX IMVI | -88.40 |
| MALME-3M | 117.11 |
| M14 | 81.92 |
| MDA-MB-435 | 56.40 |
| SK-MEL-2 | 110.09 |
| SK-MEL-28 | 118.39 |
| SK-MEL-5 | 23.44 |
| UACC-257 | 104.16 |
| UACC-62 | 82.74 |
| Ovarian Cancer | |
| IGROV1 | 40.58 |
| OVCAR-3 | 88.70 |
| OVCAR-4 | 102.60 |
| OVCAR-5 | 126.51 |
| OVCAR-8 | 3.72 |
| NCI/ADR-RES | 43.91 |
| SK-OV-3 | 93.01 |
| Renal Cancer | |
| 786-0 | 5.72 |
| ACHN | 92.59 |
| CAKI-1 | 96.75 |
| RXF 393 | 90.26 |
| SN12C | 75.85 |
| TK-10 | 110.51 |
| UO-31 | 9.13 |
| Prostate Cancer | |
| DU-145 | 109.11 |
| Breast Cancer | |
| MCF7 | 6.49 |
| MDA-MB-231/ATCC | 48.47 |
| HS 578T | 105.74 |
| BT-549 | 91.95 |
| T-47D | 79.92 |
| MDA-MB-468 | 102.72 |
| Mean | 63.00 |
| Delta | 151.40 |
| Range | 214.91 |



**6-Hydroxy-12-(6-morpholino-6-oxohexyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 314**

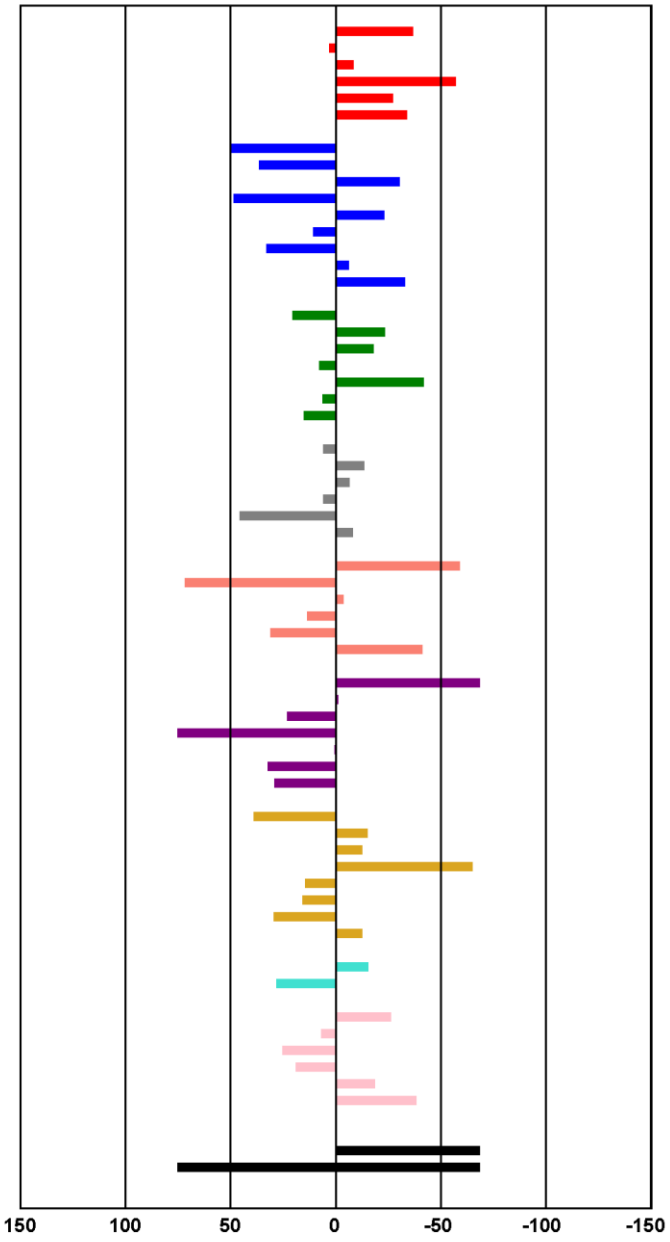



**Developmental Therapeutics Program**

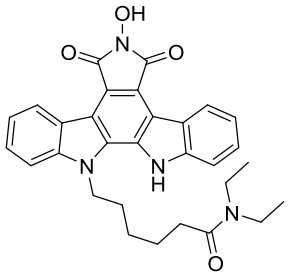
**One Dose Mean Graph**

| NSC: D-783514 / 1 | Conc: 1.00E-5 Molar | Test Date: Mar 09, 2015 |
|---|---|---|
| Experiment ID: 1503OS44 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 5.19 |
| HL-60(TB) | 44.58 |
| K-562 | 33.56 |
| MOLT-4 | -14.88 |
| RPMI-8226 | 14.88 |
| SR | 8.26 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 91.37 |
| EKVX | 77.66 |
| HOP-62 | 11.59 |
| HOP-92 | 89.84 |
| NCI-H226 | 19.05 |
| NCI-H23 | 52.04 |
| NCI-H322M | 74.39 |
| NCI-H460 | 35.78 |
| NCI-H522 | 9.19 |
| Colon Cancer | |
| COLO 205 | 62.05 |
| HCC-2998 | 18.78 |
| HCT-116 | 24.26 |
| HCT-15 | 49.24 |
| HT29 | 0.24 |
| KM12 | 47.64 |
| SW-620 | 56.44 |
| CNS Cancer | |
| SF-268 | 47.35 |
| SF-295 | 28.48 |
| SF-539 | 35.61 |
| SNB-19 | 47.32 |
| SNB-75 | 87.03 |
| U251 | 33.90 |
| Melanoma | |
| LOX IMVI | -16.80 |
| MALME-3M | 113.08 |
| M14 | 38.56 |
| MDA-MB-435 | 54.87 |
| SK-MEL-2 | 72.47 |
| SK-MEL-5 | 0.76 |
| Ovarian Cancer | |
| IGROV1 | -26.47 |
| OVCAR-3 | 40.87 |
| OVCAR-4 | 64.53 |
| OVCAR-5 | 116.43 |
| OVCAR-8 | 41.73 |
| NCI/ADR-RES | 73.67 |
| SK-OV-3 | 70.43 |
| Renal Cancer | |
| 786-0 | 80.34 |
| A498 | 26.88 |
| ACHN | 29.45 |
| CAKI-1 | -22.89 |
| RXF 393 | 55.98 |
| SN12C | 57.14 |
| TK-10 | 70.71 |
| UO-31 | 29.63 |
| Prostate Cancer | |
| PC-3 | 26.51 |
| DU-145 | 69.55 |
| Breast Cancer | |
| MCF7 | 15.71 |
| MDA-MB-231/ATCC | 48.17 |
| HS 578T | 66.54 |
| BT-549 | 60.35 |
| T-47D | 23.58 |
| MDA-MB-468 | 3.68 |
| Mean | 41.69 |
| Delta | 68.16 |
| Range | 142.90 |



**_N_,_N_-Diethyl-6-(6-hydroxy-5,7-dioxo-5,6,7,13-tetrahydro-12_H_-indolo[2,3-_a_]pyrrolo[3,4-_c_]carbazol-12-yl)hexanamide 315**

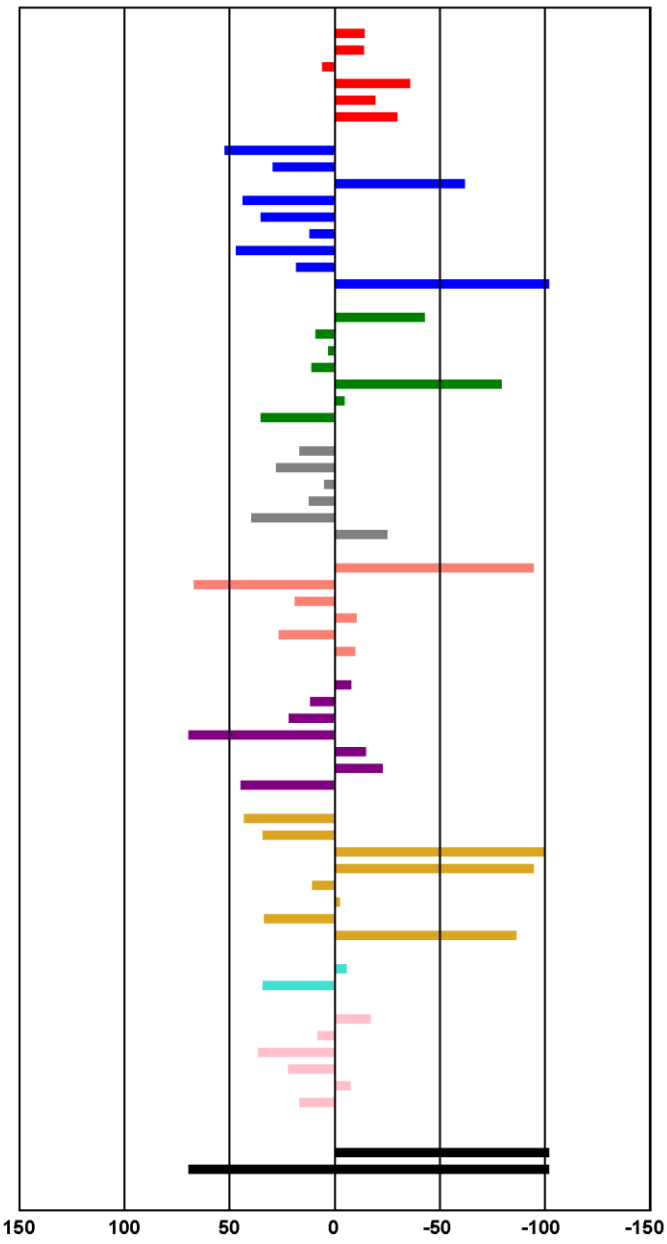



**Developmental Therapeutics Program**

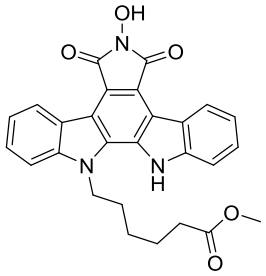
**One Dose Mean Graph**

| NSC: D-783515 / 1 | Conc: 1.00E-5 Molar | Test Date: Mar 09, 2015 |
|---|---|---|
| Experiment ID: 1503OS44 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 21.30 |
| HL-60(TB) | 21.83 |
| K-562 | 40.75 |
| MOLT-4 | -0.33 |
| RPMI-8226 | 16.13 |
| SR | 5.80 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 87.19 |
| EKVX | 64.12 |
| HOP-62 | -26.36 |
| HOP-92 | 78.48 |
| NCI-H226 | 69.90 |
| NCI-H23 | 46.75 |
| NCI-H322M | 81.84 |
| NCI-H460 | 53.07 |
| NCI-H522 | -66.53 |
| Colon Cancer | |
| COLO 205 | -7.19 |
| HCC-2998 | 44.01 |
| HCT-116 | 37.78 |
| HCT-15 | 45.95 |
| HT29 | -43.72 |
| KM12 | 30.83 |
| SW-620 | 70.10 |
| CNS Cancer | |
| SF-268 | 51.58 |
| SF-295 | 62.53 |
| SF-539 | 39.70 |
| SNB-19 | 47.20 |
| SNB-75 | 74.41 |
| U251 | 10.47 |
| Melanoma | |
| LOX IMVI | -58.97 |
| MALME-3M | 101.86 |
| M14 | 53.91 |
| MDA-MB-435 | 25.26 |
| SK-MEL-2 | 61.44 |
| SK-MEL-5 | 25.87 |
| Ovarian Cancer | |
| IGROV1 | 27.57 |
| OVCAR-3 | 46.53 |
| OVCAR-4 | 56.64 |
| OVCAR-5 | 104.30 |
| OVCAR-8 | 20.79 |
| NCI/ADR-RES | 12.67 |
| SK-OV-3 | 79.51 |
| Renal Cancer | |
| 786-0 | 78.06 |
| A498 | 69.13 |
| ACHN | -64.85 |
| CAKI-1 | -58.97 |
| RXF 393 | 45.59 |
| SN12C | 33.23 |
| TK-10 | 68.29 |
| UO-31 | -50.66 |
| Prostate Cancer | |
| PC-3 | 29.99 |
| DU-145 | 68.97 |
| Breast Cancer | |
| MCF7 | 18.54 |
| MDA-MB-231/ATCC | 42.90 |
| HS 578T | 71.09 |
| BT-549 | 56.83 |
| T-47D | 28.16 |
| MDA-MB-468 | 51.53 |
| Mean | 35.14 |
| Delta | 101.67 |
| Range | 170.83 |



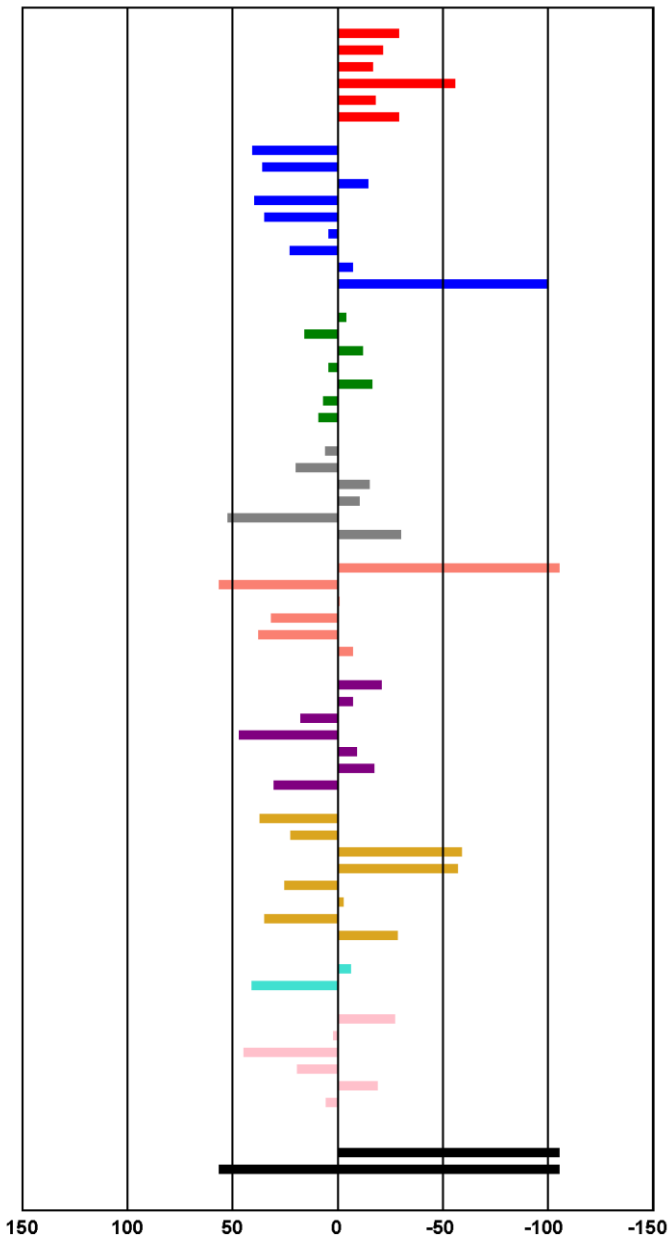
**Methyl 6-(6-hydroxy-5,7-dioxo-5,6,7,13-tetrahydro-12*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12-yl)hexanoate 316**



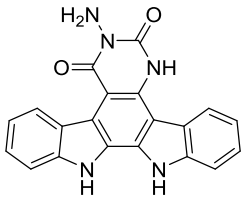

| Developmental Therapeutics Program | NSC: D-783513 / 1 | Conc: 1.00E-5 Molar | Test Date: Mar 09, 2015 |
|---|---|---|---|
| One Dose Mean Graph | Experiment ID: 1503OS44 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 11.40 |
| HL-60(TB) | 19.05 |
| K-562 | 23.81 |
| MOLT-4 | -15.56 |
| RPMI-8226 | 22.36 |
| SR | 11.27 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 80.32 |
| EKVX | 75.34 |
| HOP-62 | 26.03 |
| HOP-92 | 79.38 |
| NCI-H226 | 74.43 |
| NCI-H23 | 44.12 |
| NCI-H322M | 62.42 |
| NCI-H460 | 33.36 |
| NCI-H522 | -60.04 |
| Colon Cancer | |
| COLO 205 | 36.47 |
| HCC-2998 | 55.44 |
| HCT-116 | 28.37 |
| HCT-15 | 44.13 |
| HT29 | 24.00 |
| KM12 | 46.53 |
| SW-620 | 48.74 |
| CNS Cancer | |
| SF-268 | 45.64 |
| SF-295 | 59.64 |
| SF-539 | 25.28 |
| SNB-19 | 29.91 |
| SNB-75 | 92.04 |
| U251 | 10.25 |
| Melanoma | |
| LOX IMVI | -65.04 |
| MALME-3M | 95.99 |
| M14 | 39.49 |
| MDA-MB-435 | 71.23 |
| SK-MEL-2 | 77.33 |
| SK-MEL-5 | 33.10 |
| Ovarian Cancer | |
| IGROV1 | 19.49 |
| OVCAR-3 | 33.16 |
| OVCAR-4 | 57.48 |
| OVCAR-5 | 86.61 |
| OVCAR-8 | 31.23 |
| NCI/ADR-RES | 23.10 |
| SK-OV-3 | 70.17 |
| Renal Cancer | |
| 786-0 | 76.63 |
| A498 | 61.99 |
| ACHN | -18.53 |
| CAKI-1 | -16.83 |
| RXF 393 | 64.85 |
| SN12C | 37.82 |
| TK-10 | 74.37 |
| UO-31 | 11.83 |
| Prostate Cancer | |
| PC-3 | 34.30 |
| DU-145 | 80.55 |
| Breast Cancer | |
| MCF7 | 13.10 |
| MDA-MB-231/ATCC | 41.84 |
| HS 578T | 84.50 |
| BT-549 | 59.00 |
| T-47D | 21.58 |
| MDA-MB-468 | 45.27 |
| Mean | 40.00 |
| Delta | 105.04 |
| Range | 161.03 |

Mean Growth Percent - Growth Percent



**7-Amino-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione**
**345**

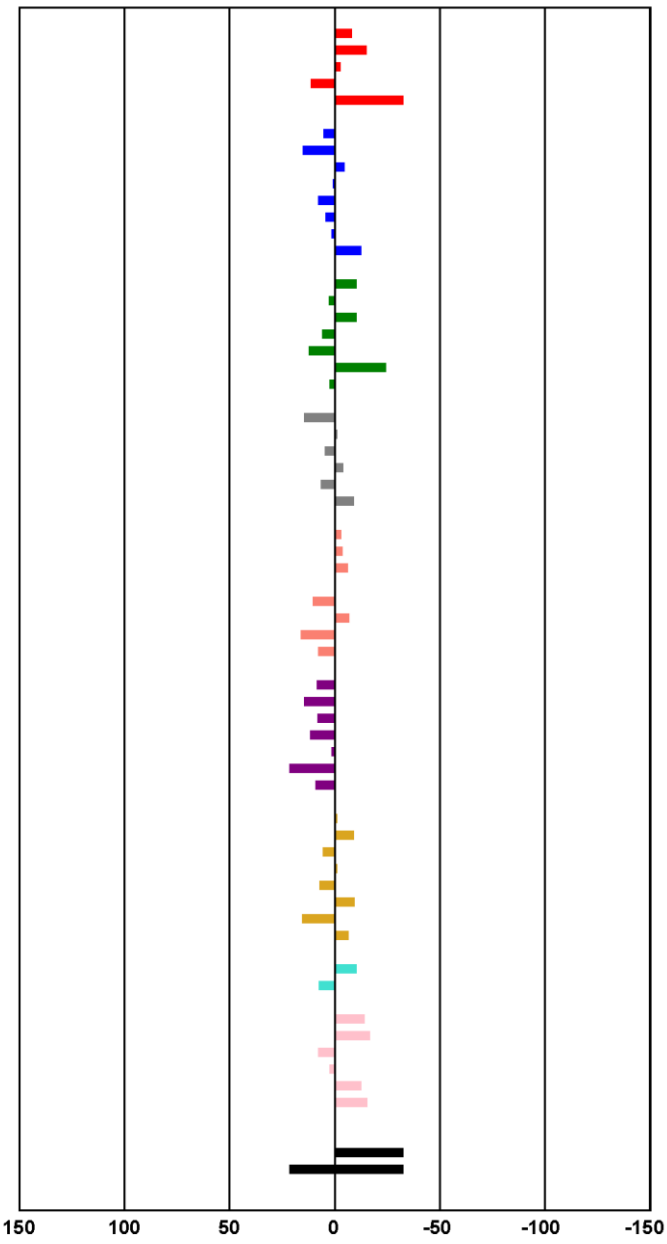



**Developmental Therapeutics Program**
**One Dose Mean Graph**

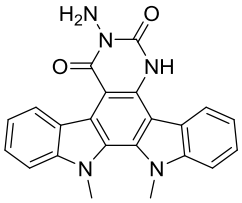
| NSC: D-778802 / 1 | Conc: 1.00E-5 Molar | Test Date: Jan 06, 2014 |
|---|---|---|
| Experiment ID: 1401OS14 | | Report Date: Jun 04, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 80.92 |
| K-562 | 73.97 |
| MOLT-4 | 86.36 |
| RPMI-8226 | 99.82 |
| SR | 56.51 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 93.88 |
| HOP-62 | 103.75 |
| HOP-92 | 84.68 |
| NCI-H226 | 89.42 |
| NCI-H23 | 96.23 |
| NCI-H322M | 92.84 |
| NCI-H460 | 89.94 |
| NCI-H522 | 76.73 |
| Colon Cancer | |
| COLO 205 | 78.97 |
| HCC-2998 | 91.20 |
| HCT-116 | 78.79 |
| HCT-15 | 94.51 |
| HT29 | 100.72 |
| KM12 | 64.86 |
| SW-620 | 91.02 |
| CNS Cancer | |
| SF-268 | 103.02 |
| SF-295 | 88.15 |
| SF-539 | 93.21 |
| SNB-19 | 85.18 |
| SNB-75 | 94.88 |
| U251 | 80.01 |
| Melanoma | |
| LOX IMVI | 86.01 |
| MALME-3M | 85.65 |
| M14 | 82.89 |
| MDA-MB-435 | 88.68 |
| SK-MEL-28 | 98.69 |
| SK-MEL-5 | 82.34 |
| UACC-257 | 104.56 |
| UACC-62 | 96.26 |
| Ovarian Cancer | |
| IGROV1 | 96.91 |
| OVCAR-3 | 102.91 |
| OVCAR-4 | 96.66 |
| OVCAR-5 | 100.06 |
| OVCAR-8 | 89.97 |
| NCI/ADR-RES | 110.00 |
| SK-OV-3 | 97.54 |
| Renal Cancer | |
| 786-0 | 88.08 |
| A498 | 80.15 |
| ACHN | 94.11 |
| CAKI-1 | 87.96 |
| RXF 393 | 95.68 |
| SN12C | 79.75 |
| TK-10 | 104.04 |
| UO-31 | 82.61 |
| Prostate Cancer | |
| PC-3 | 78.94 |
| DU-145 | 95.99 |
| Breast Cancer | |
| MCF7 | 74.91 |
| MDA-MB-231/ATCC | 72.54 |
| HS 578T | 96.24 |
| BT-549 | 90.85 |
| T-47D | 76.66 |
| MDA-MB-468 | 73.63 |
| Mean | 88.79 |
| Delta | 32.28 |
| Range | 53.49 |

Mean Growth Percent - Growth Percent



**7-Amino-13,14-dimethyl-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 349**

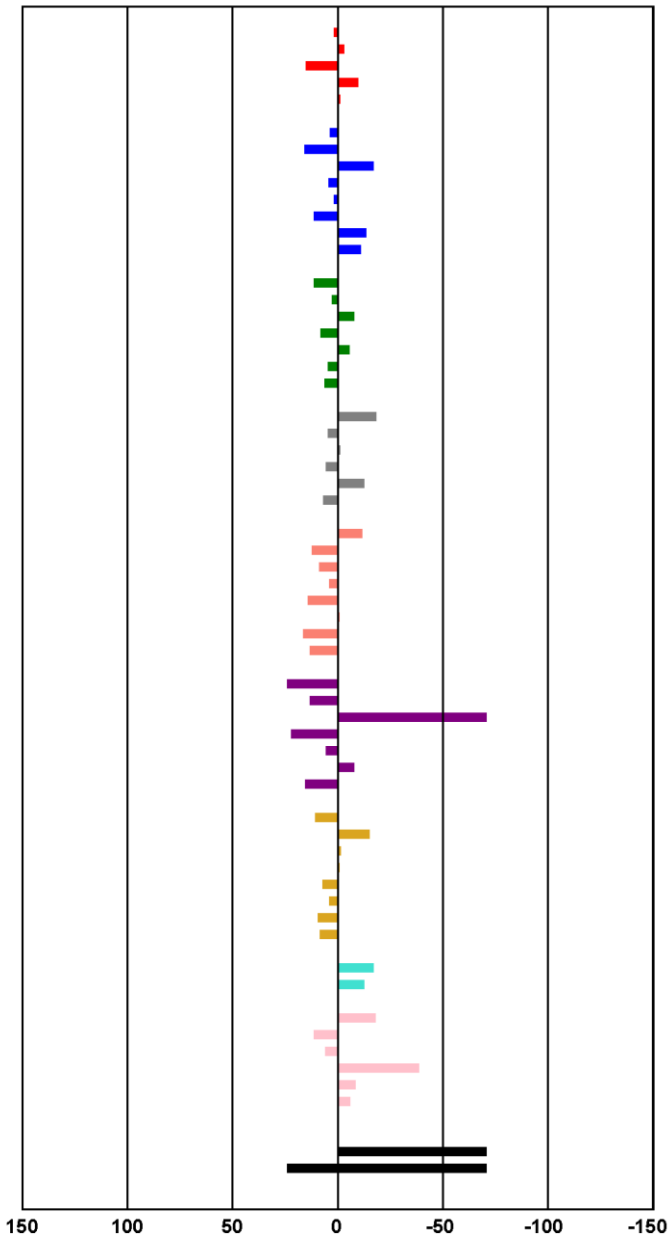


**Developmental Therapeutics Program**

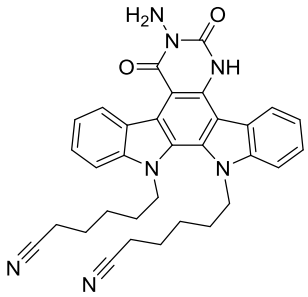
**One Dose Mean Graph**

| NSC: D-778803 / 1 | Conc: 1.00E-5 Molar | Test Date: Jan 06, 2014 |
|---|---|---|
| Experiment ID: 1401OS14 | | Report Date: Jun 04, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 85.94 |
| K-562 | 81.87 |
| MOLT-4 | 99.24 |
| RPMI-8226 | 75.21 |
| SR | 83.65 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 87.87 |
| HOP-62 | 99.86 |
| HOP-92 | 67.85 |
| NCI-H226 | 88.51 |
| NCI-H23 | 85.89 |
| NCI-H322M | 95.55 |
| NCI-H460 | 71.47 |
| NCI-H522 | 73.75 |
| Colon Cancer | |
| COLO 205 | 95.53 |
| HCC-2998 | 87.01 |
| HCT-116 | 77.11 |
| HCT-15 | 92.32 |
| HT29 | 79.38 |
| KM12 | 88.92 |
| SW-620 | 90.45 |
| CNS Cancer | |
| SF-268 | 66.68 |
| SF-295 | 88.77 |
| SF-539 | 83.63 |
| SNB-19 | 89.79 |
| SNB-75 | 72.28 |
| U251 | 90.89 |
| Melanoma | |
| LOX IMVI | 73.26 |
| MALME-3M | 96.41 |
| M14 | 93.08 |
| MDA-MB-435 | 88.07 |
| SK-MEL-28 | 98.31 |
| SK-MEL-5 | 83.92 |
| UACC-257 | 100.60 |
| UACC-62 | 97.40 |
| Ovarian Cancer | |
| IGROV1 | 108.09 |
| OVCAR-3 | 97.51 |
| OVCAR-4 | 14.17 |
| OVCAR-5 | 106.28 |
| OVCAR-8 | 89.73 |
| NCI/ADR-RES | 76.97 |
| SK-OV-3 | 99.71 |
| Renal Cancer | |
| 786-0 | 94.94 |
| A498 | 69.71 |
| ACHN | 83.40 |
| CAKI-1 | 84.00 |
| RXF 393 | 91.20 |
| SN12C | 88.28 |
| TK-10 | 93.55 |
| UO-31 | 92.75 |
| Prostate Cancer | |
| PC-3 | 67.85 |
| DU-145 | 72.20 |
| Breast Cancer | |
| MCF7 | 67.01 |
| MDA-MB-231/ATCC | 95.40 |
| HS 578T | 90.22 |
| BT-549 | 46.19 |
| T-47D | 76.39 |
| MDA-MB-468 | 78.96 |
| Mean | 84.47 |
| Delta | 70.30 |
| Range | 93.92 |



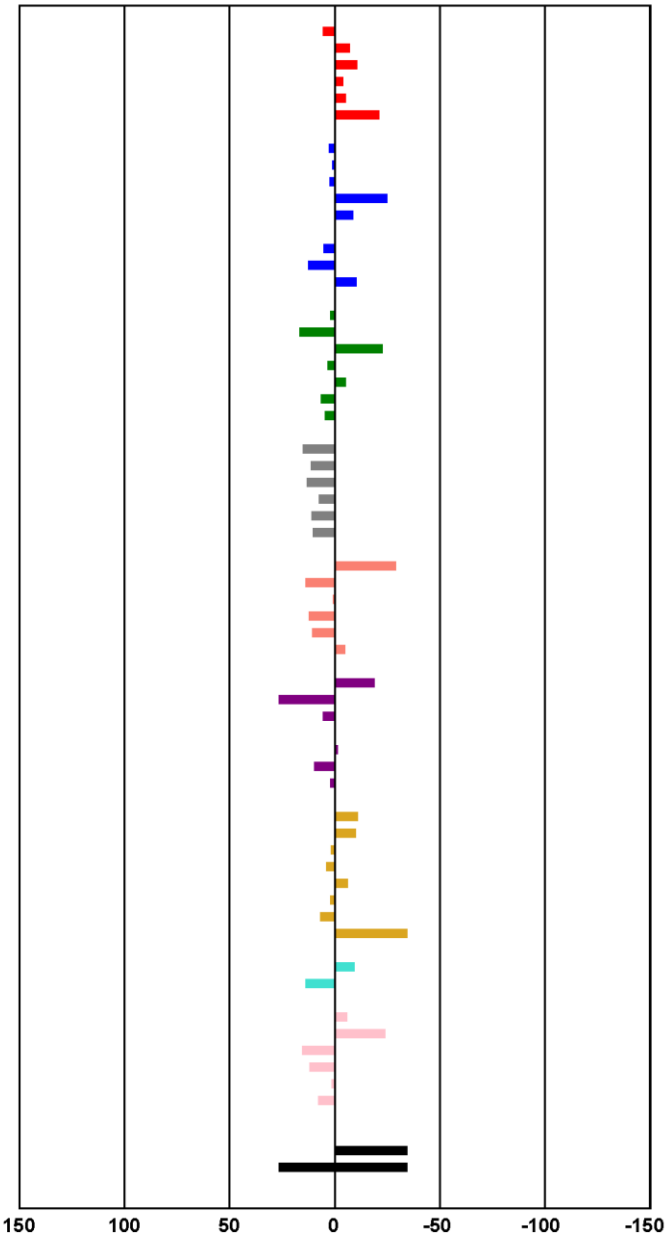
**6,6'-(7-Amino-6,8-dioxo-5,6,7,8-tetrahydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-13,14-diyl)dihexanenitrile 351**



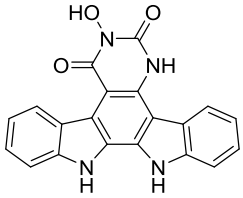
| Developmental Therapeutics Program | NSC: D-783511 / 1 | Conc: 1.00E-5 Molar | Test Date: Mar 09, 2015 |
|---|---|---|---|
| One Dose Mean Graph | Experiment ID: 1503OS44 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 93.84 |
| HL-60(TB) | 81.62 |
| K-562 | 78.13 |
| MOLT-4 | 84.87 |
| RPMI-8226 | 83.60 |
| SR | 67.82 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 91.04 |
| EKVX | 89.41 |
| HOP-62 | 90.73 |
| HOP-92 | 63.97 |
| NCI-H226 | 80.09 |
| NCI-H23 | 88.33 |
| NCI-H322M | 93.46 |
| NCI-H460 | 100.73 |
| NCI-H522 | 78.68 |
| Colon Cancer | |
| COLO 205 | 90.20 |
| HCC-2998 | 104.93 |
| HCT-116 | 66.23 |
| HCT-15 | 91.69 |
| HT29 | 83.65 |
| KM12 | 94.75 |
| SW-620 | 92.76 |
| CNS Cancer | |
| SF-268 | 103.47 |
| SF-295 | 99.48 |
| SF-539 | 101.43 |
| SNB-19 | 95.55 |
| SNB-75 | 99.08 |
| U251 | 98.53 |
| Melanoma | |
| LOX IMVI | 59.74 |
| MALME-3M | 102.06 |
| M14 | 89.14 |
| MDA-MB-435 | 100.61 |
| SK-MEL-2 | 98.77 |
| SK-MEL-5 | 83.81 |
| Ovarian Cancer | |
| IGROV1 | 69.85 |
| OVCAR-3 | 114.70 |
| OVCAR-4 | 93.75 |
| OVCAR-5 | 88.46 |
| OVCAR-8 | 87.51 |
| NCI/ADR-RES | 97.76 |
| SK-OV-3 | 90.40 |
| Renal Cancer | |
| 786-0 | 77.82 |
| A498 | 79.00 |
| ACHN | 90.11 |
| CAKI-1 | 92.13 |
| RXF 393 | 82.65 |
| SN12C | 90.33 |
| TK-10 | 94.94 |
| UO-31 | 54.35 |
| Prostate Cancer | |
| PC-3 | 79.49 |
| DU-145 | 101.90 |
| Breast Cancer | |
| MCF7 | 83.10 |
| MDA-MB-231/ATCC | 64.93 |
| HS 578T | 103.54 |
| BT-549 | 100.23 |
| T-47D | 89.59 |
| MDA-MB-468 | 96.10 |
| Mean | 88.51 |
| Delta | 34.16 |
| Range | 60.35 |

Mean Growth Percent - Growth Percent



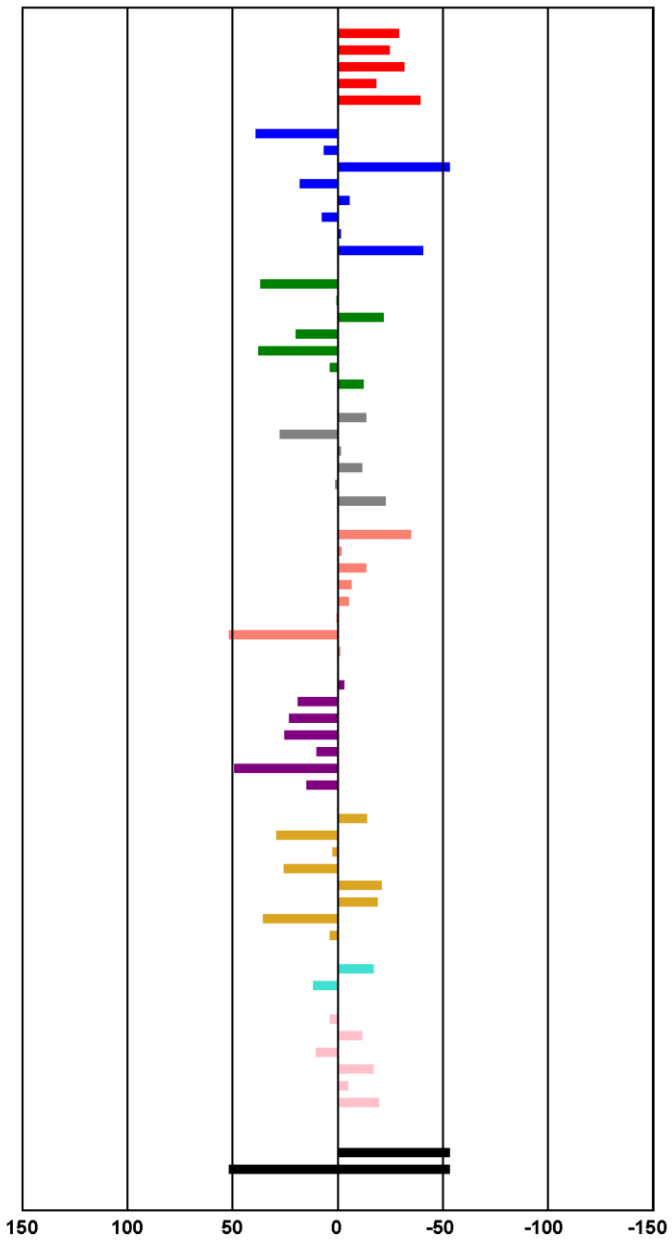
**7-Hydroxy-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione**
**346**



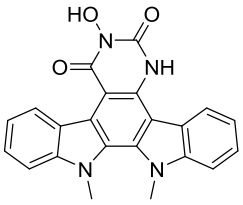

**Developmental Therapeutics Program**
**One Dose Mean Graph**

| NSC: D-778801 / 1 | Conc: 1.00E-5 Molar | Test Date: Jan 06, 2014 |
|---|---|---|
| Experiment ID: 1401OS14 | | Report Date: Jun 04, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 15.75 |
| K-562 | 20.39 |
| MOLT-4 | 13.45 |
| RPMI-8226 | 26.50 |
| SR | 5.61 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 83.19 |
| HOP-62 | 50.64 |
| HOP-92 | -8.31 |
| NCI-H226 | 62.23 |
| NCI-H23 | 39.49 |
| NCI-H322M | 51.59 |
| NCI-H460 | 43.41 |
| NCI-H522 | 4.34 |
| Colon Cancer | |
| COLO 205 | 80.95 |
| HCC-2998 | 44.86 |
| HCT-116 | 23.06 |
| HCT-15 | 64.19 |
| HT29 | 81.94 |
| KM12 | 48.01 |
| SW-620 | 32.71 |
| CNS Cancer | |
| SF-268 | 31.40 |
| SF-295 | 71.91 |
| SF-539 | 43.45 |
| SNB-19 | 33.36 |
| SNB-75 | 45.52 |
| U251 | 22.14 |
| Melanoma | |
| LOX IMVI | 9.99 |
| MALME-3M | 43.30 |
| M14 | 31.48 |
| MDA-MB-435 | 38.57 |
| SK-MEL-28 | 39.76 |
| SK-MEL-5 | 44.80 |
| UACC-257 | 96.06 |
| UACC-62 | 43.96 |
| Ovarian Cancer | |
| IGROV1 | 41.89 |
| OVCAR-3 | 63.10 |
| OVCAR-4 | 67.28 |
| OVCAR-5 | 69.53 |
| OVCAR-8 | 54.34 |
| NCI/ADR-RES | 93.34 |
| SK-OV-3 | 58.94 |
| Renal Cancer | |
| 786-0 | 31.11 |
| A498 | 73.30 |
| ACHN | 46.80 |
| CAKI-1 | 69.87 |
| RXF 393 | 24.26 |
| SN12C | 25.93 |
| TK-10 | 79.73 |
| UO-31 | 47.86 |
| Prostate Cancer | |
| PC-3 | 28.06 |
| DU-145 | 55.74 |
| Breast Cancer | |
| MCF7 | 47.87 |
| MDA-MB-231/ATCC | 33.32 |
| HS 578T | 54.71 |
| BT-549 | 27.99 |
| T-47D | 39.86 |
| MDA-MB-468 | 25.25 |
| Mean | 44.56 |
| Delta | 52.87 |
| Range | 104.37 |

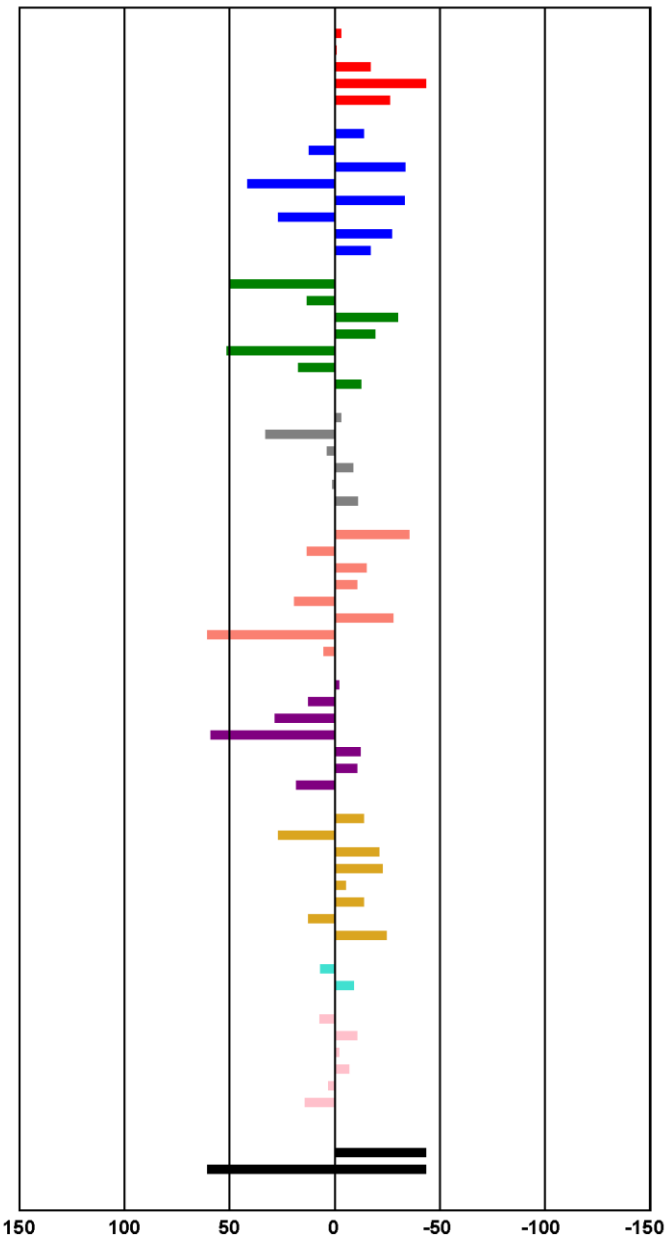


**7-Hydroxy-13,14-dimethyl-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 350**

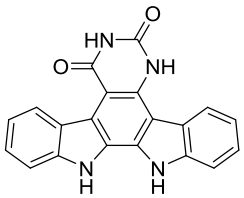



| Developmental Therapeutics Program | NSC: D-778804 / 1 | Conc: 1.00E-5 Molar | Test Date: Jan 06, 2014 |
|---|---|---|---|
| One Dose Mean Graph | Experiment ID: 1401OS14 | | Report Date: Jun 04, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 27.63 |
| K-562 | 29.98 |
| MOLT-4 | 13.61 |
| RPMI-8226 | -12.61 |
| SR | 4.45 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 16.87 |
| HOP-62 | 42.35 |
| HOP-92 | -2.93 |
| NCI-H226 | 71.58 |
| NCI-H23 | -2.72 |
| NCI-H322M | 56.79 |
| NCI-H460 | 3.60 |
| NCI-H522 | 13.71 |
| Colon Cancer | |
| COLO 205 | 80.16 |
| HCC-2998 | 43.19 |
| HCT-116 | 0.54 |
| HCT-15 | 11.56 |
| HT29 | 81.24 |
| KM12 | 47.43 |
| SW-620 | 18.14 |
| CNS Cancer | |
| SF-268 | 27.56 |
| SF-295 | 62.91 |
| SF-539 | 33.70 |
| SNB-19 | 21.77 |
| SNB-75 | 31.09 |
| U251 | 19.75 |
| Melanoma | |
| LOX IMVI | -4.92 |
| MALME-3M | 43.23 |
| M14 | 15.69 |
| MDA-MB-435 | 20.00 |
| SK-MEL-28 | 49.17 |
| SK-MEL-5 | 2.73 |
| UACC-257 | 90.55 |
| UACC-62 | 35.32 |
| Ovarian Cancer | |
| IGROV1 | 28.45 |
| OVCAR-3 | 42.41 |
| OVCAR-4 | 58.58 |
| OVCAR-5 | 88.93 |
| OVCAR-8 | 18.40 |
| NCI/ADR-RES | 19.95 |
| SK-OV-3 | 48.31 |
| Renal Cancer | |
| 786-0 | 16.79 |
| A498 | 56.87 |
| ACHN | 9.37 |
| CAKI-1 | 7.84 |
| RXF 393 | 25.43 |
| SN12C | 16.90 |
| TK-10 | 42.66 |
| UO-31 | 6.10 |
| Prostate Cancer | |
| PC-3 | 36.82 |
| DU-145 | 21.68 |
| Breast Cancer | |
| MCF7 | 37.27 |
| MDA-MB-231/ATCC | 19.85 |
| HS 578T | 28.58 |
| BT-549 | 23.80 |
| T-47D | 33.04 |
| MDA-MB-468 | 44.26 |
| Mean | 30.27 |
| Delta | 42.88 |
| Range | 103.16 |



## 13,14-Dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 348



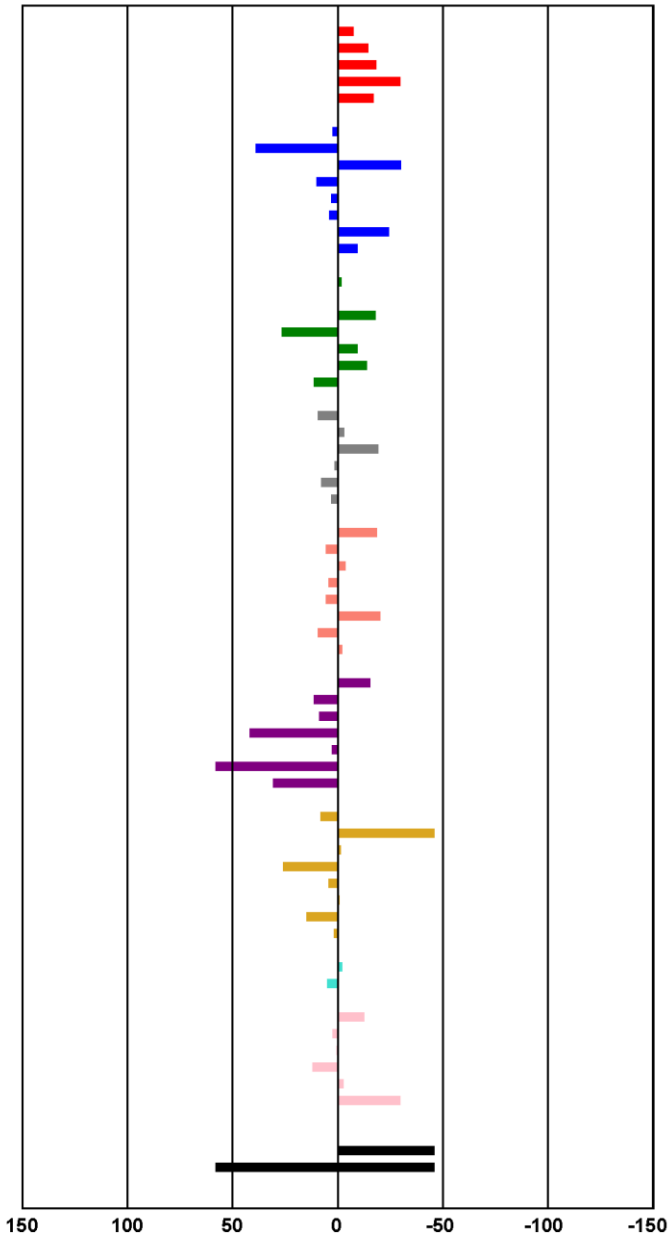

**Developmental Therapeutics Program**

**One Dose Mean Graph**

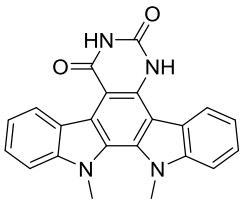
| NSC: D-778805 / 1 | Conc: 1.00E-5 Molar | Test Date: Jan 06, 2014 |
|---|---|---|
| Experiment ID: 1401OS14 | | Report Date: Jun 04, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 28.11 |
| K-562 | 21.32 |
| MOLT-4 | 17.42 |
| RPMI-8226 | 6.02 |
| SR | 18.76 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 37.53 |
| HOP-62 | 74.03 |
| HOP-92 | 5.70 |
| NCI-H226 | 45.05 |
| NCI-H23 | 38.16 |
| NCI-H322M | 38.99 |
| NCI-H460 | 11.29 |
| NCI-H522 | 26.25 |
| Colon Cancer | |
| COLO 205 | 34.10 |
| HCC-2998 | 35.25 |
| HCT-116 | 17.86 |
| HCT-15 | 61.52 |
| HT29 | 26.46 |
| KM12 | 22.00 |
| SW-620 | 46.28 |
| CNS Cancer | |
| SF-268 | 44.40 |
| SF-295 | 32.66 |
| SF-539 | 16.35 |
| SNB-19 | 36.42 |
| SNB-75 | 42.70 |
| U251 | 37.96 |
| Melanoma | |
| LOX IMVI | 17.16 |
| MALME-3M | 40.62 |
| M14 | 32.03 |
| MDA-MB-435 | 39.21 |
| SK-MEL-28 | 40.68 |
| SK-MEL-5 | 15.64 |
| UACC-257 | 44.34 |
| UACC-62 | 33.67 |
| Ovarian Cancer | |
| IGROV1 | 20.43 |
| OVCAR-3 | 46.29 |
| OVCAR-4 | 43.79 |
| OVCAR-5 | 76.80 |
| OVCAR-8 | 37.77 |
| NCI/ADR-RES | 92.99 |
| SK-OV-3 | 65.78 |
| Renal Cancer | |
| 786-0 | 43.02 |
| A498 | -10.09 |
| ACHN | 34.30 |
| CAKI-1 | 61.12 |
| RXF 393 | 39.39 |
| SN12C | 34.86 |
| TK-10 | 49.69 |
| UO-31 | 36.77 |
| Prostate Cancer | |
| PC-3 | 33.51 |
| DU-145 | 40.09 |
| Breast Cancer | |
| MCF7 | 23.14 |
| MDA-MB-231/ATCC | 37.45 |
| HS 578T | 35.58 |
| BT-549 | 46.89 |
| T-47D | 32.95 |
| MDA-MB-468 | 6.03 |
| Mean | 35.34 |
| Delta | 45.43 |
| Range | 103.08 |

Mean Growth Percent - Growth Percent



**13,14-Dimethyl-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 352**

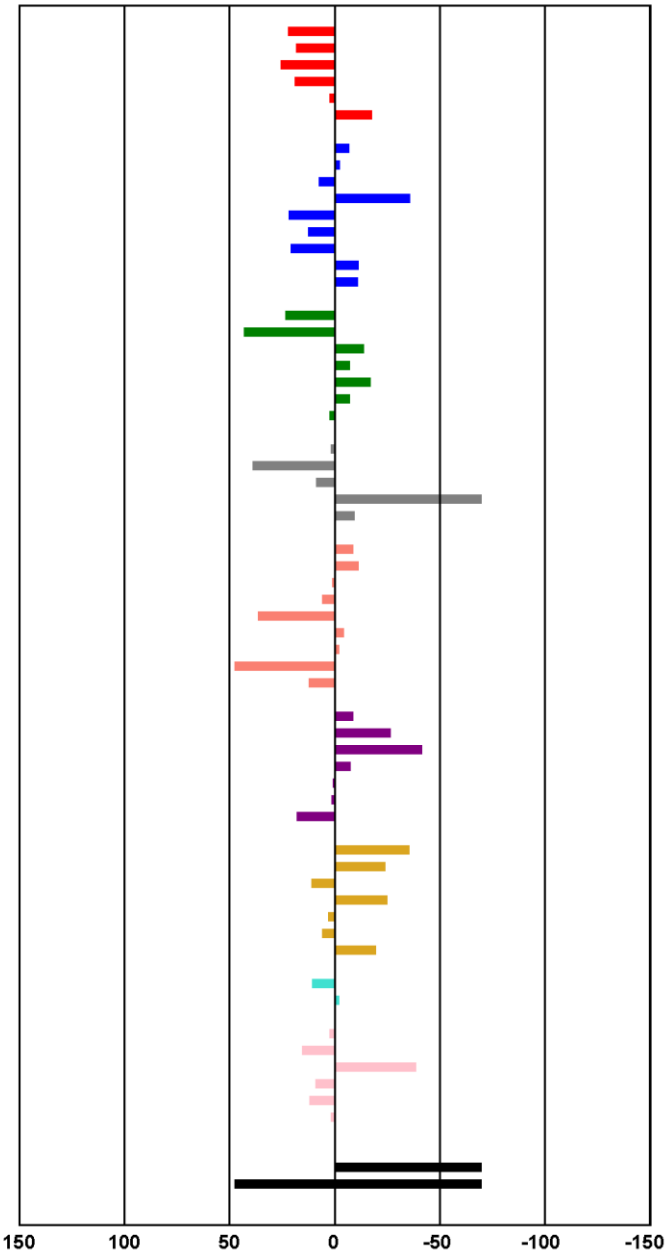



**Developmental Therapeutics Program**

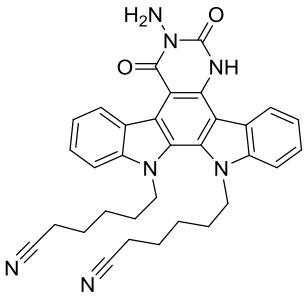
**One Dose Mean Graph**

| NSC: D-781339 / 1 | Conc: 1.00E-5 Molar | Test Date: Jun 30, 2014 |
|---|---|---|
| Experiment ID: 1406OS12 | | Report Date: Jul 25, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 52.87 |
| HL-60(TB) | 48.90 |
| K-562 | 56.08 |
| MOLT-4 | 49.42 |
| RPMI-8226 | 33.13 |
| SR | 13.71 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 24.31 |
| EKVX | 28.98 |
| HOP-62 | 38.15 |
| HOP-92 | -4.38 |
| NCI-H226 | 52.55 |
| NCI-H23 | 43.29 |
| NCI-H322M | 51.44 |
| NCI-H460 | 20.05 |
| NCI-H522 | 20.39 |
| Colon Cancer | |
| COLO 205 | 53.84 |
| HCC-2998 | 73.86 |
| HCT-116 | 17.62 |
| HCT-15 | 24.21 |
| HT29 | 14.38 |
| KM12 | 24.16 |
| SW-620 | 33.13 |
| CNS Cancer | |
| SF-268 | 32.37 |
| SF-295 | 69.62 |
| SF-539 | 39.50 |
| SNB-75 | -38.43 |
| U251 | 21.88 |
| Melanoma | |
| LOX IMVI | 22.55 |
| MALME-3M | 20.03 |
| M14 | 31.80 |
| MDA-MB-435 | 36.45 |
| SK-MEL-2 | 67.04 |
| SK-MEL-28 | 27.15 |
| SK-MEL-5 | 29.26 |
| UACC-257 | 78.07 |
| UACC-62 | 43.03 |
| Ovarian Cancer | |
| IGROV1 | 22.49 |
| OVCAR-3 | 4.89 |
| OVCAR-4 | -10.11 |
| OVCAR-5 | 23.80 |
| OVCAR-8 | 31.31 |
| NCI/ADR-RES | 32.20 |
| SK-OV-3 | 48.62 |
| Renal Cancer | |
| A498 | -4.05 |
| ACHN | 7.31 |
| CAKI-1 | 41.47 |
| RXF 393 | 6.48 |
| SN12C | 33.71 |
| TK-10 | 36.44 |
| UO-31 | 11.78 |
| Prostate Cancer | |
| PC-3 | 41.13 |
| DU-145 | 29.25 |
| Breast Cancer | |
| MCF7 | 32.87 |
| MDA-MB-231/ATCC | 46.02 |
| HS 578T | -7.29 |
| BT-549 | 39.73 |
| T-47D | 42.55 |
| MDA-MB-468 | 32.36 |
| Mean | 30.92 |
| Delta | 69.35 |
| Range | 116.50 |



**6,6'-(6,8-Dioxo-5,6,7,8-tetrahydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-13,14-diyl)dihexanenitrile 353**



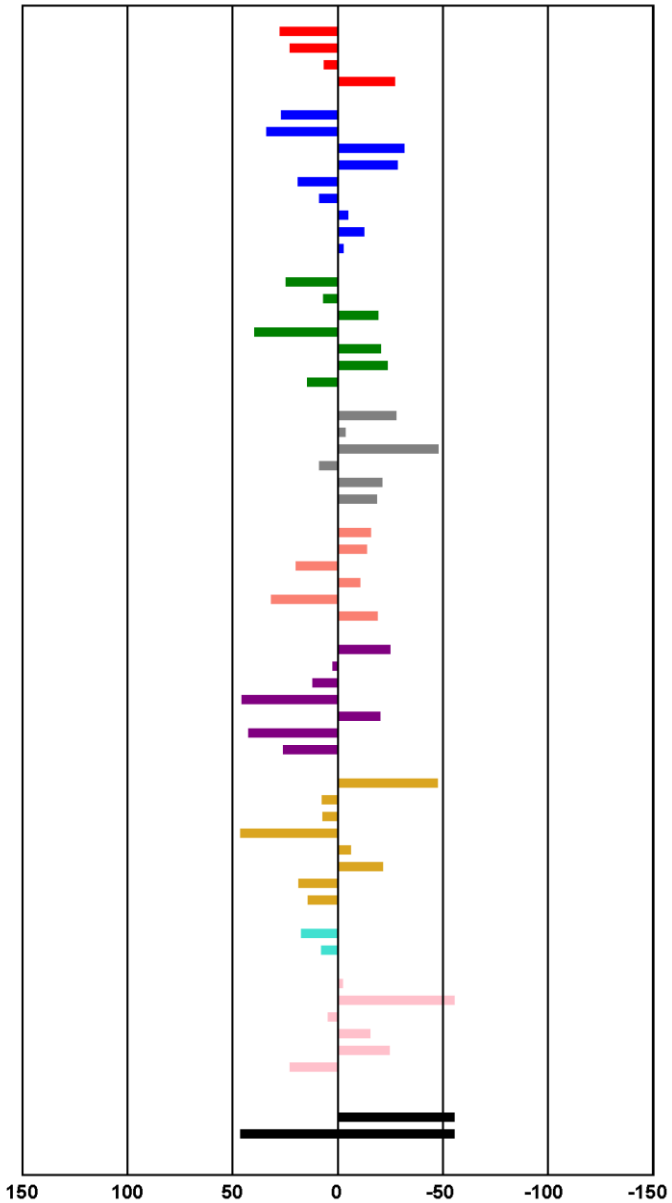

**Developmental Therapeutics Program**

**One Dose Mean Graph**

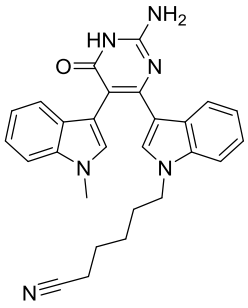
| NSC: D-783512 / 1 | Conc: 1.00E-5 Molar | Test Date: Mar 09, 2015 |
|---|---|---|
| Experiment ID: 1503OS44 | | Report Date: Sep 02, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 74.45 |
| MOLT-4 | 69.50 |
| RPMI-8226 | 53.45 |
| SR | 20.28 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 73.71 |
| EKVX | 80.69 |
| HOP-62 | 15.77 |
| HOP-92 | 19.21 |
| NCI-H226 | 65.75 |
| NCI-H23 | 55.59 |
| NCI-H322M | 42.55 |
| NCI-H460 | 34.92 |
| NCI-H522 | 44.95 |
| Colon Cancer | |
| COLO 205 | 71.58 |
| HCC-2998 | 53.78 |
| HCT-116 | 28.20 |
| HCT-15 | 86.55 |
| HT29 | 27.00 |
| KM12 | 23.82 |
| SW-620 | 61.41 |
| CNS Cancer | |
| SF-268 | 19.59 |
| SF-295 | 43.74 |
| SF-539 | -0.19 |
| SNB-19 | 55.60 |
| SNB-75 | 26.28 |
| U251 | 28.87 |
| Melanoma | |
| LOX IMVI | 31.86 |
| MALME-3M | 33.68 |
| M14 | 66.79 |
| MDA-MB-435 | 36.84 |
| SK-MEL-2 | 78.47 |
| SK-MEL-5 | 28.64 |
| Ovarian Cancer | |
| IGROV1 | 22.43 |
| OVCAR-3 | 49.16 |
| OVCAR-4 | 58.73 |
| OVCAR-5 | 92.38 |
| OVCAR-8 | 27.43 |
| NCI/ADR-RES | 89.38 |
| SK-OV-3 | 72.67 |
| Renal Cancer | |
| 786-0 | 0.17 |
| A498 | 54.46 |
| ACHN | 53.98 |
| CAKI-1 | 93.22 |
| RXF 393 | 41.43 |
| SN12C | 26.24 |
| TK-10 | 65.55 |
| UO-31 | 61.11 |
| Prostate Cancer | |
| PC-3 | 64.28 |
| DU-145 | 54.68 |
| Breast Cancer | |
| MCF7 | 45.29 |
| MDA-MB-231/ATCC | -7.94 |
| HS 578T | 51.62 |
| BT-549 | 31.97 |
| T-47D | 22.86 |
| MDA-MB-468 | 69.74 |
| Mean | 47.17 |
| Delta | 55.11 |
| Range | 101.16 |

Mean Growth Percent - Growth Percent



**6-{3-[2-Amino-5-(1-methyl-1*H*-indol-3-yl)-6-oxo-1,6-dihydropyrimidin-4-yl]-1*H*-indol-1-yl}hexanenitrile 365**



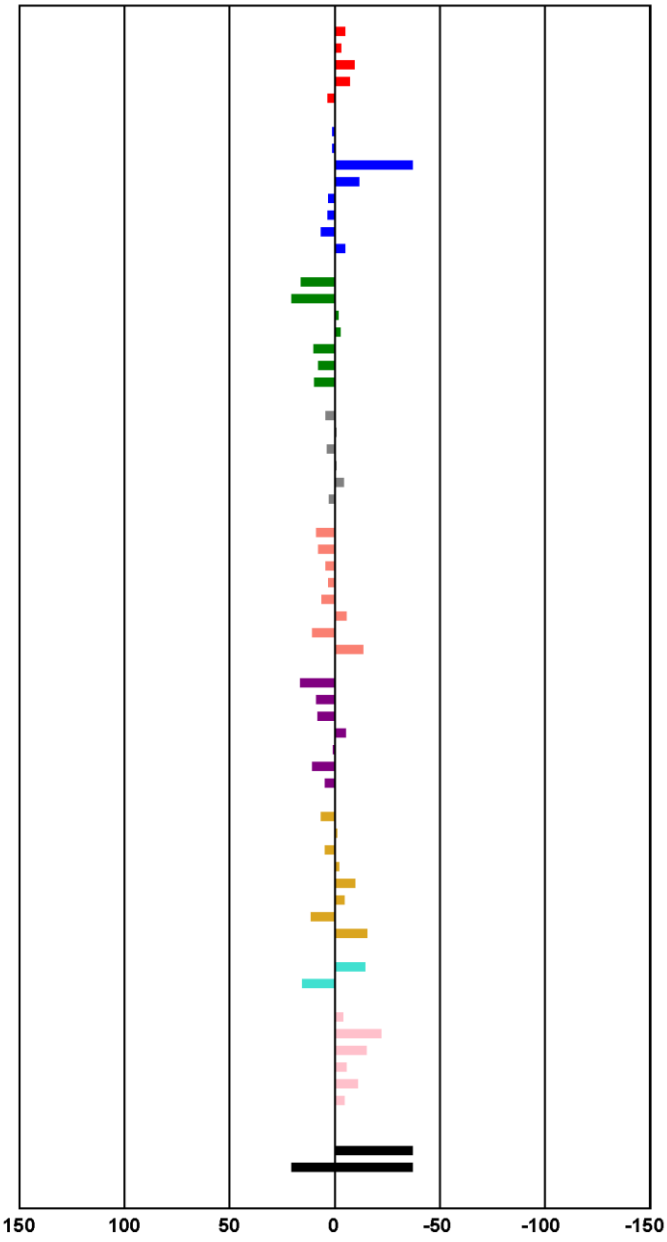

**Developmental Therapeutics Program**

**One Dose Mean Graph**

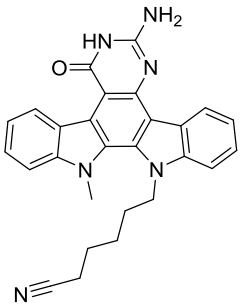
| NSC: D-778800 / 1 | Conc: 1.00E-5 Molar | Test Date: Jan 06, 2014 |
|---|---|---|
| Experiment ID: 1401OS14 | | Report Date: Jun 04, 2014 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 93.32 |
| K-562 | 95.10 |
| MOLT-4 | 88.74 |
| RPMI-8226 | 91.23 |
| SR | 100.81 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 98.80 |
| HOP-62 | 98.60 |
| HOP-92 | 61.15 |
| NCI-H226 | 86.54 |
| NCI-H23 | 100.74 |
| NCI-H322M | 101.03 |
| NCI-H460 | 104.02 |
| NCI-H522 | 93.23 |
| Colon Cancer | |
| COLO 205 | 113.79 |
| HCC-2998 | 118.01 |
| HCT-116 | 96.58 |
| HCT-15 | 95.68 |
| HT29 | 107.73 |
| KM12 | 105.38 |
| SW-620 | 107.37 |
| CNS Cancer | |
| SF-268 | 102.02 |
| SF-295 | 97.35 |
| SF-539 | 101.15 |
| SNB-19 | 97.48 |
| SNB-75 | 94.04 |
| U251 | 100.31 |
| Melanoma | |
| LOX IMVI | 106.26 |
| MALME-3M | 105.33 |
| M14 | 101.97 |
| MDA-MB-435 | 100.76 |
| SK-MEL-28 | 103.86 |
| SK-MEL-5 | 92.71 |
| UACC-257 | 108.23 |
| UACC-62 | 84.72 |
| Ovarian Cancer | |
| IGROV1 | 113.86 |
| OVCAR-3 | 106.47 |
| OVCAR-4 | 105.70 |
| OVCAR-5 | 92.90 |
| OVCAR-8 | 98.56 |
| NCI/ADR-RES | 108.28 |
| SK-OV-3 | 102.30 |
| Renal Cancer | |
| 786-0 | 104.16 |
| A498 | 97.22 |
| ACHN | 102.24 |
| CAKI-1 | 96.10 |
| RXF 393 | 88.69 |
| SN12C | 93.66 |
| TK-10 | 108.82 |
| UO-31 | 82.94 |
| Prostate Cancer | |
| PC-3 | 83.70 |
| DU-145 | 112.94 |
| Breast Cancer | |
| MCF7 | 94.42 |
| MDA-MB-231/ATCC | 76.28 |
| HS 578T | 83.11 |
| BT-549 | 92.56 |
| T-47D | 87.41 |
| MDA-MB-468 | 93.74 |
| Mean | 97.90 |
| Delta | 36.75 |
| Range | 56.86 |

Mean Growth Percent - Growth Percent



**6-[6-Amino-13-methyl-8-oxo-8,13-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazol-14(7*H*)-yl]hexanenitrile 366**



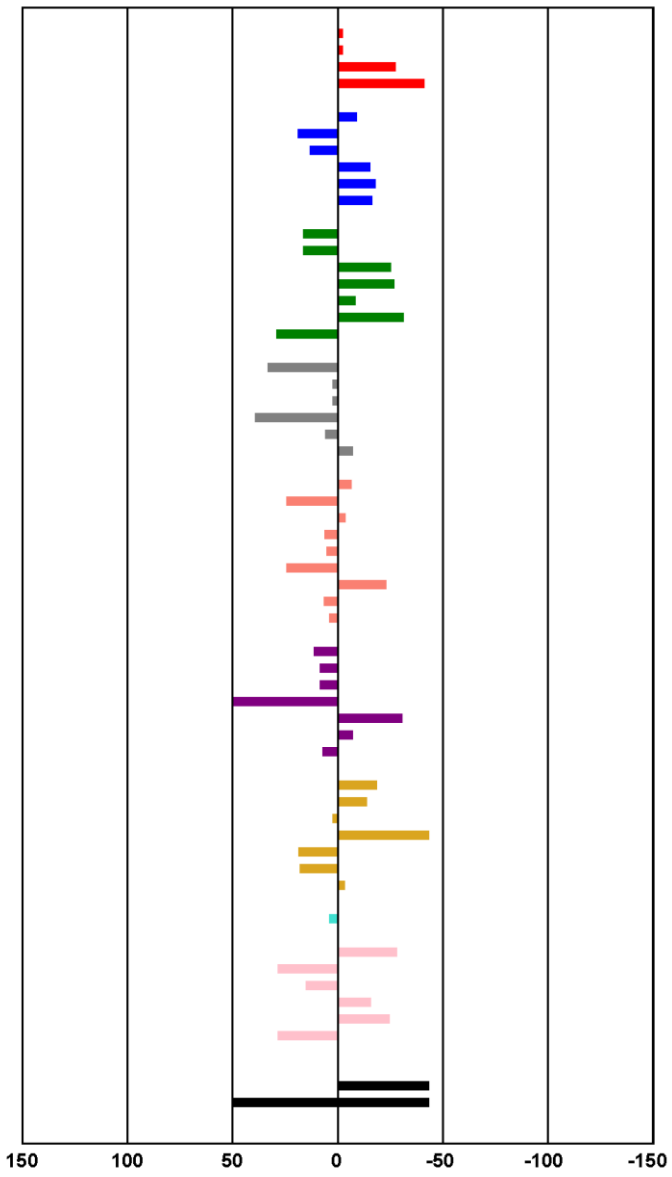

**Developmental Therapeutics Program**

**One Dose Mean Graph**

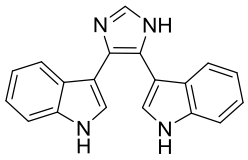
| NSC: D-785684 / 1 | Conc: 1.00E-5 Molar | Test Date: Jul 27, 2015 |
|---|---|---|
| Experiment ID: 1507OS29 | | Report Date: Aug 14, 2015 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| HL-60(TB) | 74.38 |
| K-562 | 74.32 |
| MOLT-4 | 49.13 |
| SR | 35.47 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 67.77 |
| EKVX | 94.95 |
| NCI-H226 | 89.41 |
| NCI-H23 | 61.16 |
| NCI-H460 | 58.69 |
| NCI-H522 | 60.43 |
| Colon Cancer | |
| COLO 205 | 92.45 |
| HCC-2998 | 92.44 |
| HCT-116 | 51.44 |
| HCT-15 | 49.91 |
| HT29 | 68.22 |
| KM12 | 45.44 |
| SW-620 | 105.14 |
| CNS Cancer | |
| SF-268 | 109.44 |
| SF-295 | 78.51 |
| SF-539 | 78.56 |
| SNB-19 | 115.45 |
| SNB-75 | 81.85 |
| U251 | 69.61 |
| Melanoma | |
| LOX IMVI | 70.32 |
| MALME-3M | 100.43 |
| M14 | 73.08 |
| MDA-MB-435 | 82.13 |
| SK-MEL-2 | 81.38 |
| SK-MEL-28 | 100.24 |
| SK-MEL-5 | 53.69 |
| UACC-257 | 82.56 |
| UACC-62 | 80.15 |
| Ovarian Cancer | |
| IGROV1 | 87.44 |
| OVCAR-3 | 84.48 |
| OVCAR-4 | 84.65 |
| OVCAR-5 | 126.47 |
| OVCAR-8 | 45.99 |
| NCI/ADR-RES | 69.44 |
| SK-OV-3 | 83.26 |
| Renal Cancer | |
| 786-0 | 58.20 |
| ACHN | 62.84 |
| CAKI-1 | 78.32 |
| RXF 393 | 33.46 |
| SN12C | 94.65 |
| TK-10 | 93.99 |
| UO-31 | 73.41 |
| Prostate Cancer | |
| DU-145 | 80.16 |
| Breast Cancer | |
| MCF7 | 48.61 |
| MDA-MB-231/ATCC | 104.66 |
| HS 578T | 91.06 |
| BT-549 | 60.99 |
| T-47D | 51.99 |
| MDA-MB-468 | 104.63 |
| Mean | 76.36 |
| Delta | 42.90 |
| Range | 93.01 |

Mean Growth Percent - Growth Percent



## 3,3'-(1*H*-Imidazole-4,5-diyl)bis(1*H*-indole) 369

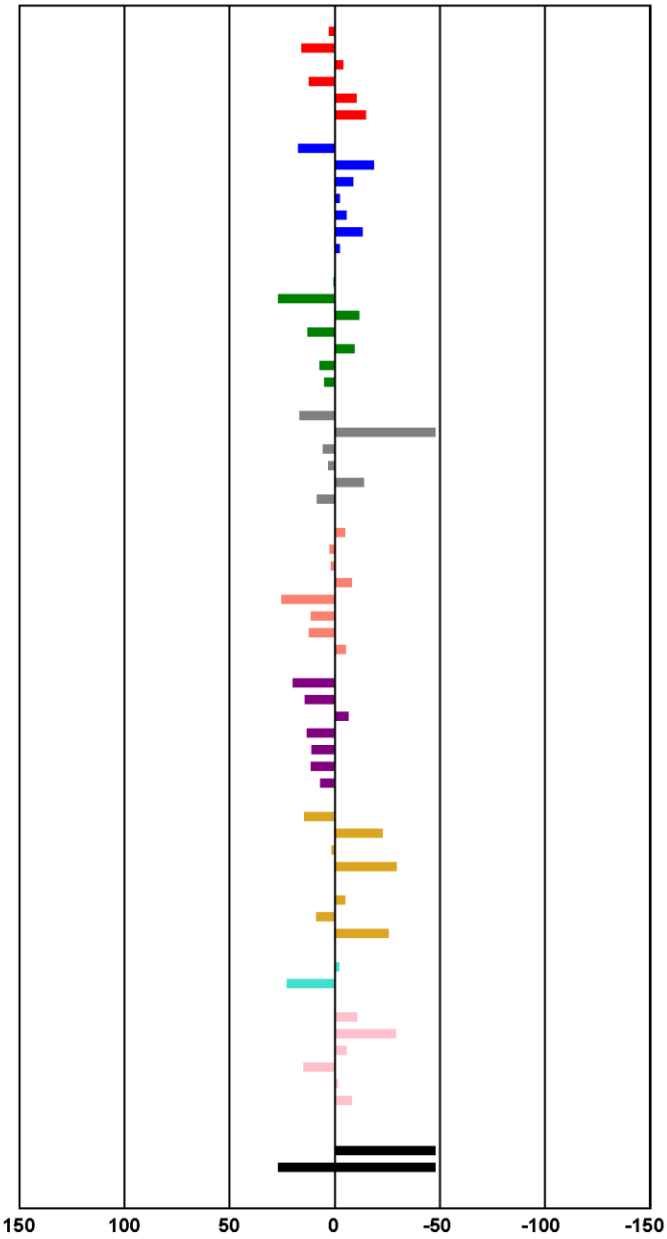


**Developmental Therapeutics Program**
**One Dose Mean Graph**

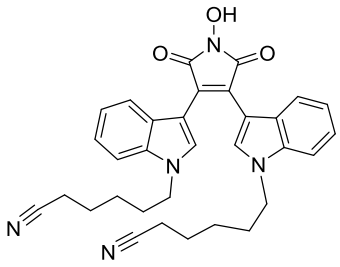
| NSC: D-763911 / 1 | Conc: 1.00E-5 Molar | Test Date: Feb 27, 2012 |
|---|---|---|
| Experiment ID: 1202OS23 | | Report Date: Apr 02, 2012 |

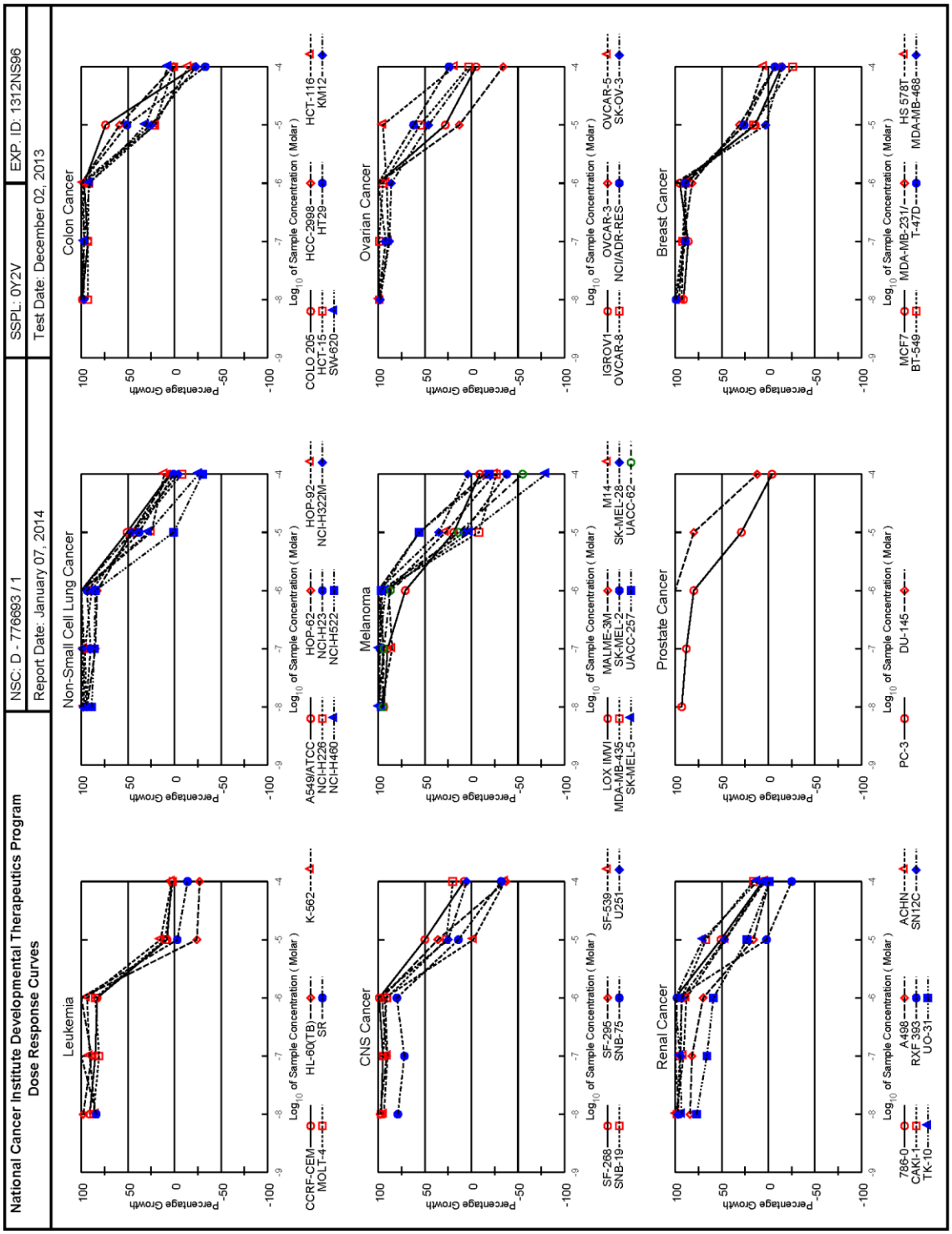
| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 80.00 |
| HL-60(TB) | 93.10 |
| K-562 | 74.04 |
| MOLT-4 | 89.63 |
| RPMI-8226 | 67.56 |
| SR | 63.10 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 94.74 |
| EKVX | 59.38 |
| HOP-62 | 69.34 |
| NCI-H23 | 75.80 |
| NCI-H322M | 72.46 |
| NCI-H460 | 64.98 |
| NCI-H522 | 75.64 |
| Colon Cancer | |
| COLO 205 | 77.72 |
| HCC-2998 | 104.09 |
| HCT-116 | 66.34 |
| HCT-15 | 90.32 |
| HT29 | 68.66 |
| KM12 | 84.42 |
| SW-620 | 82.22 |
| CNS Cancer | |
| SF-268 | 94.02 |
| SF-295 | 30.16 |
| SF-539 | 82.85 |
| SNB-19 | 80.32 |
| SNB-75 | 64.28 |
| U251 | 85.84 |
| Melanoma | |
| LOX IMVI | 72.99 |
| MALME-3M | 79.83 |
| M14 | 78.99 |
| MDA-MB-435 | 70.03 |
| SK-MEL-2 | 102.76 |
| SK-MEL-28 | 88.78 |
| UACC-257 | 89.73 |
| UACC-62 | 72.89 |
| Ovarian Cancer | |
| IGROV1 | 97.14 |
| OVCAR-3 | 91.66 |
| OVCAR-4 | 71.52 |
| OVCAR-5 | 90.65 |
| OVCAR-8 | 88.41 |
| NCI/ADR-RES | 88.58 |
| SK-OV-3 | 84.22 |
| Renal Cancer | |
| 786-0 | 91.76 |
| A498 | 55.24 |
| ACHN | 78.80 |
| CAKI-1 | 48.67 |
| RXF 393 | 77.46 |
| SN12C | 73.03 |
| TK-10 | 86.09 |
| UO-31 | 52.53 |
| Prostate Cancer | |
| PC-3 | 75.84 |
| DU-145 | 100.24 |
| Breast Cancer | |
| MCF7 | 67.40 |
| MDA-MB-231/ATCC | 48.95 |
| HS 578T | 72.49 |
| BT-549 | 92.30 |
| T-47D | 76.54 |
| MDA-MB-468 | 69.88 |
| Mean | 77.66 |
| Delta | 47.50 |
| Range | 73.93 |

Mean Growth Percent - Growth Percent

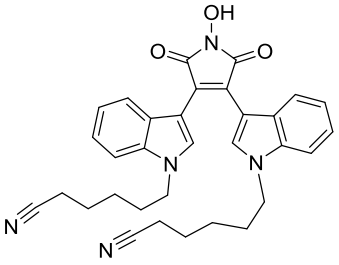


**6,6'-[3,3'-(1-Hydroxy-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile 282**



**6,6'-[3,3'-(1-Hydroxy-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(1*H*-indole-3,1-diyl)]dihexanenitrile 282**



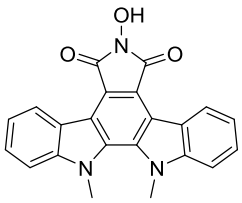
National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

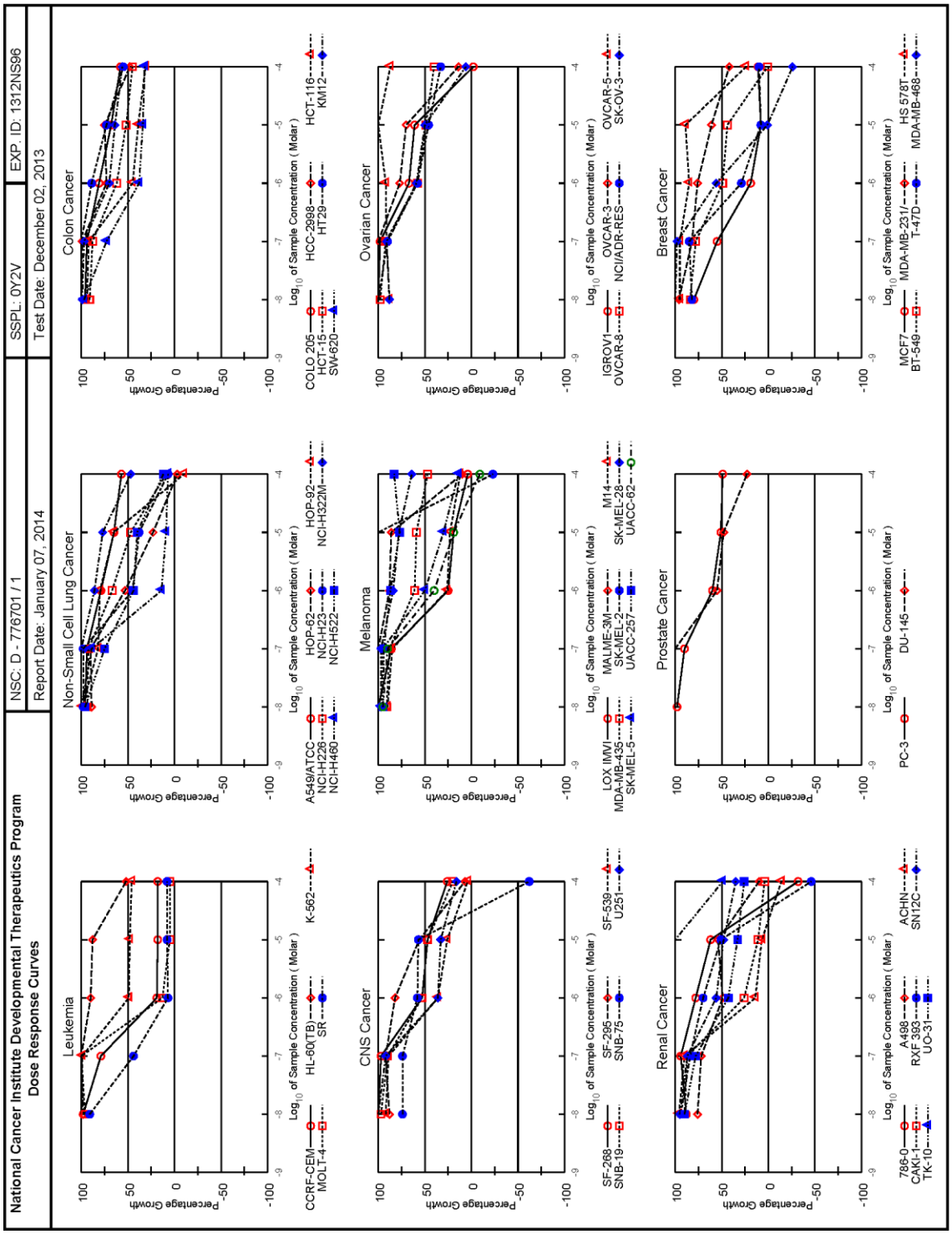
| NSC : D - 776693 / 1 | Experiment ID : 1312NS96 | Test Type : 08 | Units : Molar |
|---|---|---|---|
| Report Date : January 07, 2014 | Test Date : December 02, 2013 | QNS : | MC : |
| COMI : HW-4-354-1 (132326) | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

Log10 Concentration

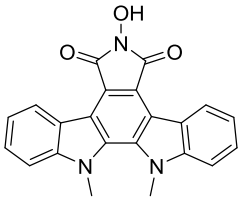
| Panel/Cell Line | Time Zero | Mean Optical Densities Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | Percent Growth -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.522 | 2.357 | 2.195 | 2.074 | 2.040 | 0.665 | 0.582 | 91 | 85 | 83 | 8 | 3 | 2.73E-6 | > 1.00E-4 | > 1.00E-4 |
| HL-60(TB) | 1.473 | 3.080 | 3.049 | 2.902 | 3.079 | 1.125 | 1.077 | 98 | 89 | 100 | -24 | -27 | 2.54E-6 | 6.44E-6 | > 1.00E-4 |
| K-562 | 0.204 | 1.328 | 1.161 | 1.228 | 1.256 | 0.375 | 0.237 | 85 | 91 | 94 | 15 | 3 | 3.59E-6 | > 1.00E-4 | > 1.00E-4 |
| MOLT-4 | 0.650 | 2.315 | 2.099 | 2.003 | 2.067 | 0.815 | 0.679 | 87 | 81 | 85 | 10 | 2 | 2.93E-6 | > 1.00E-4 | > 1.00E-4 |
| SR | 0.388 | 1.478 | 1.309 | 1.478 | 1.635 | 0.378 | 0.334 | 84 | 100 | 114 | -3 | -14 | 3.55E-6 | 9.48E-6 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.339 | 1.846 | 1.848 | 1.879 | 1.848 | 1.105 | 0.413 | 100 | 102 | 100 | 51 | 5 | 1.04E-5 | > 1.00E-4 | > 1.00E-4 |
| HOP-62 | 0.830 | 2.187 | 2.106 | 1.977 | 1.958 | 1.459 | 0.879 | 94 | 85 | 83 | 46 | 4 | 7.94E-6 | > 1.00E-4 | > 1.00E-4 |
| HOP-92 | 0.903 | 1.328 | 1.315 | 1.314 | 1.365 | 1.009 | 0.950 | 97 | 97 | 109 | 25 | 11 | 5.03E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H226 | 1.084 | 2.478 | 2.406 | 2.426 | 2.326 | 1.693 | 1.001 | 95 | 96 | 89 | 44 | -8 | 7.26E-6 | 7.08E-5 | > 1.00E-4 |
| NCI-H23 | 0.609 | 1.845 | 1.782 | 1.725 | 1.776 | 1.076 | 0.619 | 95 | 90 | 94 | 38 | 1 | 6.09E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H322M | 0.787 | 1.695 | 1.681 | 1.690 | 1.698 | 1.207 | 0.759 | 98 | 99 | 100 | 46 | -4 | 8.53E-6 | 8.46E-5 | > 1.00E-4 |
| NCI-H460 | 0.278 | 2.704 | 2.811 | 2.802 | 2.739 | 0.965 | 0.205 | 104 | 104 | 101 | 28 | -26 | 5.05E-6 | 3.30E-5 | > 1.00E-4 |
| NCI-H522 | 0.886 | 2.121 | 1.984 | 1.946 | 1.934 | 0.894 | 0.620 | 89 | 86 | 85 | 1 | -30 | 2.59E-6 | 1.05E-5 | > 1.00E-4 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.478 | 1.835 | 1.824 | 1.740 | 1.790 | 1.481 | 0.372 | 99 | 93 | 97 | 74 | -22 | 1.77E-5 | 5.87E-5 | > 1.00E-4 |
| HCC-2998 | 0.667 | 1.980 | 1.953 | 1.994 | 2.081 | 1.438 | 0.684 | 98 | 101 | 108 | 59 | 1 | 1.42E-5 | > 1.00E-4 | > 1.00E-4 |
| HCT-116 | 0.307 | 2.579 | 2.617 | 2.508 | 2.531 | 0.777 | 0.261 | 102 | 97 | 98 | 21 | -15 | 4.17E-6 | 3.78E-5 | > 1.00E-4 |
| HCT-15 | 0.365 | 2.630 | 2.482 | 2.475 | 2.444 | 0.837 | 0.377 | 93 | 93 | 92 | 21 | 1 | 3.88E-6 | > 1.00E-4 | > 1.00E-4 |
| HT29 | 0.241 | 1.238 | 1.234 | 1.270 | 1.298 | 0.747 | 0.163 | 100 | 103 | 106 | 51 | -33 | 1.02E-5 | 4.07E-5 | > 1.00E-4 |
| KM12 | 0.396 | 2.259 | 2.209 | 2.201 | 2.298 | 0.870 | 0.303 | 97 | 97 | 102 | 25 | -23 | 4.78E-6 | 3.31E-5 | > 1.00E-4 |
| SW-620 | 0.365 | 2.750 | 2.746 | 2.673 | 2.533 | 1.094 | 0.542 | 100 | 97 | 91 | 31 | 7 | 4.76E-6 | > 1.00E-4 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.553 | 1.848 | 1.808 | 1.768 | 1.834 | 1.204 | 0.653 | 97 | 95 | 99 | 50 | 8 | 1.01E-5 | > 1.00E-4 | > 1.00E-4 |
| SF-295 | 0.825 | 2.986 | 2.942 | 2.782 | 2.903 | 1.613 | 0.520 | 98 | 91 | 96 | 36 | -37 | 5.93E-6 | 3.14E-5 | > 1.00E-4 |
| SF-539 | 0.584 | 1.964 | 1.883 | 1.822 | 1.868 | 0.570 | 0.377 | 94 | 90 | 93 | -2 | -36 | 2.82E-6 | 9.42E-6 | > 1.00E-4 |
| SNB-19 | 0.636 | 2.209 | 2.155 | 2.128 | 2.064 | 1.062 | 0.956 | 97 | 95 | 91 | 27 | 20 | 4.36E-6 | > 1.00E-4 | > 1.00E-4 |
| SNB-75 | 0.767 | 1.420 | 1.284 | 1.234 | 1.289 | 0.859 | 0.519 | 79 | 72 | 80 | 14 | -32 | 2.84E-6 | 2.00E-5 | > 1.00E-4 |
| U251 | 0.487 | 2.053 | 2.087 | 2.051 | 2.056 | 0.879 | 0.565 | 102 | 100 | 100 | 25 | 5 | 4.65E-6 | > 1.00E-4 | > 1.00E-4 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.447 | 2.379 | 2.263 | 2.182 | 1.812 | 0.823 | 0.407 | 94 | 90 | 71 | 19 | -9 | 2.53E-6 | 4.81E-5 | > 1.00E-4 |
| MALME-3M | 0.447 | 0.717 | 0.717 | 0.700 | 0.736 | 0.597 | 0.384 | 100 | 94 | 107 | 56 | -14 | 1.20E-5 | 6.26E-5 | > 1.00E-4 |
| M14 | 0.555 | 2.063 | 2.018 | 1.836 | 1.904 | 0.982 | 0.417 | 97 | 85 | 89 | 28 | -25 | 4.42E-6 | 3.40E-5 | > 1.00E-4 |
| MDA-MB-435 | 0.539 | 2.620 | 2.520 | 2.526 | 2.527 | 0.494 | 0.394 | 95 | 95 | 96 | -8 | -27 | 2.74E-6 | 8.31E-6 | > 1.00E-4 |
| SK-MEL-2 | 1.239 | 2.379 | 2.384 | 2.361 | 2.347 | 1.329 | 0.772 | 100 | 98 | 97 | 8 | -38 | 3.38E-6 | 1.49E-5 | > 1.00E-4 |
| SK-MEL-28 | 0.552 | 1.546 | 1.563 | 1.567 | 1.575 | 0.904 | 0.594 | 102 | 102 | 103 | 35 | 4 | 6.07E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-5 | 0.756 | 3.071 | 3.048 | 3.059 | 2.881 | 0.803 | 0.149 | 99 | 99 | 92 | 2 | -80 | 2.92E-6 | 1.06E-5 | 4.28E-5 |
| UACC-257 | 1.097 | 2.351 | 2.315 | 2.345 | 2.319 | 1.801 | 0.887 | 97 | 100 | 97 | 56 | -19 | 1.21E-5 | 5.56E-5 | > 1.00E-4 |
| UACC-62 | 0.531 | 2.489 | 2.387 | 2.365 | 2.241 | 0.806 | 0.239 | 95 | 94 | 87 | 14 | -55 | 3.23E-6 | 1.60E-5 | 8.44E-5 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.706 | 1.996 | 2.051 | 2.010 | 2.042 | 1.063 | 0.672 | 104 | 101 | 104 | 28 | -5 | 5.08E-6 | 7.08E-5 | > 1.00E-4 |
| OVCAR-3 | 0.500 | 1.537 | 1.523 | 1.544 | 1.550 | 0.636 | 0.331 | 99 | 101 | 101 | 13 | -34 | 3.81E-6 | 1.90E-5 | > 1.00E-4 |
| OVCAR-5 | 0.744 | 1.511 | 1.505 | 1.425 | 1.445 | 1.471 | 0.884 | 99 | 89 | 91 | 95 | 18 | 3.84E-5 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-8 | 0.473 | 2.147 | 2.131 | 2.124 | 2.071 | 1.390 | 0.517 | 99 | 99 | 95 | 55 | 3 | 1.23E-5 | > 1.00E-4 | > 1.00E-4 |
| NCI/ADR-RES | 0.594 | 1.995 | 1.993 | 1.883 | 2.023 | 1.457 | 0.932 | 100 | 92 | 102 | 62 | 24 | 2.04E-5 | > 1.00E-4 | > 1.00E-4 |
| SK-OV-3 | 0.993 | 1.899 | 1.883 | 1.789 | 1.774 | 1.406 | 0.996 | 98 | 88 | 86 | 46 | . | 7.78E-6 | > 1.00E-4 | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.540 | 2.022 | 2.068 | 2.032 | 2.022 | 1.291 | 0.628 | 103 | 101 | 100 | 51 | 6 | 1.03E-5 | > 1.00E-4 | > 1.00E-4 |
| A498 | 1.702 | 2.614 | 2.470 | 2.446 | 2.340 | 1.851 | 1.683 | 84 | 82 | 70 | 16 | -1 | 2.36E-6 | 8.60E-5 | > 1.00E-4 |
| ACHN | 0.483 | 2.133 | 2.119 | 1.991 | 1.929 | 1.243 | 0.551 | 99 | 91 | 88 | 46 | 4 | 8.04E-6 | > 1.00E-4 | > 1.00E-4 |
| CAKI-1 | 0.661 | 2.858 | 2.812 | 2.749 | 2.704 | 2.129 | 1.003 | 98 | 95 | 93 | 67 | 16 | 2.13E-5 | > 1.00E-4 | > 1.00E-4 |
| RXF 393 | 1.123 | 1.665 | 1.642 | 1.674 | 1.653 | 1.136 | 0.842 | 96 | 102 | 98 | 2 | -25 | 3.16E-6 | 1.22E-5 | > 1.00E-4 |
| SN12C | 0.530 | 2.075 | 2.029 | 1.980 | 1.971 | 1.251 | 0.607 | 97 | 94 | 93 | 47 | 5 | 8.47E-6 | > 1.00E-4 | > 1.00E-4 |
| TK-10 | 1.092 | 2.232 | 2.151 | 2.177 | 2.312 | 1.894 | 1.224 | 93 | 95 | 107 | 70 | 12 | 2.22E-5 | > 1.00E-4 | > 1.00E-4 |
| UO-31 | 0.743 | 1.992 | 1.706 | 1.568 | 1.479 | 1.027 | 0.735 | 77 | 66 | 59 | 23 | -1 | 1.76E-6 | 9.01E-5 | > 1.00E-4 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.611 | 1.918 | 1.831 | 1.756 | 1.657 | 0.996 | 0.584 | 93 | 88 | 80 | 29 | -4 | 3.92E-6 | 7.40E-5 | > 1.00E-4 |
| DU-145 | 0.516 | 1.843 | 1.857 | 1.882 | 1.938 | 1.584 | 0.674 | 101 | 103 | 107 | 80 | 12 | 2.78E-5 | > 1.00E-4 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.359 | 1.964 | 1.823 | 1.735 | 1.886 | 0.576 | 0.308 | 91 | 86 | 95 | 14 | -14 | 3.57E-6 | 3.07E-5 | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.700 | 1.601 | 1.568 | 1.522 | 1.442 | 0.979 | 0.650 | 96 | 91 | 82 | 31 | -7 | 4.26E-6 | 6.47E-5 | > 1.00E-4 |
| HS 578T | 1.189 | 2.255 | 2.179 | 2.155 | 2.124 | 1.453 | 1.247 | 93 | 91 | 88 | 25 | 5 | 3.97E-6 | > 1.00E-4 | > 1.00E-4 |
| BT-549 | 1.266 | 2.304 | 2.316 | 2.222 | 2.188 | 1.437 | 0.939 | 101 | 92 | 89 | 16 | -26 | 3.44E-6 | 2.45E-5 | > 1.00E-4 |
| T-47D | 0.738 | 1.661 | 1.655 | 1.557 | 1.557 | 0.978 | 0.685 | 99 | 89 | 89 | 26 | -7 | 4.15E-6 | 6.05E-5 | > 1.00E-4 |
| MDA-MB-468 | 0.774 | 1.311 | 1.344 | 1.342 | 1.343 | 0.788 | 0.656 | 106 | 106 | 106 | 3 | -15 | 3.47E-6 | 1.39E-5 | > 1.00E-4 |

**6-Hydroxy-12,13-dimethyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 305**



## 6-Hydroxy-12,13-dimethyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 305

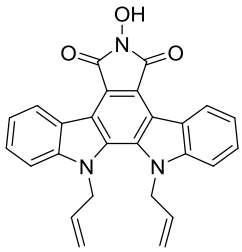


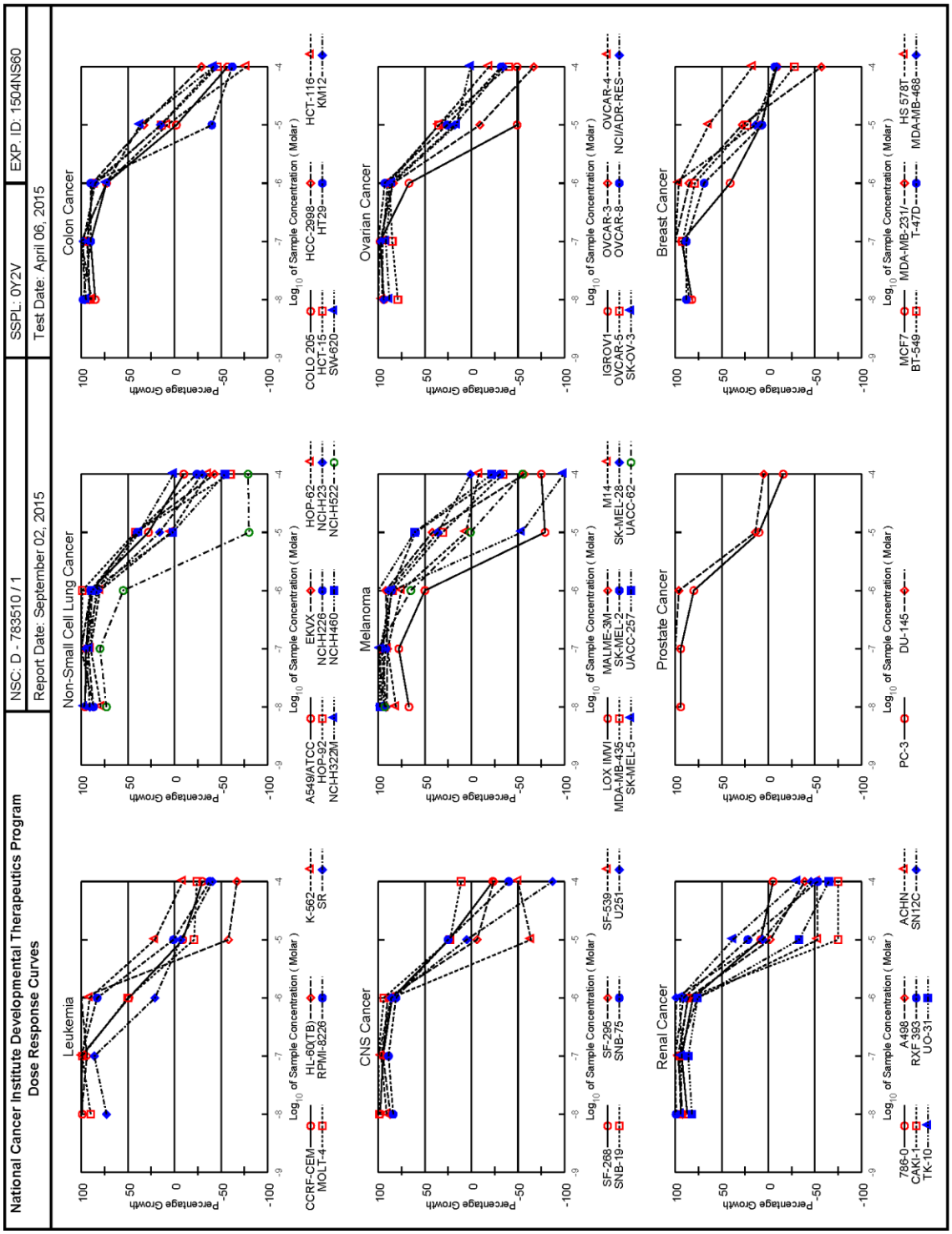

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

| | | | |
|---|---|---|---|
| NSC : D - 776701 / 1 | Experiment ID : 1312NS96 | Test Type : 08 | Units : Molar |
| Report Date : January 07, 2014 | Test Date : December 02, 2013 | QNS : | MC : |
| COMI : HW-4-371-3 (132705) | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

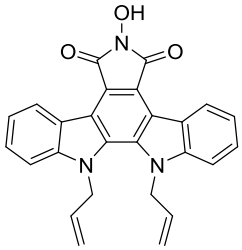
| | | Log10 Concentration | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | Mean Optical Densities | | | | | | Percent Growth | | | | | | |
| Panel/Cell Line | Zero | Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.522 | 2.357 | 2.322 | 1.973 | 0.863 | 0.851 | 0.857 | 98 | 79 | 19 | 18 | 18 | 3.02E-7 | > 1.00E-4 | > 1.00E-4 |
| HL-60(TB) | 1.473 | 3.080 | 3.051 | 3.178 | 2.920 | 2.890 | 2.314 | 98 | 106 | 90 | 88 | 52 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| K-562 | 0.204 | 1.328 | 1.357 | 1.315 | 0.750 | 0.742 | 0.726 | 103 | 99 | 49 | 48 | 46 | 9.35E-7 | > 1.00E-4 | > 1.00E-4 |
| MOLT-4 | 0.650 | 2.315 | 2.242 | 2.318 | 0.865 | 0.738 | 0.736 | 96 | 100 | 13 | 5 | 5 | 3.76E-7 | > 1.00E-4 | > 1.00E-4 |
| SR | 0.388 | 1.478 | 1.377 | 0.864 | 0.467 | 0.478 | 0.479 | 91 | 44 | 7 | 8 | 8 | 7.33E-8 | > 1.00E-4 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.339 | 1.846 | 1.783 | 1.755 | 1.522 | 1.323 | 1.204 | 96 | 94 | 79 | 65 | 57 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| HOP-62 | 0.830 | 2.187 | 2.033 | 2.047 | 1.555 | 1.140 | 0.807 | 89 | 90 | 53 | 23 | -3 | 1.29E-6 | 7.76E-5 | > 1.00E-4 |
| HOP-92 | 0.903 | 1.328 | 1.323 | 1.250 | 1.244 | 1.179 | 0.814 | 99 | 82 | 80 | 65 | -10 | 1.58E-5 | 7.38E-5 | > 1.00E-4 |
| NCI-H226 | 1.084 | 2.478 | 2.395 | 2.365 | 2.021 | 1.743 | 1.216 | 94 | 92 | 67 | 47 | 9 | 7.27E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H23 | 0.609 | 1.845 | 1.818 | 1.820 | 1.165 | 1.077 | 0.691 | 98 | 98 | 45 | 38 | 7 | 8.05E-7 | > 1.00E-4 | > 1.00E-4 |
| NCI-H322M | 0.787 | 1.695 | 1.754 | 1.757 | 1.569 | 1.487 | 1.213 | 106 | 107 | 86 | 77 | 47 | 7.88E-5 | > 1.00E-4 | > 1.00E-4 |
| NCI-H460 | 0.278 | 2.704 | 2.777 | 2.449 | 0.614 | 0.500 | 0.451 | 103 | 89 | 14 | 9 | 7 | 3.33E-7 | > 1.00E-4 | > 1.00E-4 |
| NCI-H522 | 0.886 | 2.121 | 2.077 | 1.813 | 1.426 | 1.382 | 1.039 | 96 | 75 | 44 | 40 | 12 | 6.29E-7 | > 1.00E-4 | > 1.00E-4 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.478 | 1.835 | 1.778 | 1.750 | 1.574 | 1.396 | 1.259 | 96 | 94 | 81 | 68 | 58 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| HCC-2998 | 0.667 | 1.980 | 1.997 | 1.965 | 1.631 | 1.646 | 1.297 | 101 | 99 | 73 | 75 | 48 | 8.40E-5 | > 1.00E-4 | > 1.00E-4 |
| HCT-116 | 0.307 | 2.579 | 2.436 | 2.400 | 1.330 | 1.199 | 1.014 | 94 | 92 | 45 | 39 | 31 | 7.84E-7 | > 1.00E-4 | > 1.00E-4 |
| HCT-15 | 0.365 | 2.630 | 2.430 | 2.352 | 1.763 | 1.539 | 1.392 | 91 | 88 | 62 | 52 | 45 | 1.91E-5 | > 1.00E-4 | > 1.00E-4 |
| HT29 | 0.241 | 1.238 | 1.274 | 1.235 | 1.124 | 0.989 | 0.794 | 104 | 100 | 89 | 73 | 55 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| KM12 | 0.396 | 2.259 | 2.237 | 2.200 | 1.691 | 1.591 | 1.431 | 99 | 97 | 70 | 64 | 56 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| SW-620 | 0.365 | 2.750 | 2.667 | 2.108 | 1.274 | 1.187 | 1.119 | 97 | 73 | 38 | 34 | 32 | 4.57E-7 | > 1.00E-4 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.553 | 1.848 | 1.900 | 1.808 | 1.234 | 1.158 | 0.895 | 104 | 97 | 53 | 47 | 26 | 2.74E-6 | > 1.00E-4 | > 1.00E-4 |
| SF-295 | 0.825 | 2.986 | 2.738 | 2.821 | 2.608 | 2.030 | 0.981 | 88 | 92 | 82 | 56 | 7 | 1.31E-5 | > 1.00E-4 | > 1.00E-4 |
| SF-539 | 0.584 | 1.964 | 1.852 | 1.813 | 1.075 | 0.949 | 0.644 | 92 | 89 | 36 | 26 | 4 | 5.38E-7 | > 1.00E-4 | > 1.00E-4 |
| SNB-19 | 0.636 | 2.209 | 2.169 | 2.053 | 1.468 | 1.370 | 0.956 | 97 | 90 | 53 | 47 | 20 | 2.87E-6 | > 1.00E-4 | > 1.00E-4 |
| SNB-75 | 0.767 | 1.420 | 1.248 | 1.249 | 1.147 | 1.136 | 0.292 | 74 | 74 | 58 | 57 | -62 | 1.13E-5 | 3.00E-5 | 7.92E-5 |
| U251 | 0.487 | 2.053 | 2.056 | 1.928 | 1.047 | 0.997 | 0.736 | 100 | 92 | 36 | 33 | 16 | 5.57E-7 | > 1.00E-4 | > 1.00E-4 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.447 | 2.379 | 2.407 | 2.111 | 0.921 | 0.817 | 0.528 | 101 | 86 | 25 | 19 | 4 | 3.86E-7 | > 1.00E-4 | > 1.00E-4 |
| MALME-3M | 0.447 | 0.717 | 0.735 | 0.732 | 0.684 | 0.681 | 0.475 | 107 | 105 | 88 | 86 | 10 | 3.02E-5 | > 1.00E-4 | > 1.00E-4 |
| M14 | 0.555 | 2.063 | 1.906 | 1.830 | 0.947 | 0.901 | 0.731 | 90 | 85 | 26 | 23 | 12 | 3.89E-7 | > 1.00E-4 | > 1.00E-4 |
| MDA-MB-435 | 0.539 | 2.620 | 2.437 | 2.368 | 1.802 | 1.763 | 1.521 | 91 | 88 | 61 | 59 | 47 | 5.73E-5 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-2 | 1.239 | 2.379 | 2.393 | 2.500 | 2.442 | 2.519 | 0.954 | 101 | 111 | 106 | 112 | -23 | 2.89E-5 | 6.76E-5 | > 1.00E-4 |
| SK-MEL-28 | 0.552 | 1.546 | 1.512 | 1.516 | 1.383 | 1.325 | 1.185 | 97 | 97 | 84 | 78 | 64 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-5 | 0.756 | 3.071 | 3.001 | 2.947 | 1.920 | 1.460 | 1.089 | 97 | 95 | 50 | 30 | 14 | 1.03E-6 | > 1.00E-4 | > 1.00E-4 |
| UACC-257 | 1.097 | 2.351 | 2.288 | 2.275 | 2.191 | 2.057 | 2.139 | 95 | 94 | 87 | 77 | 83 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| UACC-62 | 0.531 | 2.489 | 2.400 | 2.290 | 1.306 | 0.900 | 0.482 | 95 | 90 | 40 | 19 | -9 | 6.20E-7 | 4.69E-5 | > 1.00E-4 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.706 | 1.996 | 2.049 | 1.969 | 1.576 | 1.488 | 0.690 | 104 | 98 | 67 | 61 | -2 | 1.47E-5 | 9.20E-5 | > 1.00E-4 |
| OVCAR-3 | 0.500 | 1.537 | 1.584 | 1.554 | 1.297 | 1.231 | 0.648 | 105 | 102 | 77 | 70 | 14 | 2.31E-5 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-5 | 0.744 | 1.511 | 1.422 | 1.439 | 1.446 | 1.566 | 1.412 | 88 | 91 | 92 | 107 | 87 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-8 | 0.473 | 2.147 | 2.114 | 2.008 | 1.438 | 1.301 | 1.143 | 98 | 92 | 58 | 49 | 40 | 8.56E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI/ADR-RES | 0.594 | 1.995 | 2.016 | 1.853 | 1.400 | 1.242 | 1.055 | 102 | 90 | 58 | 46 | 33 | 4.67E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-OV-3 | 0.993 | 1.899 | 1.787 | 1.815 | 1.532 | 1.444 | 1.052 | 88 | 91 | 59 | 50 | 6 | 9.49E-6 | > 1.00E-4 | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.540 | 2.022 | 1.843 | 1.940 | 1.692 | 1.457 | 0.365 | 88 | 94 | 78 | 62 | -32 | 1.34E-5 | 4.53E-5 | > 1.00E-4 |
| A498 | 1.702 | 2.614 | 2.389 | 2.359 | 2.152 | 2.198 | 1.794 | 76 | 72 | 49 | 54 | 10 | . | > 1.00E-4 | > 1.00E-4 |
| ACHN | 0.483 | 2.133 | 2.065 | 1.965 | 0.713 | 0.593 | 0.416 | 96 | 90 | 14 | 7 | -14 | 3.34E-7 | 2.11E-5 | > 1.00E-4 |
| CAKI-1 | 0.661 | 2.858 | 2.648 | 2.566 | 1.231 | 0.904 | 0.740 | 90 | 87 | 26 | 11 | 4 | 4.02E-7 | > 1.00E-4 | > 1.00E-4 |
| RXF 393 | 1.123 | 1.665 | 1.631 | 1.577 | 1.502 | 1.399 | 0.609 | 94 | 84 | 70 | 51 | -46 | 1.02E-5 | 3.36E-5 | > 1.00E-4 |
| SN12C | 0.530 | 2.075 | 1.993 | 1.878 | 1.389 | 1.266 | 1.066 | 95 | 87 | 56 | 48 | 35 | 5.03E-6 | > 1.00E-4 | > 1.00E-4 |
| TK-10 | 1.092 | 2.232 | 2.269 | 2.307 | 2.308 | 2.283 | 1.647 | 103 | 107 | 107 | 104 | 49 | 9.47E-5 | > 1.00E-4 | > 1.00E-4 |
| UO-31 | 0.743 | 1.992 | 1.865 | 1.715 | 1.285 | 1.154 | 1.086 | 90 | 78 | 43 | 33 | 26 | 6.42E-7 | > 1.00E-4 | > 1.00E-4 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.611 | 1.918 | 1.887 | 1.791 | 1.395 | 1.281 | 1.253 | 98 | 90 | 60 | 51 | 49 | 3.77E-5 | > 1.00E-4 | > 1.00E-4 |
| DU-145 | 0.516 | 1.843 | 1.939 | 1.933 | 1.249 | 1.148 | 0.822 | 107 | 107 | 55 | 48 | 23 | 4.82E-6 | > 1.00E-4 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.359 | 1.964 | 1.636 | 1.235 | 0.668 | 0.490 | 0.532 | 80 | 55 | 19 | 8 | 11 | 1.35E-7 | > 1.00E-4 | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.700 | 1.601 | 1.562 | 1.441 | 1.381 | 1.251 | 1.075 | 96 | 82 | 76 | 61 | 42 | 3.73E-5 | > 1.00E-4 | > 1.00E-4 |
| HS 578T | 1.189 | 2.255 | 2.201 | 2.200 | 2.094 | 2.133 | 1.448 | 95 | 95 | 85 | 89 | 24 | 3.98E-5 | > 1.00E-4 | > 1.00E-4 |
| BT-549 | 1.266 | 2.304 | 2.142 | 2.073 | 1.770 | 1.720 | 1.278 | 84 | 78 | 49 | 44 | 1 | 8.93E-7 | > 1.00E-4 | > 1.00E-4 |
| T-47D | 0.738 | 1.661 | 1.492 | 1.519 | 1.010 | 0.810 | 0.833 | 82 | 85 | 29 | 8 | 10 | 4.24E-7 | > 1.00E-4 | > 1.00E-4 |
| MDA-MB-468 | 0.774 | 1.311 | 1.331 | 1.300 | 1.076 | 0.779 | 0.572 | 104 | 98 | 56 | 1 | -26 | 1.29E-6 | 1.07E-5 | > 1.00E-4 |

**12,13-Diallyl-6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 306**



**12,13-Diallyl-6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 306**



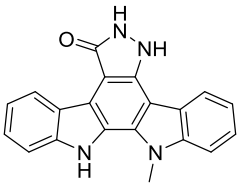

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

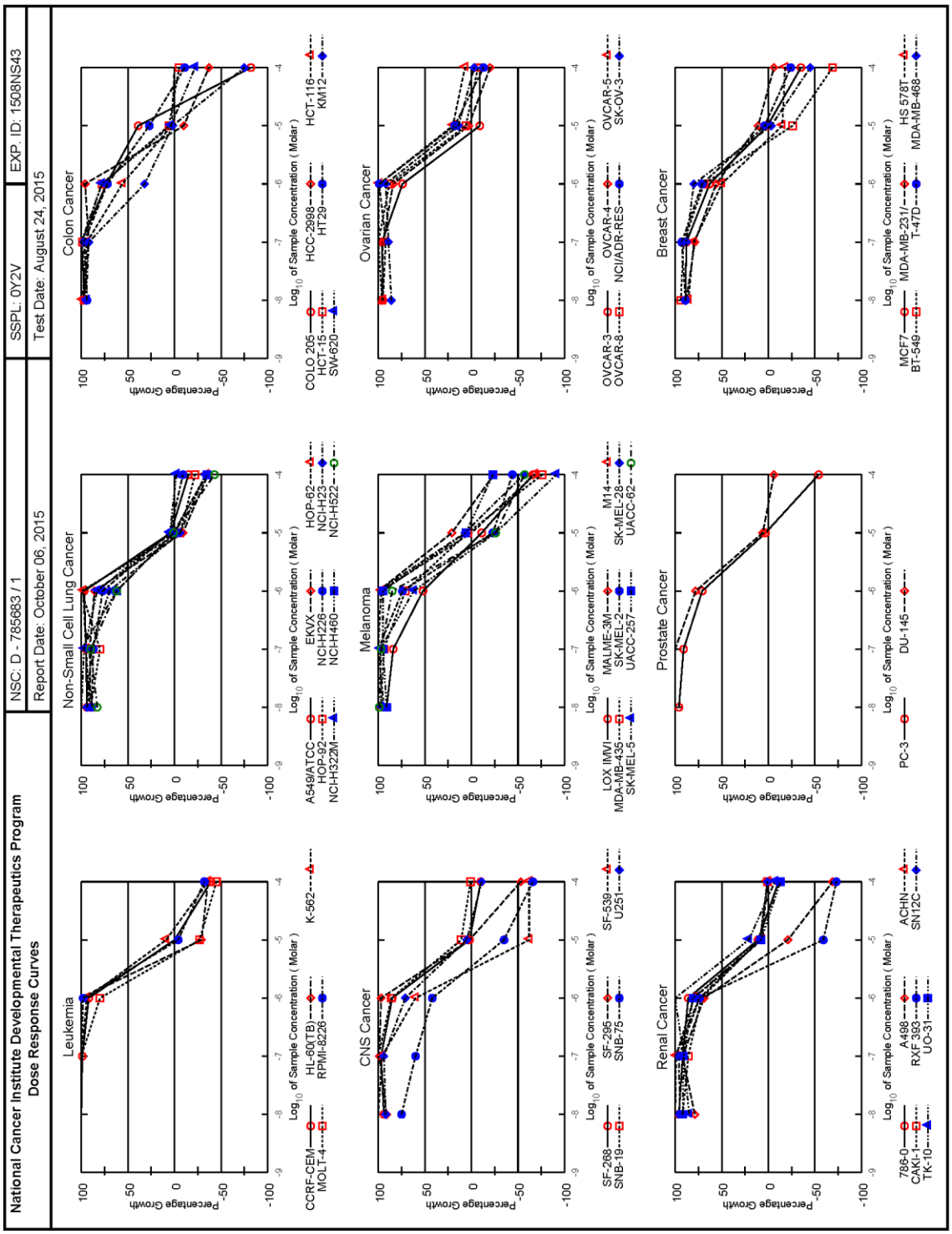
| | | | |
|---|---|---|---|
| NSC : D - 783510 / 1 | Experiment ID : 1504NS60 | Test Type : 08 | Units : Molar |
| Report Date : September 02, 2015 | Test Date : April 06, 2015 | QNS : | MC : |
| COMI : HW7-616-1 | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

Log10 Concentration

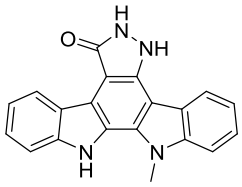
| | Time | Mean Optical Densities | | | | | | Percent Growth | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Panel/Cell Line | Zero | Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.552 | 1.672 | 1.659 | 1.836 | 1.103 | 0.505 | 0.389 | 99 | 115 | 49 | -9 | -30 | 9.71E-7 | 7.10E-6 | > 1.00E-4 |
| HL-60(TB) | 0.933 | 2.800 | 2.820 | 2.662 | 2.824 | 0.389 | 0.309 | 101 | 94 | 101 | -58 | -67 | 2.09E-6 | 4.31E-6 | 8.86E-6 |
| K-562 | 0.373 | 1.891 | 2.139 | 1.873 | 1.756 | 0.699 | 0.339 | 116 | 99 | 91 | 21 | -9 | 3.89E-6 | 5.00E-5 | > 1.00E-4 |
| MOLT-4 | 0.706 | 2.435 | 2.255 | 2.416 | 1.579 | 0.560 | 0.536 | 90 | 99 | 50 | -21 | -24 | 1.02E-6 | 5.12E-6 | > 1.00E-4 |
| RPMI-8226 | 1.307 | 2.906 | 2.964 | 3.032 | 2.629 | 1.324 | 0.808 | 104 | 108 | 83 | 1 | -38 | 2.51E-6 | 1.06E-5 | > 1.00E-4 |
| SR | 0.266 | 1.004 | 0.802 | 0.904 | 0.423 | 0.248 | 0.156 | 73 | 86 | 21 | -7 | -41 | 3.63E-7 | 5.67E-6 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.454 | 1.779 | 1.724 | 1.710 | 1.649 | 0.831 | 0.410 | 96 | 95 | 90 | 28 | -10 | 4.47E-6 | 5.54E-5 | > 1.00E-4 |
| EKVX | 0.784 | 1.909 | 1.768 | 1.829 | 1.774 | 1.241 | 0.447 | 87 | 93 | 88 | 41 | -43 | 6.34E-6 | 3.06E-5 | > 1.00E-4 |
| HOP-62 | 0.649 | 1.308 | 1.169 | 1.243 | 1.177 | 0.677 | 0.414 | 79 | 90 | 80 | 4 | -36 | 2.49E-6 | 1.27E-5 | > 1.00E-4 |
| HOP-92 | 1.053 | 1.463 | 1.468 | 1.477 | 1.459 | 1.224 | 0.416 | 101 | 103 | 99 | 42 | -60 | 7.13E-6 | 2.55E-5 | 7.89E-5 |
| NCI-H226 | 1.073 | 2.515 | 2.335 | 2.549 | 2.367 | 1.654 | 0.815 | 87 | 102 | 90 | 40 | -24 | 6.36E-6 | 4.22E-5 | > 1.00E-4 |
| NCI-H23 | 0.779 | 2.315 | 2.173 | 2.233 | 2.033 | 1.025 | 0.545 | 91 | 95 | 82 | 16 | -30 | 3.03E-6 | 2.22E-5 | > 1.00E-4 |
| NCI-H322M | 1.029 | 2.383 | 2.336 | 2.263 | 2.171 | 1.543 | 1.040 | 97 | 91 | 84 | 38 | 1 | 5.50E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H460 | 0.403 | 3.167 | 3.189 | 3.262 | 2.851 | 0.450 | 0.184 | 101 | 103 | 89 | 2 | -54 | 2.78E-6 | 1.07E-5 | 8.37E-5 |
| NCI-H522 | 0.915 | 2.362 | 1.967 | 2.067 | 1.710 | 0.188 | 0.193 | 73 | 80 | 55 | -80 | -79 | 1.09E-6 | 2.56E-6 | 6.03E-6 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.451 | 1.490 | 1.333 | 1.392 | 1.208 | 0.441 | 0.192 | 85 | 90 | 73 | -2 | -57 | 2.01E-6 | 9.31E-6 | 7.33E-5 |
| HCC-2998 | 0.691 | 2.233 | 2.098 | 2.125 | 2.053 | 1.202 | 0.490 | 91 | 93 | 88 | 33 | -29 | 4.94E-6 | 3.40E-5 | > 1.00E-4 |
| HCT-116 | 0.286 | 2.011 | 1.829 | 2.025 | 1.553 | 0.428 | 0.085 | 89 | 101 | 73 | 8 | -77 | 2.29E-6 | 1.25E-5 | 4.78E-5 |
| HCT-15 | 0.253 | 1.769 | 1.632 | 1.678 | 1.566 | 0.462 | 0.138 | 91 | 94 | 87 | 14 | -46 | 3.18E-6 | 1.70E-5 | > 1.00E-4 |
| HT29 | 0.272 | 1.601 | 1.573 | 1.467 | 1.467 | 0.162 | 0.104 | 98 | 90 | 90 | -40 | -62 | 2.02E-6 | 4.90E-6 | 2.78E-5 |
| KM12 | 0.499 | 2.661 | 2.564 | 2.626 | 2.344 | 0.834 | 0.284 | 96 | 98 | 85 | 15 | -43 | 3.20E-6 | 1.84E-5 | > 1.00E-4 |
| SW-620 | 0.282 | 1.903 | 1.816 | 1.907 | 1.463 | 0.889 | 0.163 | 95 | 100 | 73 | 37 | -42 | 4.42E-6 | 2.95E-5 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.707 | 2.353 | 2.315 | 2.269 | 2.104 | 1.100 | 0.545 | 98 | 95 | 85 | 24 | -23 | 3.72E-6 | 3.24E-5 | > 1.00E-4 |
| SF-295 | 0.923 | 2.768 | 2.526 | 2.668 | 2.592 | 0.866 | 0.699 | 87 | 95 | 90 | -6 | -24 | 2.62E-6 | 8.62E-6 | > 1.00E-4 |
| SF-539 | 0.660 | 2.140 | 2.009 | 2.103 | 1.940 | 0.238 | 0.332 | 91 | 97 | 86 | -64 | -50 | 1.75E-6 | 3.76E-6 | |
| SNB-19 | 0.824 | 2.322 | 2.303 | 2.399 | 2.231 | 1.171 | 0.983 | 99 | 105 | 94 | 23 | 11 | 4.18E-6 | > 1.00E-4 | > 1.00E-4 |
| SNB-75 | 0.418 | 0.959 | 0.873 | 0.901 | 0.856 | 0.552 | 0.253 | 84 | 89 | 81 | 25 | -40 | 3.55E-6 | 2.43E-5 | > 1.00E-4 |
| U251 | 0.598 | 2.058 | 2.082 | 2.137 | 1.851 | 0.676 | 0.079 | 102 | 105 | 86 | 5 | -87 | 2.79E-6 | 1.14E-5 | 3.98E-5 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.436 | 2.506 | 1.819 | 2.059 | 1.463 | 0.092 | 0.108 | 67 | 78 | 50 | -79 | -75 | 9.67E-7 | 2.43E-6 | 5.95E-6 |
| MALME-3M | 0.738 | 1.321 | 1.269 | 1.264 | 1.276 | 0.983 | 0.321 | 91 | 90 | 92 | 42 | -57 | 6.91E-6 | 2.67E-5 | 8.59E-5 |
| M14 | 0.630 | 2.248 | 1.940 | 2.083 | 1.846 | 0.729 | 0.577 | 81 | 90 | 75 | 6 | -8 | 2.31E-6 | 2.62E-5 | > 1.00E-4 |
| MDA-MB-435 | 0.469 | 2.457 | 2.343 | 2.371 | 2.168 | 1.081 | 0.308 | 94 | 96 | 85 | 31 | -34 | 4.45E-6 | 2.96E-5 | > 1.00E-4 |
| SK-MEL-2 | 0.985 | 2.260 | 2.226 | 2.157 | 2.085 | 1.756 | 0.683 | 97 | 92 | 86 | 60 | -31 | 1.30E-5 | 4.61E-5 | > 1.00E-4 |
| SK-MEL-28 | 0.843 | 2.550 | 2.418 | 2.462 | 2.360 | 1.461 | 0.865 | 92 | 95 | 89 | 36 | 1 | 5.47E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-5 | 0.726 | 3.137 | 3.061 | 3.079 | 2.777 | 0.335 | 0.006 | 97 | 98 | 85 | -54 | -99 | 1.79E-6 | 4.10E-6 | 9.38E-6 |
| UACC-257 | 0.825 | 1.494 | 1.478 | 1.545 | 1.549 | 1.231 | 0.646 | 98 | 108 | 108 | 61 | -22 | 1.35E-5 | 5.45E-5 | > 1.00E-4 |
| UACC-62 | 1.098 | 2.839 | 2.717 | 2.844 | 2.238 | 1.121 | 0.496 | 93 | 100 | 65 | 1 | -55 | 1.74E-6 | 1.05E-5 | 8.20E-5 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.559 | 2.231 | 2.135 | 2.192 | 1.678 | 0.288 | 0.286 | 94 | 98 | 67 | -49 | -49 | 1.40E-6 | 3.80E-6 | > 1.00E-4 |
| OVCAR-3 | 0.484 | 1.767 | 1.800 | 1.748 | 1.558 | 0.443 | 0.160 | 103 | 98 | 84 | -9 | -67 | 2.32E-6 | 8.07E-6 | 5.13E-5 |
| OVCAR-4 | 0.665 | 1.542 | 1.506 | 1.502 | 1.457 | 0.973 | 0.540 | 96 | 95 | 90 | 35 | -19 | 5.38E-6 | 4.48E-5 | > 1.00E-4 |
| OVCAR-5 | 0.836 | 1.909 | 1.688 | 1.749 | 1.785 | 1.215 | 0.505 | 79 | 85 | 88 | 35 | -40 | 5.29E-6 | 2.96E-5 | > 1.00E-4 |
| OVCAR-8 | 0.536 | 1.973 | 2.003 | 2.099 | 1.873 | 0.914 | 0.365 | 102 | 109 | 93 | 26 | -32 | 4.41E-6 | 2.83E-5 | > 1.00E-4 |
| NCI/ADR-RES | 0.493 | 1.826 | 1.741 | 1.781 | 1.656 | 0.728 | 0.325 | 94 | 97 | 87 | 18 | -34 | 3.43E-6 | 2.19E-5 | > 1.00E-4 |
| SK-OV-3 | 0.884 | 1.779 | 1.674 | 1.710 | 1.641 | 1.032 | 0.891 | 88 | 92 | 85 | 16 | 1 | 3.22E-6 | > 1.00E-4 | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.631 | 2.140 | 1.951 | 2.036 | 1.859 | 0.745 | 0.600 | 87 | 93 | 81 | 8 | -5 | 2.66E-6 | 4.04E-5 | > 1.00E-4 |
| A498 | 1.406 | 2.166 | 2.107 | 2.145 | 2.056 | 1.378 | 0.853 | 92 | 97 | 86 | -2 | -39 | 2.55E-6 | 9.48E-6 | > 1.00E-4 |
| ACHN | 0.367 | 1.601 | 1.532 | 1.546 | 1.310 | 0.173 | 0.176 | 94 | 96 | 76 | -53 | -52 | 1.60E-6 | 3.89E-6 | 9.48E-6 |
| CAKI-1 | 0.913 | 3.235 | 3.087 | 3.118 | 2.931 | 0.230 | 0.229 | 94 | 95 | 87 | -75 | -75 | 1.69E-6 | 3.45E-6 | 7.02E-6 |
| RXF 393 | 0.805 | 1.604 | 1.595 | 1.629 | 1.536 | 0.980 | 0.379 | 99 | 103 | 91 | 22 | -53 | 3.94E-6 | 1.96E-5 | 9.12E-5 |
| SN12C | 0.842 | 2.531 | 2.566 | 2.538 | 2.506 | 0.948 | 0.456 | 102 | 100 | 99 | 6 | -46 | 3.36E-6 | 1.32E-5 | > 1.00E-4 |
| TK-10 | 0.908 | 1.500 | 1.462 | 1.461 | 1.468 | 1.134 | 0.628 | 94 | 93 | 95 | 38 | -31 | 6.16E-6 | 3.57E-5 | > 1.00E-4 |
| UO-31 | 1.156 | 2.999 | 2.668 | 2.738 | 2.566 | 0.776 | 0.405 | 82 | 86 | 76 | -33 | -65 | 1.75E-6 | 5.01E-6 | 3.42E-5 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.919 | 2.348 | 2.258 | 2.268 | 2.059 | 1.069 | 0.777 | 94 | 94 | 80 | 10 | -16 | 2.69E-6 | 2.53E-5 | > 1.00E-4 |
| DU-145 | 0.446 | 1.875 | 1.940 | 1.914 | 1.816 | 0.852 | 0.512 | 105 | 103 | 96 | 14 | 5 | 3.65E-6 | > 1.00E-4 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.395 | 2.112 | 1.807 | 1.995 | 1.100 | 0.533 | 0.360 | 82 | 93 | 41 | 8 | -9 | 6.74E-7 | 2.99E-5 | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.777 | 1.608 | 1.629 | 1.659 | 1.480 | 1.013 | 0.332 | 103 | 106 | 85 | 28 | -57 | 4.12E-6 | 2.14E-5 | 8.21E-5 |
| HS 578T | 1.247 | 2.362 | 2.357 | 2.365 | 2.315 | 1.956 | 1.437 | 100 | 100 | 96 | 64 | 17 | 1.96E-5 | > 1.00E-4 | > 1.00E-4 |
| BT-549 | 1.081 | 2.314 | 2.128 | 2.218 | 2.053 | 1.363 | 0.774 | 85 | 92 | 79 | 23 | -28 | 3.27E-6 | 2.79E-5 | > 1.00E-4 |
| T-47D | 0.728 | 1.334 | 1.265 | 1.262 | 1.147 | 0.771 | 0.681 | 88 | 88 | 69 | 7 | -7 | 2.03E-6 | 3.32E-5 | > 1.00E-4 |
| MDA-MB-468 | 0.793 | 1.615 | 1.616 | 1.634 | 1.626 | 0.910 | 0.725 | 100 | 102 | 101 | 14 | -9 | 3.88E-6 | 4.19E-5 | > 1.00E-4 |

**13-Methyl-5,6,12,13-tetrahydro-7*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-7-one 360**





## 13-Methyl-5,6,12,13-tetrahydro-7*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-7-one 360

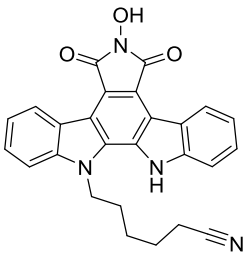


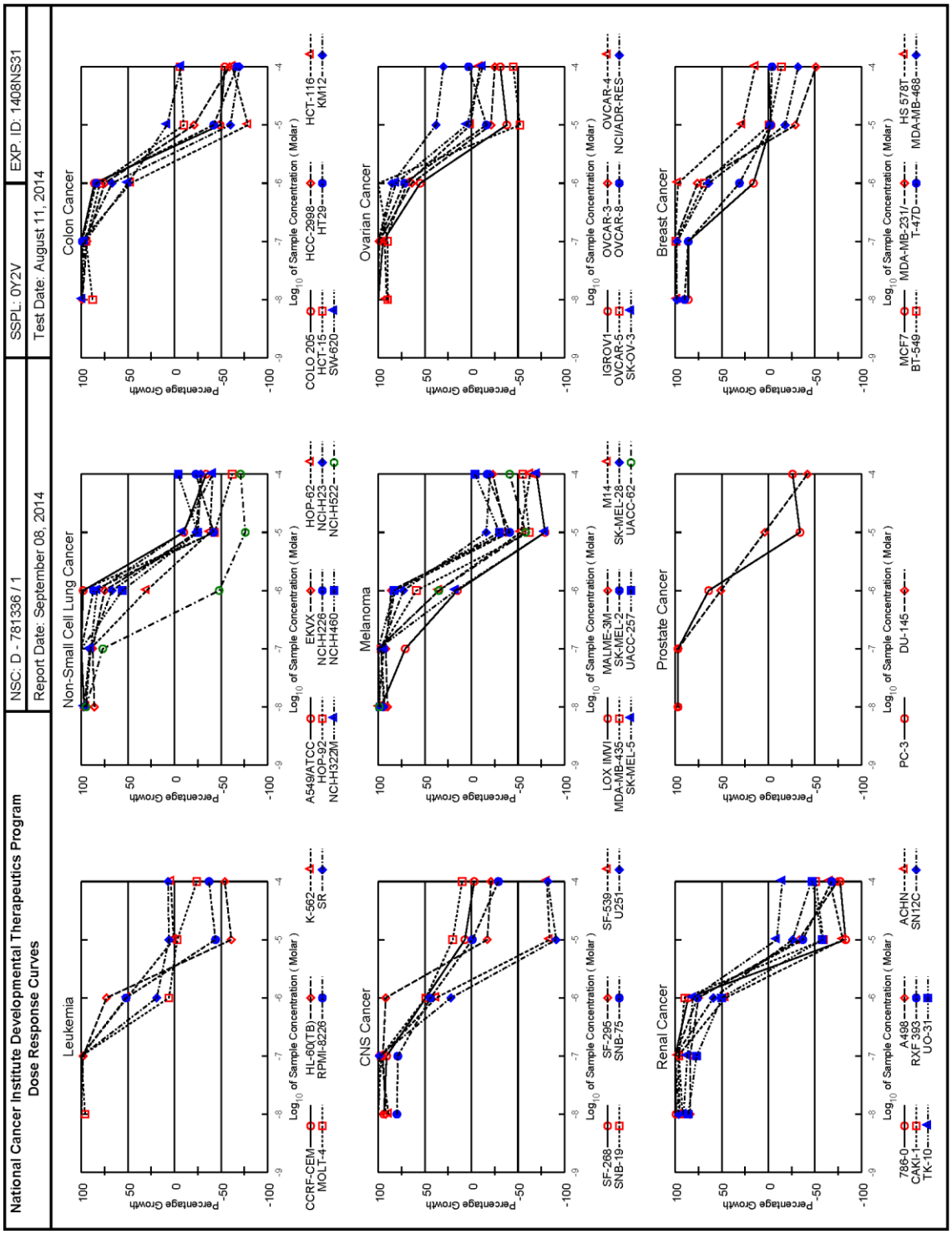
National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

| | | | |
|---|---|---|---|
| NSC : D - 785683 / 1 | Experiment ID : 1508NS43 | Test Type : 08 | Units : Molar |
| Report Date : October 06, 2015 | Test Date : August 24, 2015 | QNS : | MC : |
| COMI : HW 8-674-2 | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

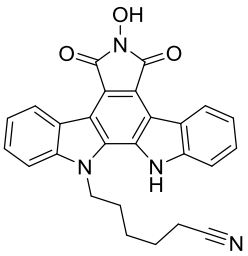
| | | Log10 Concentration | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | Mean Optical Densities | | | | | | Percent Growth | | | | | | |
| Panel/Cell Line | Zero | Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.889 | 3.311 | 3.302 | 3.294 | 3.126 | 0.898 | 0.538 | 100 | 99 | 92 | . | -39 | 2.89E-6 | 1.02E-5 | > 1.00E-4 |
| HL-60(TB) | 0.868 | 3.104 | 3.166 | 3.226 | 3.114 | 0.620 | 0.555 | 103 | 105 | 100 | -29 | -36 | 2.46E-6 | 6.01E-6 | > 1.00E-4 |
| K-562 | 0.325 | 2.419 | 2.444 | 2.458 | 2.294 | 0.514 | 0.208 | 101 | 102 | 94 | 9 | -36 | 3.30E-6 | 1.59E-5 | > 1.00E-4 |
| MOLT-4 | 0.711 | 2.740 | 2.829 | 2.856 | 2.329 | 0.516 | 0.392 | 104 | 106 | 80 | -27 | -45 | 1.89E-6 | 5.55E-6 | > 1.00E-4 |
| RPMI-8226 | 0.613 | 2.721 | 2.814 | 2.848 | 2.677 | 0.591 | 0.416 | 104 | 106 | 98 | -4 | -32 | 2.96E-6 | 9.20E-6 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.373 | 1.904 | 1.814 | 1.829 | 1.860 | 0.439 | 0.318 | 94 | 95 | 97 | 4 | -15 | 3.22E-6 | 1.68E-5 | > 1.00E-4 |
| EKVX | 0.662 | 1.567 | 1.477 | 1.486 | 1.444 | 0.605 | 0.422 | 90 | 91 | 86 | -9 | -36 | 2.41E-6 | 8.10E-6 | > 1.00E-4 |
| HOP-62 | 0.872 | 2.226 | 2.105 | 2.158 | 2.199 | 0.880 | 0.574 | 91 | 95 | 98 | 1 | -34 | 3.11E-6 | 1.04E-5 | > 1.00E-4 |
| HOP-92 | 1.197 | 1.762 | 1.708 | 1.650 | 1.903 | 1.124 | 0.939 | 90 | 80 | 125 | -6 | -22 | 3.73E-6 | 8.98E-6 | > 1.00E-4 |
| NCI-H226 | 0.528 | 1.726 | 1.592 | 1.570 | 1.445 | 0.585 | 0.482 | 89 | 87 | 77 | 5 | -9 | 2.34E-6 | 2.26E-5 | > 1.00E-4 |
| NCI-H23 | 0.596 | 2.191 | 2.098 | 2.016 | 1.718 | 0.561 | 0.401 | 94 | 89 | 70 | -6 | -33 | 1.85E-6 | 8.37E-6 | > 1.00E-4 |
| NCI-H322M | 0.498 | 1.743 | 1.746 | 1.704 | 1.527 | 0.566 | 0.491 | 100 | 97 | 83 | 5 | -2 | 2.65E-6 | 6.08E-5 | > 1.00E-4 |
| NCI-H460 | 0.215 | 2.330 | 2.372 | 2.336 | 1.525 | 0.240 | 0.140 | 102 | 100 | 62 | 1 | -35 | 1.57E-6 | 1.08E-5 | > 1.00E-4 |
| NCI-H522 | 1.040 | 2.512 | 2.269 | 2.360 | 1.949 | 1.052 | 0.591 | 83 | 90 | 62 | 1 | -43 | 1.56E-6 | 1.04E-5 | > 1.00E-4 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.436 | 1.692 | 1.644 | 1.695 | 1.350 | 0.927 | 0.077 | 96 | 100 | 73 | 39 | -82 | 4.74E-6 | 2.10E-5 | 5.41E-5 |
| HCC-2998 | 0.721 | 2.314 | 2.283 | 2.181 | 2.257 | 0.651 | 0.457 | 98 | 92 | 96 | -10 | -37 | 2.74E-6 | 8.10E-6 | > 1.00E-4 |
| HCT-116 | 0.418 | 3.037 | 3.001 | 2.867 | 1.894 | 0.507 | 0.418 | 99 | 94 | 56 | 3 | . | 1.32E-6 | 9.25E-5 | > 1.00E-4 |
| HCT-15 | 0.295 | 1.829 | 1.787 | 1.819 | 1.495 | 0.395 | 0.280 | 97 | 99 | 78 | 6 | -5 | 2.47E-6 | 3.57E-5 | > 1.00E-4 |
| HT29 | 0.313 | 1.940 | 1.845 | 1.899 | 1.490 | 0.757 | 0.278 | 94 | 97 | 72 | 27 | -11 | 3.13E-6 | 5.08E-5 | > 1.00E-4 |
| KM12 | 0.453 | 2.716 | 2.610 | 2.526 | 1.167 | 0.489 | 0.114 | 95 | 92 | 32 | 2 | -75 | 4.93E-7 | 1.05E-5 | 4.72E-5 |
| SW-620 | 0.229 | 1.754 | 1.669 | 1.765 | 1.421 | 0.291 | 0.179 | 94 | 101 | 78 | 4 | -22 | 2.40E-6 | 1.43E-5 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.599 | 2.137 | 2.050 | 2.088 | 1.901 | 0.828 | 0.540 | 94 | 97 | 85 | 2 | -10 | 2.62E-6 | 1.44E-5 | > 1.00E-4 |
| SF-295 | 0.517 | 1.824 | 1.711 | 1.807 | 1.789 | 0.545 | 0.245 | 91 | 99 | 97 | 2 | -53 | 3.14E-6 | 1.09E-5 | 8.92E-5 |
| SF-539 | 0.652 | 2.363 | 2.370 | 2.302 | 1.687 | 0.246 | 0.246 | 100 | 96 | 60 | -62 | -62 | 1.22E-6 | 3.12E-6 | 7.99E-6 |
| SNB-19 | 0.372 | 1.700 | 1.794 | 1.829 | 1.517 | 0.524 | 0.390 | 107 | 110 | 86 | 11 | 1 | 3.05E-6 | > 1.00E-4 | > 1.00E-4 |
| SNB-75 | 0.827 | 1.587 | 1.399 | 1.284 | 1.144 | 0.542 | 0.280 | 75 | 60 | 42 | -35 | -66 | 3.55E-7 | 3.53E-6 | 3.09E-5 |
| U251 | 0.475 | 1.857 | 1.750 | 1.772 | 1.459 | 0.546 | 0.424 | 92 | 94 | 71 | 5 | -11 | 2.09E-6 | 2.11E-5 | > 1.00E-4 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.319 | 2.395 | 2.202 | 2.053 | 1.408 | 0.283 | 0.109 | 91 | 84 | 52 | -11 | -66 | 1.09E-6 | 6.62E-6 | 5.09E-5 |
| MALME-3M | 0.451 | 1.014 | 1.036 | 1.015 | 1.144 | 0.572 | 0.343 | 104 | 100 | 123 | 21 | -24 | 5.24E-6 | 2.96E-5 | > 1.00E-4 |
| M14 | 0.438 | 1.997 | 2.013 | 2.048 | 1.947 | 0.439 | 0.139 | 101 | 103 | 97 | . | -68 | 3.05E-6 | 1.00E-5 | 5.40E-5 |
| MDA-MB-435 | 0.435 | 2.280 | 2.227 | 2.166 | 1.736 | 0.504 | 0.107 | 97 | 94 | 71 | 4 | -76 | 2.03E-6 | 1.11E-5 | 4.76E-5 |
| SK-MEL-2 | 0.815 | 1.586 | 1.537 | 1.599 | 1.386 | 0.626 | 0.458 | 94 | 102 | 74 | -23 | -44 | 1.77E-6 | 5.78E-6 | > 1.00E-4 |
| SK-MEL-28 | 0.738 | 2.463 | 2.550 | 2.556 | 2.595 | 0.858 | 0.324 | 105 | 105 | 108 | 7 | -56 | 3.73E-6 | 1.29E-5 | 7.99E-5 |
| SK-MEL-5 | 0.808 | 3.245 | 3.175 | 3.187 | 2.320 | 0.596 | 0.075 | 97 | 98 | 62 | -26 | -91 | 1.37E-6 | 5.04E-6 | 2.34E-5 |
| UACC-257 | 0.925 | 2.172 | 2.059 | 2.106 | 2.106 | 0.994 | 0.717 | 91 | 95 | 95 | 6 | -23 | 3.17E-6 | 1.57E-5 | > 1.00E-4 |
| UACC-62 | 0.681 | 2.515 | 2.503 | 2.465 | 2.246 | 0.502 | 0.295 | 99 | 97 | 85 | -26 | -57 | 2.07E-6 | 5.81E-6 | 5.99E-5 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| OVCAR-3 | 0.594 | 1.849 | 1.783 | 1.803 | 1.519 | 0.541 | 0.539 | 95 | 96 | 74 | -9 | -9 | 1.94E-6 | 7.80E-6 | > 1.00E-4 |
| OVCAR-4 | 0.608 | 1.606 | 1.571 | 1.555 | 1.443 | 0.635 | 0.488 | 96 | 95 | 84 | 3 | -20 | 2.61E-6 | 1.32E-5 | > 1.00E-4 |
| OVCAR-5 | 0.613 | 1.701 | 1.643 | 1.609 | 1.693 | 0.835 | 0.685 | 95 | 92 | 99 | 20 | 7 | 4.21E-6 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-8 | 0.459 | 2.084 | 2.018 | 2.105 | 1.887 | 0.555 | 0.426 | 96 | 101 | 88 | 6 | -7 | 2.90E-6 | 2.80E-5 | > 1.00E-4 |
| NCI/ADR-RES | 0.422 | 1.751 | 1.792 | 1.751 | 1.740 | 0.658 | 0.367 | 103 | 100 | 99 | 18 | -13 | 4.01E-6 | 3.75E-5 | > 1.00E-4 |
| SK-OV-3 | 0.767 | 1.496 | 1.394 | 1.416 | 1.437 | 0.872 | 0.742 | 86 | 89 | 92 | 14 | -3 | 3.46E-6 | 6.48E-5 | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.532 | 2.227 | 2.153 | 2.085 | 1.998 | 0.726 | 0.471 | 96 | 92 | 86 | 11 | -11 | 3.06E-6 | 3.16E-5 | > 1.00E-4 |
| A498 | 1.317 | 2.002 | 1.858 | 1.947 | 1.787 | 1.036 | 0.414 | 79 | 92 | 69 | -21 | -69 | 1.61E-6 | 5.79E-6 | 4.04E-5 |
| ACHN | 0.455 | 2.151 | 2.190 | 2.126 | 1.816 | 0.581 | 0.465 | 102 | 99 | 80 | 7 | 1 | 2.60E-6 | > 1.00E-4 | > 1.00E-4 |
| CAKI-1 | 0.558 | 2.647 | 2.481 | 2.353 | 2.054 | 0.753 | 0.573 | 92 | 86 | 72 | 9 | 1 | 2.22E-6 | > 1.00E-4 | > 1.00E-4 |
| RXF 393 | 0.617 | 1.263 | 1.230 | 1.236 | 1.093 | 0.250 | 0.170 | 95 | 96 | 74 | -59 | -73 | 1.51E-6 | 3.57E-6 | 8.49E-6 |
| SN12C | 0.454 | 1.947 | 1.958 | 1.996 | 1.554 | 0.593 | 0.487 | 101 | 103 | 74 | 9 | 1 | 2.33E-6 | > 1.00E-4 | > 1.00E-4 |
| TK-10 | 0.699 | 1.480 | 1.362 | 1.407 | 1.570 | 0.860 | 0.653 | 85 | 91 | 111 | 21 | -7 | 4.74E-6 | 5.72E-5 | > 1.00E-4 |
| UO-31 | 0.529 | 2.229 | 2.088 | 2.081 | 1.911 | 0.668 | 0.461 | 92 | 91 | 81 | 8 | -13 | 2.68E-6 | 2.43E-5 | > 1.00E-4 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.615 | 2.484 | 2.411 | 2.319 | 1.941 | 0.670 | 0.283 | 96 | 91 | 71 | 3 | -54 | 2.03E-6 | 1.13E-5 | 8.49E-5 |
| DU-145 | 0.355 | 1.721 | 1.749 | 1.757 | 1.423 | 0.432 | 0.332 | 102 | 103 | 78 | 6 | -6 | 2.45E-6 | 2.91E-5 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.391 | 2.257 | 2.061 | 2.036 | 1.574 | 0.402 | 0.254 | 89 | 88 | 63 | 1 | -35 | 1.63E-6 | 1.04E-5 | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.397 | 1.331 | 1.216 | 1.139 | 0.924 | 0.502 | 0.375 | 88 | 79 | 56 | 11 | -6 | 1.39E-6 | 4.61E-5 | > 1.00E-4 |
| HS 578T | 0.978 | 1.957 | 1.822 | 1.762 | 1.467 | 0.832 | 0.802 | 86 | 80 | 50 | -15 | -18 | 9.93E-7 | 5.88E-6 | > 1.00E-4 |
| BT-549 | 1.146 | 2.293 | 2.229 | 2.192 | 1.942 | 0.844 | 0.351 | 94 | 91 | 69 | -26 | -69 | 1.59E-6 | 5.30E-6 | 3.54E-5 |
| T-47D | 0.859 | 1.925 | 1.806 | 1.853 | 1.617 | 0.897 | 0.654 | 89 | 93 | 71 | 4 | -24 | 2.05E-6 | 1.34E-5 | > 1.00E-4 |
| MDA-MB-468 | 0.977 | 2.232 | 2.091 | 2.081 | 1.977 | 0.948 | 0.538 | 89 | 88 | 80 | -3 | -45 | 2.28E-6 | 9.19E-6 | > 1.00E-4 |

**6-[6-Hydroxy-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12(13*H*)-yl]hexanenitrile 308**



**6-[6-Hydroxy-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12(13*H*)-yl]hexanenitrile 308**



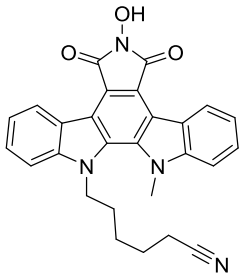

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

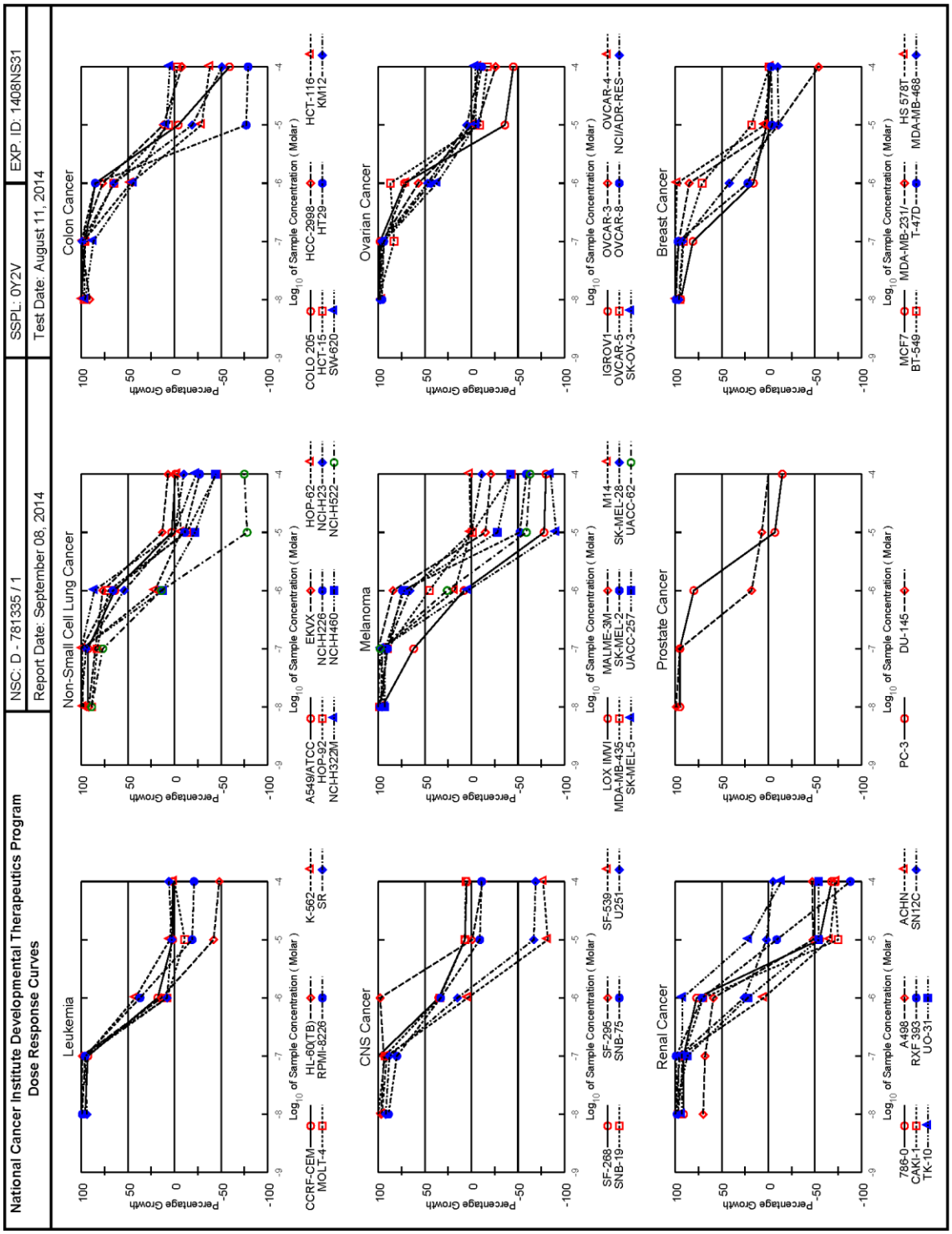
| | | | |
|---|---|---|---|
| NSC : D - 781336 / 1 | Experiment ID : 1408NS31 | Test Type : 08 | Units : Molar |
| Report Date : September 08, 2014 | Test Date : August 11, 2014 | QNS : | MC : |
| COMI : HW6-547-2 | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

Log10 Concentration

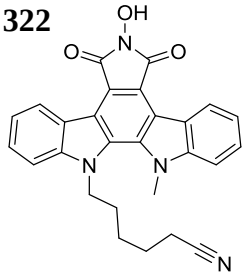
| Panel/Cell Line | Time Zero | Ctrl | Mean Optical Densities -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | Percent Growth -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.532 | 2.162 | . | . | . | . | . | . | . | . | . | . | . | . | . |
| HL-60(TB) | 0.953 | 3.013 | 3.030 | 2.967 | 2.451 | 0.373 | 0.434 | 101 | 98 | 73 | -61 | -54 | 1.48E-6 | 3.50E-6 | 8.29E-6 |
| K-562 | 0.270 | 2.055 | 2.181 | 2.161 | 1.188 | 0.283 | 0.346 | 107 | 106 | 51 | 1 | 4 | 1.07E-6 | > 1.00E-4 | > 1.00E-4 |
| MOLT-4 | 0.789 | 2.864 | 2.783 | 3.028 | 0.914 | 0.762 | 0.598 | 96 | 108 | 6 | -3 | -24 | 3.70E-7 | 4.33E-6 | > 1.00E-4 |
| RPMI-8226 | 0.955 | 2.558 | 2.567 | 2.696 | 1.782 | 0.536 | 0.601 | 101 | 109 | 52 | -44 | -37 | 1.04E-6 | 3.47E-6 | > 1.00E-4 |
| SR | 0.632 | 2.595 | 2.645 | 2.681 | 1.007 | 0.754 | 0.760 | 103 | 104 | 19 | 6 | 7 | 4.34E-7 | > 1.00E-4 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.482 | 2.358 | 2.310 | 2.366 | 2.318 | 0.435 | 0.319 | 97 | 100 | 98 | -10 | -34 | 2.78E-6 | 8.12E-6 | > 1.00E-4 |
| EKVX | 0.786 | 1.688 | 1.559 | 1.577 | 1.458 | 0.591 | 0.558 | 86 | 88 | 75 | -25 | -29 | 1.77E-6 | 5.63E-6 | > 1.00E-4 |
| HOP-62 | 0.578 | 2.019 | 1.942 | 1.878 | 1.013 | 0.361 | 0.339 | 95 | 90 | 30 | -38 | -41 | 4.68E-7 | 2.79E-6 | > 1.00E-4 |
| HOP-92 | 1.431 | 1.867 | 1.892 | 1.894 | 1.908 | 0.821 | 0.537 | 106 | 106 | 109 | -43 | -62 | 2.46E-6 | 5.24E-6 | 2.35E-5 |
| NCI-H226 | 0.545 | 1.668 | 1.717 | 1.686 | 1.517 | 0.319 | 0.420 | 104 | 102 | 87 | -42 | -23 | 1.93E-6 | 4.74E-6 | > 1.00E-4 |
| NCI-H23 | 0.675 | 2.514 | 2.450 | 2.342 | 1.910 | 0.519 | 0.485 | 97 | 91 | 67 | -23 | -28 | 1.55E-6 | 5.54E-6 | > 1.00E-4 |
| NCI-H322M | 1.153 | 2.703 | 2.651 | 2.547 | 2.443 | 1.045 | 0.681 | 97 | 90 | 83 | -9 | -41 | 2.28E-6 | 7.91E-6 | > 1.00E-4 |
| NCI-H460 | 0.417 | 3.090 | 3.148 | 3.203 | 1.920 | 0.315 | 0.399 | 102 | 104 | 56 | -25 | -4 | 1.19E-6 | 4.96E-6 | > 1.00E-4 |
| NCI-H522 | 1.063 | 2.688 | 2.612 | 2.310 | 0.550 | 0.258 | 0.303 | 95 | 77 | -48 | -76 | -71 | 1.64E-7 | 4.11E-7 | 1.16E-6 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.434 | 1.780 | 1.796 | 1.785 | 1.591 | 0.224 | 0.201 | 101 | 100 | 86 | -49 | -54 | 1.85E-6 | 4.36E-6 | 1.92E-5 |
| HCC-2998 | 0.694 | 2.271 | 2.263 | 2.175 | 1.874 | 0.550 | 0.285 | 99 | 94 | 75 | -21 | -59 | 1.82E-6 | 6.06E-6 | 5.82E-5 |
| HCT-116 | 0.311 | 2.306 | 2.317 | 2.316 | 1.257 | 0.086 | 0.119 | 101 | 101 | 47 | -79 | -62 | 8.94E-7 | 2.37E-6 | 5.90E-6 |
| HCT-15 | 0.378 | 2.635 | 2.374 | 2.530 | 2.175 | 0.342 | 0.355 | 88 | 95 | 80 | -10 | -6 | 2.15E-6 | 7.82E-6 | > 1.00E-4 |
| HT29 | 0.303 | 1.786 | 1.806 | 1.768 | 1.544 | 0.177 | 0.099 | 101 | 99 | 84 | -42 | -67 | 1.86E-6 | 4.66E-6 | 2.12E-5 |
| KM12 | 0.609 | 2.992 | 3.007 | 2.938 | 2.210 | 0.246 | 0.180 | 101 | 98 | 67 | -60 | -70 | 1.37E-6 | 3.39E-6 | 8.40E-6 |
| SW-620 | 0.358 | 2.300 | 2.286 | 2.215 | 1.332 | 0.509 | 0.335 | 99 | 96 | 50 | 8 | -7 | 1.01E-6 | 3.49E-5 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.741 | 2.152 | 2.063 | 2.022 | 1.435 | 0.845 | 0.716 | 94 | 91 | 49 | 7 | -3 | 9.57E-7 | 4.81E-5 | > 1.00E-4 |
| SF-295 | 0.977 | 3.026 | 2.862 | 2.914 | 2.872 | 0.808 | 0.770 | 92 | 95 | 92 | -17 | -21 | 2.44E-6 | 6.95E-6 | > 1.00E-4 |
| SF-539 | 0.914 | 2.339 | 2.189 | 2.301 | 1.459 | 0.146 | 0.174 | 89 | 97 | 38 | -84 | -81 | 6.32E-7 | 2.05E-6 | 5.26E-6 |
| SNB-19 | 0.847 | 2.375 | 2.373 | 2.334 | 1.598 | 1.153 | 0.995 | 100 | 97 | 49 | 20 | 10 | 9.59E-7 | > 1.00E-4 | > 1.00E-4 |
| SNB-75 | 0.784 | 1.582 | 1.421 | 1.418 | 1.137 | 0.777 | 0.559 | 80 | 79 | 44 | -1 | -29 | 6.84E-7 | 9.55E-6 | > 1.00E-4 |
| U251 | 0.492 | 2.506 | 2.549 | 2.495 | 0.934 | 0.047 | 0.089 | 102 | 99 | 22 | -91 | -82 | 4.35E-7 | 1.57E-6 | 4.36E-6 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.406 | 2.567 | 2.495 | 1.948 | 0.731 | 0.084 | 0.121 | 97 | 71 | 15 | -79 | -70 | 2.40E-7 | 1.44E-6 | 4.88E-6 |
| MALME-3M | 0.674 | 1.126 | 1.082 | 1.089 | 1.063 | 0.425 | 0.518 | 90 | 92 | 86 | -37 | -23 | 1.96E-6 | 5.01E-6 | > 1.00E-4 |
| M14 | 0.396 | 1.535 | 1.518 | 1.536 | 0.800 | 0.185 | 0.147 | 99 | 100 | 35 | -53 | -63 | 5.95E-7 | 2.51E-6 | 9.18E-6 |
| MDA-MB-435 | 0.594 | 2.705 | 2.565 | 2.643 | 1.845 | 0.228 | 0.266 | 93 | 97 | 59 | -62 | -55 | 1.19E-6 | 3.09E-6 | 8.02E-6 |
| SK-MEL-2 | 0.944 | 1.900 | 1.919 | 1.842 | 1.730 | 0.560 | 0.783 | 102 | 94 | 82 | -41 | -17 | 1.83E-6 | 4.67E-6 | > 1.00E-4 |
| SK-MEL-28 | 0.736 | 2.179 | 2.098 | 2.134 | 1.784 | 0.619 | 0.588 | 94 | 97 | 73 | -16 | -20 | 1.80E-6 | 6.61E-6 | > 1.00E-4 |
| SK-MEL-5 | 0.728 | 2.773 | 2.805 | 2.791 | 1.073 | 0.151 | 0.217 | 102 | 101 | 17 | -79 | -70 | 4.03E-7 | 1.50E-6 | 4.96E-6 |
| UACC-257 | 1.091 | 2.434 | 2.405 | 2.480 | 2.200 | 0.768 | 1.046 | 98 | 103 | 83 | -30 | -4 | 1.95E-6 | 5.45E-6 | > 1.00E-4 |
| UACC-62 | 1.034 | 2.859 | 2.846 | 2.900 | 1.670 | 0.439 | 0.609 | 99 | 102 | 35 | -58 | -41 | 5.96E-7 | 2.38E-6 | . |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.630 | 2.254 | 2.293 | 2.177 | 1.517 | 0.391 | 0.435 | 102 | 95 | 55 | -38 | -31 | 1.12E-6 | 3.89E-6 | > 1.00E-4 |
| OVCAR-3 | 0.486 | 1.697 | 1.726 | 1.682 | 1.263 | 0.386 | 0.363 | 102 | 99 | 64 | -21 | -25 | 1.47E-6 | 5.72E-6 | > 1.00E-4 |
| OVCAR-4 | 0.688 | 1.532 | 1.456 | 1.471 | 1.269 | 0.698 | 0.625 | 91 | 93 | 69 | 1 | -9 | 1.90E-6 | 1.30E-5 | > 1.00E-4 |
| OVCAR-5 | 0.693 | 1.617 | 1.524 | 1.524 | 1.624 | 0.335 | 0.383 | 90 | 90 | 101 | -52 | -45 | 2.15E-6 | 4.58E-6 | . |
| OVCAR-8 | 0.588 | 2.516 | 2.566 | 2.609 | 1.977 | 0.494 | 0.643 | 103 | 105 | 72 | -16 | 3 | 1.78E-6 | . | > 1.00E-4 |
| NCI/ADR-RES | 0.663 | 2.365 | 2.374 | 2.379 | 2.106 | 1.309 | 1.180 | 100 | 101 | 85 | 38 | 30 | 5.52E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-OV-3 | 0.635 | 1.594 | 1.629 | 1.592 | 1.410 | 0.883 | 0.556 | 104 | 100 | 81 | 5 | -12 | 2.55E-6 | 1.94E-5 | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.700 | 2.403 | 2.390 | 2.412 | 2.173 | 0.118 | 0.161 | 99 | 101 | 86 | -83 | -77 | 1.64E-6 | 3.23E-6 | 6.37E-6 |
| A498 | 1.366 | 1.952 | 1.859 | 1.858 | 1.808 | 0.911 | 0.353 | 84 | 84 | 75 | -33 | -74 | 1.72E-6 | 4.94E-6 | 2.56E-5 |
| ACHN | 0.508 | 2.120 | 2.042 | 2.074 | 1.257 | 0.103 | 0.174 | 95 | 97 | 46 | -80 | -66 | 8.51E-7 | 2.33E-6 | 5.81E-6 |
| CAKI-1 | 1.025 | 3.205 | 3.034 | 3.109 | 2.981 | 0.421 | 0.507 | 92 | 96 | 90 | -59 | -51 | 1.85E-6 | 4.01E-6 | 8.70E-6 |
| RXF 393 | 0.755 | 1.372 | 1.370 | 1.380 | 1.224 | 0.474 | 0.240 | 100 | 101 | 76 | -37 | -68 | 1.70E-6 | 4.69E-6 | 2.58E-5 |
| SN12C | 0.743 | 2.809 | 2.735 | 2.844 | 1.971 | 0.547 | 0.400 | 96 | 102 | 59 | -26 | -46 | 1.29E-6 | 4.92E-6 | > 1.00E-4 |
| TK-10 | 1.103 | 1.964 | 1.889 | 1.852 | 1.799 | 1.000 | 0.934 | 91 | 87 | 81 | -9 | -15 | 2.20E-6 | 7.88E-6 | > 1.00E-4 |
| UO-31 | 0.963 | 2.460 | 2.252 | 2.110 | 1.705 | 0.401 | 0.513 | 86 | 77 | 50 | -58 | -47 | 9.65E-7 | 2.88E-6 | . |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.765 | 2.460 | 2.411 | 2.410 | 1.853 | 0.506 | 0.570 | 97 | 97 | 64 | -34 | -26 | 1.39E-6 | 4.51E-6 | > 1.00E-4 |
| DU-145 | 0.398 | 1.683 | 1.643 | 1.644 | 1.056 | 0.450 | 0.233 | 97 | 97 | 51 | 4 | -42 | 1.06E-6 | 1.22E-5 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.371 | 2.198 | 1.943 | 1.942 | 0.671 | 0.361 | 0.372 | 86 | 86 | 16 | -3 | . | 3.29E-7 | . | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.689 | 1.638 | 1.710 | 1.658 | 1.415 | 0.491 | 0.335 | 108 | 102 | 76 | -29 | -51 | 1.78E-6 | 5.32E-6 | 8.89E-5 |
| HS 578T | 1.018 | 2.066 | 2.043 | 2.088 | 2.037 | 1.308 | 1.165 | 98 | 102 | 97 | 28 | 14 | 4.77E-6 | > 1.00E-4 | > 1.00E-4 |
| BT-549 | 1.310 | 2.604 | 2.665 | 2.593 | 2.197 | 1.291 | 1.133 | 105 | 99 | 69 | -1 | -14 | 1.84E-6 | 9.53E-6 | > 1.00E-4 |
| T-47D | 0.669 | 1.469 | 1.386 | 1.360 | 0.919 | 0.655 | 0.643 | 90 | 86 | 31 | -2 | -4 | 4.55E-7 | 8.65E-6 | > 1.00E-4 |
| MDA-MB-468 | 0.923 | 1.881 | 1.865 | 1.858 | 1.535 | 0.754 | 0.631 | 98 | 98 | 64 | -18 | -32 | 1.48E-6 | 5.98E-6 | > 1.00E-4 |

**6-[6-Hydroxy-13-methyl-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12(13*H*)-yl]hexanenitrile 322**



**6-[6-Hydroxy-13-methyl-5,7-dioxo-6,7-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12(13*H*)-yl]hexanenitrile 322**



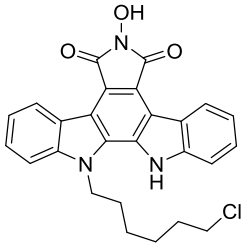
National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

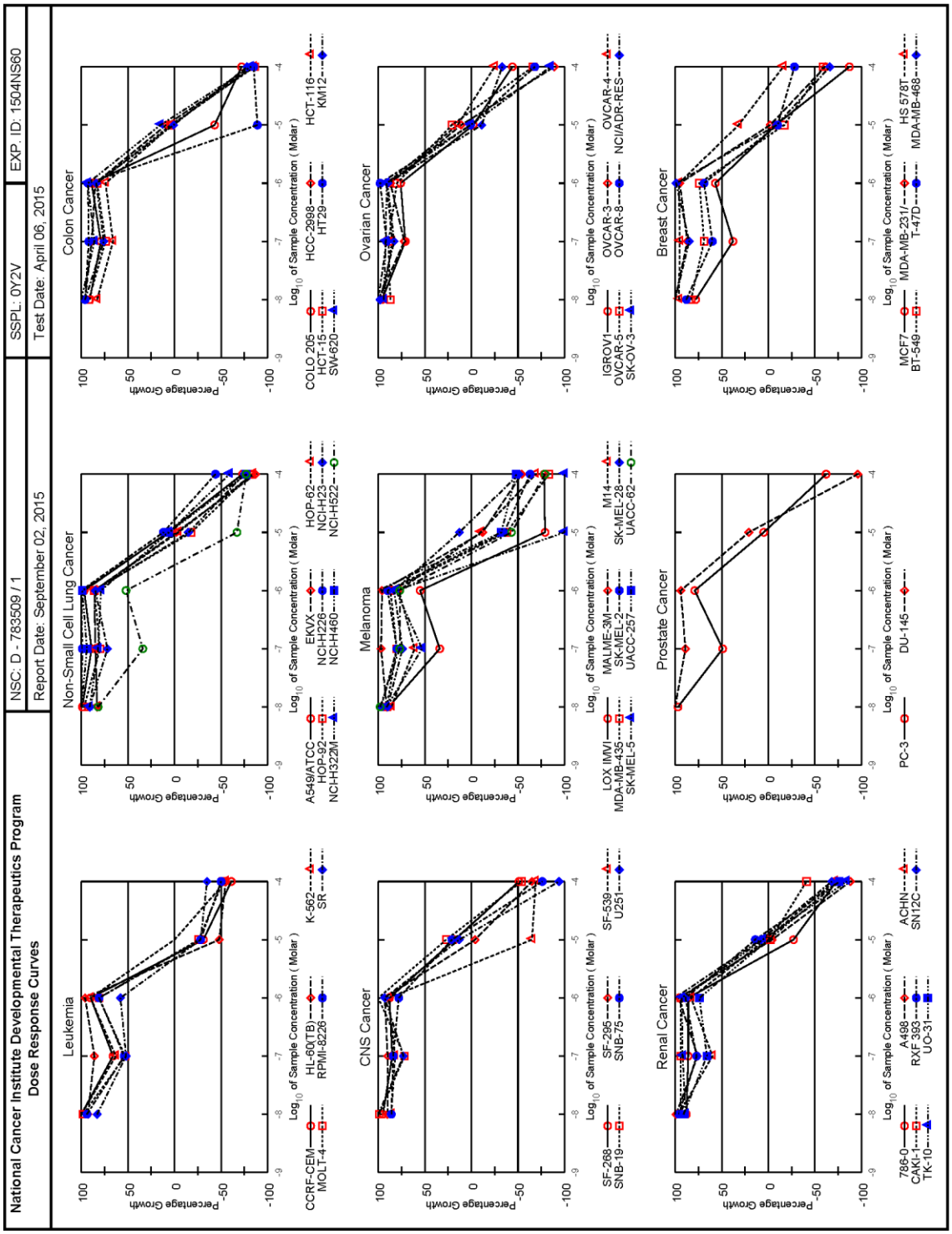
| | | | |
|---|---|---|---|
| NSC : D - 781336 / 1 | Experiment ID : 1408NS31 | Test Type : 08 | Units : Molar |
| Report Date : September 08, 2014 | Test Date : August 11, 2014 | QNS : | MC : |
| COMI : HW6-547-2 | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

Log10 Concentration

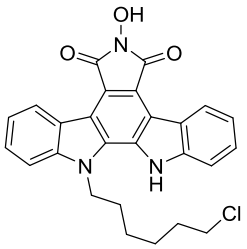
| Panel/Cell Line | Time Zero | Ctrl | Mean Optical Densities -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | Percent Growth -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.532 | 2.162 | | | | | | | | | | | | | |
| HL-60(TB) | 0.953 | 3.013 | 3.030 | 2.967 | 2.451 | 0.373 | 0.434 | 101 | 98 | 73 | -61 | -54 | 1.48E-6 | 3.50E-6 | 8.29E-6 |
| K-562 | 0.270 | 2.055 | 2.181 | 2.161 | 1.188 | 0.283 | 0.346 | 107 | 106 | 51 | 1 | 4 | 1.07E-6 | > 1.00E-4 | > 1.00E-4 |
| MOLT-4 | 0.789 | 2.864 | 2.783 | 3.028 | 0.914 | 0.762 | 0.598 | 96 | 108 | 6 | -3 | -24 | 3.70E-7 | 4.33E-6 | > 1.00E-4 |
| RPMI-8226 | 0.955 | 2.558 | 2.567 | 2.696 | 1.782 | 0.536 | 0.601 | 101 | 109 | 52 | -44 | -37 | 1.04E-6 | 3.47E-6 | > 1.00E-4 |
| SR | 0.632 | 2.595 | 2.645 | 2.681 | 1.007 | 0.754 | 0.760 | 103 | 104 | 19 | 6 | 7 | 4.34E-7 | > 1.00E-4 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.482 | 2.358 | 2.310 | 2.368 | 2.318 | 0.435 | 0.319 | 97 | 100 | 98 | -10 | -34 | 2.78E-6 | 8.12E-6 | > 1.00E-4 |
| EKVX | 0.786 | 1.688 | 1.559 | 1.577 | 1.458 | 0.591 | 0.558 | 86 | 88 | 75 | -25 | -29 | 1.77E-6 | 5.63E-6 | > 1.00E-4 |
| HOP-62 | 0.578 | 2.019 | 1.942 | 1.878 | 1.013 | 0.361 | 0.339 | 95 | 90 | 30 | -38 | -41 | 4.68E-7 | 2.79E-6 | > 1.00E-4 |
| HOP-92 | 1.431 | 1.867 | 1.892 | 1.894 | 1.908 | 0.821 | 0.537 | 106 | 106 | 109 | -43 | -62 | 2.46E-6 | 5.24E-6 | 2.35E-5 |
| NCI-H226 | 0.545 | 1.668 | 1.717 | 1.686 | 1.517 | 0.319 | 0.420 | 104 | 102 | 87 | -42 | -23 | 1.93E-6 | 4.74E-6 | > 1.00E-4 |
| NCI-H23 | 0.675 | 2.514 | 2.450 | 2.342 | 1.910 | 0.519 | 0.485 | 97 | 91 | 67 | -23 | -28 | 1.55E-6 | 5.54E-6 | > 1.00E-4 |
| NCI-H322M | 1.153 | 2.703 | 2.651 | 2.547 | 2.443 | 1.045 | 0.681 | 97 | 90 | 83 | -9 | -41 | 2.28E-6 | 7.91E-6 | > 1.00E-4 |
| NCI-H460 | 0.417 | 3.090 | 3.148 | 3.203 | 1.920 | 0.315 | 0.399 | 102 | 104 | 56 | -25 | -4 | 1.19E-6 | 4.96E-6 | > 1.00E-4 |
| NCI-H522 | 1.063 | 2.688 | 2.612 | 2.310 | 0.550 | 0.258 | 0.303 | 95 | 77 | -48 | -76 | -71 | 1.64E-7 | 4.11E-7 | 1.16E-6 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.434 | 1.780 | 1.796 | 1.785 | 1.591 | 0.224 | 0.201 | 101 | 100 | 86 | -49 | -54 | 1.85E-6 | 4.36E-6 | 1.92E-5 |
| HCC-2998 | 0.694 | 2.271 | 2.263 | 2.175 | 1.874 | 0.550 | 0.285 | 99 | 94 | 75 | -21 | -59 | 1.82E-6 | 6.06E-6 | 5.82E-5 |
| HCT-116 | 0.311 | 2.306 | 2.317 | 2.316 | 1.257 | 0.066 | 0.119 | 101 | 101 | 47 | -79 | -62 | 8.94E-7 | 2.37E-6 | 5.90E-6 |
| HCT-15 | 0.378 | 2.635 | 2.374 | 2.530 | 2.175 | 0.342 | 0.355 | 88 | 95 | 80 | -10 | -6 | 2.15E-6 | 7.82E-6 | > 1.00E-4 |
| HT29 | 0.303 | 1.786 | 1.806 | 1.768 | 1.544 | 0.177 | 0.099 | 101 | 99 | 84 | -42 | -67 | 1.86E-6 | 4.66E-6 | 2.12E-5 |
| KM12 | 0.609 | 2.992 | 3.007 | 2.938 | 2.210 | 0.246 | 0.180 | 101 | 98 | 67 | -60 | -70 | 1.37E-6 | 3.39E-6 | 8.40E-6 |
| SW-620 | 0.358 | 2.300 | 2.286 | 2.215 | 1.332 | 0.509 | 0.335 | 99 | 96 | 50 | 8 | -7 | 1.01E-6 | 3.49E-5 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.741 | 2.152 | 2.063 | 2.022 | 1.435 | 0.845 | 0.716 | 94 | 91 | 49 | 7 | -3 | 9.57E-7 | 4.81E-5 | > 1.00E-4 |
| SF-295 | 0.977 | 3.026 | 2.862 | 2.914 | 2.872 | 0.808 | 0.770 | 92 | 95 | 92 | -17 | -21 | 2.44E-6 | 6.95E-6 | > 1.00E-4 |
| SF-539 | 0.914 | 2.339 | 2.189 | 2.301 | 1.459 | 0.146 | 0.174 | 89 | 97 | 38 | -84 | -81 | 6.32E-7 | 2.05E-6 | 5.26E-6 |
| SNB-19 | 0.847 | 2.375 | 2.373 | 2.334 | 1.598 | 1.153 | 0.995 | 100 | 97 | 49 | 20 | 10 | 9.59E-7 | > 1.00E-4 | > 1.00E-4 |
| SNB-75 | 0.784 | 1.582 | 1.421 | 1.418 | 1.137 | 0.777 | 0.559 | 80 | 79 | 44 | -1 | -29 | 6.84E-7 | 9.55E-6 | > 1.00E-4 |
| U251 | 0.492 | 2.506 | 2.549 | 2.495 | 0.934 | 0.047 | 0.089 | 102 | 99 | 22 | -91 | -82 | 4.35E-7 | 1.57E-6 | 4.36E-6 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.406 | 2.567 | 2.495 | 1.948 | 0.731 | 0.084 | 0.121 | 97 | 71 | 15 | -79 | -70 | 2.40E-7 | 1.44E-6 | 4.88E-6 |
| MALME-3M | 0.674 | 1.126 | 1.082 | 1.089 | 1.063 | 0.425 | 0.518 | 90 | 92 | 86 | -37 | -23 | 1.96E-6 | 5.01E-6 | > 1.00E-4 |
| M14 | 0.396 | 1.535 | 1.518 | 1.536 | 0.800 | 0.185 | 0.147 | 99 | 100 | 35 | -53 | -63 | 5.95E-7 | 2.51E-6 | 9.18E-6 |
| MDA-MB-435 | 0.594 | 2.705 | 2.565 | 2.643 | 1.845 | 0.228 | 0.266 | 93 | 97 | 59 | -62 | -55 | 1.19E-6 | 3.09E-6 | 8.02E-6 |
| SK-MEL-2 | 0.944 | 1.900 | 1.919 | 1.842 | 1.730 | 0.560 | 0.783 | 102 | 94 | 82 | -41 | -17 | 1.83E-6 | 4.67E-6 | > 1.00E-4 |
| SK-MEL-28 | 0.736 | 2.179 | 2.098 | 2.134 | 1.784 | 0.619 | 0.588 | 94 | 97 | 73 | -16 | -20 | 1.80E-6 | 6.61E-6 | > 1.00E-4 |
| SK-MEL-5 | 0.728 | 2.773 | 2.805 | 2.791 | 1.073 | 0.151 | 0.217 | 102 | 101 | 17 | -79 | -70 | 4.03E-7 | 1.50E-6 | 4.96E-6 |
| UACC-257 | 1.091 | 2.434 | 2.405 | 2.480 | 2.200 | 0.768 | 1.046 | 98 | 103 | 83 | -30 | -4 | 1.95E-6 | 5.45E-6 | > 1.00E-4 |
| UACC-62 | 1.034 | 2.859 | 2.846 | 2.900 | 1.670 | 0.439 | 0.609 | 99 | 102 | 35 | -58 | -41 | 5.96E-7 | 2.38E-6 | |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.630 | 2.254 | 2.293 | 2.177 | 1.517 | 0.391 | 0.435 | 102 | 95 | 55 | -38 | -31 | 1.12E-6 | 3.89E-6 | > 1.00E-4 |
| OVCAR-3 | 0.486 | 1.697 | 1.728 | 1.682 | 1.263 | 0.386 | 0.363 | 102 | 99 | 64 | -21 | -25 | 1.47E-6 | 5.72E-6 | > 1.00E-4 |
| OVCAR-4 | 0.688 | 1.532 | 1.456 | 1.471 | 1.269 | 0.698 | 0.625 | 91 | 93 | 69 | 1 | -9 | 1.90E-6 | 1.30E-5 | > 1.00E-4 |
| OVCAR-5 | 0.693 | 1.617 | 1.524 | 1.524 | 1.624 | 0.335 | 0.383 | 90 | 90 | 101 | -52 | -45 | 2.15E-6 | 4.58E-6 | |
| OVCAR-8 | 0.588 | 2.516 | 2.586 | 2.609 | 1.977 | 0.494 | 0.643 | 103 | 105 | 72 | -16 | 3 | 1.78E-6 | | > 1.00E-4 |
| NCI/ADR-RES | 0.663 | 2.365 | 2.374 | 2.379 | 2.106 | 1.309 | 1.180 | 100 | 101 | 85 | 38 | 30 | 5.52E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-OV-3 | 0.635 | 1.594 | 1.629 | 1.592 | 1.410 | 0.683 | 0.556 | 104 | 100 | 81 | 5 | -12 | 2.55E-6 | 1.94E-5 | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.700 | 2.403 | 2.390 | 2.412 | 2.173 | 0.118 | 0.161 | 99 | 101 | 86 | -83 | -77 | 1.64E-6 | 3.23E-6 | 6.37E-6 |
| A498 | 1.366 | 1.952 | 1.859 | 1.858 | 1.808 | 0.911 | 0.353 | 84 | 84 | 75 | -33 | -74 | 1.72E-6 | 4.94E-6 | 2.56E-5 |
| ACHN | 0.508 | 2.120 | 2.042 | 2.074 | 1.257 | 0.103 | 0.174 | 95 | 97 | 46 | -80 | -66 | 8.51E-7 | 2.33E-6 | 5.81E-6 |
| CAKI-1 | 1.025 | 3.205 | 3.034 | 3.109 | 2.981 | 0.421 | 0.507 | 92 | 96 | 90 | -59 | -51 | 1.85E-6 | 4.01E-6 | 8.70E-6 |
| RXF 393 | 0.755 | 1.372 | 1.370 | 1.380 | 1.224 | 0.474 | 0.240 | 100 | 101 | 76 | -37 | -68 | 1.70E-6 | 4.69E-6 | 2.58E-5 |
| SN12C | 0.743 | 2.809 | 2.735 | 2.844 | 1.971 | 0.547 | 0.400 | 96 | 102 | 59 | -26 | -46 | 1.29E-6 | 4.92E-6 | > 1.00E-4 |
| TK-10 | 1.103 | 1.964 | 1.889 | 1.852 | 1.799 | 1.000 | 0.934 | 91 | 87 | 81 | -9 | -15 | 2.20E-6 | 7.88E-6 | > 1.00E-4 |
| UO-31 | 0.963 | 2.460 | 2.252 | 2.110 | 1.705 | 0.401 | 0.513 | 86 | 77 | 50 | -58 | -47 | 9.65E-7 | 2.88E-6 | |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.765 | 2.460 | 2.411 | 2.410 | 1.853 | 0.506 | 0.570 | 97 | 97 | 64 | -34 | -26 | 1.39E-6 | 4.51E-6 | > 1.00E-4 |
| DU-145 | 0.398 | 1.683 | 1.643 | 1.644 | 1.056 | 0.450 | 0.233 | 97 | 97 | 51 | 4 | -42 | 1.06E-6 | 1.22E-5 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.371 | 2.198 | 1.943 | 1.942 | 0.671 | 0.361 | 0.372 | 86 | 86 | 16 | -3 | | 3.29E-7 | | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.689 | 1.638 | 1.710 | 1.658 | 1.415 | 0.491 | 0.335 | 108 | 102 | 76 | -29 | -51 | 1.78E-6 | 5.32E-6 | 8.89E-5 |
| HS 578T | 1.018 | 2.066 | 2.043 | 2.068 | 2.037 | 1.308 | 1.165 | 98 | 102 | 97 | 28 | 14 | 4.77E-6 | > 1.00E-4 | > 1.00E-4 |
| BT-549 | 1.310 | 2.604 | 2.665 | 2.593 | 2.197 | 1.291 | 1.133 | 105 | 99 | 69 | -1 | -14 | 1.84E-6 | 9.53E-6 | > 1.00E-4 |
| T-47D | 0.669 | 1.469 | 1.386 | 1.360 | 0.919 | 0.655 | 0.643 | 90 | 86 | 31 | -2 | -4 | 4.55E-7 | 8.65E-6 | > 1.00E-4 |
| MDA-MB-468 | 0.923 | 1.881 | 1.865 | 1.858 | 1.535 | 0.754 | 0.631 | 98 | 98 | 64 | -18 | -32 | 1.48E-6 | 5.98E-6 | > 1.00E-4 |

**12-(6-Chlorohexyl)-6-hydroxy-12,13-dihydro-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione 336**



**12-(6-Chlorohexyl)-6-hydroxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 336**



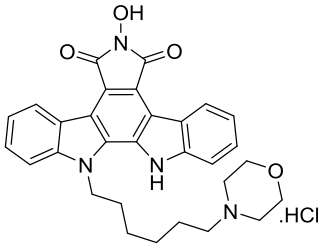

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

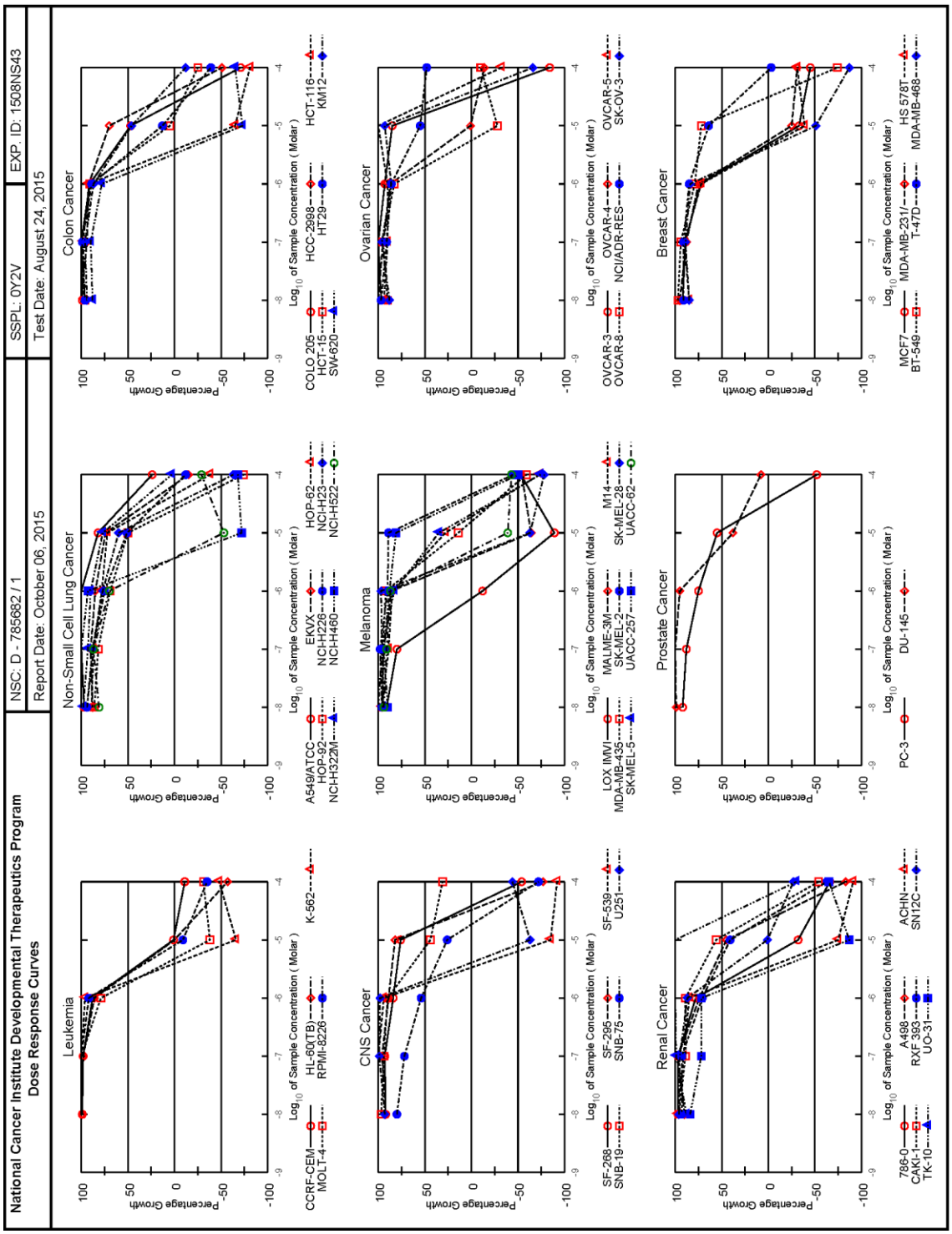
| | | | |
|---|---|---|---|
| NSC : D - 783509 / 1 | Experiment ID : 1504NS60 | Test Type : 08 | Units : Molar |
| Report Date : September 02, 2015 | Test Date : April 06, 2015 | QNS : | MC : |
| COMI : HW7-565-3 | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

Log10 Concentration

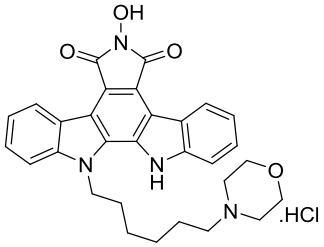
| Panel/Cell Line | Time Zero | Mean Optical Densities Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | Percent Growth -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.552 | 1.519 | 1.554 | 1.188 | 1.425 | 0.379 | 0.213 | 104 | 66 | 90 | -31 | -61 | 2.14E-6 | 5.53E-6 | 4.17E-5 |
| HL-60(TB) | 0.933 | 2.558 | 2.440 | 2.324 | 2.492 | 0.483 | 0.416 | 93 | 86 | 96 | -48 | -55 | 2.08E-6 | 4.63E-6 | 1.74E-5 |
| K-562 | 0.373 | 1.908 | 1.913 | 1.341 | 1.757 | 0.379 | 0.167 | 100 | 63 | 90 | . | -55 | 2.80E-6 | 1.02E-5 | 8.01E-5 |
| MOLT-4 | 0.706 | 2.307 | 2.269 | 1.555 | 1.999 | 0.520 | 0.338 | 98 | 53 | 81 | -26 | -52 | 1.94E-6 | 5.68E-6 | 8.22E-5 |
| RPMI-8226 | 1.307 | 2.774 | 2.693 | 2.105 | 2.491 | 0.948 | 0.651 | 94 | 54 | 81 | -28 | -50 | 1.92E-6 | 5.57E-6 | 9.77E-5 |
| SR | 0.266 | 0.915 | 0.807 | 0.606 | 0.641 | 0.192 | 0.173 | 83 | 52 | 58 | -28 | -35 | 1.23E-6 | 4.71E-6 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.454 | 1.837 | 1.828 | 1.690 | 1.795 | 0.546 | 0.125 | 99 | 89 | 97 | 7 | -73 | 3.31E-6 | 1.21E-5 | 5.19E-5 |
| EKVX | 0.784 | 1.895 | 1.708 | 1.732 | 1.742 | 0.771 | 0.101 | 83 | 85 | 86 | -2 | -87 | 2.58E-6 | 9.57E-6 | 3.67E-5 |
| HOP-62 | 0.649 | 1.300 | 1.188 | 1.203 | 1.210 | 0.624 | 0.107 | 83 | 85 | 86 | -4 | -84 | 2.52E-6 | 9.06E-6 | 3.80E-5 |
| HOP-92 | 1.053 | 1.471 | 1.459 | 1.387 | 1.413 | 0.862 | 0.188 | 97 | 80 | 86 | -18 | -82 | 2.22E-6 | 6.70E-6 | 3.15E-5 |
| NCI-H226 | 1.073 | 2.485 | 2.481 | 2.472 | 2.488 | 1.241 | 0.606 | 100 | 99 | 100 | 12 | -44 | 3.70E-6 | 1.64E-5 | > 1.00E-4 |
| NCI-H23 | 0.779 | 2.322 | 2.179 | 1.898 | 2.088 | 0.863 | 0.195 | 91 | 72 | 85 | -15 | -75 | 2.23E-6 | 7.09E-6 | 3.84E-5 |
| NCI-H322M | 1.029 | 2.397 | 2.278 | 2.145 | 2.110 | 1.114 | 0.424 | 91 | 82 | 79 | 6 | -59 | 2.50E-6 | 1.25E-5 | 7.31E-5 |
| NCI-H460 | 0.403 | 3.155 | 3.182 | 2.994 | 3.120 | 0.567 | 0.094 | 101 | 94 | 99 | 6 | -77 | 3.35E-6 | 1.18E-5 | 4.76E-5 |
| NCI-H522 | 0.915 | 2.487 | 2.200 | 1.443 | 1.727 | 0.305 | 0.214 | 82 | 34 | 52 | -67 | -77 | . | 2.73E-6 | 7.23E-6 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.451 | 1.470 | 1.380 | 1.254 | 1.360 | 0.255 | 0.126 | 91 | 79 | 89 | -43 | -72 | 1.97E-6 | 4.70E-6 | 1.69E-5 |
| HCC-2998 | 0.691 | 2.261 | 2.201 | 2.069 | 2.019 | 0.833 | 0.102 | 96 | 89 | 85 | 9 | -85 | 2.87E-6 | 1.25E-5 | 4.23E-5 |
| HCT-116 | 0.286 | 2.063 | 1.763 | 1.467 | 1.593 | 0.336 | 0.044 | 83 | 66 | 74 | 3 | -85 | 2.15E-6 | 1.08E-5 | 4.02E-5 |
| HCT-15 | 0.253 | 1.742 | 1.625 | 1.349 | 1.490 | 0.320 | 0.035 | 92 | 74 | 83 | 4 | -86 | 2.64E-6 | 1.12E-5 | 3.99E-5 |
| HT29 | 0.272 | 1.628 | 1.576 | 1.518 | 1.535 | 0.031 | 0.040 | 96 | 92 | 93 | -89 | -85 | 1.73E-6 | 3.25E-6 | 6.12E-6 |
| KM12 | 0.499 | 2.687 | 2.605 | 2.155 | 2.342 | 0.527 | 0.110 | 96 | 76 | 84 | 1 | -78 | 2.59E-6 | 1.04E-5 | 4.44E-5 |
| SW-620 | 0.282 | 1.915 | 1.942 | 1.684 | 1.804 | 0.526 | 0.052 | 102 | 86 | 93 | 15 | -82 | 3.56E-6 | 1.43E-5 | 4.70E-5 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.707 | 2.316 | 2.239 | 2.075 | 2.118 | 0.998 | 0.349 | 95 | 85 | 88 | 18 | -51 | 3.48E-6 | 1.83E-5 | 9.79E-5 |
| SF-295 | 0.923 | 2.780 | 2.592 | 2.595 | 2.643 | 0.883 | 0.323 | 90 | 90 | 93 | -4 | -65 | 2.75E-6 | 9.01E-6 | 5.65E-5 |
| SF-539 | 0.660 | 2.149 | 1.935 | 1.878 | 1.978 | 0.231 | 0.207 | 86 | 82 | 89 | -65 | -69 | 1.78E-6 | 3.77E-6 | 7.99E-6 |
| SNB-19 | 0.824 | 2.303 | 2.294 | 1.889 | 2.346 | 1.225 | 0.378 | 99 | 72 | 103 | 27 | -54 | 4.98E-6 | 2.15E-5 | 8.90E-5 |
| SNB-75 | 0.418 | 1.002 | 0.918 | 0.908 | 0.876 | 0.542 | 0.099 | 86 | 84 | 78 | 21 | -76 | 3.14E-6 | 1.65E-5 | 5.36E-5 |
| U251 | 0.598 | 2.112 | 2.144 | 1.707 | 2.009 | 0.798 | 0.035 | 102 | 73 | 93 | 13 | -94 | 3.47E-6 | 1.33E-5 | 3.88E-5 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.436 | 2.541 | 2.319 | 1.154 | 1.603 | 0.093 | 0.094 | 89 | 34 | 55 | -79 | -78 | . | 2.59E-6 | 6.11E-6 |
| MALME-3M | 0.738 | 1.348 | 1.291 | 1.332 | 1.321 | 0.850 | 0.336 | 91 | 97 | 96 | -12 | -54 | 2.65E-6 | 7.74E-6 | 7.85E-5 |
| M14 | 0.630 | 2.231 | 2.006 | 1.609 | 1.875 | 0.567 | 0.195 | 86 | 61 | 78 | -10 | -69 | 2.07E-6 | 7.68E-6 | 4.75E-5 |
| MDA-MB-435 | 0.469 | 2.490 | 2.317 | 2.096 | 2.226 | 0.292 | 0.079 | 91 | 80 | 87 | -38 | -83 | 1.98E-6 | 4.97E-6 | 1.85E-5 |
| SK-MEL-2 | 0.985 | 2.235 | 2.203 | 1.960 | 2.108 | 0.844 | 0.361 | 97 | 80 | 90 | -35 | -63 | 2.09E-6 | 5.27E-6 | 3.42E-5 |
| SK-MEL-28 | 0.843 | 2.489 | 2.325 | 2.054 | 2.211 | 1.050 | 0.410 | 90 | 74 | 83 | 13 | -51 | 2.95E-6 | 1.57E-5 | 9.50E-5 |
| SK-MEL-5 | 0.726 | 2.925 | 2.731 | 1.900 | 2.507 | -0.001 | -0.014 | 91 | 53 | 81 | -100 | -100 | 1.48E-6 | 2.80E-6 | 5.29E-6 |
| UACC-257 | 0.825 | 1.491 | 1.497 | 1.561 | 1.584 | 0.562 | 0.426 | 101 | 110 | 114 | -32 | -48 | 2.74E-6 | 6.04E-6 | > 1.00E-4 |
| UACC-62 | 1.098 | 2.792 | 2.762 | 2.401 | 2.411 | 0.631 | 0.236 | 98 | 77 | 77 | -43 | -79 | 1.69E-6 | 4.42E-6 | 1.61E-5 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.559 | 2.244 | 2.176 | 1.751 | 1.832 | 0.547 | 0.312 | 96 | 71 | 76 | -2 | -44 | 2.13E-6 | 9.38E-6 | > 1.00E-4 |
| OVCAR-3 | 0.484 | 1.775 | 1.795 | 1.409 | 1.602 | 0.631 | 0.051 | 102 | 72 | 87 | 11 | -89 | 3.06E-6 | 1.30E-5 | 4.06E-5 |
| OVCAR-4 | 0.665 | 1.615 | 1.572 | 1.474 | 1.530 | 0.805 | 0.498 | 95 | 85 | 91 | 15 | -25 | 3.45E-6 | 2.34E-5 | > 1.00E-4 |
| OVCAR-5 | 0.836 | 1.855 | 1.721 | 1.739 | 1.651 | 1.050 | 0.282 | 87 | 89 | 80 | 21 | -66 | 3.22E-6 | 1.74E-5 | 6.51E-5 |
| OVCAR-8 | 0.536 | 1.939 | 1.974 | 1.811 | 1.909 | 0.547 | 0.170 | 103 | 91 | 98 | 1 | -68 | 3.11E-6 | 1.03E-5 | 5.42E-5 |
| NCI/ADR-RES | 0.493 | 1.770 | 1.742 | 1.553 | 1.640 | 0.440 | 0.330 | 98 | 83 | 90 | -11 | -33 | 2.49E-6 | 7.80E-6 | > 1.00E-4 |
| SK-OV-3 | 0.884 | 1.720 | 1.672 | 1.658 | 1.645 | 0.896 | 0.131 | 94 | 92 | 91 | 1 | -85 | 2.87E-6 | 1.04E-5 | 3.92E-5 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.631 | 2.110 | 1.927 | 1.907 | 1.903 | 0.464 | 0.170 | 88 | 86 | 86 | -27 | -73 | 2.09E-6 | 5.81E-6 | 3.19E-5 |
| A498 | 1.406 | 2.167 | 2.156 | 1.988 | 2.139 | 1.351 | 0.173 | 99 | 76 | 96 | -4 | -88 | 2.89E-6 | 9.14E-6 | 3.55E-5 |
| ACHN | 0.367 | 1.485 | 1.438 | 1.042 | 1.298 | 0.405 | 0.107 | 96 | 60 | 83 | 3 | -71 | 2.61E-6 | 1.11E-5 | 5.24E-5 |
| CAKI-1 | 0.913 | 3.266 | 3.124 | 3.121 | 3.120 | 0.894 | 0.543 | 94 | 94 | 94 | -2 | -41 | 2.86E-6 | 9.50E-6 | > 1.00E-4 |
| RXF 393 | 0.805 | 1.637 | 1.608 | 1.444 | 1.542 | 0.926 | 0.196 | 96 | 77 | 89 | 14 | -76 | 3.32E-6 | 1.45E-5 | 5.19E-5 |
| SN12C | 0.842 | 2.473 | 2.467 | 2.498 | 2.548 | 0.974 | 0.273 | 100 | 102 | 105 | 8 | -68 | 3.68E-6 | 1.28E-5 | 5.85E-5 |
| TK-10 | 0.908 | 1.529 | 1.458 | 1.480 | 1.486 | 0.947 | 0.148 | 89 | 92 | 93 | 6 | -84 | 3.13E-6 | 1.17E-5 | 4.21E-5 |
| UO-31 | 1.156 | 3.002 | 2.836 | 2.383 | 2.521 | 1.162 | 0.281 | 91 | 66 | 74 | . | -76 | 2.11E-6 | 1.01E-5 | 4.59E-5 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.919 | 2.326 | 2.290 | 1.609 | 2.025 | 0.988 | 0.351 | 97 | 49 | 79 | 5 | -62 | . | 1.18E-5 | 6.64E-5 |
| DU-145 | 0.446 | 1.868 | 1.934 | 1.709 | 1.782 | 0.747 | 0.020 | 105 | 89 | 94 | 21 | -96 | 4.01E-6 | 1.52E-5 | 4.07E-5 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.395 | 2.130 | 1.747 | 1.056 | 1.377 | 0.362 | 0.052 | 78 | 38 | 57 | -8 | -87 | . | 7.41E-6 | 3.39E-5 |
| MDA-MB-231/ATCC | 0.777 | 1.867 | 1.693 | 1.553 | 1.620 | 0.761 | 0.306 | 103 | 87 | 95 | -2 | -61 | 2.90E-6 | 9.52E-6 | 6.57E-5 |
| HS 578T | 1.247 | 2.309 | 2.263 | 2.251 | 2.262 | 1.582 | 1.046 | 96 | 95 | 96 | 32 | -16 | 5.14E-6 | 4.59E-5 | > 1.00E-4 |
| BT-549 | 1.081 | 2.277 | 2.078 | 1.909 | 1.965 | 0.900 | 0.445 | 83 | 69 | 74 | -17 | -59 | 1.83E-6 | 6.53E-6 | 6.16E-5 |
| T-47D | 0.728 | 1.365 | 1.289 | 1.113 | 1.168 | 0.654 | 0.522 | 88 | 60 | 69 | -10 | -28 | 1.74E-6 | 7.44E-6 | > 1.00E-4 |
| MDA-MB-468 | 0.793 | 1.538 | 1.564 | 1.425 | 1.528 | 0.718 | 0.268 | 103 | 85 | 99 | -9 | -66 | 2.82E-6 | 8.18E-6 | 5.17E-5 |

**4-[6-(6-hydroxy-5,7-dioxo-5,6,7,13-tetrahydro-12*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12-yl)hexyl]morpholin-4-ium chloride 338**



**4-[6-(6-hydroxy-5,7-dioxo-5,6,7,13-tetrahydro-12*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12-yl)hexyl]morpholin-4-ium chloride 338**



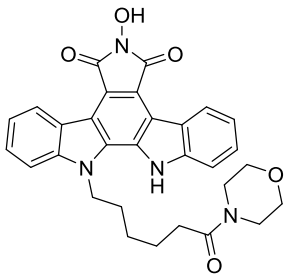

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

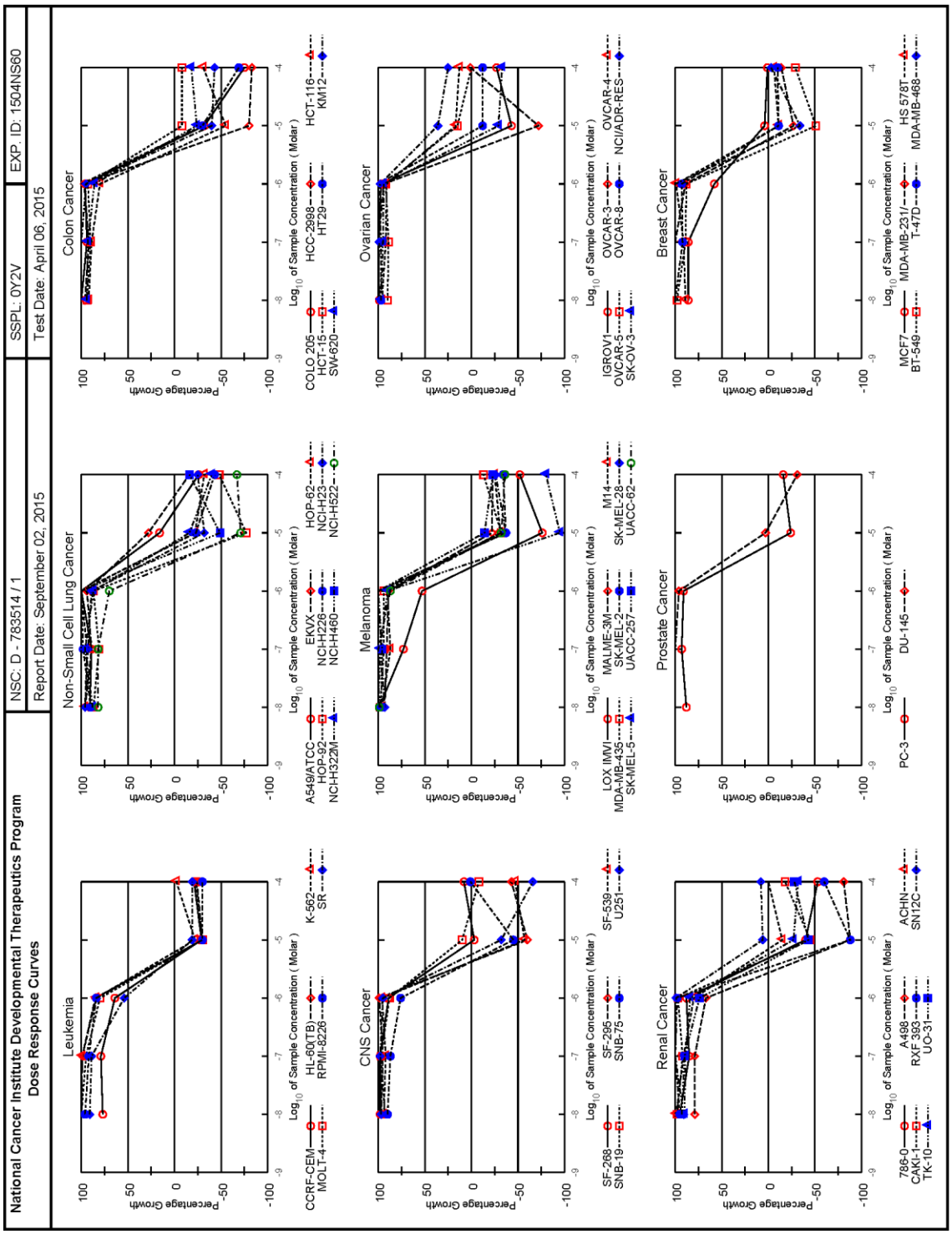
| | | | |
|---|---|---|---|
| NSC : D - 785682 / 1 | Experiment ID : 1508NS43 | Test Type : 08 | Units : Molar |
| Report Date : October 06, 2015 | Test Date : August 24, 2015 | QNS : | MC : |
| COMI : HW8-666-1 | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

Log10 Concentration

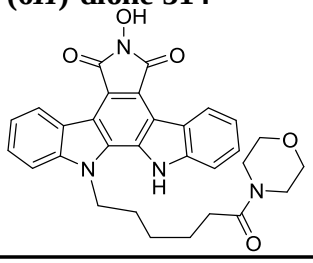
| Panel/Cell Line | Time Zero | Mean Optical Densities Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | Percent Growth -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.889 | 3.314 | 3.299 | 3.276 | 3.028 | 0.904 | 0.789 | 99 | 98 | 88 | 1 | -11 | 2.73E-6 | 1.13E-5 | > 1.00E-4 |
| HL-60(TB) | 0.868 | 3.198 | 3.171 | 3.155 | 2.873 | 0.873 | 0.373 | 99 | 98 | 86 | . | -57 | 2.63E-6 | 1.01E-5 | 7.54E-5 |
| K-562 | 0.325 | 2.415 | 2.517 | 2.425 | 2.321 | 0.109 | 0.170 | 105 | 101 | 96 | -66 | -48 | 1.91E-6 | 3.89E-6 | . |
| MOLT-4 | 0.711 | 2.826 | 2.817 | 2.844 | 2.389 | 0.442 | 0.491 | 100 | 101 | 79 | -38 | -31 | 1.78E-6 | 4.75E-6 | > 1.00E-4 |
| RPMI-8226 | 0.613 | 2.593 | 2.593 | 2.589 | 2.431 | 0.556 | 0.397 | 100 | 100 | 92 | -9 | -35 | 2.59E-6 | 8.08E-6 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.373 | 1.803 | 1.754 | 1.821 | 1.828 | 1.542 | 0.712 | 97 | 101 | 102 | 82 | 24 | 3.52E-5 | > 1.00E-4 | > 1.00E-4 |
| EKVX | 0.662 | 1.515 | 1.413 | 1.432 | 1.385 | 1.293 | 0.564 | 88 | 90 | 85 | 74 | -15 | 1.86E-5 | 6.81E-5 | > 1.00E-4 |
| HOP-62 | 0.872 | 2.241 | 2.093 | 2.081 | 1.943 | 1.858 | 0.541 | 89 | 87 | 78 | 72 | -38 | 1.59E-5 | 4.51E-5 | > 1.00E-4 |
| HOP-92 | 1.197 | 1.732 | 1.656 | 1.635 | 1.564 | 1.465 | 0.316 | 86 | 82 | 69 | 50 | -74 | 1.00E-5 | 2.54E-5 | 6.44E-5 |
| NCI-H226 | 0.528 | 1.641 | 1.569 | 1.499 | 1.359 | 1.101 | 0.465 | 94 | 87 | 75 | 51 | -12 | 1.06E-5 | 6.47E-5 | > 1.00E-4 |
| NCI-H23 | 0.596 | 2.224 | 2.120 | 2.032 | 1.851 | 1.567 | 0.223 | 94 | 88 | 77 | 60 | -63 | 1.20E-5 | 3.08E-5 | 7.89E-5 |
| NCI-H322M | 0.498 | 1.716 | 1.689 | 1.629 | 1.597 | 1.432 | 0.537 | 98 | 93 | 90 | 77 | 3 | 2.30E-5 | > 1.00E-4 | > 1.00E-4 |
| NCI-H460 | 0.215 | 2.196 | 2.235 | 2.249 | 2.049 | 0.061 | 0.089 | 102 | 103 | 93 | -72 | -58 | 1.81E-6 | 3.66E-6 | 7.38E-6 |
| NCI-H522 | 1.040 | 2.546 | 2.262 | 2.357 | 2.101 | 0.488 | 0.742 | 81 | 87 | 70 | -53 | -29 | 1.46E-6 | 3.72E-6 | . |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.436 | 1.662 | 1.653 | 1.680 | 1.557 | 1.016 | 0.126 | 99 | 101 | 91 | 47 | -71 | 8.67E-6 | 2.51E-5 | 6.63E-5 |
| HCC-2998 | 0.721 | 2.427 | 2.424 | 2.319 | 2.283 | 1.922 | 0.357 | 100 | 94 | 92 | 70 | -51 | 1.47E-5 | 3.82E-5 | 9.89E-5 |
| HCT-116 | 0.418 | 3.023 | 2.956 | 2.959 | 2.803 | 0.148 | 0.080 | 97 | 98 | 92 | -65 | -81 | 1.85E-6 | 3.86E-6 | 8.06E-6 |
| HCT-15 | 0.295 | 1.754 | 1.690 | 1.780 | 1.617 | 0.374 | 0.221 | 96 | 102 | 91 | 5 | -25 | 3.00E-6 | 1.50E-5 | > 1.00E-4 |
| HT29 | 0.313 | 1.907 | 1.844 | 1.896 | 1.727 | 0.523 | 0.191 | 96 | 99 | 89 | 13 | -39 | 3.25E-6 | 1.78E-5 | > 1.00E-4 |
| KM12 | 0.453 | 2.634 | 2.502 | 2.553 | 2.353 | 1.451 | 0.400 | 94 | 96 | 87 | 46 | -12 | 7.89E-6 | 6.26E-5 | > 1.00E-4 |
| SW-620 | 0.229 | 1.690 | 1.522 | 1.546 | 1.363 | 0.061 | 0.081 | 88 | 90 | 78 | -73 | -65 | 1.52E-6 | 3.27E-6 | 7.00E-6 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.599 | 2.108 | 1.986 | 2.001 | 1.871 | 1.752 | 0.274 | 92 | 93 | 84 | 76 | -54 | 1.59E-5 | 3.84E-5 | 9.28E-5 |
| SF-295 | 0.517 | 1.797 | 1.699 | 1.746 | 1.659 | 1.572 | 0.117 | 92 | 96 | 89 | 82 | -77 | 1.59E-5 | 3.28E-5 | 6.73E-5 |
| SF-539 | 0.652 | 2.256 | 2.276 | 2.252 | 2.164 | 0.098 | 0.052 | 101 | 100 | 94 | -85 | -92 | 1.77E-6 | 3.36E-6 | 6.38E-6 |
| SNB-19 | 0.372 | 1.693 | 1.659 | 1.614 | 1.557 | 0.950 | 0.784 | 97 | 94 | 90 | 44 | 31 | 7.31E-6 | > 1.00E-4 | > 1.00E-4 |
| SNB-75 | 0.827 | 1.853 | 1.485 | 1.424 | 1.277 | 1.044 | 0.233 | 80 | 72 | 54 | 26 | -72 | 1.44E-6 | 1.85E-5 | 5.99E-5 |
| U251 | 0.475 | 1.771 | 1.686 | 1.760 | 1.740 | 0.176 | 0.264 | 93 | 99 | 98 | -63 | -44 | 1.98E-6 | 4.05E-6 | . |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.319 | 2.326 | 2.154 | 1.916 | 0.280 | 0.036 | 0.149 | 91 | 80 | -12 | -89 | -53 | 2.10E-7 | 7.36E-7 | 3.11E-6 |
| MALME-3M | 0.451 | 1.004 | 0.983 | 0.973 | 0.928 | 0.164 | 0.209 | 96 | 94 | 86 | -64 | -54 | 1.74E-6 | 3.76E-6 | 8.10E-6 |
| M14 | 0.438 | 2.010 | 1.945 | 1.837 | 1.768 | 0.872 | 0.120 | 96 | 89 | 85 | 28 | -73 | 4.05E-6 | 1.88E-5 | 5.94E-5 |
| MDA-MB-435 | 0.435 | 2.287 | 2.199 | 2.186 | 2.138 | 0.693 | 0.179 | 95 | 95 | 92 | 14 | -59 | 3.45E-6 | 1.55E-5 | 7.56E-5 |
| SK-MEL-2 | 0.815 | 1.543 | 1.514 | 1.530 | 1.472 | 1.462 | 0.447 | 96 | 98 | 90 | 89 | -45 | 1.95E-5 | 4.60E-5 | > 1.00E-4 |
| SK-MEL-28 | 0.738 | 2.520 | 2.544 | 2.447 | 2.463 | 0.270 | 0.163 | 101 | 96 | 97 | -63 | -78 | 1.96E-6 | 4.02E-6 | 8.25E-6 |
| SK-MEL-5 | 0.808 | 3.179 | 3.089 | 3.093 | 2.765 | 1.830 | 0.212 | 95 | 96 | 83 | 35 | -74 | 4.78E-6 | 2.09E-5 | 6.04E-5 |
| UACC-257 | 0.925 | 2.137 | 2.022 | 2.060 | 1.995 | 1.909 | 0.458 | 90 | 94 | 88 | 81 | -50 | 1.72E-5 | 4.14E-5 | 9.92E-5 |
| UACC-62 | 0.681 | 2.499 | 2.394 | 2.341 | 2.271 | 0.417 | 0.390 | 94 | 91 | 87 | -39 | -43 | 1.98E-6 | 4.93E-6 | > 1.00E-4 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| OVCAR-3 | 0.594 | 1.793 | 1.760 | 1.805 | 1.706 | 1.608 | 0.095 | 97 | 101 | 93 | 85 | -84 | 1.60E-5 | 3.17E-5 | 6.28E-5 |
| OVCAR-4 | 0.608 | 1.611 | 1.614 | 1.564 | 1.483 | 0.615 | 0.524 | 100 | 95 | 87 | 1 | -14 | 2.69E-6 | 1.11E-5 | > 1.00E-4 |
| OVCAR-5 | 0.613 | 1.704 | 1.642 | 1.592 | 1.594 | 1.723 | 0.415 | 94 | 90 | 90 | 102 | -32 | 2.43E-5 | 5.74E-5 | > 1.00E-4 |
| OVCAR-8 | 0.459 | 2.158 | 1.992 | 2.068 | 1.875 | 0.332 | 0.414 | 90 | 95 | 83 | -28 | -10 | 2.00E-6 | 5.62E-6 | > 1.00E-4 |
| NCI/ADR-RES | 0.422 | 1.738 | 1.701 | 1.617 | 1.558 | 1.144 | 1.051 | 97 | 91 | 86 | 55 | 48 | 4.87E-5 | > 1.00E-4 | > 1.00E-4 |
| SK-OV-3 | 0.767 | 1.450 | 1.371 | 1.423 | 1.354 | 1.400 | 0.264 | 88 | 96 | 86 | 93 | -66 | 1.86E-5 | 3.85E-5 | 7.97E-5 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.532 | 2.201 | 2.144 | 2.159 | 1.838 | 0.360 | 0.182 | 97 | 97 | 78 | -32 | -66 | 1.80E-6 | 5.09E-6 | 3.35E-5 |
| A498 | 1.317 | 2.013 | 1.945 | 1.995 | 1.832 | 1.631 | 0.225 | 90 | 97 | 74 | 45 | -63 | 6.74E-6 | 2.25E-5 | 5.53E-5 |
| ACHN | 0.455 | 2.245 | 2.194 | 2.076 | 1.971 | 0.115 | 0.040 | 97 | 91 | 85 | -75 | -91 | 1.65E-6 | 3.40E-6 | 7.00E-6 |
| CAKI-1 | 0.558 | 2.650 | 2.576 | 2.420 | 2.409 | 1.721 | 0.257 | 96 | 89 | 89 | 56 | -54 | 1.12E-5 | 3.22E-5 | 9.20E-5 |
| RXF 393 | 0.617 | 1.222 | 1.177 | 1.181 | 1.046 | 0.864 | 0.226 | 93 | 93 | 71 | 41 | -63 | 4.94E-6 | 2.46E-5 | 7.44E-5 |
| SN12C | 0.454 | 1.933 | 1.881 | 1.854 | 1.735 | 0.466 | 0.331 | 96 | 95 | 87 | 1 | -27 | 2.67E-6 | 1.07E-5 | > 1.00E-4 |
| TK-10 | 0.699 | 1.423 | 1.339 | 1.416 | 1.487 | 1.651 | 0.488 | 88 | 99 | 109 | 131 | -30 | 3.19E-5 | 6.50E-5 | > 1.00E-4 |
| UO-31 | 0.529 | 2.211 | 1.937 | 1.736 | 1.746 | 0.089 | 0.184 | 84 | 72 | 72 | -87 | -65 | 1.38E-6 | 2.85E-6 | 5.86E-6 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.615 | 2.483 | 2.329 | 2.262 | 2.017 | 1.639 | 0.293 | 92 | 88 | 75 | 55 | -52 | 1.11E-5 | 3.25E-5 | 9.51E-5 |
| DU-145 | 0.355 | 1.663 | 1.656 | 1.666 | 1.594 | 0.858 | 0.458 | 99 | 100 | 95 | 38 | 8 | 6.23E-6 | > 1.00E-4 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.391 | 2.193 | 2.053 | 1.987 | 1.702 | 0.263 | 0.215 | 92 | 89 | 73 | -33 | -45 | 1.64E-6 | 4.88E-6 | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.397 | 1.326 | 1.281 | 1.218 | 1.090 | 0.296 | 0.275 | 95 | 88 | 75 | -25 | -31 | 1.76E-6 | 5.57E-6 | > 1.00E-4 |
| HS 578T | 0.978 | 2.034 | 1.879 | 1.926 | 1.741 | 0.600 | 0.673 | 85 | 90 | 72 | -39 | -31 | 1.59E-6 | 4.48E-6 | > 1.00E-4 |
| BT-549 | 1.146 | 2.317 | 2.281 | 2.250 | 2.075 | 1.987 | 0.296 | 97 | 94 | 79 | 72 | -74 | 1.41E-5 | 3.10E-5 | 6.83E-5 |
| T-47D | 0.859 | 1.850 | 1.759 | 1.760 | 1.702 | 1.494 | 0.831 | 91 | 91 | 85 | 64 | -3 | 1.62E-5 | 8.94E-5 | > 1.00E-4 |
| MDA-MB-468 | 0.977 | 2.096 | 1.928 | 1.969 | 1.918 | 0.478 | 0.131 | 85 | 89 | 84 | -51 | -87 | 1.79E-6 | 4.19E-6 | 9.82E-6 |

**6-Hydroxy-12-(6-morpholino-6-oxohexyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 314**



**6-Hydroxy-12-(6-morpholino-6-oxohexyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione 314**



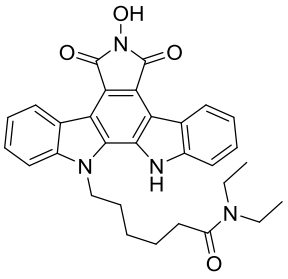
National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

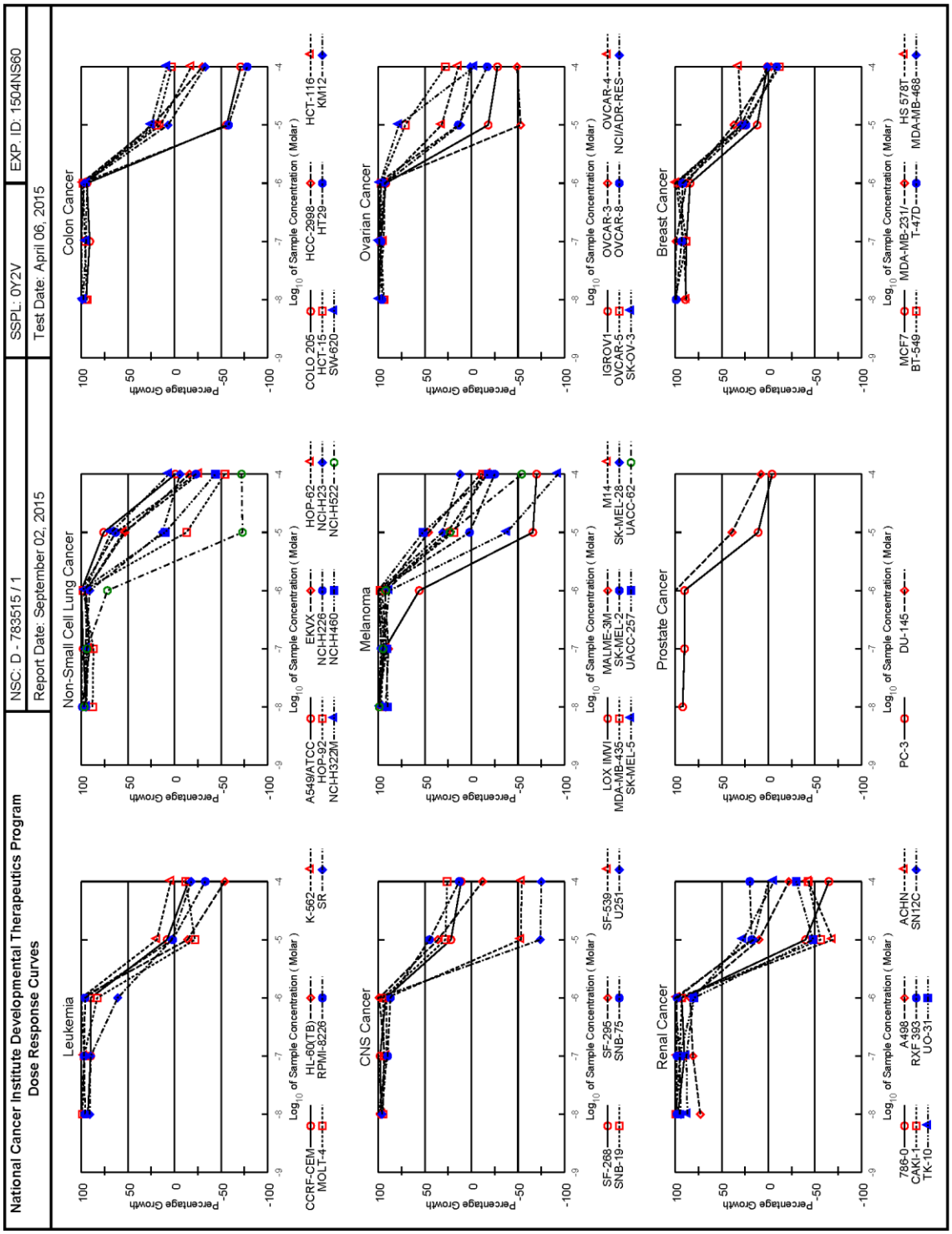
| | | | |
|---|---|---|---|
| NSC : D - 783514 / 1 | Experiment ID : 1504NS60 | Test Type : 08 | Units : Molar |
| Report Date : September 02, 2015 | Test Date : April 06, 2015 | QNS : | MC : |
| COMI : HW8-657-2 | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

Log10 Concentration

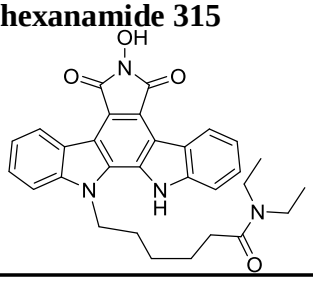
| Panel/Cell Line | Time Zero | Mean Optical Densities Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | Percent Growth -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.552 | 1.637 | 1.386 | 1.412 | 1.251 | 0.395 | 0.436 | 77 | 79 | 64 | -29 | -21 | 1.43E-6 | 4.93E-6 | > 1.00E-4 |
| HL-60(TB) | 0.933 | 2.640 | 2.686 | 2.628 | 2.385 | 0.649 | 0.709 | 103 | 99 | 85 | -30 | -24 | 2.01E-6 | 5.45E-6 | > 1.00E-4 |
| K-562 | 0.373 | 1.899 | 1.970 | 1.877 | 1.663 | 0.292 | 0.366 | 105 | 99 | 84 | -22 | -2 | 2.11E-6 | 6.23E-6 | > 1.00E-4 |
| MOLT-4 | 0.706 | 2.485 | 2.482 | 2.393 | 2.134 | 0.496 | 0.544 | 100 | 95 | 80 | -30 | -23 | 1.88E-6 | 5.36E-6 | > 1.00E-4 |
| RPMI-8226 | 1.307 | 2.894 | 2.835 | 2.775 | 2.633 | 0.913 | 0.918 | 96 | 92 | 84 | -30 | -30 | 1.97E-6 | 5.43E-6 | > 1.00E-4 |
| SR | 0.266 | 0.985 | 0.924 | 0.910 | 0.651 | 0.215 | 0.216 | 91 | 89 | 54 | -19 | -19 | 1.12E-6 | 5.45E-6 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.454 | 1.715 | 1.603 | 1.578 | 1.806 | 0.852 | 0.338 | 91 | 89 | 107 | 16 | -26 | 4.22E-6 | 2.40E-5 | > 1.00E-4 |
| EKVX | 0.784 | 1.935 | 1.889 | 1.811 | 1.866 | 1.104 | 0.660 | 96 | 89 | 94 | 28 | -16 | 4.62E-6 | 4.33E-5 | > 1.00E-4 |
| HOP-62 | 0.649 | 1.329 | 1.305 | 1.291 | 1.378 | 0.505 | 0.441 | 96 | 94 | 107 | -22 | -32 | 2.77E-6 | 6.74E-6 | > 1.00E-4 |
| HOP-92 | 1.053 | 1.500 | 1.442 | 1.414 | 1.444 | 0.240 | 0.548 | 87 | 81 | 87 | -77 | -48 | 1.69E-6 | 3.39E-6 | . |
| NCI-H226 | 1.073 | 2.491 | 2.356 | 2.469 | 2.507 | 0.824 | 0.617 | 90 | 98 | 101 | -23 | -43 | 2.58E-6 | 6.51E-6 | > 1.00E-4 |
| NCI-H23 | 0.779 | 2.348 | 2.292 | 2.239 | 2.180 | 0.532 | 0.586 | 96 | 93 | 89 | -32 | -25 | 2.11E-6 | 5.47E-6 | > 1.00E-4 |
| NCI-H322M | 1.029 | 2.488 | 2.319 | 2.424 | 2.299 | 0.866 | 0.614 | 88 | 96 | 87 | -16 | -40 | 2.29E-6 | 7.02E-6 | > 1.00E-4 |
| NCI-H460 | 0.403 | 3.199 | 3.196 | 3.217 | 3.186 | 0.207 | 0.340 | 100 | 101 | 100 | -49 | -16 | 2.16E-6 | 4.69E-6 | > 1.00E-4 |
| NCI-H522 | 0.915 | 2.013 | 1.815 | 1.817 | 1.679 | 0.270 | 0.305 | 82 | 82 | 70 | -71 | -67 | 1.38E-6 | 3.14E-6 | 7.14E-6 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.451 | 1.574 | 1.594 | 1.504 | 1.533 | 0.308 | 0.111 | 102 | 94 | 96 | -32 | -75 | 2.30E-6 | 5.65E-6 | 2.81E-5 |
| HCC-2998 | 0.691 | 2.263 | 2.196 | 2.145 | 2.216 | 0.142 | 0.118 | 96 | 92 | 97 | -80 | -83 | 1.85E-6 | 3.54E-6 | 6.80E-6 |
| HCT-116 | 0.286 | 2.140 | 1.992 | 1.932 | 1.769 | 0.128 | 0.197 | 92 | 89 | 80 | -55 | -31 | 1.67E-6 | 3.90E-6 | . |
| HCT-15 | 0.253 | 1.893 | 1.789 | 1.724 | 1.784 | 0.233 | 0.232 | 94 | 90 | 93 | -8 | -8 | 2.67E-6 | 8.32E-6 | > 1.00E-4 |
| HT29 | 0.272 | 1.502 | 1.561 | 1.501 | 1.499 | 0.191 | 0.085 | 105 | 100 | 100 | -30 | -69 | 2.42E-6 | 5.88E-6 | 3.29E-5 |
| KM12 | 0.499 | 2.678 | 2.678 | 2.750 | 2.559 | 0.298 | 0.282 | 100 | 103 | 95 | -40 | -43 | 2.14E-6 | 5.03E-6 | > 1.00E-4 |
| SW-620 | 0.282 | 1.942 | 1.845 | 1.830 | 1.710 | 0.214 | 0.231 | 94 | 93 | 86 | -24 | -18 | 2.12E-6 | 6.02E-6 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.707 | 2.307 | 2.279 | 2.277 | 2.126 | 0.686 | 0.843 | 98 | 98 | 89 | -3 | 8 | 2.64E-6 | . | > 1.00E-4 |
| SF-295 | 0.923 | 2.827 | 2.764 | 2.732 | 2.803 | 0.369 | 0.522 | 97 | 95 | 99 | -60 | -43 | 2.03E-6 | 4.18E-6 | . |
| SF-539 | 0.660 | 2.236 | 2.134 | 2.109 | 2.179 | 0.281 | 0.348 | 94 | 92 | 96 | -57 | -47 | 2.00E-6 | 4.23E-6 | . |
| SNB-19 | 0.824 | 2.375 | 2.265 | 2.325 | 2.185 | 0.976 | 0.762 | 93 | 97 | 88 | 10 | -8 | 3.05E-6 | 3.68E-5 | > 1.00E-4 |
| SNB-75 | 0.418 | 0.986 | 0.929 | 0.911 | 0.849 | 0.231 | 0.424 | 90 | 87 | 76 | -45 | 1 | 1.64E-6 | . | > 1.00E-4 |
| U251 | 0.598 | 2.203 | 2.152 | 2.164 | 2.125 | 0.406 | 0.206 | 97 | 98 | 95 | -32 | -66 | 2.26E-6 | 5.59E-6 | 3.42E-5 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.436 | 2.617 | 2.601 | 2.037 | 1.591 | 0.104 | 0.211 | 99 | 73 | 53 | -76 | -52 | 1.05E-6 | 2.57E-6 | 6.27E-6 |
| MALME-3M | 0.738 | 1.356 | 1.316 | 1.299 | 1.291 | 0.575 | 0.572 | 93 | 91 | 89 | -22 | -23 | 2.26E-6 | 6.34E-6 | > 1.00E-4 |
| M14 | 0.630 | 2.221 | 2.153 | 2.009 | 2.065 | 0.433 | 0.470 | 96 | 87 | 90 | -31 | -25 | 2.14E-6 | 5.52E-6 | > 1.00E-4 |
| MDA-MB-435 | 0.469 | 2.470 | 2.439 | 2.351 | 2.359 | 0.319 | 0.408 | 98 | 94 | 94 | -32 | -13 | 2.25E-6 | 5.58E-6 | > 1.00E-4 |
| SK-MEL-2 | 0.985 | 2.085 | 2.140 | 2.084 | 2.120 | 0.824 | 0.651 | 105 | 100 | 103 | -37 | -34 | 2.40E-6 | 5.47E-6 | > 1.00E-4 |
| SK-MEL-28 | 0.843 | 2.508 | 2.399 | 2.450 | 2.505 | 0.535 | 0.623 | 93 | 97 | 100 | -37 | -26 | 2.32E-6 | 5.40E-6 | > 1.00E-4 |
| SK-MEL-5 | 0.726 | 2.974 | 3.001 | 2.919 | 2.748 | 0.036 | 0.145 | 101 | 98 | 90 | -95 | -80 | 1.64E-6 | 3.06E-6 | 5.71E-6 |
| UACC-257 | 0.825 | 1.515 | 1.497 | 1.490 | 1.620 | 0.708 | 0.638 | 97 | 96 | 115 | -14 | -23 | 3.19E-6 | 7.76E-6 | > 1.00E-4 |
| UACC-62 | 1.098 | 2.816 | 2.791 | 2.814 | 2.591 | 0.749 | 0.706 | 99 | 100 | 87 | -32 | -36 | 2.05E-6 | 5.40E-6 | > 1.00E-4 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.559 | 2.285 | 2.276 | 2.334 | 2.232 | 0.318 | 0.409 | 99 | 103 | 97 | -43 | -27 | 2.16E-6 | 4.92E-6 | > 1.00E-4 |
| OVCAR-3 | 0.484 | 1.718 | 1.782 | 1.810 | 1.648 | 0.134 | 0.495 | 105 | 107 | 94 | -72 | 1 | 1.85E-6 | . | . |
| OVCAR-4 | 0.665 | 1.618 | 1.583 | 1.538 | 1.536 | 0.823 | 0.787 | 96 | 92 | 91 | 17 | 13 | 3.57E-6 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-5 | 0.836 | 1.952 | 1.845 | 1.833 | 1.860 | 1.002 | 0.837 | 90 | 89 | 92 | 15 | . | 3.49E-6 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-8 | 0.536 | 2.101 | 2.056 | 2.049 | 2.122 | 0.473 | 0.472 | 97 | 97 | 101 | -12 | -12 | 2.84E-6 | 7.86E-6 | > 1.00E-4 |
| NCI/ADR-RES | 0.493 | 1.800 | 1.834 | 1.784 | 1.771 | 0.964 | 0.816 | 103 | 99 | 98 | 36 | 25 | 5.93E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-OV-3 | 0.884 | 1.842 | 1.815 | 1.795 | 1.782 | 0.628 | 0.592 | 97 | 95 | 94 | -29 | -33 | 2.27E-6 | 5.81E-6 | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.631 | 2.194 | 2.109 | 1.964 | 2.011 | 0.382 | 0.297 | 95 | 85 | 88 | -40 | -53 | 1.99E-6 | 4.91E-6 | 6.04E-5 |
| A498 | 1.406 | 2.118 | 1.971 | 1.968 | 1.885 | 0.162 | 0.267 | 79 | 79 | 67 | -88 | -81 | 1.29E-6 | 2.70E-6 | 5.66E-6 |
| ACHN | 0.367 | 1.607 | 1.598 | 1.501 | 1.360 | 0.312 | 0.371 | 99 | 91 | 80 | -15 | . | 2.07E-6 | . | > 1.00E-4 |
| CAKI-1 | 0.913 | 3.232 | 3.186 | 3.058 | 3.155 | 0.502 | 0.749 | 98 | 92 | 97 | -45 | -18 | 2.13E-6 | 4.81E-6 | > 1.00E-4 |
| RXF 393 | 0.805 | 1.678 | 1.632 | 1.678 | 1.660 | 0.097 | 0.318 | 95 | 100 | 98 | -88 | -60 | 1.81E-6 | 3.37E-6 | 6.25E-6 |
| SN12C | 0.842 | 2.663 | 2.491 | 2.676 | 2.647 | 0.954 | 0.995 | 91 | 101 | 99 | 6 | 8 | 3.37E-6 | > 1.00E-4 | > 1.00E-4 |
| TK-10 | 0.908 | 1.523 | 1.464 | 1.458 | 1.427 | 0.663 | 0.620 | 90 | 89 | 84 | -27 | -32 | 2.03E-6 | 5.72E-6 | > 1.00E-4 |
| UO-31 | 1.156 | 3.048 | 2.920 | 2.868 | 2.552 | 0.659 | 0.836 | 93 | 90 | 74 | -43 | -28 | 1.60E-6 | 4.28E-6 | > 1.00E-4 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.919 | 2.272 | 2.108 | 2.183 | 2.153 | 0.702 | 0.775 | 88 | 93 | 91 | -24 | -16 | 2.28E-6 | 6.22E-6 | > 1.00E-4 |
| DU-145 | 0.446 | 1.895 | 1.990 | 1.960 | 1.835 | 0.489 | 0.309 | 107 | 105 | 96 | 3 | -31 | 3.12E-6 | 1.22E-5 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.395 | 2.193 | 1.950 | 1.944 | 1.439 | 0.466 | 0.420 | 86 | 86 | 58 | 4 | 1 | 1.41E-6 | > 1.00E-4 | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.777 | 1.697 | 1.756 | 1.703 | 1.593 | 0.570 | 0.670 | 108 | 101 | 89 | -27 | -14 | 2.16E-6 | 5.87E-6 | > 1.00E-4 |
| HS 578T | 1.247 | 2.262 | 2.148 | 2.174 | 2.247 | 1.111 | 1.177 | 89 | 91 | 99 | -11 | -6 | 2.78E-6 | 7.95E-6 | > 1.00E-4 |
| BT-549 | 1.081 | 2.309 | 2.290 | 2.175 | 2.166 | 0.531 | 0.764 | 98 | 89 | 85 | -51 | -29 | 1.89E-6 | 4.31E-6 | . |
| T-47D | 0.728 | 1.425 | 1.451 | 1.368 | 1.379 | 0.650 | 0.655 | 104 | 92 | 93 | -11 | -10 | 2.61E-6 | 7.89E-6 | > 1.00E-4 |
| MDA-MB-468 | 0.793 | 1.564 | 1.586 | 1.674 | 1.673 | 0.524 | 0.769 | 103 | 114 | 114 | -34 | -3 | 2.71E-6 | 5.90E-6 | > 1.00E-4 |

**_N_,_N_-Diethyl-6-(6-hydroxy-5,7-dioxo-5,6,7,13-tetrahydro-12_H_-indolo[2,3-_a_]pyrrolo[3,4-_c_]carbazol-12-yl)hexanamide 315**





## *N*,*N*-Diethyl-6-(6-hydroxy-5,7-dioxo-5,6,7,13-tetrahydro-12*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-12-yl)hexanamide 315



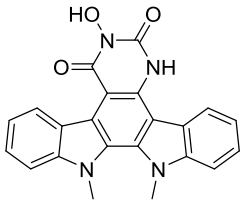
National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

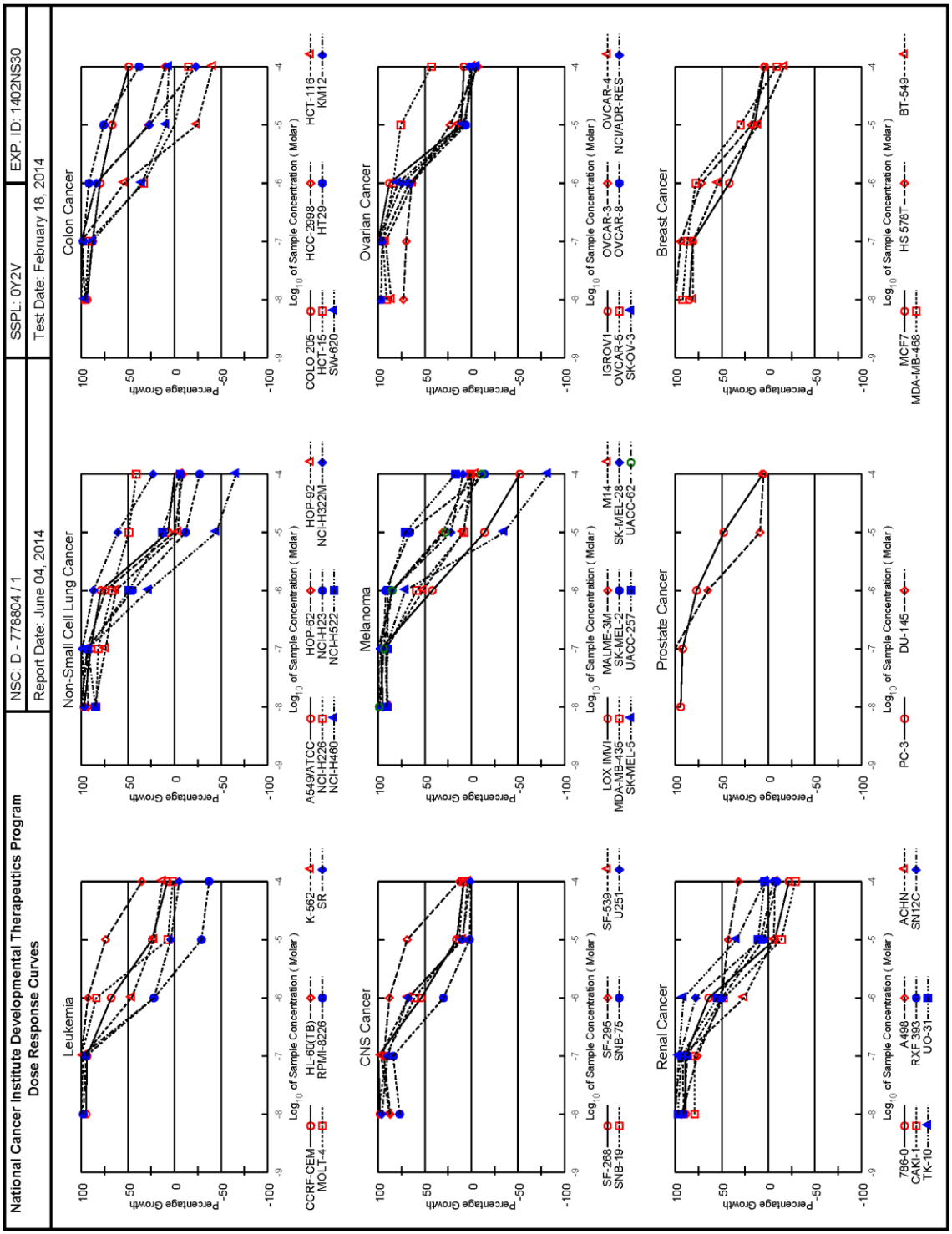
| | | | |
|---|---|---|---|
| NSC : D - 783515 / 1 | Experiment ID : 1504NS60 | Test Type : 08 | Units : Molar |
| Report Date : September 02, 2015 | Test Date : April 06, 2015 | QNS : | MC : |
| COMI : HW8-660-1 | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

Log10 Concentration

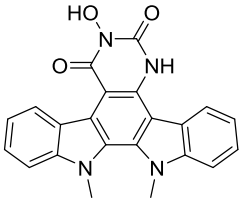
| Panel/Cell Line | Time Zero | Mean Optical Densities Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | Percent Growth -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.552 | 1.625 | 1.550 | 1.523 | 1.522 | 0.634 | 0.471 | 93 | 90 | 90 | 8 | -15 | 3.07E-6 | 2.19E-5 | > 1.00E-4 |
| HL-60(TB) | 0.933 | 2.600 | 2.689 | 2.748 | 2.625 | 0.807 | 0.432 | 105 | 109 | 101 | -14 | -54 | 2.80E-6 | 7.63E-6 | 8.07E-5 |
| K-562 | 0.373 | 1.858 | 1.901 | 1.808 | 1.862 | 0.660 | 0.430 | 103 | 97 | 100 | 19 | 4 | 4.18E-6 | > 1.00E-4 | > 1.00E-4 |
| MOLT-4 | 0.706 | 2.596 | 2.585 | 2.541 | 2.272 | 0.549 | 0.621 | 99 | 97 | 83 | -22 | -12 | 2.05E-6 | 6.14E-6 | > 1.00E-4 |
| RPMI-8226 | 1.307 | 2.877 | 2.814 | 2.831 | 2.807 | 1.337 | 0.873 | 96 | 97 | 96 | 2 | -33 | 3.06E-6 | 1.13E-5 | > 1.00E-4 |
| SR | 0.266 | 1.061 | 0.987 | 0.978 | 0.754 | 0.289 | 0.217 | 91 | 90 | 61 | 3 | -18 | 1.56E-6 | 1.36E-5 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.454 | 1.603 | 1.547 | 1.578 | 1.658 | 1.327 | 0.452 | 95 | 98 | 105 | 76 | -1 | 2.18E-5 | 9.84E-5 | > 1.00E-4 |
| EKVX | 0.784 | 1.894 | 1.882 | 1.793 | 1.844 | 1.369 | 0.658 | 99 | 91 | 95 | 53 | -16 | 1.09E-5 | 5.84E-5 | > 1.00E-4 |
| HOP-62 | 0.649 | 1.296 | 1.266 | 1.243 | 1.264 | 1.007 | 0.480 | 95 | 92 | 95 | 55 | -26 | 1.16E-5 | 4.78E-5 | > 1.00E-4 |
| HOP-92 | 1.053 | 1.465 | 1.415 | 1.412 | 1.459 | 0.919 | 0.482 | 88 | 87 | 96 | -13 | -54 | 2.73E-6 | 7.66E-6 | 7.91E-5 |
| NCI-H226 | 1.073 | 2.419 | 2.400 | 2.432 | 2.503 | 1.916 | 0.832 | 99 | 101 | 106 | 63 | -23 | 1.41E-5 | 5.44E-5 | > 1.00E-4 |
| NCI-H23 | 0.779 | 2.296 | 2.225 | 2.231 | 2.153 | 0.976 | 0.731 | 95 | 96 | 91 | 13 | -6 | 3.33E-6 | 4.74E-5 | > 1.00E-4 |
| NCI-H322M | 1.029 | 2.434 | 2.356 | 2.348 | 2.380 | 1.981 | 1.108 | 94 | 94 | 96 | 68 | 6 | 1.93E-5 | > 1.00E-4 | > 1.00E-4 |
| NCI-H460 | 0.403 | 3.166 | 3.164 | 3.183 | 3.180 | 0.682 | 0.228 | 100 | 101 | 100 | 10 | -44 | 3.62E-6 | 1.54E-5 | > 1.00E-4 |
| NCI-H522 | 0.915 | 2.253 | 2.207 | 2.176 | 1.872 | 0.248 | 0.260 | 97 | 94 | 72 | -73 | -72 | 1.41E-6 | 3.13E-6 | 6.94E-6 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.451 | 1.471 | 1.420 | 1.380 | 1.412 | 0.198 | 0.130 | 95 | 91 | 94 | -56 | -71 | 1.97E-6 | 4.23E-6 | 9.09E-6 |
| HCC-2998 | 0.691 | 2.167 | 2.196 | 2.237 | 2.266 | 1.024 | 0.487 | 102 | 105 | 107 | 23 | -30 | 4.72E-6 | 2.71E-5 | > 1.00E-4 |
| HCT-116 | 0.286 | 1.922 | 1.987 | 1.918 | 1.885 | 0.524 | 0.235 | 104 | 100 | 98 | 15 | -18 | 3.75E-6 | 2.81E-5 | > 1.00E-4 |
| HCT-15 | 0.253 | 1.820 | 1.721 | 1.762 | 1.795 | 0.550 | 0.306 | 94 | 96 | 98 | 19 | 3 | 4.07E-6 | > 1.00E-4 | > 1.00E-4 |
| HT29 | 0.272 | 1.529 | 1.550 | 1.574 | 1.619 | 0.114 | 0.059 | 102 | 104 | 107 | -58 | -78 | 2.21E-6 | 4.44E-6 | 8.91E-6 |
| KM12 | 0.499 | 2.606 | 2.593 | 2.635 | 2.545 | 0.637 | 0.336 | 99 | 101 | 97 | 7 | -33 | 3.31E-6 | 1.47E-5 | > 1.00E-4 |
| SW-620 | 0.282 | 1.865 | 1.826 | 1.792 | 1.765 | 0.672 | 0.409 | 98 | 95 | 94 | 25 | 8 | 4.29E-6 | > 1.00E-4 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.707 | 2.294 | 2.247 | 2.266 | 2.161 | 1.058 | 0.887 | 97 | 98 | 92 | 22 | 11 | 3.97E-6 | > 1.00E-4 | > 1.00E-4 |
| SF-295 | 0.923 | 2.834 | 2.793 | 2.704 | 2.822 | 1.618 | 0.809 | 98 | 93 | 99 | 36 | -12 | 6.07E-6 | 5.57E-5 | > 1.00E-4 |
| SF-539 | 0.660 | 2.258 | 2.193 | 2.117 | 2.113 | 0.309 | 0.304 | 96 | 91 | 91 | -53 | -54 | 1.92E-6 | 4.27E-6 | 9.49E-6 |
| SNB-19 | 0.824 | 2.332 | 2.261 | 2.244 | 2.260 | 1.246 | 1.209 | 95 | 94 | 95 | 28 | 26 | 4.70E-6 | > 1.00E-4 | > 1.00E-4 |
| SNB-75 | 0.418 | 0.988 | 0.994 | 0.929 | 0.917 | 0.676 | 0.490 | 101 | 90 | 87 | 45 | 13 | 7.71E-6 | > 1.00E-4 | > 1.00E-4 |
| U251 | 0.598 | 2.030 | 1.973 | 2.035 | 2.048 | 0.155 | 0.149 | 96 | 100 | 101 | -74 | -75 | 1.96E-6 | 3.78E-6 | 7.29E-6 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.436 | 2.557 | 2.547 | 2.418 | 1.631 | 0.148 | 0.131 | 100 | 93 | 56 | -66 | -70 | 1.13E-6 | 2.88E-6 | 7.38E-6 |
| MALME-3M | 0.738 | 1.357 | 1.306 | 1.292 | 1.317 | 1.022 | 0.663 | 92 | 89 | 93 | 46 | -10 | 8.20E-6 | 6.57E-5 | > 1.00E-4 |
| M14 | 0.630 | 2.179 | 2.199 | 2.045 | 2.116 | 1.026 | 0.520 | 101 | 91 | 96 | 26 | -17 | 4.49E-6 | 3.93E-5 | > 1.00E-4 |
| MDA-MB-435 | 0.469 | 2.549 | 2.494 | 2.424 | 2.515 | 0.855 | 0.412 | 97 | 94 | 98 | 19 | -12 | 4.04E-6 | 4.00E-5 | > 1.00E-4 |
| SK-MEL-2 | 0.985 | 2.047 | 2.079 | 2.009 | 2.086 | 1.009 | 0.738 | 103 | 96 | 104 | 2 | -25 | 3.38E-6 | 1.21E-5 | > 1.00E-4 |
| SK-MEL-28 | 0.843 | 2.544 | 2.606 | 2.501 | 2.559 | 1.375 | 1.050 | 104 | 97 | 101 | 31 | 12 | 5.38E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-5 | 0.726 | 3.107 | 3.071 | 2.999 | 2.818 | 0.454 | 0.048 | 98 | 95 | 88 | -38 | -93 | 2.00E-6 | 5.02E-6 | 1.67E-5 |
| UACC-257 | 0.825 | 1.523 | 1.453 | 1.462 | 1.648 | 1.189 | 0.675 | 90 | 91 | 118 | 52 | -18 | 1.07E-5 | 5.51E-5 | > 1.00E-4 |
| UACC-62 | 1.098 | 2.868 | 2.842 | 2.773 | 2.728 | 1.514 | 0.504 | 99 | 95 | 92 | 23 | -54 | 4.10E-6 | 2.01E-5 | 8.85E-5 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.559 | 2.259 | 2.296 | 2.215 | 2.123 | 0.459 | 0.402 | 102 | 97 | 92 | -18 | -28 | 2.41E-6 | 6.87E-6 | > 1.00E-4 |
| OVCAR-3 | 0.484 | 1.677 | 1.741 | 1.771 | 1.642 | 0.226 | 0.245 | 105 | 108 | 97 | -53 | -49 | 2.06E-6 | 4.42E-6 | . |
| OVCAR-4 | 0.665 | 1.602 | 1.617 | 1.545 | 1.533 | 0.966 | 0.798 | 102 | 94 | 93 | 32 | 14 | 5.06E-6 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-5 | 0.836 | 1.903 | 1.839 | 1.873 | 1.876 | 1.597 | 1.136 | 94 | 97 | 97 | 71 | 28 | 3.11E-5 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-8 | 0.536 | 1.951 | 1.893 | 1.911 | 2.050 | 0.734 | 0.445 | 96 | 97 | 107 | 14 | -17 | 4.10E-6 | 2.83E-5 | > 1.00E-4 |
| NCI/ADR-RES | 0.493 | 1.803 | 1.825 | 1.791 | 1.718 | 0.647 | 0.503 | 102 | 99 | 93 | 12 | 1 | 3.40E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-OV-3 | 0.884 | 1.725 | 1.721 | 1.716 | 1.712 | 1.539 | 0.859 | 99 | 99 | 98 | 78 | -3 | 2.22E-5 | 9.21E-5 | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.631 | 2.037 | 2.000 | 1.890 | 1.943 | 0.382 | 0.222 | 97 | 90 | 93 | -40 | -65 | 2.12E-6 | 5.04E-6 | 2.59E-5 |
| A498 | 1.406 | 2.198 | 1.988 | 2.052 | 2.069 | 1.485 | 1.092 | 73 | 81 | 84 | 10 | -22 | 2.86E-6 | 2.03E-5 | > 1.00E-4 |
| ACHN | 0.367 | 1.551 | 1.575 | 1.531 | 1.522 | 0.114 | 0.209 | 102 | 98 | 98 | -69 | -43 | 1.93E-6 | 3.85E-6 | . |
| CAKI-1 | 0.913 | 3.266 | 3.239 | 3.141 | 3.110 | 0.400 | 0.517 | 99 | 95 | 93 | -56 | -43 | 1.95E-6 | 4.21E-6 | . |
| RXF 393 | 0.805 | 1.608 | 1.592 | 1.591 | 1.594 | 0.951 | 0.963 | 98 | 98 | 98 | 18 | 20 | 4.01E-6 | > 1.00E-4 | > 1.00E-4 |
| SN12C | 0.842 | 2.561 | 2.459 | 2.477 | 2.604 | 1.115 | 0.851 | 94 | 95 | 102 | 16 | . | 4.03E-6 | > 1.00E-4 | > 1.00E-4 |
| TK-10 | 0.908 | 1.530 | 1.450 | 1.456 | 1.405 | 1.074 | 0.850 | 87 | 88 | 80 | 27 | -6 | 3.64E-6 | 6.41E-5 | > 1.00E-4 |
| UO-31 | 1.156 | 2.983 | 2.900 | 2.899 | 2.620 | 0.602 | 0.809 | 95 | 95 | 80 | -48 | -30 | 1.72E-6 | 4.22E-6 | > 1.00E-4 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.919 | 2.339 | 2.231 | 2.203 | 2.198 | 1.089 | 0.882 | 92 | 90 | 90 | 11 | -4 | 3.19E-6 | 5.29E-5 | > 1.00E-4 |
| DU-145 | 0.446 | 1.771 | 1.854 | 1.814 | 1.770 | 0.957 | 0.558 | 106 | 103 | 100 | 39 | 8 | 6.50E-6 | > 1.00E-4 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.395 | 2.188 | 1.984 | 1.981 | 1.898 | 0.602 | 0.418 | 89 | 88 | 84 | 12 | 1 | 2.94E-6 | > 1.00E-4 | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.777 | 1.716 | 1.747 | 1.710 | 1.808 | 1.121 | 0.751 | 103 | 99 | 88 | 37 | -3 | 5.52E-6 | 8.25E-5 | > 1.00E-4 |
| HS 578T | 1.247 | 2.292 | 2.170 | 2.195 | 2.283 | 1.553 | 1.582 | 88 | 91 | 99 | 29 | 32 | 5.05E-6 | > 1.00E-4 | > 1.00E-4 |
| BT-549 | 1.081 | 2.229 | 2.249 | 2.089 | 2.197 | 1.386 | 0.950 | 102 | 88 | 97 | 27 | -12 | 4.66E-6 | 4.85E-5 | > 1.00E-4 |
| T-47D | 0.728 | 1.380 | 1.374 | 1.333 | 1.327 | 0.884 | 0.660 | 99 | 93 | 92 | 24 | -9 | 4.12E-6 | 5.21E-5 | > 1.00E-4 |
| MDA-MB-468 | 0.793 | 1.582 | 1.615 | 1.630 | 1.709 | 1.026 | 0.804 | 104 | 106 | 116 | 29 | 1 | 5.79E-6 | > 1.00E-4 | > 1.00E-4 |

**7-Hydroxy-13,14-dimethyl-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 350**



## 7-Hydroxy-13,14-dimethyl-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 350



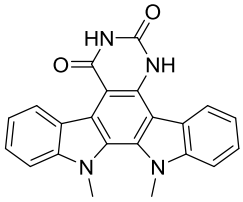

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

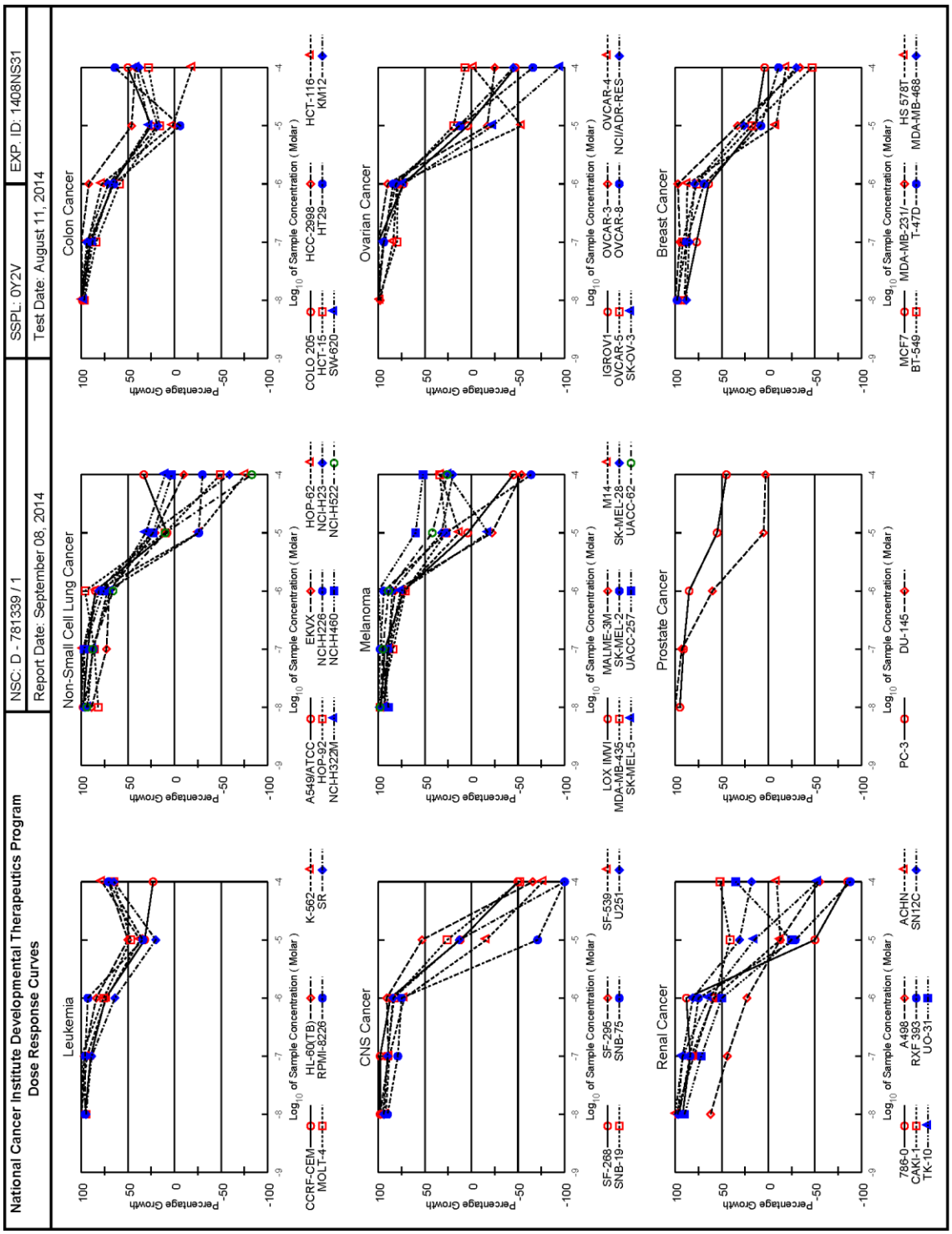
| | | | |
|---|---|---|---|
| NSC : D - 778804 / 1 | Experiment ID : 1402NS30 | Test Type : 08 | Units : Molar |
| Report Date : June 04, 2014 | Test Date : February 18, 2014 | QNS : | MC : |
| COMI : HW-6-466-3 (136284) | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

Log10 Concentration

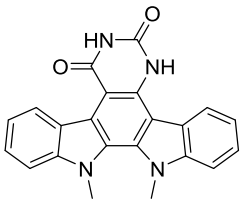
| Panel/Cell Line | Time Zero | Mean Optical Densities Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | Percent Growth -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.364 | 1.942 | 1.868 | 1.844 | 1.438 | 0.735 | 0.485 | 95 | 94 | 68 | 24 | 8 | 2.54E-6 | > 1.00E-4 | > 1.00E-4 |
| HL-60(TB) | 0.610 | 1.941 | 1.966 | 1.977 | 1.851 | 1.589 | 1.081 | 102 | 103 | 93 | 74 | 35 | 4.14E-5 | > 1.00E-4 | > 1.00E-4 |
| K-562 | 0.133 | 0.779 | 0.881 | 0.768 | 0.429 | 0.275 | 0.217 | 116 | 98 | 46 | 22 | 13 | 8.34E-7 | > 1.00E-4 | > 1.00E-4 |
| MOLT-4 | 0.893 | 2.531 | 2.774 | 2.751 | 2.265 | 1.010 | 0.921 | 115 | 113 | 84 | 7 | 2 | 2.76E-6 | > 1.00E-4 | > 1.00E-4 |
| RPMI-8226 | 0.676 | 2.086 | 2.061 | 2.012 | 0.988 | 0.483 | 0.428 | 98 | 95 | 22 | -29 | -37 | 4.13E-7 | 2.73E-6 | > 1.00E-4 |
| SR | 0.273 | 0.852 | 0.915 | 0.850 | 0.400 | 0.296 | 0.261 | 111 | 100 | 22 | 4 | -5 | 4.35E-7 | 2.92E-5 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.450 | 1.877 | 1.829 | 1.729 | 1.584 | 0.554 | 0.456 | 97 | 90 | 79 | 7 | . | 2.56E-6 | > 1.00E-4 | > 1.00E-4 |
| HOP-62 | 0.770 | 1.864 | 1.791 | 1.803 | 1.581 | 0.771 | 0.702 | 93 | 94 | 74 | . | -9 | 2.12E-6 | 1.02E-5 | > 1.00E-4 |
| HOP-92 | 1.093 | 1.647 | 1.571 | 1.505 | 1.441 | 1.051 | 1.026 | 86 | 74 | 63 | -4 | -6 | 1.56E-6 | 8.74E-6 | > 1.00E-4 |
| NCI-H226 | 0.854 | 1.595 | 1.474 | 1.460 | 1.352 | 1.216 | 1.160 | 84 | 82 | 67 | 49 | 41 | 8.57E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H23 | 0.556 | 1.698 | 1.714 | 1.629 | 1.069 | 0.490 | 0.405 | 101 | 94 | 45 | -12 | -27 | 7.87E-7 | 6.18E-6 | > 1.00E-4 |
| NCI-H322M | 0.797 | 2.011 | 1.973 | 1.997 | 1.848 | 1.533 | 1.074 | 97 | 99 | 87 | 61 | 23 | 1.91E-5 | > 1.00E-4 | > 1.00E-4 |
| NCI-H460 | 0.268 | 2.714 | 2.735 | 2.554 | 0.944 | 0.147 | 0.092 | 101 | 93 | 28 | -45 | -66 | 4.57E-7 | 2.39E-6 | 1.69E-5 |
| NCI-H522 | 0.919 | 1.883 | 1.738 | 1.819 | 1.388 | 1.042 | 0.864 | 85 | 93 | 49 | 13 | -6 | 9.34E-7 | 4.77E-5 | > 1.00E-4 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.507 | 1.538 | 1.474 | 1.413 | 1.329 | 1.203 | 1.012 | 94 | 88 | 80 | 67 | 49 | 8.83E-5 | > 1.00E-4 | > 1.00E-4 |
| HCC-2998 | 0.609 | 2.237 | 2.173 | 2.312 | 1.981 | 1.072 | 0.765 | 96 | 105 | 84 | 28 | 10 | 4.11E-6 | > 1.00E-4 | > 1.00E-4 |
| HCT-116 | 0.237 | 1.620 | 1.616 | 1.689 | 0.985 | 0.181 | 0.140 | 100 | 105 | 54 | -24 | -41 | 1.13E-6 | 4.96E-6 | > 1.00E-4 |
| HCT-15 | 0.265 | 1.748 | 1.682 | 1.632 | 0.761 | 0.267 | 0.226 | 96 | 92 | 33 | . | -15 | 5.23E-7 | 1.02E-5 | > 1.00E-4 |
| HT29 | 0.212 | 1.152 | 1.224 | 1.138 | 1.075 | 0.922 | 0.573 | 108 | 98 | 92 | 76 | 38 | 4.86E-5 | > 1.00E-4 | > 1.00E-4 |
| KM12 | 0.483 | 2.545 | 2.614 | 2.560 | 2.215 | 1.048 | 0.372 | 103 | 102 | 84 | 27 | -23 | 3.99E-6 | 3.50E-5 | > 1.00E-4 |
| SW-620 | 0.272 | 2.058 | 1.981 | 1.849 | 0.898 | 0.432 | 0.382 | 96 | 88 | 35 | 9 | 6 | 5.24E-7 | > 1.00E-4 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.543 | 1.951 | 1.924 | 1.975 | 1.300 | 0.764 | 0.659 | 98 | 102 | 54 | 16 | 8 | 1.26E-6 | > 1.00E-4 | > 1.00E-4 |
| SF-295 | 0.850 | 3.014 | 2.737 | 2.880 | 2.761 | 2.350 | 1.138 | 87 | 94 | 85 | 69 | 13 | 2.21E-5 | > 1.00E-4 | > 1.00E-4 |
| SF-539 | 0.924 | 2.619 | 2.424 | 2.567 | 2.078 | 1.085 | 0.984 | 88 | 97 | 66 | 9 | 4 | 2.03E-6 | > 1.00E-4 | > 1.00E-4 |
| SNB-19 | 0.543 | 2.008 | 2.005 | 1.897 | 1.432 | 0.735 | 0.673 | 100 | 92 | 61 | 13 | 9 | 1.68E-6 | > 1.00E-4 | > 1.00E-4 |
| SNB-75 | 0.696 | 1.316 | 1.171 | 1.217 | 0.882 | 0.709 | 0.698 | 77 | 84 | 30 | 2 | . | 4.26E-7 | > 1.00E-4 | > 1.00E-4 |
| U251 | 0.521 | 1.934 | 1.879 | 1.788 | 1.476 | 0.668 | 0.531 | 96 | 90 | 68 | 10 | 1 | 2.03E-6 | > 1.00E-4 | > 1.00E-4 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.229 | 1.758 | 1.685 | 1.716 | 0.868 | 0.197 | 0.109 | 95 | 97 | 42 | -14 | -52 | 7.10E-7 | 5.58E-6 | 8.65E-5 |
| MALME-3M | 0.571 | 1.191 | 1.185 | 1.199 | 1.101 | 0.763 | 0.580 | 99 | 101 | 85 | 31 | 1 | 4.47E-6 | > 1.00E-4 | > 1.00E-4 |
| M14 | 0.441 | 1.346 | 1.259 | 1.276 | 0.910 | 0.526 | 0.425 | 90 | 92 | 52 | 9 | -4 | 1.10E-6 | 5.19E-5 | > 1.00E-4 |
| MDA-MB-435 | 0.452 | 2.134 | 1.967 | 1.986 | 1.442 | 0.583 | 0.475 | 90 | 91 | 59 | 8 | 1 | 1.49E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-2 | 1.184 | 2.427 | 2.403 | 2.358 | 2.324 | 2.000 | 1.023 | 98 | 94 | 92 | 66 | -14 | 1.58E-5 | 6.73E-5 | > 1.00E-4 |
| SK-MEL-28 | 0.605 | 1.818 | 1.758 | 1.799 | 1.635 | 0.875 | 0.709 | 95 | 98 | 85 | 22 | 9 | 3.61E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-5 | 0.842 | 2.957 | 2.765 | 2.796 | 2.346 | 0.544 | 0.154 | 91 | 92 | 71 | -35 | -82 | 1.58E-6 | 4.65E-6 | 2.06E-5 |
| UACC-257 | 1.119 | 2.241 | 2.139 | 2.125 | 2.133 | 1.913 | 1.307 | 91 | 90 | 90 | 71 | 17 | 2.42E-5 | > 1.00E-4 | > 1.00E-4 |
| UACC-62 | 0.838 | 2.687 | 2.669 | 2.576 | 2.412 | 1.347 | 0.750 | 99 | 94 | 85 | 28 | -11 | 4.07E-6 | 5.28E-5 | > 1.00E-4 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.641 | 2.195 | 2.301 | 2.242 | 2.008 | 0.815 | 0.766 | 107 | 103 | 88 | 11 | 8 | 3.12E-6 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-3 | 0.459 | 1.507 | 1.225 | 1.193 | 1.128 | 0.701 | 0.427 | 73 | 70 | 64 | 23 | -7 | 2.18E-6 | 5.82E-5 | > 1.00E-4 |
| OVCAR-4 | 0.536 | 1.049 | 0.975 | 1.010 | 0.860 | 0.614 | 0.531 | 86 | 92 | 63 | 15 | -1 | 1.88E-6 | 8.64E-5 | > 1.00E-4 |
| OVCAR-5 | 0.647 | 1.751 | 1.655 | 1.697 | 1.574 | 1.482 | 1.118 | 91 | 95 | 84 | 76 | 43 | 5.97E-5 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-8 | 0.612 | 2.278 | 2.230 | 2.204 | 1.728 | 0.713 | 0.622 | 97 | 96 | 67 | 6 | 1 | 1.90E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI/ADR-RES | 0.643 | 2.079 | 2.114 | 2.074 | 1.702 | 0.756 | 0.622 | 102 | 100 | 74 | 8 | -3 | 2.29E-6 | 5.09E-5 | > 1.00E-4 |
| SK-OV-3 | 0.642 | 1.132 | 1.196 | 1.181 | 1.031 | 0.687 | 0.607 | 113 | 110 | 79 | 9 | -5 | 2.62E-6 | 4.24E-5 | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.602 | 1.985 | 1.827 | 1.827 | 1.492 | 0.560 | 0.467 | 89 | 89 | 64 | -7 | -22 | 1.59E-6 | 7.98E-6 | > 1.00E-4 |
| A498 | 1.389 | 2.317 | 2.251 | 2.091 | 1.849 | 1.784 | 1.687 | 93 | 76 | 50 | 43 | 32 | 9.65E-7 | > 1.00E-4 | > 1.00E-4 |
| ACHN | 0.502 | 1.806 | 1.689 | 1.706 | 0.847 | 0.472 | 0.468 | 91 | 92 | 26 | -6 | -7 | 4.39E-7 | 6.54E-6 | > 1.00E-4 |
| CAKI-1 | 0.609 | 1.626 | 1.416 | 1.416 | 1.094 | 0.521 | 0.431 | 79 | 79 | 48 | -14 | -29 | 8.46E-7 | 5.85E-6 | > 1.00E-4 |
| RXF 393 | 0.786 | 1.337 | 1.287 | 1.267 | 1.060 | 0.815 | 0.713 | 91 | 87 | 50 | 5 | -9 | 9.78E-7 | 2.30E-5 | > 1.00E-4 |
| SN12C | 0.861 | 2.572 | 2.672 | 2.493 | 2.194 | 0.994 | 0.820 | 108 | 95 | 78 | 8 | -5 | 2.50E-6 | 4.17E-5 | > 1.00E-4 |
| TK-10 | 0.870 | 1.900 | 1.903 | 1.863 | 1.810 | 1.215 | 0.913 | 100 | 96 | 91 | 34 | 4 | 5.18E-6 | > 1.00E-4 | > 1.00E-4 |
| UO-31 | 0.884 | 2.777 | 2.717 | 2.645 | 1.922 | 1.095 | 0.959 | 97 | 93 | 55 | 11 | 4 | 1.29E-6 | > 1.00E-4 | > 1.00E-4 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.585 | 2.097 | 2.011 | 1.971 | 1.745 | 1.304 | 0.663 | 94 | 92 | 77 | 48 | 6 | 8.67E-6 | > 1.00E-4 | > 1.00E-4 |
| DU-145 | 0.409 | 1.804 | 1.856 | 1.852 | 1.315 | 0.537 | 0.484 | 104 | 103 | 65 | 9 | 5 | 1.85E-6 | > 1.00E-4 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.429 | 2.338 | 2.050 | 1.984 | 1.238 | 0.717 | 0.511 | 85 | 81 | 42 | 15 | 4 | 6.38E-7 | > 1.00E-4 | > 1.00E-4 |
| HS 578T | 0.885 | 1.876 | 1.872 | 1.813 | 1.595 | 1.063 | 0.939 | 100 | 94 | 72 | 18 | 5 | 2.53E-6 | > 1.00E-4 | > 1.00E-4 |
| BT-549 | 0.934 | 1.824 | 1.653 | 1.659 | 1.419 | 1.034 | 0.777 | 81 | 81 | 54 | 11 | -17 | 1.27E-6 | 2.50E-5 | > 1.00E-4 |
| MDA-MB-468 | 0.773 | 1.654 | 1.583 | 1.543 | 1.463 | 1.037 | 0.700 | 92 | 87 | 78 | 30 | -9 | 3.85E-6 | 5.76E-5 | > 1.00E-4 |

**13,14-Dimethyl-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 352**



**13,14-Dimethyl-13,14-dihydroindolo[2,3-*a*]pyrimido[4,5-*c*]carbazole-6,8(5*H*,7*H*)-dione 352**



National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

| NSC : D - 781339 / 1 | Experiment ID : 1408NS31 | Test Type : 08 | Units : Molar |
|---|---|---|---|
| Report Date : September 08, 2014 | Test Date : August 11, 2014 | QNS : | MC : |
| COMI : HW6-534-1 | Stain Reagent : SRB Dual-Pass Related | SSPL : 0Y2V | |

Log10 Concentration

| Panel/Cell Line | Time Zero | Mean Optical Densities Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | Percent Growth -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.532 | 2.428 | 2.360 | 2.229 | 1.933 | 1.141 | 0.965 | 96 | 90 | 74 | 32 | 23 | 3.73E-6 | > 1.00E-4 | > 1.00E-4 |
| HL-60(TB) | 0.953 | 2.898 | 2.976 | 2.843 | 2.592 | 1.932 | 2.337 | 104 | 97 | 84 | 50 | 71 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| K-562 | 0.270 | 2.063 | 2.110 | 1.964 | 1.587 | 0.940 | 1.662 | 103 | 94 | 73 | 37 | 78 | . | > 1.00E-4 | > 1.00E-4 |
| MOLT-4 | 0.789 | 2.739 | 2.651 | 2.772 | 2.263 | 1.703 | 2.086 | 95 | 102 | 76 | 47 | 65 | . | > 1.00E-4 | > 1.00E-4 |
| RPMI-8226 | 0.955 | 2.654 | 2.578 | 2.578 | 2.542 | 1.540 | 2.167 | 96 | 96 | 93 | 34 | 71 | . | > 1.00E-4 | > 1.00E-4 |
| SR | 0.632 | 2.633 | 2.525 | 2.418 | 1.907 | 1.035 | 1.931 | 95 | 89 | 64 | 20 | 65 | . | > 1.00E-4 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.482 | 2.378 | 2.339 | 2.249 | 2.093 | 0.842 | 1.108 | 98 | 93 | 85 | 8 | 33 | 2.86E-6 | > 1.00E-4 | > 1.00E-4 |
| EKVX | 0.786 | 1.711 | 1.607 | 1.459 | 1.435 | 0.976 | 0.708 | 89 | 73 | 70 | 21 | -10 | 2.54E-6 | 4.72E-5 | > 1.00E-4 |
| HOP-62 | 0.578 | 1.975 | 1.994 | 1.946 | 1.747 | 0.430 | 0.141 | 101 | 98 | 84 | -26 | -76 | 2.03E-6 | 5.83E-6 | 3.07E-5 |
| HOP-92 | 1.431 | 1.889 | 1.805 | 1.827 | 1.870 | 1.475 | 0.733 | 82 | 86 | 96 | 10 | -49 | 3.40E-6 | 1.46E-5 | > 1.00E-4 |
| NCI-H226 | 0.545 | 1.651 | 1.624 | 1.503 | 1.337 | 0.401 | 0.379 | 97 | 87 | 72 | -26 | -30 | 1.66E-6 | 5.37E-6 | > 1.00E-4 |
| NCI-H23 | 0.675 | 2.413 | 2.344 | 2.367 | 2.077 | 1.090 | 0.275 | 96 | 97 | 81 | 24 | -59 | 3.47E-6 | 1.94E-5 | 7.72E-5 |
| NCI-H322M | 1.153 | 2.694 | 2.588 | 2.504 | 2.212 | 1.628 | 1.306 | 93 | 88 | 69 | 31 | 10 | 3.11E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H460 | 0.417 | 3.152 | 3.138 | 3.074 | 2.493 | 1.047 | 0.508 | 100 | 97 | 76 | 23 | 3 | 3.09E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H522 | 1.063 | 2.697 | 2.601 | 2.494 | 2.133 | 1.225 | 0.183 | 94 | 88 | 66 | 10 | -83 | 1.90E-6 | 1.28E-5 | 4.43E-5 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.434 | 1.740 | 1.730 | 1.616 | 1.291 | 0.786 | 1.085 | 99 | 90 | 66 | 25 | 50 | 2.44E-6 | > 1.00E-4 | > 1.00E-4 |
| HCC-2998 | 0.694 | 2.022 | 1.986 | 2.067 | 1.910 | 1.308 | 1.250 | 98 | 103 | 92 | 46 | 42 | 8.25E-6 | > 1.00E-4 | > 1.00E-4 |
| HCT-116 | 0.311 | 2.229 | 2.210 | 2.101 | 1.816 | 0.357 | 0.252 | 99 | 93 | 78 | 2 | -19 | 2.37E-6 | 1.29E-5 | > 1.00E-4 |
| HCT-15 | 0.378 | 2.649 | 2.591 | 2.264 | 1.719 | 0.732 | 1.015 | 97 | 84 | 59 | 16 | 28 | 1.62E-6 | > 1.00E-4 | > 1.00E-4 |
| HT29 | 0.303 | 1.807 | 1.822 | 1.680 | 1.287 | 0.284 | 1.269 | 101 | 92 | 65 | -6 | 64 | . | . | > 1.00E-4 |
| KM12 | 0.609 | 2.945 | 2.944 | 2.803 | 2.272 | 1.033 | 1.495 | 100 | 94 | 71 | 18 | 38 | 2.51E-6 | > 1.00E-4 | > 1.00E-4 |
| SW-620 | 0.358 | 2.243 | 2.179 | 2.018 | 1.618 | 0.874 | 1.125 | 97 | 88 | 67 | 27 | 41 | 2.67E-6 | > 1.00E-4 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.741 | 2.150 | 2.124 | 2.115 | 1.970 | 0.911 | 0.371 | 98 | 98 | 87 | 12 | -50 | 3.12E-6 | 1.56E-5 | > 1.00E-4 |
| SF-295 | 0.977 | 2.958 | 2.915 | 2.745 | 2.788 | 2.019 | 0.332 | 98 | 89 | 91 | 53 | -66 | 1.05E-5 | 2.78E-5 | 7.32E-5 |
| SF-539 | 0.914 | 2.437 | 2.318 | 2.250 | 2.010 | 0.770 | 0.212 | 92 | 88 | 72 | -16 | -77 | 1.78E-6 | 6.61E-6 | 3.63E-5 |
| SNB-19 | 0.847 | 2.467 | 2.386 | 2.311 | 2.199 | 1.264 | 0.406 | 95 | 90 | 83 | 26 | -52 | 3.80E-6 | 2.14E-5 | 9.41E-5 |
| SNB-75 | 0.784 | 1.568 | 1.492 | 1.400 | 1.374 | 0.228 | -0.024 | 90 | 79 | 75 | -71 | -100 | 1.49E-6 | 3.27E-6 | 7.19E-6 |
| U251 | 0.492 | 2.586 | 2.467 | 2.373 | 2.257 | 0.759 | -0.053 | 94 | 90 | 84 | 13 | -100 | 3.02E-6 | 1.30E-5 | 3.60E-5 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.406 | 2.322 | 2.267 | 2.189 | 1.800 | 0.474 | 0.225 | 97 | 93 | 73 | 4 | -45 | 2.13E-6 | 1.19E-5 | > 1.00E-4 |
| MALME-3M | 0.674 | 1.177 | 1.146 | 1.125 | 1.087 | 0.525 | 0.307 | 94 | 90 | 82 | -22 | -54 | 2.03E-6 | 6.14E-6 | 7.28E-5 |
| M14 | 0.396 | 1.585 | 1.513 | 1.433 | 1.267 | 0.552 | 0.789 | 94 | 87 | 73 | 13 | 33 | 2.43E-6 | > 1.00E-4 | > 1.00E-4 |
| MDA-MB-435 | 0.594 | 2.662 | 2.603 | 2.332 | 2.059 | 1.160 | 1.287 | 98 | 84 | 71 | 27 | 34 | 3.05E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-2 | 0.944 | 1.888 | 1.921 | 1.868 | 1.833 | 1.195 | 0.345 | 104 | 98 | 94 | 27 | -64 | 4.50E-6 | 1.97E-5 | 7.08E-5 |
| SK-MEL-28 | 0.736 | 2.256 | 2.244 | 2.091 | 2.023 | 1.216 | 1.036 | 99 | 89 | 85 | 32 | 20 | 4.50E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-5 | 0.728 | 2.817 | 2.678 | 2.579 | 2.307 | 0.599 | 1.245 | 93 | 89 | 76 | -18 | 25 | 1.88E-6 | . | > 1.00E-4 |
| UACC-257 | 1.091 | 2.436 | 2.293 | 2.381 | 2.473 | 1.901 | 1.795 | 89 | 96 | 103 | 60 | 52 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| UACC-62 | 1.034 | 2.906 | 2.867 | 2.813 | 2.700 | 1.812 | 1.524 | 98 | 95 | 89 | 42 | 26 | 6.64E-6 | > 1.00E-4 | > 1.00E-4 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.630 | 2.222 | 2.377 | 2.265 | 1.789 | 0.694 | 0.336 | 110 | 103 | 73 | 4 | -47 | 2.14E-6 | 1.20E-5 | > 1.00E-4 |
| OVCAR-3 | 0.486 | 1.674 | 1.647 | 1.675 | 1.559 | 0.406 | 0.366 | 98 | 100 | 90 | -17 | -25 | 2.38E-6 | 7.00E-6 | > 1.00E-4 |
| OVCAR-4 | 0.688 | 1.502 | 1.483 | 1.374 | 1.338 | 0.319 | 0.675 | 98 | 84 | 80 | -54 | -2 | 1.67E-6 | 3.97E-6 | . |
| OVCAR-5 | 0.693 | 1.882 | 1.682 | 1.489 | 1.470 | 0.878 | 0.764 | 100 | 80 | 79 | 19 | 7 | 3.00E-6 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-8 | 0.588 | 2.524 | 2.554 | 2.413 | 2.214 | 0.811 | 0.202 | 102 | 94 | 84 | 12 | -66 | 2.94E-6 | 1.41E-5 | 6.26E-5 |
| NCI/ADR-RES | 0.663 | 2.257 | 2.266 | 2.184 | 1.959 | 0.877 | 0.365 | 101 | 95 | 81 | 13 | -45 | 2.89E-6 | 1.70E-5 | > 1.00E-4 |
| SK-OV-3 | 0.635 | 1.645 | 1.665 | 1.588 | 1.376 | 0.490 | 0.032 | 102 | 94 | 73 | -23 | -95 | 1.75E-6 | 5.79E-6 | 2.38E-5 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.700 | 2.316 | 2.183 | 2.060 | 2.115 | 0.352 | 0.104 | 92 | 84 | 88 | -50 | -85 | 1.88E-6 | 4.34E-6 | 1.01E-5 |
| A498 | 1.366 | 1.946 | 1.726 | 1.623 | 1.497 | 1.193 | 0.617 | 62 | 44 | 23 | -13 | -55 | 4.75E-6 | 4.37E-6 | 7.68E-5 |
| ACHN | 0.508 | 2.198 | 2.175 | 1.856 | 1.505 | 0.443 | 0.462 | 99 | 80 | 59 | -13 | -9 | 1.33E-6 | 6.62E-6 | > 1.00E-4 |
| CAKI-1 | 1.025 | 3.143 | 3.060 | 2.785 | 2.249 | 1.894 | 2.122 | 96 | 83 | 58 | 41 | 52 | . | > 1.00E-4 | > 1.00E-4 |
| RXF 393 | 0.755 | 1.328 | 1.307 | 1.234 | 1.186 | 0.535 | 0.093 | 96 | 84 | 75 | -29 | -88 | 1.74E-6 | 5.25E-6 | 2.27E-5 |
| SN12C | 0.743 | 2.822 | 2.751 | 2.665 | 2.422 | 1.397 | 1.112 | 97 | 92 | 81 | 31 | 18 | 4.21E-6 | > 1.00E-4 | > 1.00E-4 |
| TK-10 | 1.103 | 1.934 | 1.943 | 1.870 | 1.634 | 1.226 | 0.514 | 101 | 92 | 64 | 15 | -53 | 1.91E-6 | 1.65E-5 | 8.90E-5 |
| UO-31 | 0.963 | 2.513 | 2.355 | 2.086 | 1.735 | 0.723 | 1.506 | 90 | 72 | 50 | -25 | 35 | 9.81E-7 | . | > 1.00E-4 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.765 | 2.536 | 2.451 | 2.377 | 2.265 | 1.738 | 1.556 | 95 | 91 | 85 | 55 | 45 | 3.01E-5 | > 1.00E-4 | > 1.00E-4 |
| DU-145 | 0.398 | 1.673 | 1.690 | 1.584 | 1.160 | 0.466 | 0.434 | 101 | 93 | 60 | 5 | 3 | 1.51E-6 | > 1.00E-4 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.371 | 2.212 | 2.021 | 1.780 | 1.548 | 0.629 | 0.450 | 90 | 77 | 64 | 14 | 4 | 1.90E-6 | > 1.00E-4 | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.689 | 1.671 | 1.694 | 1.624 | 1.638 | 1.014 | 0.458 | 102 | 95 | 97 | 33 | -34 | 5.41E-6 | 3.14E-5 | > 1.00E-4 |
| HS 578T | 1.018 | 2.005 | 1.907 | 1.886 | 1.874 | 0.936 | 0.821 | 90 | 88 | 87 | -8 | -19 | 2.44E-6 | 8.21E-6 | > 1.00E-4 |
| BT-549 | 1.310 | 2.495 | 2.454 | 2.395 | 2.218 | 1.520 | 0.691 | 97 | 92 | 77 | 18 | -47 | 2.83E-6 | 1.87E-5 | > 1.00E-4 |
| T-47D | 0.669 | 1.423 | 1.405 | 1.334 | 1.190 | 0.730 | 0.599 | 98 | 88 | 69 | 8 | -11 | 2.05E-6 | 2.71E-5 | > 1.00E-4 |
| MDA-MB-468 | 0.923 | 1.838 | 1.725 | 1.703 | 1.643 | 1.162 | 0.646 | 88 | 85 | 79 | 26 | -30 | 3.51E-6 | 2.91E-5 | > 1.00E-4 |