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Synthesis and Evaluation of Novel Quinolines and Quinazolinediones as Potential Anti-Cancer Agents

Kieran Greaney, B.Sc.

Thesis presented for the degree of Doctor of Philosophy to National University of Ireland, Cork.

Department of Chemistry

Supervisor: Dr. Florence McCarthy

Head of Department: Prof. Martyn Pemble

May 2014
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To express my thanks through words seems such an injustice but I’ll give it my best shot. First and foremost I would like to express my deepest gratitude to Dr. Florence McCarthy whose guidance and support during this project has been nothing short of immense, and for having me as part of such a great research group.

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To my many mates, both at home and in college who made this journey so much easier with all the brews, biscuits (which I mostly got stung for!!), beer and banter - it’s been real! However Conor it will never be ok to leave the teabag in the cup while you’re drinking it.

Most of all I would like to thank my immediate and extended family for their constant love and support throughout this project. Mum and Dad I constantly fail to find the words to express my love for you both, thank you so very much for everything!
Declaration

I hereby confirm that the body of work described within this thesis for the degree of Doctor of Philosophy, is my own research work, and has not been submitted for any other degree, either at University College Cork or elsewhere.

_________________________________________              Date: ________________
For Mum and Dad
Abstract

This thesis outlines the design and effectuation of novel chemical routes towards a nascent class of functionalised quinoline-5,8-diones and the expansion of a series of contemporary quinazolinediones towards an innovative family of pyridinoquinazolinetetrone derivatives. This fragment based approach is envisaged to lead to advancements in the three scaffolds, expanding the SAR pool of both quinolines and quinazolinediones with subsequent evaluation of chemotherapeutic potential as well as furnishing a new class of tricycle for biological investigation.

Development of novel quinoline-5,8-diones is provided for by expanding on existing methodology. By using a variety of selected nucleophiles on a critical intermediate, a broad range of novel compounds was afforded which serve as molecular probes into the chemotherapeutic potency of this class of compounds, while also serving as integral intermediates for accomplishing novel pyridinoquinazolinetetrone congeners using contemporary cyclisation methodology.

In order to incorporate functionality into our quinazolinedione template, an efficient synthetic strategy was constructed which provided a robust route to effectuate a highly derivatised pyrimidinedione ring from simple starting materials. As derivatisation of this template is unreported our chief priority was to synthesise a range of diverse quinazolinediones. The application of annulation methodology using functionalised precursors provided a library of N-3 derivatised quinazolinedione analogues. These, along with their N-1 functionalised derivatives provide a wide scope from which to construct a series of pyridinoquinazolinetetrone derivatives while also serving as a unique class of molecules whose biological potential is uncharted.

Although the actualisation of the pyridinoquinazolinetetrone was ultimately unsuccessful, our work has led to the development of novel quinoline-5,8-diones which were found to possess excellent anti-cancer activity when assessed by the NCI screen. Preliminary results indicated appreciable cytotoxicity across several tumour types.
Of the quinazolinediones synthesised eight compounds were accepted for screening by the NCI. Results from the single-dose tests however indicated that these compounds possessed little cytotoxic activity at 10 µM. The development of this novel template in conjunction with the highly active quinolinediones serves as an excellent rostrum for future synthetic endeavours.
**Abbreviations**

Anhyd.  Anhydrous  
ATP  Adenosine triphosphate  
s  Singlet  
bs  Broad singlet  
CAK  CdK-activating kinase  
Cdc  Cell-division cycle  
CDCl₃  Deuterated chloroform  
CdK  Cyclin-dependant kinase  
d  Doublet  
DCM  Dichloromethane  
DBU  1,8-Diazabicyclo[5.4.0]undec-7-ene  
dd  Doublet of doublets  
ddt  Doublet of doublet of triplets  
dt  Doublet of triplets  
DEPT  Distortionless enhancement of polarisation transfer  
DMF  N,N-Dimethylformamide  
DMF-d₇  Deuterated N,N-Dimethylformamide  
DMSO-d₆  Deuterated dimethyl sulfoxide  
DNA  Deoxyribonucleic acid  
DPP-4  Dipeptidyl peptidase-4  
DSP  Dual specificity phosphatase
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GGPP</td>
<td>Geranylgeranyldiphosphate</td>
</tr>
<tr>
<td>GI₅₀</td>
<td>Concentration at which growth is inhibited to 50%</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>50% Inhibition concentration</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
</tr>
<tr>
<td>LC₅₀</td>
<td>Concentration required for 50% cell death</td>
</tr>
<tr>
<td>lit.</td>
<td>Literature</td>
</tr>
<tr>
<td>LNCaP</td>
<td>Androgen-sensitive human prostate adenocarcinoma cell-line</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>MiaPaCa2</td>
<td>Human pancreatic carcinoma cell-line</td>
</tr>
<tr>
<td>MIC₉₀</td>
<td>Minimum concentration which inhibits 90% of organisms</td>
</tr>
<tr>
<td>m.p.</td>
<td>Melting point</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
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<tr>
<td>Symbol</td>
<td>Definition</td>
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<td>------------</td>
</tr>
<tr>
<td>µM</td>
<td>Micromolar</td>
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<tr>
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<tr>
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<tr>
<td>m</td>
<td>Multiplet</td>
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<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
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<td>Nanomolar</td>
</tr>
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<td>NMP</td>
<td>N-Methyl-2-pyrrolidone</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>PDE3</td>
<td>Phosphodiesterase type 3</td>
</tr>
<tr>
<td>PDE4</td>
<td>Phosphodiesterase type 4</td>
</tr>
<tr>
<td>PGGTase</td>
<td>Protein geranylgeranyl transferase</td>
</tr>
<tr>
<td>p-TsOH</td>
<td>para-Toluenesulfonic acid</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>POCl₃</td>
<td>Phosphorous oxychloride</td>
</tr>
<tr>
<td>q</td>
<td>Quartet</td>
</tr>
<tr>
<td>qt</td>
<td>Quintet</td>
</tr>
<tr>
<td>r.t.</td>
<td>Room temperature</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure-activity relationship</td>
</tr>
<tr>
<td>sep</td>
<td>Septet</td>
</tr>
<tr>
<td>st</td>
<td>Sextet</td>
</tr>
<tr>
<td>1,1,3,3-TMP</td>
<td>1,1,3,3-Tetramethoxypropane</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TGI</td>
<td>Total growth inhibition</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>Thr</td>
<td>Threonine</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>td</td>
<td>Triplet of doublets</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilane</td>
</tr>
<tr>
<td>Tyr</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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</table>
1.0 Biological Introduction
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1.3 Perspectives ............................................................... 36
1.0 Biological introduction

The following chapter is divided into two sections based on the parent bicyclic fragment which pyridinoquinazolinetetrone 1 is derived from. Each section opens by briefly outlining the applications each pharmacophore has in medicinal chemistry followed by a detailed description of the biological profile of the scaffolds most relevant to this work.

Fig 1.1.1 Illustrates both bicycles from which 1 is inferred. Highlighted in orange is the quinoline pharmacophore (Section 1.1) and the quinazoline fragment is highlighted in blue (Section 1.2).

1.1 Quinolines

The quinoline 2 scaffold which is comprised of a benzene ring fused with pyridine at two adjacent carbon atoms forms the foundation of a vast array of diverse compounds with extensive pharmacological properties. The quinoline structure is perhaps most notably associated with anti-malarial drugs arising from the isolation of the natural alkaloid quinine. Until the 1940’s it was the drug of choice for treatment of malaria until it was superseded by chloroquine, another quinoline, which possessed a more favourable pharmacological profile.¹
Due to much work being carried out on effective syntheses of quinoline compounds as well as diversification, a range of quinoline type compounds now possess among others, anti-tubercular, anti-hypertensive and anti-Alzheimer activity. Quinolines have also been shown to exhibit anti-cancer activity, highlighted in a recent review by Solomon et al. One subclass in the vast pedigree of quinolines which have received attention as antineoplastics in the last ten to fifteen years are quinoline-5,8-diones due to their ability to inhibit Cdc25 phosphatase, a key regulator of the eukaryotic cell cycle, with some derivatives exhibiting nanomolar activity in in vitro assays.

The following section outlines the role that quinoline-5,8-diones have in generating effective chemotherapeutic agents against Cdc25 phosphatase which has been shown to be overexpressed in a multitude of cancers, Table 1.1.1 (page 18). This focus stems from the direct relationship of 3 with the quinoline fragment of 1, Fig 1.1.1 (page 14).
1.1.1 Introduction to regulation of the cell cycle

Common to all cancers is a disordered cell cycle and irregularities such as overexpression, deletion or mutations in the molecules which govern the cell cycle. A family of proteins known collectively as cell division cycle 25 (Cdc25) proteins are highly conserved dual specificity (acting on tyrosine or serine/threonine residues) phosphatases (DSP) which activate cyclin-dependant kinase (CdK) complexes, by dephosphorylating the Thr 14 and Tyr 15 residues of CdK. A consequent phosphorylation of Thr 161 by CdK-activating kinase (CAK) results in complete activation leading to cell-cycle progression Fig 1.1.5 (page 19). Cdc25 phosphatases also play a role in checkpoint pathways, (e.g. G1/S or G2/M) which are activated as a result of DNA damage. When DNA damage occurs the cell responds by activating a relevant checkpoint mechanism, resulting in cell-cycle arrest which either leads to repair of the damaged DNA or apoptosis. Overexpression of Cdc25 is thought to lead to a loss of cell cycle checkpoint control, uncontrolled cell proliferation and a loss of genome integrity. From this it is easy to see that Cdc25 phosphatases make ideal targets for cancer therapy.
Fig. 1.1.4 Illustrates the four stages of the cell cycle. Thr 14 is represented as the yellow P and Tyr 15 is represented by the grey P. During the gap 1 (G1) phase (blue) cells increase in mass and synthesise mRNA and proteins for DNA synthesis. Synthesis (S) phase (brown) is where DNA synthesis occurs. Following completion of DNA replication the cell enters the gap 2 (G2) phase (grey) where cells continue to grow and synthesise proteins necessary for mitosis. The mitosis (M) phase, (yellow) involves cells duplicating into two identical cells.

1.1.2 Cdc25 in cell-cycle control

In the human genome three Cdc25 genes have been identified. These three isoforms are Cdc25A, Cdc25B and Cdc25C. Each gene can produce alternative splicing variants which generate two Cdc25A variants and five variants each for Cdc25B and Cdc25C. Of the three isoforms it is the overexpression of Cdc25A and Cdc25B which are linked to a variety of human malignancies in the majority of cases. Table 1.1.1 illustrates the percentage of cancers which show the overexpression of Cdc25 proteins.
<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Cdc25 A %</th>
<th>Cdc25 B %</th>
<th>Cdc25 C %</th>
</tr>
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<tbody>
<tr>
<td>Thyroid</td>
<td>17-69</td>
<td>36-64</td>
<td>ND</td>
</tr>
<tr>
<td>Breast</td>
<td>70</td>
<td>57</td>
<td>ND</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>56</td>
<td>20</td>
<td>ND</td>
</tr>
<tr>
<td>Ovarian</td>
<td>30</td>
<td>30</td>
<td>ND</td>
</tr>
<tr>
<td>Colorectal</td>
<td>47-53</td>
<td>43-67</td>
<td>27</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>50</td>
<td>65</td>
<td>15</td>
</tr>
<tr>
<td>Gliomas</td>
<td>ND</td>
<td>47</td>
<td>ND</td>
</tr>
<tr>
<td>Laryngeal</td>
<td>41</td>
<td>57</td>
<td>ND</td>
</tr>
<tr>
<td>Oesophageal</td>
<td>46-66</td>
<td>48-79</td>
<td>ND</td>
</tr>
<tr>
<td>Gastric</td>
<td>ND</td>
<td>78</td>
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<tr>
<td>Endometrial</td>
<td>ND</td>
<td>73</td>
<td>13</td>
</tr>
<tr>
<td>Prostate</td>
<td>ND</td>
<td>30</td>
<td>ND</td>
</tr>
</tbody>
</table>

Table 1.1.1 Percentage of tumours which exhibit overexpression of Cdc25A, Cdc25B or Cdc25C proteins, ND-not determined. 9.

Cdc25 proteins are responsible for activating CdKs which are a family of highly conserved serine/threonine protein kinases associated with regulatory cyclin subunits. CdKs are held in an inactive state by WEE1 and MYT1 kinases which phosphorylate the Thr14 and Tyr15 residues of CdK1, located within the ATP binding loop of CdK. 16 When CdK activity is necessary for the progression of the cell-cycle to the next phase the dual specificity phosphatases (Cdc25s) dephosphorylate both residues thereby activating the CdK-cyclin complex, Fig 1.1.5. CAK then phosphorylates Thr 161 yielding the fully active complex. 8
In mammalian cells the three isoforms of Cdc25 are implicated in cell cycle regulation as a result of their ability to dephosphorylate CdK1 and CdK2. Although initially it was thought that there was a specific role for each Cdc25 phosphatase at defined stages of the cell cycle in reality it is emerging that all the Cdc25 isoforms are involved in phosphorylating CdK-cyclin complexes Fig 1.1.4 (page 17).

**1.1.3 Structure and catalytic mechanism of Cdc25 phosphatase**

Cdc25 phosphatases are between 470 and 566 residues long and consist of two primary domains, an N-terminus and a C-terminus. The N-terminus contains a regulatory domain which modulates the activity of the enzyme. Contained within the C-terminus is the catalytic domain which is highly conserved among the three Cdc25 proteins. Within the catalytic domain is a phosphate binding loop (P-loop) also known as a HCX₅R motif where H is a highly conserved histidine residue, C is the catalytic cysteine, X₅ are the five residues which create the loop in which all amide nitrogens bond to the phosphate of the substrate and R is a highly conserved arginine which hydrogen bonds to the phosphorylated amino acid of the substrate, Fig 1.1.6.
This motif is common to all tyrosine phosphatases. The structures of Cdc25A and Cdc25B have been solved by X-ray crystallography and showed similar catalytic domains.\textsuperscript{19,20} A key difference between these two isoforms is the relatively shallow active site of Cdc25A compared to Cdc25B whose active site is similar to other DSPs. The Cdc25A catalytic domain also contains no flexible peptide loops proximal to the active site that might facilitate substrate binding.

Cdc25B contains a flat active site within a shallow pocket. Adjacent to the active site is a cavity known as the “swimming pool” due to the abundance of well-ordered water molecules contained within the pocket.\textsuperscript{21} The mechanism of catalysis occurs in two distinct steps.\textsuperscript{22} Firstly the catalytic cysteine acts as a nucleophile to the phosphate ester substrate, generating a thiophosphorylated intermediate. A proton transfer to the leaving group also occurs in this step however the origin of this proton is debatable. In other DSP proteins an Asp residue located in a mobile loop distal to the active site acts as the proton source (Cdc25s lack this catalytic site residue). The final step involves hydrolysis of the thiophosphorylated intermediate to regenerate the free enzyme as a free CdK-cyclin

\textit{Fig 1.1.6 Catalytic site P-loop bound to a tungstate anion}\textsuperscript{19}
complex which promotes cell cycle progression, Fig. 1.1.7. From this it is obvious to see that regulating this transfer would lead to control of the cell cycle.

**Fig. 1.1.7 Catalytic cycle of Cdc25B**

1.1.4 Inhibition of Cdc25 in anti-cancer therapy

The main families of compounds which have been identified to be potent Cdc25 inhibitors include quinoline-5,8-diones, phosphomimetics and electrophilic entities. Quinoline-5,8-dione compounds which are congeners of vitamin K are some of the most numerous and active compounds **Fig. 1.1.8**.

**Fig. 1.1.8 Illustrates the similarity between the vitamin K₁ and quinoline-5,8-dione pharmacophores.**
To date there has been no crystal structure of quinoline-5,8-diones docked in the Cdc25 active site which hampers the establishment of a definite mechanism of action, however it is thought to involve either covalent adduct formation with a serine residue adjacent to the catalytic site,\textsuperscript{24} or irreversible oxidation of the cysteine residue in the catalytic domain to a sulphonic acid (Cys-SO$_3^-$).\textsuperscript{24} The two most potent compounds in this family are JUN1111 and its 6-chloro derivative NSC663284 (DA3003-1) which were synthesised by Lazo and co-workers.\textsuperscript{25,26} Adociaquinone B which is a derivative of a marine sponge extract also showed an excellent inhibition profile. Collaborative work between IPSEN pharmaceuticals and the research group of Boutros resulted in the discovery of two potent Cdc25 inhibitors, BN82685 and IRC083864, the latter displaying the most efficacy to date.\textsuperscript{9} Table 1.1.2 illustrates the nanomolar activity of the five most potent quinone derivatives against Cdc25 in an enzyme assay. Both BN82685 and IRC083864 were found to possess excellent inhibition properties against MiaPaCa2 (0.1 µM) and LNCaP (0.02 µM) cell lines respectively \textit{in vitro}. Furthermore \textit{in vivo} testing also showed encouraging results with both compounds showing activity against their respective cell lines in xenografted tumours in nude mice.

Given the infancy of this research the selectivity of these compounds is an issue which requires future SAR development. It is envisaged that Cdc25 phosphatase inhibitors are not selective for tumour cell lines and would inhibit the cell-cycle progression of any cell type but the upregulation of Cdc25s in various cancers means increased sensitivity may exist. For example, both colon adenocarcinoma (HCT116) and pancreatic ductal adenocarcinoma show increased expression of Cdc25B and also show increased sensitivity to chemical inhibition of Cdc25 phosphatase activity.\textsuperscript{9,27}
<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; in vitro µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>JUN1111</td>
<td><img src="image" alt="Structure" /></td>
<td>0.38-1.8</td>
</tr>
<tr>
<td>NSC663284 (DA3003-1)</td>
<td><img src="image" alt="Structure" /></td>
<td>0.2-0.9</td>
</tr>
<tr>
<td>BN82685</td>
<td><img src="image" alt="Structure" /></td>
<td>0.17-0.25</td>
</tr>
<tr>
<td>Adociaquinone B</td>
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<td>0.07</td>
</tr>
<tr>
<td>IRC083864</td>
<td><img src="image" alt="Structure" /></td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Table 1.1.2** In vitro IC<sub>50</sub> values for five of the most potent compounds identified so far against the three Cdc25 isoforms.

DA3003-1 was first reported by Lazo et al. in 2001 and showed sub micromolar inhibition of Cdc25, *Fig. 1.1.9*. Owing to the encouraging results from this study further elaboration of the pharmacophore was investigated by Wipf et al. in 2008. This study was based on inverting the six and seven positions of DA3003-1 type structures leading to the synthesis and biological evaluation of analogous quinoline-5,8-diones.

![Inverse quinoline-5,8-diones](image)

*Fig. 1.1.9* Inverse quinoline-5,8-diones WDP1079 and WDP1149 synthesised by Wipf et al..
The IC$_{50}$ values of WDP1079 and WDP1149 against the Cdc25B catalytic domain as well as subsequent cytotoxicity assays against the Cdc25B expressing lung cancer cell line A549 are detailed below in Table 1.1.3. As a comparison the IC$_{50}$ of DA3003-1 against A549 is also shown.$^{24}$ Of the two quinolines synthesised by Wipf et al. it is apparent that halogenation of both the four and seven position results in greater activity against Cdc25B and A549. These encouraging preliminary findings provide the rationale to diversify this inchoate branch of quinolines and form the basis of our synthetic venture into quinoline-5,8-diones as Cdc25 phosphatase inhibitors Section 6.2.1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cdc25B IC$_{50}$ ± SEM/µM</th>
<th>A549 IC$_{50}$ ± SEM/µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA3003-1</td>
<td>0.91 ± 0.36</td>
<td>1.48 ± 0.04</td>
</tr>
<tr>
<td>WDP1079</td>
<td>1.10 ± 0.1</td>
<td>2.69 ± 0.08</td>
</tr>
<tr>
<td>WDP1149</td>
<td>5.30 ± 0.6</td>
<td>9.52 ± 0.33</td>
</tr>
</tbody>
</table>

Table 1.1.3 Comparison of in vitro efficacy of inverted quinoline-5,8-diones versus DA3003-1 against Cdc25B and human lung carcinoma cell line (A549).

1.1.5 Conclusion

The pivotal role CdKs play in cell cycle regulation makes them an attractive target for the ontogenesis of antineoplastic agents. As CdK activators, Cdc25 phosphatases are discernible targets for the development of novel approaches to indirectly inhibit CdKs and their ramifications on cell cycle regulation. The quinoline-5,8-dione pharmacophore represents a privileged template which serves as a molecular probe to investigate the consequence of Cdc25 phosphatase inhibition.

To date, the exact role and mechanism of Cdc25 phosphatases remains vague largely due to the paucity of suitable exploratory templates, a niche where quinoline-5,8-diones apply. Given the importance of Cdc25 inhibition and its subsequent effects in cell cycle
control, the elaboration of the quinoline-5,8-dione pharmacophore is of eminent importance in order to expatiate the biological knowledge of this key process.

1.2 Quinazolines

The quinazoline scaffold, consisting of a core bicyclic structure 4, represents a family of molecules containing diverse pharmacophores which possess a broad spectrum of activity. Up until the late 1960’s, only two quinazolines were used medically, methaqualone 5, a soporific and anti-convulsant, and the diuretic quinethazone 6.\textsuperscript{29}

However, in recent years there have been significant advances in this field leading to the generation of quinazoline derivatives possessing a range of activities including analgesic and anti-inflammatory, anti-malarial, anti-fungal, anti-diabetic, diuretic, anti-hypertensive, sedative/soporific, anti-cancer as well as the treatment of benign prostatic hyperplasia.\textsuperscript{29} Between 2007 and 2010 alone eighty eight world patents were filed for 4-anilinoquinazolines, a family of tyrosine kinase inhibitors which are at the forefront of chemotherapy.\textsuperscript{30} Our specific interest lies in the exploration of the quinazoline2,4-(1H,3H)-dione scaffold 7 as they represent an underdeveloped domain of quinazolines.
1.2.1 Quinazoline-2,4-(1H,3H)-dione scaffold

Quinazoline-2,4-(1H,3H)-diones 7 represent a branch of quinazoline derivatives which have also been found to possess a vast array of pharmacological properties ranging from serotonin receptor antagonists, glutamate receptor antagonists, α-adrenoceptor antagonists, acetylcholine receptor antagonists, anti-bacterial and anti-cancer agents. Owing to the intrinsic nature of quinazoline-2,4-(1H,3H)-dione fragment 7, Fig 1.2.2, in pyridinoquinazolinetetrone 1, Fig 1.1.1 (page 14) the following section outlines the most prevalent biological applications of this pharmacophore.

![Fig 1.2.2 Structure and numbering sequence of quinazoline-2,4-(1H,3H)-dione.](image)

1.2.2 Anti-cancer activity

A recently published paper by Zhou et al. documents the synthesis of a range of quinazoline-2,4-(1H,3H)-dione derivatives which were found to possess significant anticancer activity when tested against the NCI 60-cell line screen. Following SAR studies a total of forty-two relevant compounds were synthesised of which seventeen exhibited anti-proliferative activity. Four of these compounds were found to possess sub-micromolar activity, Table 1.2.1 (page 27). The final two compounds (NSC D-752221/1 and NSC D-751371/1) appear to have remarkable cytotoxic activity given their similarity to other compounds assayed. Due to the lack of selectivity of these compounds for a specific cancer sub-type, no plausible mechanism of action was identified, however these compounds may be useful as leads for future SAR studies.
<table>
<thead>
<tr>
<th>Compound</th>
<th>% mean growth at 10 µM</th>
<th>GI\textsubscript{50} (µM) average value over 56 cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSC D-750471/1</td>
<td>20.39</td>
<td>0.794</td>
</tr>
<tr>
<td>NSC D-752951/1</td>
<td>14.59</td>
<td>0.741</td>
</tr>
<tr>
<td>NSC D-752221/1</td>
<td>-3.62</td>
<td>0.363</td>
</tr>
<tr>
<td>NSC D-751371/1</td>
<td>-81.75</td>
<td>0.407</td>
</tr>
</tbody>
</table>

Table 1.2.1 The four most potent compounds synthesised by Zhou et al. when tested against the NCI 60-cell line screen.

### 1.2.3 Geranylgeranyltransferase-I inhibitors

Carrico et al. outlines the employment of quinazoline-2,4-dione congeners as perspective protein geranylgeranyltransferase-I (PGGTase-I) inhibitors as a potential
Geranylgeranyltransferase-I belongs to the prenyltransferase family which catalyse the lipidation of proteins. Specifically PGGTase-I catalyses the transfer of a geranylgeranyl moiety to the cysteine residue of specific proteins. PGGTase-I is part of the CAAX prenyltransferase category where C is a cysteine residue, A represents any aliphatic amino acid moiety and X is leucine, isoleucine or phenylalanine. The precedence for this study is based on the fact that many PGGTase-I substrates were found to play a critical role in the development of tumours and subsequent metastasis.

The PGGTase-I enzyme is a heterodimer with both α and β subunits containing primarily alpha helices, Fig 1.2.3. The α-subunit forms a crescent shape around the β-subunit. The β-subunit forms a compact, α–α barrel domain which contains a central cavity. At the α–β interface is the substrate binding site which extends into the funnel-shaped cavity of the β-subunit. Contained along this funnel are hydrophobic residues with a catalytic zinc ion at the top of the funnel which binds to the cysteine of the CAAX system.

Fig 1.2.3 PGGTase-I enzyme with the α- and β-subunits highlighted in red and blue respectively. The catalytic zinc is highlighted as the magenta sphere. Highlighted in cyan is 3’azaGGPP, a non-reactive analogue of geranylgeranyldiphosphate. Also shown in yellow is the CAAX (CVIL) residue of the peptide substrate.
Design of peptidomimetics was based around the adaption of the CAAX residues. The central AA units were replaced with a rigid linker which lead to the synthesis of GGTI-2154, a potent PGGTase-I inhibitor, IC$_{50}$=21nM, Fig 1.2.4. The cysteine residue has also been replaced with an imidazole bioisostere which was previously shown to increase metabolic stability and selectivity for PGGTase-I.

![GGTI-2154 structural formula](image)

**Fig 1.2.4**

To further this work, a series of molecular modelling studies were carried out in order to explore the development of novel PGGTase-I inhibitors, which revealed that the quinazoline-2,4-dione scaffold would be an attractive alternative to the biphenyl linker present in GGTI-2154. As a result a series of quinazoline-2,4-dione congeners were furnished in order to investigate their efficacy against PGGTase-I. Of the sixteen compounds two produced IC$_{50}$ values in the nano-molar region, Fig 1.2.5.

![Compound structures](image)

**Fig 1.2.5**
Comparison of derivatives 8 and 9 shows that the phenylalanine derivative 9 exhibits superior activity when compared to the leucine derivative 8. Further studies determined that an unsubstituted imidazole was necessary for effective binding to zinc. Conversion of the amino acid residues to the D-series lead to a complete drop off in activity most likely due to size restrictions in the X pocket. Docking studies of the leucine derivative 8 revealed that the compounds bind in the CVIL site suggesting the activity of these compounds may be due to competitive inhibition of PGGTase-I substrates.

1.2.4 Anti-bacterial agents

Quinazoline-2,4-(1H,3H)-diones have also been the subject of much interest as new avenues of therapy for resistant gram-positive infections.\textsuperscript{36,37} Fig. 1.2.6 (page 31) shows two of the existing antibiotics used in the treatment of gram-positive infections. The need to develop new therapies arises from the emergence of resistant strains of bacteria namely methicillin resistant \textit{Staphlococcus aureus} (MRSA) and vancomycin resistant \textit{enterococci} (VRE) which are of particular concern. There has also been an emergence of vancomycin resistant \textit{Staphylococcus aureus} infection (VRSA), a drug which is usually associated as a last line therapy. Therefore it is easy to see the urgent need to develop original therapies to combat this serious clinical problem.
A study carried out by Huband et al. details the antibiotic activity of two novel quinazoline-2,4-(1H,3H)-diones, PD 0305970 and the 3-desamino analogue PD 0326448, which serve as next generation therapies for resistant and susceptible strains of bacteria. In vitro testing of the compounds was carried out against 1,036 clinically significant strains of bacteria. Both analogues were found to possess exceptional antibiotic activity versus gram-positive, including resistant strains.

The MIC<sub>90</sub> values of PD 0305970 ranged from 0.008-0.5 µg/ml against staphylococci, streptococci, Corynebacterium spp., while PD 0326448 exhibited values which were two- to fourfold higher in an identical study. When compared against existing treatments for gram-positive resistant strains, Streptococcus pneumonia, Enterococcus faecalis,
Enterococcus faecium and staphylococci, PD 0305970 is bestowed with an exemplary 8- to 512-fold MIC\textsubscript{90} advantage over existing treatments.

The quinazoline-2,4-(1H,3H)-diones excel in the treatment of quinolone resistant mutants with similar or superior anti-bacterial properties as current quinolones to susceptible strains of gram-positive bacteria. A study using PD 0305970 showed that this activity is most likely due to the targeting of the gyrB and parE subunits in contrast to the quinolones which targets the gyrA and parC.

In 2010 Oppegard et al. published work detailing the biological evaluation of novel quinazoline-2,4-(1H,3H)-diones as potential alternatives to quinolone type antibiotics.\textsuperscript{37} In order to be viable these compounds had to fulfil two criteria; (i) possess activity against known quinolone-resistant mutants and (ii) to display similar activity as quinolone antibiotics towards DNA gyrase and Topoisomerase IV. It is thought that dual targeting agents assist in slowing the emergence of drug resistant mutants.

In a previous study the same group demonstrated that gyrA and gyrB mutations of E. coli which are resistant to quinolone therapies displayed sensitivity to 8-methoxy-quinazoline-2,4-diones. Both 8-methoxy and 8-methyl quinazoline-2,4-diones were then tested against three mutant gyrases in order to assess the efficacy of these compounds against high, moderate and low resistance strains, Table 1.2.2. As can be seen below both of the compounds show increased activity towards mutant strains relative to wild-type gyrase than ciprofloxacin, Table 1.2.2. Another study which examined these two compounds against the catalytic activity of S. aureus gyrase and S. aureus Topo IV demonstrated comparable efficacy which suggests that quinazoline-2,4-diones may function as dual-target antibiotics. From these studies it can be seen that quinazoline-2,4-diones represent a class of drugs with promising potential in the fight against antibiotic resistance.
Table 1.2.2 Inhibition of catalytic activities of *E. coli* gyrase

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wild-type gyrase IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>GyrA S83W gyrase IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>GyrA G81C gyrase IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>GyrA A67S gyrase IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>0.45 ± 0.004</td>
<td>101 ± 1.9</td>
<td>28 ± 7.0</td>
<td>1.0 ± 0.15</td>
</tr>
<tr>
<td>8-Methoxy</td>
<td>2.8 ± 0.1</td>
<td>5.9 ± 0.9</td>
<td>4.3 ± 0.4</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>8-Methyl</td>
<td>0.95 ± 0.15</td>
<td>3.8 ± 0.6</td>
<td>1.7 ± 0.2</td>
<td>1.2 ± 0.3</td>
</tr>
</tbody>
</table>

1.2.5 Glutamate receptor antagonists

Quinazoline-2,4-diones have also garnered attention as potential glutamate receptor antagonists. Glutamate (Glu) is the primary excitatory neurotransmitter in the central and peripheral nervous system and is involved in a range of physiological processes such as learning and memory. Excess glutamate transmission has also been implicated in a range of neurological disorders such as Alzheimer’s, Parkinson’s, epilepsy, multiple sclerosis as well as the transmission of pain.

Glutamate expends its effects by acting on two sets of receptors, metabotropic (mGluRs) and ionotropic (iGluRs) receptors. The ionotropic receptors are classified into three
subsets, \( N \)-methyl-\( \alpha \)-aspartate (NMDA), \( \alpha \)-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate (KA) receptors each of which contain six, four and five subunits respectively. While many insights have been provided by research into the role of NMDA and AMPA receptors, knowledge of the role of KA receptors is sparse owing to the lack of selective antagonists.

Work carried out by Colotta et al. in 2004 detailed the synthesis of 3-hydroxy-1\( H \)-quinazoline-2,4-diones and subsequent bioassays showed that these compounds were antagonistic towards NMDA and AMPA receptors.\(^{51}\) By manipulating the substituents on the quinazoline scaffold it was found that the affinity for a specific receptor type could be effected, \textit{Fig 1.2.8}.

\[ \text{Fig. 1.2.8 NMDA antagonist 7-chloro-3-hydroxy-1H-quinazoline-2,4-dione 10 and AMPA antagonist 7-chloro-3-hydroxy-6-(4H-1,2,4-triazol-4-yl)quinazoline-2,4(1H,3H)-dione 11.} \]

With this in mind the same group published a later paper exploring the SAR of this pharmacophore with the aim of developing KA specific antagonists to use as probes for the characterisation of the KA receptor.\(^{33}\) Diversification was investigated at the three and six positions, using the compounds in \textit{Fig 1.2.8} as lead compounds. Exploration of the three position was carried out on 7-chloro-3-hydroxy-1\( H \)-quinazoline-2,4-dione 10 with a variety of ethers being synthesised however these compounds lacked any affinity for AMPA, Gly/NMDA or KA receptors illustrating the necessity of the 3-hydroxyl group.

Derivatisation of the six position led to the discovery of 6-(2-carboxybenzoylamino)-3-hydroxy-1\( H \)-quinazoline-2,4-dione, \textit{12, Fig 1.2.9}. This compound exhibits a good affinity
for both high and low-affinity KA receptors with IC$_{50}$ values of 0.62 and 1.6 µM respectively. The compound also shows good selectivity versus Gly/NMDA and AMPA receptors. Since few selective KA receptor antagonists are known, quinazoline-2,4-diones represent a family of compounds which are pivotal in the emergence of research in this field.

![Chemical structure](image)

**Fig 1.2.9**

### 1.2.6 Conclusion

The quinazoline-2,4-(1H,3H)-dione heterocycle represents a privileged pharmacophore with which to develop novel chemotherapeutic agents due to their widespread and distinct biopharmaceutical properties. The limited exploration of this moiety as antineoplastic agents has shown promising preliminary results, section 1.2.2, but much work is necessary in order to expound the mode of action of these drugs, congruous to Carrico’s work on PGGTase-I inhibitors, section 1.2.3, so as to implement more judicious investigation.$^{39}$

Quinazolinedione derivatives have also been bequeathed with excellent anti-bacterial properties mediated by the inhibition of bacterial gyrase and topoisomerase IV and represent a new avenue in the treatment of multidrug and fluoroquinolone resistant strains of bacteria.

Given the wide range of disorders implicated with excess glutamate transmission, antagonism of its receptors represents an attractive target for developing effective therapies. The quinazoline-2,4-(1H,3H)-dione scaffold represents an exemplary research tool in this field. Targeted elaboration of the quinazolinedione backbone conferred
remarkable selectivity profiles, leading to the generation of receptor specific antagonists. Owing to the sparsity of detailed knowledge of these receptors quinazoline-2,4-(1H,3H)-diones serve as principle templates to expand the biological understanding of glutamate receptor antagonists.

1.3 Perspectives

In Sections 1.1 and 1.2 critical insight into the prevalence of both pharmacophores in medicinal chemistry was highlighted. The validated bioactivity of both classes of compounds proffers the paradigm of synthesising a tricyclic hybrid in the quest for novel chemotherapeutic agents. It is foreseen that the fusion of these structures to generate the unheralded pyridinoquinazolinetetrone 1 will lead to a cogent new template with which to pioneer new avenues of drug discovery.
2.0 Quinoline Chemical Introduction
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2.1 Introduction

The quinoline moiety is of great interest to chemistry due to its prominence in biologically active natural products, in particular alkaloids, most notably as antimalarial drugs.\textsuperscript{52-54} Perhaps the most known quinoline derivative is the alkaloid quinine, 13 which occurs naturally in the bark of the cinchona tree and was the antimalarial drug of choice from the 17\textsuperscript{th} century until the 1940’s.

![Quinoline structure](image)

A wide range of quinoline derivatives display a broad range of pharmacological properties ranging from anti-cancer,\textsuperscript{55} anti-HIV,\textsuperscript{56} anti-hypertensive,\textsuperscript{57} anti-tuberculosis\textsuperscript{58} to anti-Alzheimer activities.\textsuperscript{59} Due to their importance, continuing research is focused on the development of more efficient methods of synthesis as well as derivatisation.

A key subset in the synthesis of quinolines are quinolones (hydroxyquinolines), in this chapter synthetic routes will be classified based on the substitution pattern of quinolones followed by the substitution pattern of quinolines;

- 2-Quinolones
- 4-Quinolones
- Substituted quinolines
In each case syntheses will be classified based on the named reaction. The final section addresses the synthesis of quinoline-5,8-diones, a family of quinolines central to this work.

**2.2 Synthesis of 2-quinolones**

**2.2.1 Knorr Synthesis**

The Knorr quinoline synthesis, first described in 1886 by Ludwig Knorr, is an intramolecular cyclisation reaction which converts a β-ketoanilide 14 to a 2-quinolone 15 under strongly acidic conditions.\(^{60}\) The β-ketoanilide 14 is generated from the acid-catalysed condensation of primary arylamines and β-ketoesters, *Scheme 2.2.1.1.*

![Scheme 2.2.1.1](image)

Recently, Klumpp *et al.* published an investigation into the mechanism of the cyclisation.\(^{61}\) His results, supported by low-temperature \(^1\)H, \(^{13}\)C and \(^{15}\)N NMR indicated that the β-ketoanilide 14 undergoes diprotonation at the two carbonyl oxygen atoms to form a distonic superelectrophile. Computational studies also showed that this conformation was the most stable, being at least 8 kcal mol\(^{-1}\) more stable than other dications. Klumpp synthesised a range of substituted 2-quinolones from acetoacetanilides (generated from the respective anilines and diketene) in the presence of trifluoromethanesulfonic (triflic) acid at ambient temperature in high to excellent yields for activated aryl groups e.g., *Scheme 2.2.1.2.*
This chemistry was unsuccessful for deactivated aryl groups with the exception of a para-fluoro acetanilide which gave a moderate yield of 44 % (this was increased to 69 % with the addition of 10 mol % SbF₅).

Studies on acid equivalents were also performed, Scheme 2.2.1.3, where it was found that the yield decreased with decreasing quantities of triflic acid, in accordance with an earlier paper published by Staskun who observed that the Knorr cyclisation required heating with excess strong acid which is consistent with the formation of supercaticonic species.⁵²

<table>
<thead>
<tr>
<th>Acid eq.</th>
<th>Yield%</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>97</td>
</tr>
<tr>
<td>2.5</td>
<td>86</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
</tr>
</tbody>
</table>
2.3 Synthesis of 4-quinolones

2.3.1 Conrad-Limpach synthesis

The Conrad-Limpach synthesis, first described in 1891, involves the thermal or acid-catalysed cyclisation of primary arylamines with β-ketoesters to generate the imine 16, which cyclises to 4-quinolone 17.63

\[
\text{Ph}_2\text{O} \quad 260 \text{ °C} \quad \text{EtOH}
\]

Scheme 2.3.1.1

In a search for novel PDE4 inhibitors, Billah et al. employed this synthesis to yield the 8-methoxyquinoline 18.64 o-Anisidine was condensed with ethyl 4,4,4-trifluoroacetooacetate in the presence of polyphosphoric acid to give the quinolone 19. Chlorination at the 4-position generated 20 and subsequent catalytic dehydrogenation furnished the 8-substituted quinoline 21. Bromination at the 5-position followed by carbonylation afforded 8-methoxyquinoline-5-carboxylic acid 22. Activation of the acid residue followed by reaction with the sodium salt of 4-amino-3,5-dichloropyridine gave 8-methoxyquinoline-5-carboxamide 18, Scheme 2.3.1.2. Derivatisation of 18 in a subsequent publication resulted in the synthesis of the dichloropyridine-N-oxide derivative.65 Both of these compounds showed very promising results as selective inhibitors of PDE4.
2.3.2 Camps Synthesis

Li et al. used the Camps synthesis to synthesise a range of 6,7-substituted-2-phenyl-4-quinolones 23 in a search for anticancer drug candidates due to the known anti-microtubular activity of 2-phenyl-4-quinolones, which interact with tubulin at the colchicine (Figure 2.3.2.1) site. Access to these compounds was achieved via two synthetic routes. The first, Scheme 2.3.2.1, involves reacting o-amino acetophenones 24, and benzoyl chlorides 25 to form diarylamides 26. Potassium tert-butoxide mediated cyclisation of 26 resulted in the formation of 23.
Li found that substitution at the 3'-position of 6,7-methylenedioxy derivatives is well tolerated with no significant change in activity for a range of electron-donating and electron-withdrawing substituents, the same was also true for steric bulk with OBz and H at the 3'-position showing similar results. 6-Amino derivatives (morpholine and pyrrolidine) showed high activity with IC50 values in the nano-molar range against tubulin polymerisation. Yields for the reactions ranged from 27-80% for a diverse range of substituents. All the compounds synthesised exhibited cytotoxic effects against a variety of human tumour cell lines including solid tumours. The most potent compound synthesised 27, Fig. 2.3.2.1, possessed GI50 values in the nano and subnanomolar range across the majority of cell lines tested in the NCI programme and is also a potent inhibitor of radiolabelled colchicine binding to tubulin with activity comparable to the anti-mitotic products colchicine, podophyllotoxin and combretastatin A-4.
The second method involves the acid catalysed condensation of substituted anthranilamides 28, with substituted acetophenones 29 to generate the corresponding imines 30. Lithium diisopropylamide mediated cyclisation of 30 gave the respective 2-phenyl-4-quinolones 23 in good yields, Scheme 2.3.2.2.

More recently Hadjeri et al. reported the synthesis and anti-mitotic activity of 5-hydroxy-7-methoxy-2-phenyl-4-quinolones. The compounds were synthesised in a similar fashion to Scheme 2.3.2.2. In terms of structural requirements they found that a 7-methoxy, a 5-hydroxyl group and a free N-1 were all necessary for anti-mitotic activity. The presence of a fluorine at the 3'- or 2'- position and a methoxy or chloro group at the
7-position resulted in high activity for cell cycle arrest and antiproliferation, with 31 being the most potent, Fig. 2.3.2.1.

Sui et al. used the Camps synthesis to synthesise a series of novel quinolones 32 as potential topoisomerase II inhibitors. In all twenty-six compounds were synthesised and tested against topoisomerase II using ellipticine, as a reference. The most potent of the compounds synthesised was over 400 times more potent than ellipticine.

Scheme 2.3.2.3

Ketones 33 were synthesised by regioselective electrophilic aromatic substitution at the ortho position by the corresponding nitriles using titanium tetrachloride as catalyst. N-Acylation of 33 using benzoyl chlorides under standard conditions gave the amides 34. Cyclisation of 34 under pressure in the presence of sodium ethoxide gave quinolones 35, which were demethylated using hydrogen bromide to yield quinolones 32, Scheme 2.3.2.3.

Buchwald et al. developed a novel two-step synthesis of 2-aryl quinolones via a copper catalysed amidation of o-halophenones followed by a base catalysed Camps cyclisation of the resultant N-(2-ketophenyl)amides, Scheme 2.3.2.4.
From these findings a series of $N$-(2-ketophenyl)amides were synthesised in good yield. Cyclisation in the presence of 3-3.5 equivalents of base resulted in the generation of a library of 2-aryl (phenyl, chlorophenyl, pyridyl, thiophenyl and styryl) quinolones.

### 2.4 Synthesis of substituted quinolines

#### 2.4.1 Combes synthesis

The Combes synthesis of quinolines was first described in 1888 by the French chemist Alphonse-Edmond Combes.\(^7^0\) The reaction involves the acid catalysed condensation of ortho-unsubstituted anilines with β-diketones to generate 2,4-disubstituted quinolines, 36, or β-keto aldehydes to give 4-substituted quinolines via an imine intermediate, Scheme 2.4.1.1.

![Scheme 2.4.1.1](image)

Though one of the less utilised cyclisation methods the Combes synthesis has found applications in the synthesis of benzoquinolines\(^7^1,7^2\) and pyrido[3,2'-b]carbazoles.\(^7^3\)
2.4.2 Friedländer synthesis

First described in 1882 by German chemist Paul Friedländer, the Friedländer reaction involves the condensation of α-aminoaryl aldehydes or ketones with an aldehyde or ketone possessing an α-CH₂ group under basic or acidic conditions, *Scheme 2.4.2.1*.

![Scheme 2.4.2.1](image)

The Friedländer synthesis has the advantage of being one of the simplest and most straightforward methods for synthesising polysubstituted quinolines. The reaction is catalysed by both acid and base. Brønsted acids like sulfimic acid, hydrochloric acid, sulphuric acid, p-toluene sulfonic acid and phosphoric acid are widely reported as catalysts, though reaction conditions are usually harsh and lead to reduced efficiency and hence lower yields. Fehnel *et al.* reported that under thermal or base catalysed conditions simple ketones fail to react with o-aminobenzophenone.⁷⁴

A novel synthesis of 3-(methanesulfonyl)quinolines was developed by Atechian *et al.* as previous literature reported low to mediocre yields and long reaction times. Anthranilic acid 37 was firstly cyclised to benzoxazinone 38 followed by conversion to 3-(methanesulfonyl)quinoline 39 in a 39% overall yield. The synthesis of 39 allowed access to derivatives at the 4-position. Chlorination of 39 using POCI₃ and N,N-dimethyl-p-toluidine in refluxing toluene for 7 hours gave 40, as a crystalline solid. Further derivatisation of 40 with secondary amines, like morpholine, gave 41 in an 80% yield. Elaboration of the 6-position of 41 was achieved using both Buchwald and Suzuki-Miyaura protocols to generate 42 and 43 in 78% and 56% yields respectively, *Scheme 2.4.2.2*.⁷⁵
2.4.2.1 Lewis acid catalysed Friedländer synthesis

Wu et al. published work detailing the use of molecular iodine as an efficient, mild and environmentally friendly catalyst in the Friedländer reaction. After screening several reaction conditions it was found that 1 mol% of iodine at room temperature for 16 hours produced a variety of 2,3,4-trisubstituted quinolines in good to excellent yields, Scheme 2.4.2.1.1. The reaction was also shown to tolerate a wide range of ketones both cyclic and acyclic.
A similar study was carried out by Adapa and co-workers\textsuperscript{77} where neodymium(III) nitrate hexahydrate (5 mol\%) was used as the Lewis acid catalyst for the reaction, \textit{Scheme 2.4.2.1.1}. This reaction, like Wu’s was applicable to a broad range of substrates, both cyclic and acyclic.

In 2007, Atechian and co-workers\textsuperscript{75} published work detailing the synthesis of poly-substituted quinolines \textit{via} a gold(III)catalysed Friedländer synthesis first described by Arcadi \textit{et al}.\textsuperscript{78} In this reaction the gold acts as a Lewis acid much like neodymium in Adapa’s synthesis, \textit{Scheme 2.4.2.1.1}. The general reaction is outlined in \textit{Scheme 2.4.2.1.2}.

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{scheme2.4.2.1.1.png}
\end{scheme}

\textbf{Scheme 2.4.2.1.2}

The condensation of 2-aminobenzophenones 44 with 45 led to the formation of 46 however, if the carbonyl groups of 45 had similar reactivity a regioisomeric mixture was formed as was the case where R\textsubscript{1}= i-Bu/CH\textsubscript{2}OMe and R\textsubscript{2}= Me. The reaction proceeds smoothly giving moderate to good yields.

\subsection*{2.4.2.2 Synthesis of quinolines from alcohols}

Ricardo and Yus developed an indirect Friedländer synthesis by reacting 2-aminobenzylc alcohol with a series of ketones.\textsuperscript{79} They proposed a metal free Meerwein-Ponndorf-Verley reaction between 2-aminobenzylc alcohol and benzophenone using potassium \textit{tert}-butoxide, followed by a Friedländer annulation to generate the quinoline. The mechanism was supported with deuterium labelling experiments and the fact that the reaction does not proceed in the absence of benzophenone. After optimisation using the
alcohol 47 and ketone 48 the desired quinoline 49 was isolated in a 99% yield, \textit{Scheme 2.4.2.2.1}.

![Scheme 2.4.2.2.1](image)

Expanding on this, a range of 2,3 and 4-tri-substituted quinolines were regioselectively synthesised in excellent yields, \textit{Scheme 2.4.2.2.2}.

![Scheme 2.4.2.2.2](image)

2.4.2.3 Catalyst-free Friedländer annulation in water

Wang \textit{et al.} reported the synthesis of various quinolines from 2-aminobenzaldehyde 50 in aqueous conditions without the use of catalysts.\textsuperscript{80} A series of 2,3-disubstituted and polycyclic quinolines were synthesised in excellent yields, \textit{Scheme 2.4.2.3.1}. This method is tolerant of a wide variety of substrates and is a useful, green addition to existing chemistry.

![Scheme 2.4.2.3.1](image)
2.5 Pfitzinger synthesis of quinolines

The Pfitzinger reaction involves the reaction of isatin 51 or its derivatives with methylene ketones in an alkaline medium to generate quinoline-4-carboxylic acid derivatives. The reaction was first described in 1886 by W. Pfitzinger and is the most important variant of the Friedlander synthesis. Isatin 51, in the presence of base is converted into an isatinic acid salt 52 which cyclocondenses via the α-keto function with methylene ketones. Treatment with acid results in the formation of quinoline-4-carboxylic acid derivatives 53, Scheme 2.5.1.

Scheme 2.5.1

Pfitzinger studied the reaction of isatin 51 with acetone in the presence of an aqueous base to generate 2-methyl-4-quinolinecarboxylic acid 54, Scheme 2.5.2. In further publications Pfitzinger reported optimised reaction conditions for the synthesis of 54 using 33% NaOH at 100 °C for 8 hours giving up to an 80% yield while various other groups reported a drop in yield using more dilute sodium hydroxide solutions.82,83

Scheme 2.5.2

As is shown in Scheme 2.5.2 the reaction of 51 with symmetrical ketones results in the formation of only one product, similarly only one product is formed in the case of unsymmetrical ketones containing only one methyl/methylene group, Scheme 2.5.3.
Borsche et al.\textsuperscript{84} and Braun et al.\textsuperscript{85} carried out studies of unsymmetrical ketones (e.g. butanone) with \textit{51} and found that 2,3-dimethyl-4-quinolinecarboxylic acid \textit{55} was the major product with 2-ethyl-4-quinolinecarboxylic acid \textit{56} being the minor. Palmer and McIntyre\textsuperscript{86} published a mechanism explaining these observations, \textit{Scheme 2.5.4}.

\textbf{Scheme 2.5.4}

Buu-Hoi \textit{et al}. demonstrated that 7-halogen-substituted isatins, when reacted with methyl ethyl ketone only give 2,3-dimethyl-8-haloquinoline acids.\textsuperscript{87,88} Generally the reactivity of carbanions for these reactions is $2\textdegree>1\textdegree>3\textdegree$ with the lack of $3\textdegree$ carbanion reactivity being due to steric restrictions. This rule is consistent with the findings of Palmer and McIntyre, \textit{Scheme 2.5.4}.\textsuperscript{86}

Unsymmetrical ketones which contain aryl substituents were investigated by Palmer and McIntyre\textsuperscript{86}, who found that the nature of the aryl substituent had an influence on the reaction products. Electron-withdrawing substituents at the \textit{para}-position of the aryl group exclusively gave 3-aryl quinolinecarboxylic acids, \textit{57}. Unsubstituted or electron-
donating groups at the para-position gave a mixture of compounds, 57 and 58 with the major product being 3-aryl quinolinecarboxylic acids, 57, Scheme 2.5.5.

![Scheme 2.5.5]

Synthesis of quinolinedicarboxylic acids from isatin 59 and its derivatives and α-keto acids is well documented in the literature, chiefly due to the search for anti-malarial drugs. Many groups studied the reaction of 59 and its derivatives with pyruvic acid to generate substituted quinoline dicarboxylic acids. Buchman and co-workers\textsuperscript{89} successfully synthesised 6,8-dichloroquinoline-2,4-dicarboxylic acid 60 in excellent yield using this protocol, Scheme 2.5.6.

![Scheme 2.5.6]

As can be seen in Scheme 2.5.6 the reaction conditions are quite mild. Cragoe demonstrated that when halo-substituted acids or their esters are used the reaction takes place even at room temperature, an example of which is shown in Scheme 2.5.7.\textsuperscript{90,91}
Scheme 2.5.7

The reaction of isatin 59 with chloropyruvic acid generated the dicarboxylic acid 61 which was decarboxylated in situ to give 62 in an 85% yield.\textsuperscript{92}

The reaction of isatin 51 with acetoacetic acid (\(\beta\)-keto acid) was investigated by Pfitzinger and structure 63 was proposed.\textsuperscript{83} Enhelhard later proved the structure of 63 by oxidation to 64, Scheme 2.5.8.\textsuperscript{93}

Scheme 2.5.8

Alkyl aryl ketones are common reactants used in the Pfitzinger reaction and generate 2-aryl quinoline-4-carboxylic acids exclusively.\textsuperscript{83} Similarly alkyl hetaryl ketones yield 2-hetaryl quinolinecarboxylic acids. Due to the large number of publications only one example is shown, Scheme 2.5.9. Gilman\textsuperscript{94} and Atwell\textsuperscript{95} synthesised compounds of type 65 in a search for anti-malarial drugs.

Scheme 2.5.9
The Pfitzinger reaction provides a convenient method for the synthesis of polycyclic systems when cyclic ketones/diketones are used resulting in a wide variety of fused quinoline derivatives e.g., Scheme 2.5.10. \(^{96}\)

![Scheme 2.5.10](image)

**Scheme 2.5.10**

### 2.6 Skraup type synthesis

The Skraup and Doebner-von Miller synthesis of quinolines involves the reaction of an aromatic amine containing at least one unsubstituted ortho-position with an electrophilic three carbon fragment. The archetypal Skraup synthesis involves the reaction of aniline with glycerol 66, sulphuric acid and nitrobenzene as an oxidising agent, although more recent non-organic oxidising agents such as arsenic pentoxide, boric acid, iron(III) salts and iodine have replaced nitrobenzene due to the reduction of resin formation, leading to purer isolates. \(^{97}\) The sulphuric acid acts to generate acrolein 67 *in situ* by catalysing the dehydration of 66 which then undergoes conjugate addition with aniline resulting in the formation of 68. Nitrobenzene then oxidises 68 to quinoline 69, **Scheme 2.6.1**.

![Scheme 2.6.1](image)

**Scheme 2.6.1**

The Doebner-von Miller reaction is classically described as the reaction of aniline with the crotonic condensation product of an aldehyde or ketone, in this case acetaldehyde,
generating dihydroquinoline 70 and oxidation results in 2-methylquinoline 71, Scheme 2.6.2.

Scheme 2.6.2

Due to the low-yielding nature of the Skraup and Doebner-von Miller syntheses, recent investigations have been devoted to finding optimal reaction conditions. Li et al. used a system of 12 M HCl, toluene and tetrabutylammonium chloride when synthesising 2-alkyl-8-quinolinecarboxylic acid which gave a 57% yield.98 Matsugi et al. also reported improved yields by using a mixture of 6M HCl and toluene in their syntheses.99

2.6.1 Substituent effects on cyclisation

Aromatic amines substituted at the ortho-position lead to the formation of 8-substituted quinolines, Scheme 2.6.1.1.100

Scheme 2.6.1.1

para-Substituted aminobenzenes cyclise at any symmetrical ortho-position to give 6-substituted quinolines. 2,5-Dimethyl-4-(p-nitrobenzyl)pyridine 72 was reacted under Skraup conditions to generate 6-[(2,5-dimethyl-4-pyridyl)methyl]quinoline 73. 2,5-Dimethyl-4-(p-nitrobenzyl)pyridine 74 from which 72 was synthesised also acted as the oxidising agent, Scheme 2.6.1.2.101
**Scheme 2.6.1.2**

*meta*-Substituted anilines, 75 cyclise to give a mixture of 5 and 7 substituted quinolines with the outcome dependant on the substituent at the *meta* position.

Strong electron-donating substituents preferentially give 7-substituted quinolines, 76 (78:22). Weaker electron-donating substituents also give 7-substituted quinolines with only a slight preference however (56:44). Strong electron-withdrawing groups promote cyclisation at the 2-position of 75 giving 5-substituted quinolines, 77 as the main product (78:22), *Scheme 2.6.1.3*.102-108

**Scheme 2.6.1.3**

2.7 Quinoline-5,8-diones

Central to this project is the synthesis of novel quinoline-5,8-diones. Interest in this family of compounds as antineoplastics arose from a study carried out in 2001 by Lazo and co-workers.6 The search for novel inhibitors of Cdc25 in the NCI repository lead to the identification of 30 quinolinediones, of which 8 had micromolar activity. Outlined in *Scheme 2.7.1* is the synthetic route used by Lazo to access quinoline-5,8-diones using
syntheses previously described.\textsuperscript{109-111} Oxidation and chlorination of quinoline-8-ol 78 was achieved in one step to generate 79 in a 30% yield. Amination of 79 was carried out at ambient temperature using functionalised ethyl amines in the presence of triethylamine, leading to the synthesis of a mixture of regioisomers 80 (DA3003-1) and 81 (DA3003-2) in a 2:1 mixture (measured by NMR) which were separated using column chromatography.

\textbf{Scheme 2.7.1}

More recently Wipf \textit{et al.} published work detailing the synthesis of compounds of type 81 in an effort to address the problems associated with quinoline-5,8-dione redox cycling leading to undesired off-target mechanisms. Synthesis starts from 2,5-dimethoxyanilene 82 which is refluxed with Meldrum’s acid and trimethyl orthoformate resulting in the formation of 83 in an 80% yield. Bromination of 83 at the four-position was achieved using a mixture of bromine and acetic acid to afford 84 in an 84% yield. Pyridone 85 is realised in an 81% yield by refluxing 84 in diphenyl ether at 250 °C. Treatment of 85 with POCI\textsubscript{3} results in the formation of the 4-chloroquinoline 86. Synthesis of the desired quinoline-5,8-diones was carried out in a two-step process. Firstly 86 is oxidatively demethylated using ceric ammonium nitrate. Following completion the isolated crude product is aminated at the 6-position using 4-(2-aminoethyl)morpholine to afford the functionalised quinone 87. Finally chlorination of the 7-position was achieved by treating a methanolic solution of 87 with \textit{N}-chlorosuccinimide to generate WDP1079 in a 65% yield, \textbf{Scheme 2.7.2}.\textsuperscript{28}
Scheme 2.7.2

Wipf also used this synthesis to generate the 4-methoxy-7-fluoro derivative of WDP1079, WDP1149. Generation of the methyl ether was afforded by heating 86 with sodium methoxide in methanol to generate 88. Synthesis of the 6-amino quinoline was achieved using analogous conditions to Scheme 2.7.2. Fluorination of the 7-position was achieved using Selectfluor® to afford WDP1149 albeit in low yield, Scheme 2.7.3.²⁸

Scheme 2.7.3
2.8 Conclusion

Due to their widespread applications in medicinal chemistry considerable progress has been made in the development of the efficient synthesis of the quinoline pharmacophore. While traditional methods remain firmly rooted in many syntheses, pullulating biological interest has led to the genesis of numerous novel methodologies which offer highly derivatised quinolines from simple precursors.

Given the promising preliminary biological results attributed to quinoline-5,8-diones this family of compounds make an attractive synthetic target. The sum total of literature in this area is outlined in Section 2.7 making it obvious that the scope for development of this area is tremendous. With the importance of their application highlighted in Section 1.1.4 development of this pharmacophore is imperative in order to elucidate their mode of action and ameliorate this area of medicinal chemistry.
3.0 Quinazoline Chemical Introduction


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3.7 N-1, N-3-Disubstituted quinazolinediones

3.7.1 Synthesis from anthranilate precursors

3.8 Conclusion
3.1 Quinazoline introduction

Quinazolines 89, Scheme 3.1.1 and their related derivatives can be found embedded in a wide range of biologically active compounds, for example, anti-cancer, anti-fungal, diuretic, anti-inflammatory, anti-convulsant and anti-hypertensive drugs.\textsuperscript{112-115} This class of compound forms a substantial part of heterocyclic chemistry and is of considerable interest to the pharmaceutical industry.

\textbf{Scheme 3.1.1}

The first quinazoline-based alkaloid natural product, vasicine (peganine) 90, Scheme 3.1.1 was isolated from the plant material of \textit{Adhatoda vasica} in 1888.\textsuperscript{116} Research remained dormant until the 1950’s when the effectiveness of quinazoline derivatives as antibacterial, anti-viral and anti-parasitic agents were discovered.\textsuperscript{117} It has only been the last fifteen years that research has seen significant advances, which is driven by their diverse applications.

Many literature syntheses of quinazolines require long reaction times and are often low yielding.\textsuperscript{118-120} As a result much attention has been focussed to develop more efficient methods for the construction of quinazolines. Although in the majority of cases no new chemistry was employed, the use of microwave-enhanced processes and new catalysts offer clear advantages both in yield and reaction time.

In this chapter synthetic routes will be classified based on the increasing substitution patterns of the pyrimidine ring system converging on syntheses most relevant to this work;

- 2-Substituted quinazolines
- 4-Substituted quinazolines
• 2,4-Disubstituted quinazolines
• Quinazoline-2,4-diones
• N-3 Substituted quinazoline-2,4-diones
• N-1, N-3 Substituted quinazoline-2,4-diones

3.2 Synthesis of 2-substituted quinazolines

2-Substituted quinazolines represent a medicinally important branch of quinazolines, finding uses in the treatment of essential thrombocytosis, chronic myeloid leukaemia, hypertension and heart disease. Anagrelide is used in the treatment of essential thrombocytosis (overproduction of blood platelets) and has also been used for treating chronic myeloid leukaemia.\(^\text{121}\) Its congener, quazinone is a PDE3 inhibitor used for the treatment of heart disease. Quinethazone is a thiazide-type diuretic used in the treatment of hypertension,\(^\text{122}\) Fig. 3.2.1.

![Fig. 3.2.1](image-url)
3.2.1 Synthesis from triazoline intermediates

In 1999 Erba and co-workers published a method for the synthesis of 2-alkylquinazolines from triazolines, Scheme 3.2.1.1. Synthesis of the triazoline intermediates began by reacting a functionalised aldehyde with morpholine in toluene at room temperature to generate the enamine 91 and subsequent reaction with an aryl azide to generate triazoline 92.

Cyclisation to 2-alkylquinazolines 93 was afforded by treatment of 92 with either a saturated ethanolic solution of ammonia at 150 °C, or in ammonium acetate in refluxing toluene. Yields for this reaction varied from excellent 93a (92%) and 93b (95%) to moderate for 93c (38%) and 93d (37%). Overall this synthesis proved to be a robust and reliable method for the synthesis of 2-substituted quinazolines that possess electron-withdrawing groups at the 6-position.

![Scheme 3.2.1.1](image-url)
3.2.2 Reaction of amidines with 2-fluorobenzaldehydes

In 1999 Kotsuki et al. published their work on the reaction of cyano/nitro activated o-fluorobenzaldehydes with a variety of arylamidines to generate 2-aryl quinazolines in moderate yields after chromatography, Scheme 3.2.2.1. The reaction involves the condensation of o-fluorobenzaldehydes 94 with aryl amidines resulting in the formation of imines 95 followed by a nucleophilic aromatic substitution at the ortho-position in the presence of potassium carbonate in acetonitrile at reflux to generate quinazolines 96.

![Scheme 3.2.2.1]

R = 4-C_{10}H_{11}, 62%
4-(p-FC_6H_4)C_6H_4, 55%
4-BnOC_6H_4, 57%
4-BrC_6H_4, 69%
n-Bu, 67%

3.3 Synthesis of 4-substituted quinazolines

Some of the most promising 4-substituted quinazolines synthesised to date are shown in Fig. 3.3.1. In 2003 Iressa™ was the first epidermal growth factor receptor inhibitor, for the treatment of lung cancer. Similar compounds such as afatinib and dacomitinib are currently undergoing phase III clinical trials, Fig 3.3.1. The prevalence of this family of compounds in chemotherapy was previously highlighted in Section 1.2.
3.3.1 Derivatisation of 4(3H)-quinazolinones

Conversion of 4(3H)-quinazolinones to 4-chloroquinazolines is well documented in the literature and is most commonly achieved by treating a quinazolin-4-one with POCl₃ or thionyl chloride. An alternative synthesis was published by Sugimoto et al., which involves the use of a phosphonium salt of N-chlorosuccinimide, 97 in refluxing dioxane to give good yields of 4-chloroquinazolines, 98, Scheme 3.3.1. 4-Chloroquinazolines are very versatile intermediates as they can be derivatised further through nucleophilic attack at the C-4 position.
The 4-position can also be activated using a thiomethyl substituent as reported by Rewcastle et al.\textsuperscript{126} Treatment of quinazolinone \textbf{99} with Lawesson’s reagent affords quinazolinethione, \textbf{100} which is converted to the thiomethyl ether \textbf{101} by treatment with potassium hydroxide and iodomethane. Displacement of the thioether with a nucleophile affords the 4-substituted quinazoline \textbf{102}, in good yield, \textit{Scheme 3.3.1.2}. 4-Arylaminoquinazolines are of particular interest due to their potential as antitumour agents, \textit{Scheme 3.3.2.2}.\textsuperscript{113}

\begin{center}
\begin{tikzpicture}

\t\node[draw, rectangle] (a) at (0,0) {\textbf{99}}; \node[draw, rectangle, right of=a, node distance=1.5cm] (b) {\textbf{100}}; \node[draw, rectangle, right of=b, node distance=1.5cm] (c) {\textbf{101}}; \node[draw, rectangle, below of=b, node distance=1.5cm] (d) {\textbf{102}};

\t\draw[-stealth] (a) -- node[above] {P$_2$S$_5$, pyridine, reflux\hspace{1cm}16h, 59\%} (b);
\t\draw[-stealth] (b) -- node[above] {Me$_3$KOH, MeOH, r.f.\hspace{1cm}16h, 54\%} (c);
\t\draw[-stealth] (c) -- node[above] {3-BrC$_6$H$_4$NH$_2$HCl\hspace{1cm}IP$_3$OH, reflux\hspace{1cm}6h, 85\%} (d);
\end{tikzpicture}
\end{center}

\textit{Scheme 3.3.1.2}

As well as useful intermediates 4-thioquinazolines also garnered some attention as potential antifungal agents. Xu and co-workers synthesised a range of 6-fluoro-4-alkylthioquinazolines with derivatives containing 4-thioallyl, 4-thio-\textit{n}-propyl and 4-thioethyl showing good antifungal activity.\textsuperscript{129} Synthesis began by reacting 2-amino-5-fluorobenzoic acid \textbf{103} with formamide to generate the quinazolinone \textbf{104}. Thiol \textbf{105} was afforded from treatment of \textbf{104} with Lawesson’s reagent. Alkylthioquinazolines \textbf{106} were synthesised in good to excellent yield by treating \textbf{105} with a number of alkyl halides under phase-transfer conditions, \textit{Scheme 3.3.1.3}. 

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3.3.2 Reaction of anilines with 2-aminobenzonitrile

4-Anilinoquinazolines can also be synthesised from the reaction of 2-aminobenzonitrile \textbf{107} and anilines \textbf{108} as detailed by Szczepankiewicz et al.\textsuperscript{130,131} These reactions proceed via amidines \textbf{109} which are heated with 85\% formic acid to give 4-arylaminoquinazolines \textbf{110} in good yields, \textit{Scheme 3.3.2.1}.

Compounds of similar structure were reported by Foote et al. which exhibited anti-tumour activity, \textbf{111}, \textit{Fig. 3.3.2.2}.\textsuperscript{132} Vasdev and co-workers published work on \textsuperscript{18}F labelled 4-anilinoquinazolines as potential EGFR imaging probes, \textbf{112}, \textit{Fig. 3.3.2.2}.\textsuperscript{133}
4-Anilinoquinazolines have also been studied as potential tyrosine kinase inhibitors by pharmaceutical companies, AstraZeneca\textsuperscript{134} and Qilu, \textbf{Fig. 3.3.2.2}.\textsuperscript{135,136}

\textbf{Fig. 3.3.2.2}

Tsou \textit{et al.} reported an efficient method for the synthesis of 4-anilinoquinazolines where incorporation of the 4-anilino group and ring closure were achieved in one step.\textsuperscript{137} The first step of the reaction involved the condensation of 2-amino-5-nitrobenzonitrile \textbf{114} with DMF dimethylacetal. Refluxing the resultant imine \textbf{115} in acetic acid with 3-bromoaniline gave the desired compound \textbf{116} in an 89\% yield, \textbf{Scheme 3.3.2.3}. A similar method was reported by Yoon \textit{et al.} using microwave conditions to generate substituted 4-aminoquinazolines in excellent yield.\textsuperscript{138}

\textbf{Scheme 3.3.2.3}
3.3.3 Palladium mediated quinazoline synthesis

A novel method for synthesising 4-substituted quinazolines involving a palladium catalysed reaction was developed by Akazome et al.\textsuperscript{139} The reaction involves an intermolecular reductive $N$-heterocyclisation between 2-nitrophenyl ketones \textbf{117} and formamide to give a variety of 4-substituted quinazolines \textbf{118}, \textit{Scheme 3.3.3.1}. It was speculated by the authors that the reaction proceeds \textit{via} an active nitrene intermediate which was generated by selective deoxygenation of the nitro group by carbon monoxide.

![Scheme 3.3.3.1](image)

\textit{Scheme 3.3.3.1}

3.4 Synthesis of 2,4-disubstituted quinazolines

There are many examples of 2,4-disubstituted quinazolines in medicine, three of which are highlighted in \textit{Fig. 3.4.1}. Bunazosin was initially developed to treat benign prostatic hyperplasia.\textsuperscript{140} Its congener prazosin is an alpha-adrenergic antagonist used for the treatment of high blood pressure, anxiety and panic disorder.\textsuperscript{141} Linagliptin is a 2,4-disubstituted quinazoline which was approved in 2011 for the treatment of type-II diabetes, which acts by inhibiting DPP-4.\textsuperscript{142}
3.4.1 Reactivity of 2,4-dichloroquinazolines

As was mentioned in Section 3.3.1, chloroquinazolines serve as useful intermediates when derivatising quinazolines. 2,4-Dichloroquinazolines can be accessed in good yields from chlorinating the corresponding quinazoline-2,4-dione using phosphorous oxychloride or thionyl chloride. Due to the increased electrophilicity of the 4-position, regiospecific substitution can be achieved by nucleophilic substitution. Lee et al. exploited this regioselectivity in the synthesis of potential phosphodiesterase inhibitors, Scheme 3.4.1.1.143
Quinazoline-2,4-dione 119 was prepared from anthranilamide using phosgene or anthranilic acid using potassium isocyanate followed by cyclisation. 2,4-Dichloroquinazoline 120 was furnished by refluxing 119 in POCl₃. The 4-position was then selectively aminated using benzylamine. An imidazole moiety was subsequently introduced at the 2-position by heating 121 with excess imidazole to give 122 in a 63% yield. The inherent reactivity of both the 2 and 4 positions allows expansive diversification of the quinazoline pharmacophore in a regioselective manner.

Undheim et al. investigated the use of trialkylalanes in palladium catalysed coupling reactions, Fig. 3.4.1.2. Synthesis of 2-chloro-4-methylquinazoline, 123 is achieved in a 76% yield. The mechanism involves the oxidative addition at the more electrophilic 4-position of 120 to the palladium (0) complex. The methyl group is transferred to the palladium (II) complex from the aluminium and subsequent reductive elimination generates 123 in good yield. Repeating the process using tri-isobutylalane affords 2-isobutyl-4-methylquinoline, 124 in an 80% yield. This method provides a regiospecific route to bioactive 2,4-disubstituted quinazolines.

Scheme 3.4.1.1
3.4.2 Chlorination of quinazoline-2,4-dione in the presence of cyclic amines

As was alluded to in Section 3.4.1 the 4-position of 120 is more reactive towards nucleophiles than the 2-position. Based on work initially carried out by Miki, Yoshida et al. published work which demonstrated that the 2-position could be selectively substituted using a tertiary cyclic amine in the presence of POCl₃ in good yield. This work was conducted while developing a more frugal route to the potential anti-dementia drug, 2-(4’-allylpiperazin-1-yl)-4-pentyloxyquinazoline. Reaction of quinazoline-2,4-dione 119 and 1,4-diallylpiperazine 125 afforded the key intermediate, 2-(4’-allylpiperazin-1-yl)-4-chloroquinazoline 126, Scheme 3.4.2.1. If a primary or secondary amine is used in place of a tertiary the reaction proceeds typically with substitution occurring at the more favoured 4-position.
3.4.3 Synthesis of 2,4-diaminoquinazolines

Zielenski et al. reported the synthesis of 2,4-diaminoquinazolines from chloroamidines and dialkylcyanamides. The chloroamidines were synthesised in two steps from substituted phenyl isocyanates via reaction with N,N-diethylamine to afford substituted ureas. Treatment with phosphorous pentachloride generated the chloroamidines 127. Reaction with N,N-dimethylcyanamide followed by cyclisation furnished 2,4-diaminoquinazolines 128, Scheme 3.4.3.1. Although a wide variety of substituted phenylisocyanates were tolerated, purification of the quinazolines proved difficult in some cases leading to lower yields.

![Scheme 3.4.3.1](image)

**Scheme 3.4.3.1**

Wilson et al. reported the synthesis of 2,4-disubstituted quinazolines using a resin bound isothiocyanate 129 generated from the parent carboxystyrene resin. Their investigation began with the synthesis of the antihypertensive drug prazosin, which was achieved in three steps from 129. 2-Amino-4,5-dimethoxybenzonitrile was dissolved in NMP, added to the resin and stirred for 3 hours to generate 130. Treatment of the intermediate with 1-(2-furoyl)-piperazine and EDC under basic conditions to furnish the resin bound guanidine 131. Cleavage and cyclisation was achieved using a mixture of trifluoroacetic acid and water yielding prosazin 132 as the TFA salt in a 24% overall yield, Scheme
### 3.4.3.2

This method offers an alternative to sequential chlorine displacement of 2,4-dichloroquinazolines employed by Lee et al.\(^{143}\)

![Scheme 3.4.3.2](image)

### 3.4.4 Rearrangement of triazolines to 2-alkyl-4-arylaminoquinazolines

Previous to his work on 2-substituted quinazolines (Scheme 3.2.1.1) Erba et al. reported the synthesis of 2-alkyl-4-anilinoquinazolines via the cyclisation of arylamines with amidines. Refluxing triazoline 133 in xylene induces thermal elimination of nitrogen followed by rearrangement to the tertiary amidine 134. Reaction of 134 with anilines afforded a range of 2-alkyl-4-anilinoquinazolines 135 in moderate to low yields, Scheme 3.4.4.1.\(^{149}\) The low yields of 135d and 135e, which could not be improved upon with longer reaction times, is due to the lower nucleophilicity of the substituted arylamines used for the cyclisation. Conversely 135c shows a higher yield due to the increased nucleophilicity of the aryl substituent. It also seems that the steric bulk of the R group influences yields, which is illustrated when comparing 135a and 135b.
3.4.5 Microwave synthesis of 2-substituted-4-aminoquinazolines

The synthesis of 2-substituted-4-aminoquinazolines was reported by Seijas and co-workers in 2000.\textsuperscript{150} 2-Aminobenzonitrile 136 was reacted with a variety of nitriles in the presence of potassium tert-butoxide under microwave conditions to give quinazolines 137 in excellent yield, \textit{Scheme 3.4.5.1}. These reactions represent a significant improvement in methodology not only because of their short reaction time but improved yields, catalytic amount of base and absence of solvent.

\textbf{Scheme 3.4.5.1}
3.4.6 Use of Grignard reagents

Bergman et al. demonstrated that when 2-aminobenzonitrile is reacted with Grignard reagents the resulting intermediate was useful in accessing a variety of quinazoline derivatives in good to excellent yields, when quenched with suitable electrophiles (acid chlorides, formates, oxalates and Viehe’s salt).\textsuperscript{151,152} It was found that when quenched with diethyl oxalate the quinazoline product generated was susceptible to reaction with a second mole of the intermediate leading to the formation of 2,2′-coupled bis-quinazoline, accounting for the lower yield of the desired product. This general approach for synthesis of 2,4-disubstituted quinazolines is a useful addition to existing procedures, given the range of available Grignard reagents, \textit{Scheme 3.4.6.1}.

\textit{Scheme 3.4.6.1}
3.5 Synthesis of Quinazoline-2,4-diones

There are many examples of this reaction type in the literature. Some of the more recent publications are discussed below which detail more bespoke preparations that display clear advantages over conventional methods.

3.5.1 Quinazoline-2,4-diones from aminobenzonitrile precursors

Mizuno et al. first described the synthesis of quinazoline-2,4-diones in 2000 using 2-aminobenzonitrile \textbf{138} and carbon dioxide in the presence of DBU, \textit{Scheme 3.5.1.1}\textsuperscript{153}. More recently the same group used supercritical carbon dioxide in place of organic solvents to synthesise a range of quinazoline-2,4-diones \textbf{138} in good to excellent yields.\textsuperscript{154} Gao et al. published similar work detailing the use of guanidines as catalysts for this reaction with very encouraging results.\textsuperscript{155} Skibo and Sung published work which included more conventional methods where an anthranilamide is treated with phosgene to generate compounds of type \textbf{139}. The preceding anthranilic acid can also be cyclised directly upon treatment with potassium isocyanate, \textit{Scheme 3.4.1.1}\textsuperscript{156,157}.

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\textbf{138} \hspace{1cm} \textbf{139}};
\draw[->,thick] (a) -- (a) node[midway,above] {DBU};
\draw[->,thick] (a) -- (a) node[midway,above] {CO$_2$};
\end{tikzpicture}
\end{center}

\textit{Scheme 3.5.1.1}

3.6 N-3 substituted quinazolinediones

The application of N-3 substituted quinazolinediones has been previously discussed in \textit{Section 1.2.2}, which highlights the promising anti-cancer activity of some recently described N-3 substituted quinazolinediones.
3.6.1 Baeyer-Villiger oxidation to 3-arylquinazoline-2,4-diones

Azizian et al. described the synthesis of 3-arylquinazoline-2,4-diones via the rearrangement of benzoxazinones.\textsuperscript{158} The procedure involves the oxidation of 3-arylimino-2-indolinones 140 with $m$-chloroperbenzoic acid at 0 °C to generate 141. The expected quinazolinedione 142 was formed in excellent yield after separation from impurities by flash chromatography, \textit{Scheme 3.6.1.1}. A range of quinazolinediones were synthesised demonstrating the versatility of this procedure. When the reaction was carried out in methanol it was found that the carbamate, 143 was returned in high yield. Cyclisation of 143 to the corresponding quinazolinedione 142 was afforded by heating 143 to its melting point.

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {$\text{140}$};
  \node (b) at (2,0) {$\text{141}$};
  \node (c) at (4,0) {$\text{142}$};
  \node (d) at (0,-2) {$\text{143}$};
  \node (e) at (2,-2) {$\text{143}$};
  \draw[->] (a) -- node[above] {$m$-CPBA, DCM, 0 °C} (b);
  \draw[->] (b) -- node[above] {$m$-CPBA, MeOH, 0 °C} (c);
  \draw[->] (c) -- node[above] {heat} (e);
\end{tikzpicture}
\end{center}

\textbf{Scheme 3.6.1.1}
3.6.2 Preparation using Appel’s salt

Kim et al. developed a facile synthesis of a range of 3-substituted-2-cyano-4(3H)-quinazolinones. Reaction of methyl anthranilate 144 with Appel’s salt 145 in the presence of pyridine returned dithiazolium 146 in a 50% yield. 3-Substituted-2-cyano-4(3H)-quinazolinones 147 are afforded by reacting primary alkylamines with dithiazolium 146. The nitrile group can readily be displaced by a variety of nucleophiles to generate a range of corresponding 2-substituted analogues. Hydrolysis of the nitrile offers a convenient method for synthesising N-3 substituted quinazolinediones 148 in moderate to good yields (R=Me, 56% overall), Scheme 3.6.2.1.

Scheme 3.6.2.1

3.6.3 Palladium-catalysed synthesis of N-3 substituted quinazoline-2,4-diones

Willis et al. developed an efficient synthesis of N-3 substituted quinazolinediones 148 by reacting methyl o-bromobenzoate 149 with mono-N-substituted ureas 150 in the presence of a palladium catalyst, Scheme 3.6.3.1. This method is tolerant of a wide variety of substituted ureas as well as both electron-donating and electron-withdrawing substituents on the benzene ring of 149. Regioselectivity was determined by comparison.
with existing literature, 2D NMR studies and conversion to known compounds. Willis speculated that selectivity is due to an initial arylation reaction followed by a ring-closing amidation reaction, with the arylation occurring at the least hindered nitrogen of the urea.

\[
\text{Scheme 3.6.3.1}
\]

Li et al. described the synthesis of analogous compounds from methyl anthranilate 144 using microwave conditions, Scheme 3.6.3.2. Initial use of THF as the solvent in these reactions led to dimerization of the urea, so DMF and DMSO were screened, however this lead to the isolation of unicyclised product. It was thought that a nucleophilic solvent may be necessary in order to facilitate the elimination of methanol so a 1:1 DMSO:H\textsubscript{2}O solvent system was employed which lead to the formation of 148 with minimal dimerization. A range of compounds were synthesised probing substitution patterns, electron distribution and steric hindrance. A variety of N-3 aryl derivatives were all synthesised in good to excellent yields, however, N-3 alkyl analogues returned low yields. Steric hindrance did prove problematic as was evident in the use of 2,6-diisopropylphenylisocyanate where no product was formed. Overall this method was proved to be a rapid and green alternative to Willis’ synthesis.

\[
\text{Scheme 3.6.3.2}
\]
3.7 \textit{N-1, N-3-Disubstituted quinazolinediones}

\textit{Section 1.2.3} and \textit{Section 1.2.4} highlight the relevance of \textit{N-1, N-3-disubstituted quinazolinediones} in the development of prospective chemotherapeutic agents, specifically in the design of peptidomimetics for use as anti-cancer agents and also as new therapies in the fight against antibiotic resistance.

3.7.1 Synthesis from anthranilate precursors

While searching for potential immunosuppressive and anti-inflammatory agents Michne \textit{et al.} described a method for synthesising \textit{N-1, N-3-disubstituted quinazolinediones}, \textit{Scheme 3.7.1.1}. Alkylation of 151 followed by the formation of the amide using methylamine gave 152. Reaction of 152 with phenyl chloroformate in the presence of sodium hydride followed by reduction gave 153 in a 51% overall yield.

\begin{center}
\textbf{Scheme 3.7.1.1}
\end{center}

Although not the most conventional method it does provide a viable route to \textit{N-1, N-3-disubstituted quinazolinediones}. More commonly \textit{N-3-substituted quinazolinediones} are prepared and the \textit{N-1} position is substituted using an alkylating/arylating agent in the presence of a base. These reactions usually give high to excellent yields and a range of nucleophiles can be used. Michne \textit{et al.} also described this method for compounds of type 153 as did Willis, \textit{Scheme 3.7.1.2}. 

\begin{center}
\end{center}
3.8 Conclusion

Quinazolines represent a family of compounds which possess extensive and diverse biological profiles and as a result have gained significant interest in the field of medicinal chemistry. As a result of this interest much research has been carried out into the development of efficient routes of synthesis. This interest has lead to the development of many prospective novel chemotherapeutic agents, perhaps the most pronounced in this area is the development of 4-anilinoquinazolines which are currently at the cutting edge of cancer chemotherapeutics.

In contrast elaboration of the quinazoline-2,4-dione pharmacophore remains an underdeveloped demesne of the quinazoline family. Given the beseeching biological modes of action attributed to a range of quinazoline-2,4-diones, the development of novel synthetic routes as well as elaboration of the pharmacophore is imperative in order to advance this area of chemotherapeutics.
4.0 Aims and Objectives
4.0 Aims and Objectives

4.1 Overview

As previously outlined in the introductory chapters, quinazolines and quinolines are perhaps some of the best known classes of biologically active compounds. Both are common in natural products and possess a wide range of pharmacological properties and are used in the treatment of diseases such as cancer, HIV, Alzheimer’s disease and fungal infection.

In spite of this extensive research of both moieties, the elaboration of pharmacophores remains unheralded to date. The amalgamation of both moieties into a highly functionalised novel template to generate a new class of hybrid small molecules, 1, Fig. 4.1, is also an area of research which is surprisingly unreported and forms the ultimate goal of this project. It is envisaged that 1 will possess significant bioactivity given the precedence of its components outlined in Section 1.1 and Section 1.2 and also the activity of congenerous tricycles (e.g. anthraquinone Pixantrone). In order to achieve this the synthesis will be divided into two routes based on which ring is formed last.
Chapter 4 | Aims and Objectives

Fig 4.1 Structure of novel pyridinoquinazinolinetetrone (PQT) scaffold 1 derived from quinazoline precursor 154 and quinoline precursor 155

Route A involves construction of the A-ring last which necessitates the synthesis of the quinazoline template 154. To date, the overall body of research shows no investigation into derivatising the N-1 (Y) and N-3 (X) positions of 154. The goal of Chapter 5.3 is to explore the derivative potential of 154 and probe the pharmacological significance of derivatisation at these positions.

Route C is concerned with synthesising the C-ring last. Firstly the quinoline scaffold 155 will be synthesised and from here an investigation into the derivatisation of 155 at the 4, 6 and 7-positions will be carried out as to date there is a paucity of literature in this area. The goal of Chapter 5.2 therefore is the synthesis of a series of novel quinoline derivatives.

4.2 Development of synthetic targets

The generation of a novel tricyclic template forms the basis of our investigation into new innovative drug therapies. Consequently, the ultimate goal of our synthetic strategy is to develop new routes towards PQT 1 and to synthesise novel precursors of biological interest.
Route A consists of the synthesis of substituted quinazolinediones 154 eventually incorporating the potential to cyclise the A-ring on the functionalised quinazolinedione 154. Initially, efforts will concentrate on the modification of the quinazolinedione moiety 154, a key precursor, at positions 1 and 3. As eluded to earlier, previous research in this area is very limited. Willis, Li and Michne documented the substitution of structurally similar compounds however the derivatisation of 154 is uncharted. Biological analysis of any novel compounds synthesised will be utilised to determine their chemotherapeutic potential.

![Structures of quinazolinedione 154 and novel pyridinoquinazolinedione 156]

Subsequent to the population of a library of functionalised quinazolinediones, our efforts will converge on affecting the incorporation of a pyridine moiety on the B-ring of 156 using methodology previously employed within our research group to generate novel azaindoles. Finally oxidation of the B-ring to generate 1 will be investigated.

Route C will focus on the modification of quinoline 155 to generate a series of novel substituted quinolinediones 157. These key intermediates have been found to possess an exceptional chemotherapeutic profile as was eluded to in previous studies by Wipf et al., who illustrated that these compounds possess excellent CdC25B phosphatase inhibitory activity and potential as anti-cancer agents.
Fig 4.3 Structures of aminoquinolinedione 157 and WDP1079

Subsequent to investigating the derivatisation of 157, focus will then centre on the formation of the pyrimidinedione C-ring to generate compounds of type 1.

4.3 Synthetic focus and application

The first stage of synthesis will centre on affecting a range of novel quinazolinediones to generate a molecularly diverse set of novel congeners. The route chosen for the synthesis starts with 2,5-dimethoxybenzoic acid 158 which is efficiently converted to the intermediate methyl-3,6-dimethoxy-2-aminobenzoate 159 in three steps and serves as the platform from which quinazolinediones 160 can be affected, Fig 4.4.165

Fig 4.4 Synthetic scheme illustrating the route to N-3 substituted quinazolinediones 160

Having synthesised a series of novel molecules of type 160, emphasis will turn to the synthesis of N-1, N-3 disubstituted quinazolinediones 161. Using these two series of novel compounds as substrates, modification of the quinazolinedione core will generate the
scaffold 156 from which to construct the pyridine A-ring ultimately leading to the novel tricyclic template 162.

![Diagram showing the synthesis process](image)

**Fig 4.4** Structures of N-3 substituted quinazolinedione 160 and N-1, N-3 disubstituted quinazolinedione 161

The second stage of synthesis involves development of a series of novel quinolinediones 157 en route to the synthesis of 1. This synthesis starts from 1,4-dimethoxybenzene 163 and leads to the generation of intermediate 86 in six steps in high yield, **Fig 4.5.**

![Diagram showing the synthetic scheme](image)

**Fig 4.5** Synthetic scheme of Route B towards novel quinolinediones 157 and ultimately 1

From 86 a series of novel quinolinediones can be affected initially generating 6-aminoquinolinediones which can be further derivatised to generate structures of type 157. As there is limited literature on compounds of this type efforts will focus on expanding this series to generate novel derivatives.
Finally an investigation into the construction of the pyrimidinedione C-ring will be explored using new methodology with the ultimate goal of developing a new route to PQT 1.

All novel compounds synthesised will be submitted to the NCI 60-cell line screen. This service is provided by the Developmental Therapeutics Program (DTP) at the US National Cancer Institute. It involves the screening of novel natural and synthetic compounds against 60 different human tumour cell lines, representing leukaemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate and kidney. This testing provides an overall picture of the cytotoxicity of a compound as well as any specificity the drug has for a particular cancer cell line.
5.0 Chemical Results and Discussion
Chapter 5 | Chemical Results and Discussion

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5.0 Chemical Results and Discussion

5.1 Outline of approach towards novel Pyridinoquinazolinetetrone derivatives

The original aim of our project was to create a new synthetic route to novel pyridinoquinazolinetetrones (PQTs) and investigate the derivatisation potential of this template to develop novel congeners of biological interest. Retrosynthetic analysis allowed the identification of two viable synthetic routes towards 1 from which it was envisaged that it would be possible to access a range of PQT derivatives, Scheme 5.1.1.

Scheme 5.1.1 Retrosynthetic analysis to access key versatile intermediates from which PQT 1 can be synthesised

In 2008 Wipf et al. outlined a synthesis to affect quinolinediones from 1,4-dimethoxyaniline in a series of high yielding steps, Scheme 5.1.2. This synthesis forms the basis of route C which will allow us to develop novel quinolinedione structures. The synthesis will be extended by further derivatising compounds synthesised by Wipf et al. and employing a variety of amines to generate novel quinolinediones which will investigate the effects of varying the substituent at the 6-position as well as derivatising these congeners to further understand the characteristics of this pharmacophore.
Scheme 5.1.2 Route towards quinolinediones as described by Wipf et al.\textsuperscript{28}

The basis for the development of route A has its origins in work carried out by Skibo \textit{et al.} where the synthesis of 6-nitro-5,8-dimethoxyquinazoline-2,4-(1\textit{H},3\textit{H})-dione 164 was afforded from 3,6-dimethoxy-2-nitrobenzamide 165, Scheme 5.1.3.\textsuperscript{166}
This synthesis was modified by us employing the intermediate methyl-3,6-dimethoxy-2-aminobenzoate 159 in place of 2-carboamoyl-3,6-dimethoxyaniline 166, Scheme 5.3.1.1 (page 140). Using this synthesis 159 is afforded in three steps from the commercially available 2,5-dimethoxybenzoic acid 158, whereas in Skibo’s synthesis the intermediate 166 was realised in five steps from gentisic acid. From this intermediate a new route will be developed to generate novel N-3 substituted quinazolidiones with a focus on synthesising a multitude of congeners to broaden our knowledge of motifs at the 3-position as well as serving as a platform for further derivatisation.

Both instances offer versatile routes to generate novel pyridinoquinazolinonetetrones with derivatives provided for from the use of highly functionalised substrates. The amino function present in both bicyclic precursors proffers a dexterous synthetic handle to construct the respective heterocycle affecting novel tricyclic congeners.
5.2 Quinolinedione Series

5.2.1 Synthesis of quinolinedione precursors

The following section outlines in detail the synthesis of precursors towards the generation of novel quinolinediones.

5.2.1.1 Nitration of 1,4-dimethoxybenzene

The first step in this synthesis was the nitration of 1,4-dimethoxybenzene 163 to generate 2-nitro-1,4-dimethoxybenzene 167, *Scheme 5.2.1.1*. This was achieved using a mixture of copper nitrate and acetic anhydride. Over the 3 hour course of the reaction the mixture turned from blue through green to yellow. Under these Menke conditions 167 was formed in an 82% yield with no evidence of polynitration. The melting point (70 – 71 °C) is in accordance with previously reported literature values (71 – 72 °C). The peaks at 1367 cm\(^{-1}\) and 1497 cm\(^{-1}\) in the IR spectrum are characteristic of an N-O stretch (symmetrical and asymmetrical respectively) of a nitro group. Addition of the nitro group gives rise to a well-defined characteristic set of aromatic signals in the \(^1\)H NMR. Both H-6 and H-3 are represented as doublets at 7.04 and 7.38 ppm respectively, each coupling to H-5 giving rise to ortho and meta coupling respectively (9.2 and 3.1 Hz). The proton at position-5 is represented as a doublet of doublets at 7.11 ppm.

5.2.1.2 Reduction of 2-nitro-1,4-dimethoxybenzene

This reaction was initially carried out via hydrogenation using palladium on carbon as the catalyst. The reaction proceeded well in these conditions however it was later decided to reduce 167 using an iron-mediated reduction, given that the reaction could be carried out on a greater scale using this method. The reaction proved highly reliable, consistently giving 2,5-dimethoxyaniline 168 in a 94% yield as a pure off-white solid, *Scheme 5.2.1.1*. Comparison of the \(^1\)H NMR spectra of 167 and 168 reveals the emergence of a broad
singlet at 3.78 ppm (integrating for two protons) in 168 coinciding with the successful conversion to an amine function. In addition The IR spectrum of 168 showed the absence of the characteristic N-O stretches (1367 cm\(^{-1}\) and 1497 cm\(^{-1}\)) and the presence of characteristic N-H stretches at 3368 and 3459 cm\(^{-1}\).

The reaction was commenced by heating 167 to reflux followed by the simultaneous addition of iron powder and iron (III) chloride. Due to this addition being carried out at reflux temperature an oversized flask was used in order to contain the vigour of the addition. Iron (III) chloride was weighed out immediately before addition due to its hygroscopic nature.\(^{168}\)

![Scheme 5.2.1.1](image)

5.2.1.3 Incorporation of Meldrum’s acid fragment

Treatment of 82 with Meldrum’s acid in the presence of triethyl orthoformate afforded the arylamino-methylene derivative 83 in an excellent yield of 88\% without the need for further purification, \textit{Scheme 5.2.1.1}. The presence of the Meldrum’s acid fragment was evident in the \(^{1}\text{H} \text{NMR}\) and is represented by a singlet at 1.75 ppm and a doublet at 8.63 ppm corresponding to the six hydrogens of the two methyl groups and the single
methylene hydrogen respectively, Fig 5.1. A significant shift was also observed for the amine proton moving from 3.78 ppm to 11.55 ppm as a result of the delocalising capacity of the dione as well as the integration diminishing from two to one. Mass spectrum analysis provided further proof of synthesis showing the parent molecular ion at 100% [308 m/z, (M+H)+].

![Fig 5.1 1H NMR of 5-(((2,5-dimethoxyphenyl)amino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione 83 measured in CDCl3 at 300 MHz](image)

5.2.1.4 Bromination of Meldrum’s acid derivative

Bromination of 83 was achieved by suspending 83 in glacial acetic acid at 5 °C followed by the addition of a bromine/acetic acid mixture to the suspension, Scheme 5.2.1.1. After complete addition the reaction was stirred for 30 minutes followed by precipitation of the product by the slow addition of ice water. Quick addition of the water resulted in the formation of a gum which proved difficult to work with and purify. Like the previous step, 84 was isolated by suction filtration as a pure green/yellow solid in an excellent yield. Comparison of the 1H NMR spectra for these two reactions shows a distinct difference in the aromatic region going from perspicuous splitting patterns for the aromatic protons of
83 to the presence of two singlets corresponding to the aromatic C(3)H and C(6)H protons at 7.18 ppm and 6.88 ppm respectively. The absence of evident coupling in the aromatic region corroborates that substitution occurred at the 4-position.

5.2.1.5 Pyridone annulation

The penultimate step in the formation of the intermediate 6-bromo-4-chloro-5,8-dimethoxyquinoline 86 involved the thermal cyclisation of bromo derivative 84 to generate the 6-bromo-5,8-dimethoxyquinolin-4(1H)-one 85, Scheme 5.2.1.1.

This method was first described by Echavarren et al. and involves the use of the high boiling point solvent diphenyl ether to reflux the reaction at 250 – 260 °C for 30 minutes. At high temperatures Meldrum’s acid is thermally unstable, leading to a pericyclic reaction which releases acetone and carbon dioxide, producing a highly electrophilic ketene which then undergoes electrophilic aromatic substitution to generate 85.

Early attempts of this reaction were unsuccessful and returned degraded starting material with no trace of product being formed. Due to the use of an oil bath temperature fluctuations lead to intermittent reflux resulting in the degradation of the Meldrum’s acid adduct without befalling intramolecular cyclisation leading to the recovery of a black tar like substance which was unidentifiable by all analytical techniques.

In order to achieve the consistent temperatures required for this reaction a heating mantle was used as a direct heat source. The reaction was refluxed for 30 minutes followed by cooling to room temperature. When the mantle was used the reaction could not be stirred using a stirring bar however the convection current generated upon heating proved sufficient to agitate the reaction. Once cooled the reaction was poured into hexane resulting in the precipitation of light brown crystals which were collected by suction filtration returning a 75% yield.
5.2.1.6 Quinoline formation

Treatment of 85 with POCl₃ resulted in the synthesis of intermediate 86 for the use in the synthesis of quinone derivatives of 86 outlined in Section 4.1 (page 88). The reaction was then quenched by adding the mixture to crushed ice portion-wise. Complete quenching of each aliquot and maintenance of ice temperature was necessary before addition of the next portion due to the exothermic nature of the reaction. Once quenched, the solution was neutralised using 2M aqueous NaOH and the crude product was extracted. Following purification 86 was isolated in a 78% yield as a light yellow solid, Scheme 5.2.1.1 (page 100).

The melting point (90 – 92 °C) correlated well with literature reports. Confirmation of forming 86 was evident by comparing the ¹H NMR with that of 85, noting that there was an absence of a broad singlet corresponding to H(1) of the pyridone moiety. Both H-2 and H-3 appear as doublets at 8.73 and 7.52 ppm respectively, the former being more deshielded due to the proximity of the pyridine nitrogen. Also, mass spectrum analysis revealed the parent molecular ion at 90% [303 m/z, (M+H)+] correlating with the presence of ³⁵Cl and 26% [301 m/z, (M+H)+] corresponding to ³⁷Cl.

With the establishment of successful methodology to generate a versatile intermediate, it was now possible to proceed to the next stage of synthesis which involved derivatising the 6-position of 86 to allow access to a series of novel quinolinediones of biological interest.

5.2.2 6-Substituted quinolines

The following section discusses in detail the synthesis of novel 6-substituted quinolines and 6-substituted quinolinediones utilising Stille conditions and methods outlined by Wipf et al. to form new C-C and C-N bonds at the 6-position. Also discussed in this section are
attempted methods of synthesising unprecedented 6-vinyl quinolinediones as precursors to pyridinoquinazolinetetrones 1.

5.2.2.1 Synthesis via Stille conditions

To introduce functionality at the 6-position it was decided to investigate the use of carbon-carbon bond forming reactions. Previously in our group similar functionalisation was utilised in order to effectuate 5-vinyl ellipticines. This work, carried out by Miller made this reaction an obvious choice for use on this system.\textsuperscript{170} The versatility of the vinyl group also made it an attractive target for synthesising novel derivatives as well as elucidating a route to C-ring cyclisation.

In order to effect this carbon-carbon bond forming methodology to generate novel 6-alkyl quinolines using Stille conditions it was first necessary to synthesise the substrates which were required for the reaction. First described in the early 1980’s the Stille cross-coupling involves the formation of a new C-C bond between an organostannane and an organic electrophile.\textsuperscript{171} The main advantages of using this method involve the easy preparation and stability of the organotin substrates as well as their tolerance of a wide variety of functional groups. The main disadvantage of this reaction however is that removal of all traces of tin can be difficult as will be seen later in this section. Despite this the Stille reaction is very useful and has found much use in organic synthesis (e.g. the synthesis of (+)-Mycotrienol, Ircinal A and Quadrigemine C).\textsuperscript{172-174}

Tributyl(vinyl) stannane 169 was synthesised by reacting vinyl magnesium bromide with tributyltin chloride in an anhydrous, inert atmosphere for 15 hours at room temperature,\textsuperscript{175} \textit{Scheme 5.2.2.1}.

\textbf{Scheme 5.2.2.1}
The crude product was purified by column chromatography (eluting with 50% ethyl acetate in hexane) to yield a malodourous, colourless oil in a 74% yield. The $^1$H NMR was consistent with the structure of 169 and previously reported data with the three butyl groups represented by a multiplet (0.89 - 1.68ppm), the terminal vinyl protons as a pair of doublets at 5.65 and 6.15ppm corresponding to the trans and cis protons respectively and finally a multiplet (6.14 – 6.53ppm) for the vinyl proton adjacent to the tin.

It was also necessary to synthesise the tetrakis(triphenylphosphine) palladium 170 catalyst for the reaction. This was achieved by reacting palladium chloride with triphenyl phosphine in DMSO in an anhydrous, inert atmosphere (140 °C for 15 minutes). Hydrazine hydrate was then added and the reaction was allowed to cool, Scheme 5.2.2.2. The resulting precipitate was filtered under an atmosphere of nitrogen and dried under vacuum for twelve hours. The catalyst was stored under nitrogen in the refrigerator owing to its labile nature. The material was analysed by melting point (94 – 96 °C, lit. melting point 84-85 °C, 95-105 °C 113-115 °C).176-178

\[
\text{PdCl}_2 \xrightarrow{i) \text{PPh}_3, DMSO\text{(anhyd.)}} \xrightarrow{\text{ii) NH}_2\text{NH}_2\cdot\text{H}_2\text{O.}} \text{Pd(PPh}_3\text{)}_4 170 86\%
\]

Scheme 5.2.2.2

Having synthesised the materials necessary for the reaction, 86 was added to a dry round-bottomed flask under nitrogen containing toluene. Following dissolution, 169 and 170 were added and the reaction was heated at reflux for 72 hours, Scheme 5.2.2.3.

\[
\begin{align*}
\text{86} & \xrightarrow{\text{Sn(nBu)}_3} \text{171} & 42\%  \\
\text{Pd(PPh}_3\text{)}_4, & \text{Toluene, reflux, 72 hrs}
\end{align*}
\]

Scheme 5.2.2.3
The cooled reaction mixture was then filtered through Celite® to remove the spent catalyst and the liquor was reduced to a crude gum. The gum was dissolved in ethyl acetate and a 5M aqueous solution of potassium fluoride was added. The biphasic mixture was vigorously stirred for one hour to remove the generated tributyltin bromide. Potassium fluoride reacts with tributyltin bromide resulting in the formation of the insoluble solid, tributyltin fluoride. After work-up the crude product was purified by column chromatography (eluting with 5% acetone in hexane). The desired product 171 was isolated as a low melting point (33 – 35 °C) pale yellow solid in a 42% yield. The $^1$H NMR of 171 revealed that despite careful purification remnants of tin still remained, Fig 5.2. Evidence for structure 171 was provided for by the $^{13}$C NMR where the vinyl carbons were represented present at 115.48 and 129.85ppm corresponding to the terminal and internal carbons respectively. Column chromatography was carried out twice more and while a reduction in the presence of tin was seen, disappointingly it was never completely removed and in order to conserve material it was utilised with slight remnants of tin present. Future investigations could explore the use of other stannanes or palladium catalysed reactions to synthesise 171.
5.2.2.2 Attempted oxidative demethylation of 4-chloro-5,8-dimethoxy-6-vinylquinoline

Following the successful synthesis of 171 attention was then turned to effecting the oxidative demethylation to generate 172. Existing methodology to synthesise 6-amino quinolinediones was initially used to try to generate the oxidation product. Initially the reaction was attempted using acetonitrile as the solvent however 171 was found to be only sparingly soluble in this medium. Following solubility tests it was decided to attempt the reaction in methanol. Following dissolution of 171 in methanol the solution was then cooled to 0 °C. An aqueous solution of cerium ammonium nitrate was then added portionwise, Scheme 5.2.2.4.

Scheme 5.2.2.4

The reaction was then allowed to warm to room temperature over four hours. Upon workup, analysis of the crude product showed that the desired quinolinedione 172 had not been formed and only unreacted starting material was recovered despite many attempts using various reaction conditions, Section 5.3.3.4. This outcome was disappointing given this protocol works well for the 6-bromo substrate 86. Ultimately failure to convert 171 to the quinolinedione undermined the synthesis of the C-ring of 1 via this route.
5.2.3 Synthesis of 4-substituted quinolines

As attempts to generate 6-vinyl quinolinedione 172 were unsuccessful our attention turned to investigating substitution at the four position via nucleophilic displacement of the chlorine. Preliminary investigations were directed at synthesising a series of alkyl ethers in order to generate a range of congeners that investigates the molecular flexibility of the four position, Section 3.1, Fig. 5.3.

![Fig. 5.3](image)

5.2.3.1 Synthesis of 4,5,8-trimethoxy-6-vinylquinoline

For this series of reactions it was first necessary to generate the alkoxide for use in the reaction. A 25% solution of sodium methoxide in methanol was generated by adding sodium metal portionwise to methanol at 0 °C under an atmosphere of nitrogen. Once all the sodium metal had dissolved the solution was allowed to warm to room temperature. Quinoline 171 was then added and the reaction was refluxed for one hour, Scheme 5.2.3.1.

![Scheme 5.2.3.1](image)
After workup the crude product was purified by column chromatography (eluting with 100% ethyl acetate) to give an 82% yield of 173 as a pale yellow oil. Confirmation of synthesis was afforded by mass spectrometry, showing the parent molecular ion at 100% [246 m/z, (M+H)+] and NMR. The 1H NMR clearly shows the presence of three peaks between 3.70 and 4.20 ppm corresponding to nine methyl protons. The presence of an extra carbon signal in the 13C NMR at 55.98 ppm is further confirmation that 173 was afforded.

5.2.3.2 Synthesis of 4-ethoxy-5,8-dimethoxy-6-vinylquinoline

Next, sodium ethoxide was used in order to synthesise 174. For preparation of sodium ethoxide it was not necessary to cool the ethanol to 0 °C. The rate of reaction is sufficiently slow that the heat generated at room temperature was not an issue. After complete dissolution of the sodium metal, 171 was added to the 25% sodium ethoxide solution and heated at reflux for one hour, Scheme 5.2.3.2.

Following workup the crude material was purified using the same protocol as previously outlined to give 174 as a pure pale yellow oil in a moderate yield of 44%. When compared to 173 it can be seen that there is a significant drop in yield which may be attributed to the diminished nucleophilic nature of sodium ethoxide. Proof of synthesis was afforded by mass spectrometry and NMR. The 1H NMR shows a triplet (integrating for three protons) at 1.53 ppm and a quartet (integrating for two protons) at 4.17 ppm. Also the DEPT 135 13C NMR reveals two new signals, the first at 14.45 ppm and the second in the
negative phase at 64.64 ppm corresponding to the CH₃ and CH₂ of the ethoxy group respectively. The ¹H NMRs of 174 and also 173 still showed slight vestiges of tin, testament to its persistent nature.

5.2.3.3 Synthesis of 4-butoxy-5,8-dimethoxy-6-vinylquinoline

Extending the series gave rise to the n-butoxy derivative 175. Sodium metal was added portionwise to refluxing n-butanol under a nitrogen atmosphere. After complete dissolution 171 was added to the 25% sodium n-butoxide solution and heated at reflux for one hour. Purification was achieved by column chromatography (eluting with 100% ethyl acetate) to give 175 as a pale yellow oil in a low yield of 14%, Scheme 5.2.3.3.

![Scheme 5.2.3.3](image)

The low yield is not entirely surprising as the n-butoxide anion is a weak nucleophile. As well as the presence of tin residues there were also additional impurities which despite careful purification could not be removed, Fig 5.4. Highlighted in blue between 0.5 and 1.5 ppm the persistent nature of the tin substrate used in the preceding coupling can be seen. Furthermore, there are also three additional signals between 3.0 and 3.7 ppm which are not characteristic of 175, and were calculated to be present in a 10% concentration.
5.2.3.4 Synthesis of 4-isopropoxy-5,8-dimethoxy-6-vinylquinoline

The final compound of this series was generated by reacting a 25% solution of sodium isopropoxide in isopropanol and 171 at reflux for one hour. The sodium isopropoxide solution was generated by refluxing sodium metal in isopropanol until all the sodium was dissolved. Following workup the crude product was purified by column chromatography (eluting with 100% ethyl acetate) to give 176 as a pale yellow oil in a 46% yield, Scheme 5.2.3.4.
Mass spectrometry provided proof of synthesis and analysis of the $^1$H NMR revealed incorporation of the isopropoxy group due to the presence of a doublet at 1.51 ppm and a septet at 4.84 ppm integrating for six and one proton respectively. Similar to the previous reaction, careful purification failed to remove all traces of tin impurity which lead to the isolation of 176 in 90% purity (assigned by $^1$H NMR).

5.2.4 Synthesis of 6-Aminoquinolinediones

Having explicated the methodology to modify the four position attention was then turned to developing on the six position. Preliminary work carried out in this area lead to the synthesis of 6-vinyl quinoline 171 however the oxidation product could not be effected, Section 5.2.2.1 (page 104) and Section 5.2.2.2 (page 107) and hence a change of strategy was necessary. In order to generate 6-functionalised novel compounds containing a quinoline-5,8-dione backbone, the work of Wipf et al. was elaborated on to generate a series of novel 6-aminoquinolone-5,8-diones to probe the molecular tolerances of the six position, Fig. 5.5.28 These aminoquinolinediones also served as novel substrates for further derivatisation to highly bioactive antineoplastics.

![Fig. 5.5](image.png)

5.2.4.1 Synthesis of 4-chloro-6-(2-morpholin-4-yl-ethylamino)quinoline-5,8-dione

Synthesis of 87 was afforded via a two-step reaction from 86. The first step was oxidative demethylation of 86 using cerium(IV) ammonium nitrate which yielded 4-chloro-6-
bromoquinoline-5,8-dione, 177. This product was isolated crude before being immediately dissolved in ethanol and cerium(III) chloride heptahydrate was added. The golden solution immediately turned dark brown and following complete dissolution reacted with 4-(2-aminoethyl)morpholine, Scheme 5.2.4.1.

Scheme 5.2.4.1

The reaction was stirred at room temperature overnight and following work-up was purified by column chromatography to give the 6-aminoquinolinedione 87 as a red solid in a 44% yield over the two steps. The analytical data obtained was in good agreement with previously reported literature. Having duplicated the results published by Wipf et al. a series of amines was selected to serve as molecular probes.28

5.2.4.2 Synthesis of 4-chloro-6-(methylamino)quinoline-5,8-dione

The first of these novel amines was methylamine. The reaction was carried out in the same manner as previously except methylamine (33% in water) was used to generate the desired amine, Scheme 5.2.4.2.
Following work-up and purification 178 was isolated as a red solid in a 43% overall yield, comparing closely to that of 87. Structural confirmation was provided for by NMR and also high resolution mass spectrometry which showed the parent molecular ion at 40% [223.0272 m/z, (M+H)^+], corresponding to C_{10}H_{7}N_{2}O_{3}^{35}\text{Cl}.

5.2.4.3 Attempted synthesis of 4-chloro-6-(4-(2-[(4-chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino]ethyl)piperazin-1-yl)quinoline-5,8-dione

The next reaction in the syntheses of 6-aminoquinolinediones was to incorporate an amino ethyl piperazine moiety at the 6-position. The reaction was carried out in the same manner as before however the desired product was not formed. Instead what was seen was the formation of 179 as a result of the terminal nitrogen on the piperazine ring displacing the bromine on a second mole of 177 to generate 179, Scheme 5.2.4.3. This isn’t entirely surprising considering the nucleophilic nature of the terminal secondary amine.

![Scheme 5.2.4.3](image)

*Scheme 5.2.4.3*

The product was purified by column chromatography eluting with DCM:MeOH (9:1) to give a red solid in a moderate 24% overall yield, considering the maximum yield was 50%. The aromatic region of the 1H NMR showed pairs of signals for H(2)/H(2') and H(7)/H(7'). The H(7)/H(7') protons are represented by two singlets at 5.91 ppm and 6.13 ppm. A pair of doublets at 8.79 ppm and 8.93 ppm represented H(2)/H(2'). The H(3)/H(3') protons however did not resolve into two doublets instead being represented by a single doublet,
Fig. 5.6. Similar results were seen in the $^{13}$C NMR where corresponding carbons on each ring system resulted in a similar pattern.

![Chemical Structure](image)

**Fig. 5.6 $^1$H NMR of 4-chloro-6-(4-{2-[(4-chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino]ethyl)piperazin-1-yl})quinoline-5,8-dione 179 measured in CDCl$_3$ at 300 MHz**

High resolution mass spectrometry analysis provided further structural confirmation showing the parent molecular ion at 100% [512.0893 m/z, (M+H)$^+$] corresponding to C$_{24}$H$_{19}$N$_5$O$_4$Cl$_2$.

**5.2.4.4 Synthesis of 4-chloro-6-{[2-(piperidin-1-yl)ethyl]amino}quinoline-5,8-dione**

The last of the cyclic amines to be incorporated on to the 6-position was piperidine. Quinoline 180 was prepared by reacting the crude quinolinedione 177 with 1-(2-aminoethyl)piperidine in the same fashion as previously outlined, *Scheme 5.2.4.4.*

![Chemical Reaction](image)
The crude product was purified by column chromatography eluting with DCM:MeOH (9:1) to give the desired product as a red solid in a low to moderate yield of 34%. The aliphatic region of the $^1\text{H}$ NMR corresponds to the presence of the piperidine moiety showing well defined signals for each set of protons, Fig. 5.7.

![Fig. 5.7 $^1\text{H}$ NMR of 4-chloro-6-[[2-(piperidin-1-yl)ethyl]amino]quinoline-5,8-dione 180 measured in CDCl₃ at 300 MHz with the expansion showing the piperidinyl region](image)

The two C(4’) protons are expressed as a multiplet at 1.48 ppm. The quintet at 1.62 ppm represents the four C(3’) and C(5’) protons and the four C(2’) and C(6’) protons are expressed as a triplet at 2.44 ppm. Mass spectrometry analysis further confirmed the desired compound which was represented by the presence of two peaks of relative intensity 3:1 corresponding to the $^{35}\text{Cl}$ and $^{37}\text{Cl}$ isotope at 98% [320 m/z, (M+H)$^+$] and 32% [322 m/z, (M+H)$^+$].

5.2.4.5 Synthesis of 4-chloro-6-[[2-(dimethylamino)ethyl]amino]quinoline-5,8-dione

The investigation of incorporating an acyclic tertiary amine was investigated also as part of the study. This was achieved by employing $N,N$-dimethylethlenediamine as the amine,
which was added (1.1 eq.) to the cerium(III) chloride quinolinedione solution and stirred at room temperature for 16 hours, \textbf{Scheme 5.2.4.5}. Following the work-up the crude quinolinedione was purified by column chromatography to give the desired compound \textit{181} in a 24% overall yield.

\begin{center}
\includegraphics[width=0.5\textwidth]{reaction_scheme}
\end{center}

\textit{Scheme 5.2.4.5}

Structural confirmation was provided by $^1$H NMR analysis which showed a new singlet at 2.29ppm which integrated for six protons indicating the two methyl groups of the amine, as well as the characteristic signals of the four hydrogens of the ethyl chain. High resolution mass spectrometry analysis provided further structural confirmation showing the parent molecular ion at 100\% \([(M+H)^+], \text{280.0844 m/z}\) corresponding to C$_{13}$H$_{15}$N$_3$O$_2$Cl.

\textbf{5.2.4.6 Synthesis of 6-(benzylamino)-4-chloroquinoline-5,8-dione}

In order to effectuate the 6-aminobenzyl derivative \textit{182}, benzylamine was added to a stirring solution of quinolinedione \textit{177} and cerium(III) chloride, with the resulting mixture being allowed to stir for 16 hours at room temperature. Isolation of the benzyl derivative \textit{182} in a 28\% yield from the crude reaction residue was achieved by column chromatography (10\% methanol in DCM), \textbf{Scheme 5.2.4.6}. The incorporation of the benzyl moiety is clearly evident in NMR spectra. The $^1$H NMR reveals a five hydrogen multiplet between 7.30 and 7.41ppm corresponding to the five aromatic protons of the benzyl moiety, as well as a doublet at 4.41ppm integrating for two protons indicative of the CH$_2$
group. The mass spectrum provided further proof showing the parent molecular ion at 100% [299 m/z, (M+H)^+].

Scheme 5.2.4.6

Through the elaboration of work carried out by Wipf et al. a novel series of JUN-1120-2 congeners was successfully synthesised. The biological activity of these compounds will be examined by screening against the NCI 60-cell screen as well as serving as substrates for further derivatisation Section 6.2.

5.2.5 Synthesis of 7-Halo-6-aminoquinolinediones

The next stage of synthesis in the project involves utilising the novel quinolinediones which were generated as well as JUN 1120-2 to functionalise the 7-position with halides as their introduction leads to highly biologically active compounds e.g. WDP1079, Fig. 5.8. Initially syntheses will concentrate on incorporating either bromine or iodine at the seven position given the potential bioactivity and synthetic handle.

Fig. 5.8
5.2.5.1 Iodination of 4-chloro-6-(2-morpholin-4-yl-ethylamino)quinoline-5,8-dione

To synthesise the iodo derivative of WDP1079, 87 was dissolved in methanol which unlike documentation by Wipf, required heating.\(^{28}\) Once the solution returned to room temperature \(N\)-iodosuccinimide was added and stirring ensued for 16 hours, **Scheme 5.2.5.1**. Following work-up the crude product was purified by column chromatography (eluting with DCM:methanol (9:1)) to give 183 in an excellent yield of 91% as a deep red solid.

![Scheme 5.2.5.1](image)

**Scheme 5.2.5.1**

Analysis of the \(^1\)H NMR revealed that the singlet at 5.90 ppm which correlated for the H-7 proton of 87 was absent, **Fig 5.8** this coupled with high resolution mass spectrum analysis, which showed the parent molecular ion at 100% \([447.9925 \text{ m/z, (M+H)}^+]\) corresponding to \(C_{15}H_{15}N_{3}O_{3}\) \(^{35}\)Cl\(^{127}\) confirmed the incorporation of iodine.
Fig 5.8 $^1$H NMR of 4-chloro-7-iodo-6-(2-morpholin-4-yl-ethylamino)quinoline-5,8-dione carried out in CDCl$_3$ at 400 MHz

In order to assess the purity of 7-halo quinolinedione derivatives, samples were subjected to LC-MS. At the time of synthesis high resolution mass spectrometry and NMR of the reaction product determined that 183 was synthesised. Under HRMS conditions fragmentation of the iodine was observed to some extent evident by the mass peak of 320.0800 m/z. LC-MS analysis carried out some time later failed to show the presence of iodine in the structure evident by the single peak at 2.15 mins in the HPLC trace corresponding to the mass 320.0811 m/z, Fig 5.9. From this it appears that over time (> 3 months) or under HPLC-MS conditions that 183 degrades with the loss of iodine.
5.2.5.2 Iodination of 4-chloro-6-(methylamino)quinoline-5,8-dione

To investigate the effect of varying the amino side chain on the activity of these compounds the ethyl morpholine moiety of WDP1079 was replaced with a methyl group. Reaction of 178 with N-iodosuccinimide (1 eq.) at room temperature for 16 hours lead to the formation of 4-chloro-7-iodo-6-(methylamino)quinolone-5,8-dione 184. The reaction was carried out in methanol and required a large volume (120 mL) to dissolve 178 (112 mg). Following purification of the crude product by column chromatography (eluting with 100% ethyl acetate) 184 was isolated in a 40% yield as a red solid, Scheme 5.2.5.2.
TLC analysis of the crude product had revealed the presence of two products. The second product, 7-iodo-4-methoxy-6-(methylamino)quinoline-5,8-dione 185 was returned as a red solid in a 28% yield after column chromatography (eluting with DCM:methanol (9:1)). Interestingly this was not seen by Wipf et al. or when synthesising the morpholine derivative 183 of this compound, despite the use of methanol as the solvent in all cases. Structural confirmation of 184 was provided for by $^1$H NMR which revealed an absence of the singlet for the C-7 proton. Also, mass spectral analysis of both compounds revealed the presence of molecular ions at 100%, [349.0 m/z, (M+H)$^+$] and 100%, [345.0 m/z, (M+H)$^+$] corresponding to 184 and 185 respectively.

Illustrated in Fig. 5.10 is a comparative stacked plot of the $^1$H NMR spectra of 184 and 185. Although it is conclusive that the desired compounds are represented by this $^1$H NMR data it can also be seen that both compounds are not entirely pure. Due to the quantities of both congeners isolated it also proved onerous to obtain empirical $^{13}$C NMR analysis.
Given this and the fact that \textbf{183} exhibited deterioration the purity of \textbf{184} was assessed under LC-MS conditions. Analysis returned a single peak which eluted at 3.37 mins and corresponded to a mass of 205.0580 consonant with the molecular formula C_{10}H_{9}N_{2}O_{3}, arising from fragmentation of iodine and hydrolysis of the chlorine at the 4-position \textbf{Fig 5.11} and \textbf{Fig 5.12}.

\textit{Fig 5.10} Stacked $^1$H NMR plot of \textbf{184} and \textbf{185} measured in CDCl$_3$ at 300MHz.
Chemical Results and Discussion

Fig 5.11 LC-MS trace of 184

![LC-MS trace of 184](image)

Fig 5.12 Fragmentation profile of 184

![Fragmentation profile of 184](image)

LC-MS analysis was also carried out on 185 revealing the parent ion at 3.57 mins, which despite being broad was the only peak present in the trace, Fig 5.13. From this it can be seen that 185 is more stable than 184 under HPLC-MS conditions and does not undergo hydrolysis at the 4-position as a result of the diminished electrophilicity of the 4-position due to the presence of the methoxy function.
5.2.5.3 Iodination of 4-Chloro-6-(benzylamino)quinoline-5,8-dione

The synthesis of 186 was achieved by adding N-iodosuccinimide to a solution of 182 in methanol at room temperature, Scheme 5.2.5.3. Following reaction for 16 hours the crude product was purified by column chromatography eluting with 100% ethyl acetate to give the desired product in a 39% yield as a deep red solid. The incorporation of iodine was confirmed by mass spectrometry which displayed the parent molecular ion at 100% [425 m/z, (M+H)+] correlating with the presence of the $^{35}$Cl isotope, the $^{37}$Cl isotope was also present at 28% [427 m/z, (M+H)+].
High resolution mass spectrometry analysis of the second isolated product was shown to be 6-(benzylamino)-7-iodo-4-methoxyquinoline-5,8-dione 187. The parent molecular ion was present at 100% \([421.0035 \text{ m/z}, (M+H)^+]\) corresponding to \(\text{C}_{17}\text{H}_{13}\text{N}_{2}\text{O}_{3}\). Structural confirmation via NMR proved difficult with both \(^1\text{H}\) and \(^{13}\text{C}\) NMR data returning complex spectra.

Having proven by HRMS that the reaction proceeds, attention was turned to explicating the purity of both 186 and 187 by LC-MS as a complex matrix was immediately apparent. Under HPLC conditions the parent molecular ion of 186 was not observed, the first mass observed was 191.0462 \(\text{m/z}\) at 4.15 mins corresponding to \(\text{C}_9\text{H}_7\text{N}_2\text{O}_3\), as a result of the fragmentation of the benzyl group the iodine and reversion of the chloropyridine to the pyridone. Following this 316.9419 \(\text{m/z}\) was observed at 6.42 mins which was determined to be \(\text{C}_9\text{H}_6\text{N}_2\text{O}_3\text{I}\), arising from the fragmentation of the benzyl group and displacement of the chlorine with water. The final peak eluted at 8.74 mins which mass spectrometry determined to be 334.9083 \(\text{m/z}\), consistent with the molecular formula \(\text{C}_9\text{H}_5\text{N}_2\text{O}_2\text{Cl}\), due to fragmentation of the benzyl moiety, **Fig 5.14** and **Fig 5.15**.

**Scheme 5.2.5.3**

![Scheme 5.2.5.3](image)

**Fig 5.14 Fragmentation pattern of 186**
Similar fragments were observed in the LCMS trace for 187 with 191.0455 m/z and 316.9406 m/z eluting at 4.11 mins and 6.42 mins respectively. Also present were 311.1021 m/z which eluted at 7.42 mins, a structure from which proved difficult to deduce. Elution of 330.9576 m/z occurred at 7.30 mins which is consistent with the fragmentation of the benzyl group yielding a molecular formula of $\text{C}_{10}\text{H}_8\text{N}_2\text{O}_3$. Elution of 281.0920 m/z occurred at 8.28 mins, consistent with the molecular formula $\text{C}_{16}\text{H}_{13}\text{N}_2\text{O}_3$, which arises as a result of the fragmentation of iodine and displacement of the chlorine with water. Finally at 8.35 mins is 293.0926 m/z, congruent with the molecular formula $\text{C}_{17}\text{H}_{13}\text{N}_2\text{O}_3$. The most tangible structure given this formula arises from the fragmentation of the iodine at the 7-position and oxidation of the amine to generate the corresponding imine, Fig 5.16 and Fig 5.17.
Having explored iodination at the 7-position attention was turned to generating a library of bromine analogues.
5.2.5.4 Bromination of 4-Chloro-6-(2-morpholin-4-yl-ethylamino)quinoline-5,8-dione

The bromo derivative of 183 was affected by employing N-bromosuccinimide under the same conditions. The crude product was assimilated using identical chromatographic conditions to give 188 as a deep red solid in a 67% yield, *Scheme 5.2.5.4*. The incorporation of bromine at the 7-position was supported by mass spectrum analysis. The parent molecular ion was present at 100%, [402 m/z, (M+H)+] which corresponded to the C_{15}H_{16}N_{3}O_{3}^{79}Br^{37}Cl/C_{15}H_{16}N_{3}O_{3}^{81}Br^{35}Cl isotopes. Ions were also observed at 64%, [400 m/z, (M+H)+] and 20%, [404 m/z, (M+H)+] characteristic of C_{15}H_{16}N_{3}O_{3}^{79}Br^{35}Cl and C_{15}H_{16}N_{3}O_{3}^{81}Br^{37}Cl isotopes respectively.

![Scheme 5.2.5.4](image)

1H NMR analysis of 188 revealed the absence of the characteristic singlet for H-7 however it proved difficult to obtain a clean NMR, *Fig 5.18*. Similarly the 13C spectrum showed a collection of aberrant peaks.
Fig 5.18 shows the aromatic region of the $^1$H NMR of 188

In an effort to determine the impurities present further analysis was carried out. On initial inspection the diode array at 360nm showed the presence of one peak at 3.42 mins, however under closer scrutiny using LC-MS conditions it was observed that the compound undergoes hydrolysis of the chlorine to generate the 4-hydroxy derivative, represented by the peak eluting at 3.46 mins with a mass of 382 m/z which HRMS confirms to be C$_{15}$H$_{17}$N$_3$O$_4$Br. Further evidence of the 4-hydroxypyridine (4-pyridone) is seen in the impurity peaks of the $^1$H NMR, Fig 5.18.

Fig 5.19 Fragmentation pattern of 188
5.2.5.5 Bromination of 4-Chloro-6-(methylamino)quinoline-5,8-dione

The bromo congener of 184 was synthesised by reacting 178 with N-bromosuccinimide at room temperature for 16 hours, Scheme 5.2.5.5. The crude product was purified by column chromatography eluting with 100% ethyl acetate to give 189 in a 38% yield. A second product 190 was also isolated during chromatography, eluting with DCM:methanol (9:1) and was isolated in a 26% yield.

![Scheme 5.2.5.5](image)

Incorporation of bromine to generate 189 was confirmed by high resolution mass spectrum analysis. The parent ion was present at 100% \([302.9362 \text{ m/z}, (M+H)^+]\) corresponding to \(\text{C}_{10}\text{H}_6\text{N}_2\text{O}_2^{35}\text{Cl}^{81}\text{Br/C}_{10}\text{H}_6\text{N}_2\text{O}_2^{37}\text{Cl}^{79}\text{Br}\) isotopes. Ions were also present at 75% \([300.9375 \text{ m/z}, (M+H)^+]\) and 30% \([304.9363 \text{ m/z}, (M+H)^+]\) corresponding to the less abundant isotopes, \(\text{C}_{10}\text{H}_6\text{N}_2\text{O}_2^{35}\text{Cl}^{79}\text{Br}\) and \(\text{C}_{10}\text{H}_6\text{N}_2\text{O}_2^{37}\text{Cl}^{81}\text{Br}\) respectively. Analysis of the \(^1\text{H}\) NMR showed the collapse of the H-7 singlet providing further confirmation that the transformation was successful. However, procuring definitive NMR data proved difficult. Akin to the Section 5.2.5.2, the presence of the desired compound is clear however the \(^1\text{H}\) and \(^{13}\text{C}\) NMRs display additional peaks which are not characteristic of 189 and could not be removed with further purification. Analysis of the minor product by high resolution mass spectrometry provided confirmation that the chlorine was again displaced by methanol to generate the 4-methoxy derivative 190 giving rise to two parent ions of equal intensity at 98%, \([296.9865 \text{ m/z}, (M+H)^+]\) and \([298.9851 \text{ m/z}, (M+H)^+]\) characteristic of the presence of \(\text{C}_{11}\text{H}_9\text{N}_2\text{O}_3^{79}\text{Br}\) and \(\text{C}_{11}\text{H}_9\text{N}_2\text{O}_3^{81}\text{Br}\) respectively.
With similar results obtaining NMR data both 189 and 190 were analysed using LC-MS. Analysis of 189 under HPLC conditions lead to the elution of a single peak at 3.03 mins which mass spectrometric analysis determined have a mass of 282.9717 m/z, consistent with the molecular formula C\textsubscript{10}H\textsubscript{8}N\textsubscript{2}O\textsubscript{3}\textsuperscript{79}Br, arising from the displacement of the 4-chloro group by a hydroxyl moiety. The parent ion of 190 was observed under LC-MS conditions eluting at 3.03 mins, Fig 5.20.

![Fig 5.20 LC-MS of 190](image)

5.2.5.6 Bromination of 4-Chloro-6-(benzylamino)quinoline-5,8-dione

The 6-aminobenzyl congener of WDP1079 was effected by stirring 182 in the presence of N-bromosuccinimide for 16 hours at room temperature, Scheme 5.2.5.6. The crude product was purified using column chromatography which lead to the isolation of two products both as deep red solids, the first 191 eluting with 100% ethyl acetate followed by the second 192 eluting with DCM:methanol (9:1).
Scheme 5.2.5.6

High resolution mass spectrometry provided proof that incorporation of the bromine had occurred, generating the desired compound 191. The parent molecular ion was present in the spectrum at 95% \[378.9676 \text{ m/z}, \ (M+H)^+\] corresponding to \(C_{16}H_{10}N_2O_2^{35}\text{Cl}^{79}\text{Br}/C_{16}H_{10}N_2O_2^{37}\text{Cl}^{79}\text{Br}\).

The minor product 192 was identified by mass spectrum analysis with the molecular ion being present at 98% \[373 \text{ m/z}, \ (M+H)^+\]. Although evidence of the reaction occurring is provided for by mass spectrometry, NMR analysis highlighted the presence of impurities in both 191 and 192. In an effort to elucidate the composition of both fractions LCMS was carried out.

As was previously mentioned the parent ion for 191 was observed under HRMS analysis, however under HPLC-MS conditions an ion for 191 was not observed, instead what was seen was a collection of fragmentations. At 5.22 mins a peak emerged in the HPLC trace corresponding to a mass of 268.964 m/z which is consistent with the structure \(C_9H_6N_2O_3^{79}\text{Br}\) and is consistent with fragmentation of the benzyl group and displacement of the chlorine with water to generate the corresponding pyridone. The next peak in the HPLC followed at 7.07 mins and mass spectrometry revealed a mass of 282.9742 m/z with a molecular composition of \(C_{10}H_8N_2O_3^{79}\text{Br}\) which is equable with the loss of the benzyl group at the 6-position, however in place of the chlorine is a methoxy moiety. At 8.40 mins a peak elutes which mass spectrometric analysis shows to possess a mass of 286.9267 m/z, congruous to \(C_9H_5N_2O_2^{35}\text{Cl}^{79}\text{Br}\). Also present in the trace were peaks at 8.65 and 10.26 mins, corresponding to masses of 313.0986 m/z and 329.0306 m/z respectively, however it proved difficult to deduce any tangible molecular structures from this data, Fig. 5.21.
On comparison of the HPLC traces of 191 and 192, Fig 5.21 it can be seen that a similar fragmentation pattern is present for 192. In the case of 192 the parent molecular ion was observed at 10.05 mins on the HPLC trace which mass spectrometry determined to be 373.0190 m/z, concurrent with a molecular formula of C_{17}H_{14}N_{2}O_{3}^{79}Br.
Although synthesis of 7-halo derivatives was successful their inherent electrophilicity lead to difficulties in isolating the desired products. This increased reactivity arises upon substitution of the 7-position as the 6-amino derivatives discussed in Section 5.2.4 provided consistent structural data when analysed.

Generation of the 4-methoxy derivatives could have arisen during the halogenation reaction (carried out in methanol) or during purification, interestingly however this was not observed for morpholine derivatives **183** and **188**, which from a biological perspective is important Section 6.2.1. Similarly the lability of the chlorine is observable under LC-MS analysis returning pyridone derivatives after coming in contact with the solvent systems used (H₂O/HCO₂H, ACN/HCO₂H).

From the LC-MS data it also appears that the bromo congeners are more stable than the respective iodine derivatives as the analysis revealed retention of the bromine in instances where the iodine fragments using identical conditions.
In the case of the 6-aminobenzyl derivatives fragmentation of the benzyl groups was observed which was previously not seen for the aminoethylmorpholine or aminomethyl congeners.

5.2.6 Conclusion

The quinolinedione family of compounds offer tremendous scope for the development of new anti-cancer therapies given their ability to influence cellular expression. Surprisingly to date this domain remains sparsely populated given the potential of these compounds. Hence one of the aims of this project was to synthesise a range of novel substituted quinolinediones on route to affecting a novel heterocyclic tricycle for biological investigation.

Quinolinediones were identified as key intermediates towards the development of pyridinoquinazolinetetrones based on retrosynthetic analysis carried out at the outset. The chemistry employed to generate these intermediates proceeded smoothly with the synthesis of 86 allowing access to a range of novel 6-substituted quinolinediones.

Stille conditions were utilised in order to produce 171 which served as a precursor for generating the pyrimidinedione C-ring. Although this reaction proceeded well, ultimately it was the ensuing unsuccessful attempts to affect the respective quinolinedione 172 which hampered producing a suitable substrate from which to construct tricycles by this means. Following this an investigation into substituting the 4-position of 171 was carried out using a series of alkoxides. In general the reactions proceeded well returning the expected products, however obtaining pure isolates proved difficult largely due to the remnants from the previous coupling.

The synthesis of 6-aminoquinolinediones was met with much more success and led to the generation of a series of novel congeners as well as JUN-1120-2. An attempt to synthesise
the aminoethyl piperazinyl congener led to the isolation of 179 arising from the nucleophilicity of the terminal piperazine nitrogen.

Reaction of aminoquinolinediones 87, 178 and 182 with halides led to the synthesis of a range of novel 7-substituted-6-aminoquinolinediones. In all cases the desired compounds were synthesised and observed however the tendency of the chlorine at the 4-position to undergo nucleophilic displacement with the solvent was evident. The lability of 4-position was further demonstrated under HPLC conditions where displacement of the chlorine was seen over a range of compounds, along with displacement of functional groups at the 6 and 7 positions also. While it was possible to obtain NMR analysis for some of the congeners synthesised, the reactive nature of these species led to difficulties when trying to obtain empirical NMR data for others. From a biological perspective the reactivity of these compounds is a limiting factor for their utility. Synthetically the same property offers a commodious means to establish a novel route to compounds of type 1, however due to the time constraints of the project this work will be the subject of future investigation.
5.3 Quinazolinedione Series

5.3.1 Synthesis of Quinazolinedione precursors

The following section outlines the synthesis towards the generation of novel quinazolinediones, Section 5.1.

5.3.1.1 Nitration of 2,5-dimethoxybenzoic acid

In order to synthesise quinazolinedione structures it was first necessary to generate the precursor methyl-3,6-dimethoxy-2-aminobenzoate 159. In 2001 en route to the synthesis of (±)-puraquinonic acid Derrick et al. reported the fashioning of 159 in three steps from the inexpensive and commercially available substrate 2,5-dimethoxybenzoic acid 158. The first step in the synthesis involves the nitration of 158 using conc. nitric acid resulting in the synthesis of a 4:1 mixture of regioisomers 3,6-dimethoxy-2-nitrobenzoic acid, 193 and 2,5-dimethoxy-4-nitrobenzoic acid, 194, Scheme 5.3.1.1 (page 140).165

The ratio of the mixture was determined by proton NMR by comparing the integration of the singlet at 7.51 ppm which corresponded to H(6) of 194 with the ab quartet [H(4) and H(5)] of 193 at 7.36 and 7.42 ppm, Fig. 5.23. The isomeric mixture was carried forward to the next step due to their poor separation profile on TLC. This straight-forward method proved very robust and the reaction was scaled up to 30 g with no loss in purity or yield.
Methylation of the crude mixture was then carried out by dissolving the isomeric mixture in acetone and stirring at room temperature for 12 hours in the presence of potassium carbonate and dimethyl sulfate. The desired regioisomer 195 was isolated by column chromatography in a 72% yield as a bright yellow powder, Scheme 5.3.1.1. The melting point (117 – 118 °C) correlated well with reported values as did the proton NMR.\textsuperscript{165}
5.3.1.3 Reduction of methyl-3,6-dimethoxy-2-nitrobenzoic acid

Initially methyl-3,6-dimethoxy-2-aminobenzoate, 159 was prepared by hydrogenation using Pd/C as the catalyst giving a pure product in high yield (92%). The preferred method however was an iron/acetic acid reduction, Scheme 5.3.1.1, the main advantage being a reduction in reaction time (12 h vs. 1.5 h) coupled with the fact that the latter method is also less hazardous while yields were comparable. Conversion to the amine was evident from the proton NMR, revealing a 2H broad singlet at 5.41 ppm. The IR spectrum also showed a characteristic N-H stretch at 3488 cm\(^{-1}\).
5.3.2 Pyrimidinedione formation

5.3.2.1 5,8-Dimethoxyquinazoline-2,4-(1H,3H)-dione

Employing a method used by Feng et al. in the synthesis of Alogliptin, the cyclisation of 159 to 5,8-dimethoxyquinazoline-2,4-(1H,3H)-dione 154 was achieved by stirring 159 with urea in a sealed vessel at 200 °C for 1 hour. Following cooling the product was triturated with water to give quinazolinedione 154 as a pale brown solid in good yield (76%), Scheme 5.3.2.1. The melting point (dec. >300 °C) agreed with literature values and the proton NMR showed two distinct broad singlets for both of the N-H protons at 10.15 and 10.95 ppm, with the mass spectrum also showing the parent ion at 100%, [223.1 m/z, (M+H)+].

Scheme 5.3.2.1

In order to regioselectively synthesise N-3 substituted quinazolinediones a selection of commercially available isocyanates were utilised. A total of eight novel compounds were synthesised in this fashion with a further seven being furnished by derivatising 3-(2-chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 196 to give a total of 15 novel compounds. As will be seen an unexpected common side product 197 was isolated from the reaction of two unrelated isocyanate substrates. Dimerisation of isocyanates to generate 1,3-bis-substituted ureas as a result of CO extrusion was observed in some reactions and proved extremely difficult to separate from the desired product. This was largely overcome by using sub-stoichiometric quantities of isocyanate in subsequent reactions.
5.3.2.2 5,8-Dimethoxy-3-phenylquinazoline-(1H,3H)-dione

5,8-Dimethoxy-3-phenylquinazoline-(1H,3H)-dione 198 was prepared in a two-step process initially involving treatment of 159 with phenyl isocyanate in the presence of triethylamine. The mixture was heated at reflux for 20 hours in anhydrous THF to generate the phenylureido intermediate. The reaction mixture was then concentrated and cyclisation was achieved by heating the intermediate in an ethanol/2M aqueous NaOH mixture (10:1) for 4 hours at reflux. The reaction was cooled to room temperature and neutralised using 2M aqueous HCl followed by cooling on ice. This lead to the formation of a precipitate which was isolated by suction filtration to give 198 as a pure white powder in a good yield of 58% over two steps, **Scheme 5.3.2.2**.

![Scheme 5.3.2.2](image)

5.3.2.3 5,8-Dimethoxy-3-benzylquinazoline-(1H,3H)-dione

In an effort to increase the yield of this reaction the use of a higher boiling point solvent was investigated. 1,4-Dioxane was chosen due to its good solvating properties as well as its higher boiling point. This lead to the furnishing of 199 as a white powder in a 56% yield, **Scheme 5.3.2.3**. Further syntheses employed 1,4-dioxane as the solvent due to its higher boiling point.
5.3.2.4 5,8-Dimethoxy-3-(2-nitrophenyl)quinazoline-(1H,3H)-dione

Incorporation of a substituent at the ortho-position of the N-3 phenyl ring was implemented by synthesising 5,8-dimethoxy-3-(2-nitrophenyl)quinazoline-(1H,3H)-dione 200 which was isolated in a 50% yield as a bright yellow powder, Scheme 5.3.2.4. This provided a functional handle on the N-3 phenyl ring which could be derivatised at a later stage.

The introduction of the nitro substituent at the ortho position led to the resolution of the phenyl aromatic signals in the proton NMR. The aromatic regions of 198 and 200 are shown in a stacked plot as a comparison in Fig 5.24, with the signals for each proton in 200 being clearly distinguishable.
5.3.2.5 5,8-Dimethoxy-3-(3,4,5-trimethoxybenzyl)quinazoline-2,4(1H,3H)-dione

Employing 3,4,5-trimethoxybenzyl isocyanate as the substrate lead to the furnishing of 5,8-dimethoxy-3-(3,4,5-trimethoxybenzyl)quinazoline-2,4(1H,3H)-dione 201 which was isolated after drying in a moderate yield of 34% as a white powder, Scheme 5.3.2.5.

Scheme 5.3.2.5

Fig 5.24 Stacked $^1$H NMR spectra of 5,8-Dimethoxy-3-phenylquinazoline-(1H,3H)-dione 198 and 5,8-Dimethoxy-3-(2-nitrophenyl)quinazoline-(1H,3H)-dione 200 measured in DMSO-d$_6$ at 400 MHz and 300 MHz respectively

5.3.2.5 5,8-Dimethoxy-3-(3,4,5-trimethoxybenzyl)quinazoline-2,4(1H,3H)-dione

Employing 3,4,5-trimethoxybenzyl isocyanate as the substrate lead to the furnishing of 5,8-dimethoxy-3-(3,4,5-trimethoxybenzyl)quinazoline-2,4(1H,3H)-dione 201 which was isolated after drying in a moderate yield of 34% as a white powder, Scheme 5.3.2.5.

Scheme 5.3.2.5
The trimethoxybenzyl function was represented in the $^1$H NMR by a 3H singlet at 3.61 ppm corresponding to the C(4’) methoxy group, a 6H singlet at 3.72 ppm corresponding to the C(3’) and C(5’) methoxy groups, a 2H singlet at 4.96 ppm corresponding to the CH$_2$ protons and a 2H singlet at 6.62 ppm corresponding to C(2’)H and C(6’)H. In addition, mass spectrum data identified the parent ion at 100%, [403.1 m/z, (M+H)$^+$].

$^{5.3.2.6}$ 3-(3,6-Dimethoxy-2-methylbenzoate)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

The final N-3 aryl quinazolinedione arose as a result of the attempted synthesis of N-3 R/S-methyl benzyl and N-3 4-chlorobenzenesulfonyl quinazolinediones, Scheme 5.3.2.6. These three reactions resulted in the generation of the common product 197. The reaction was carried out under identical conditions to previous compounds and returned low yields (S-MeBn = 23%, R-MeBn = 24% and 4-ClPhSO$_2$ = 14%) of 197 as a pure white powder after isolation by suction filtration.

![Scheme 5.3.2.6](image-url)

Evidence for structure 197 arises from the $^1$H NMR which clearly shows five peaks between 3.50 ppm and 4.00 ppm corresponding to the presence of five methyl signals. In the aromatic region are two ab systems, the first at 6.72 ppm and 7.30 ppm corresponding to the H-6 and H-7 proton signals and the second at 7.19 ppm and 7.27 ppm corresponding to the H-4’ and H-5’ protons, Fig. 5.25.
Fig 5.25 $^1$H NMR spectrum of $3$-(3,6-dimethoxy-2-methylbenzoate)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 197 measured in DMSO-d$_6$ at 300 MHz

In order to explore the formation of 197 an investigation was carried out into the variance of the stoichiometric equivalents of the isocyanate used. The R and S-methylbenzyl isocyanates were chosen as a direct comparison for this investigation.

For the attempted synthesis of the S-methylbenzyl derivative a sub-stoichiometric quantity of the isocyanate was used (0.88 eq.) as before, which led to the synthesis of 197 in a 23% yield. In the case of the attempted synthesis of the R-methylbenzyl derivative the isocyanate was added to the reaction in excess (1.03 eq) in order to examine whether this would lead to the generation of the desired product. As previously mentioned the use of super-stoichiometric quantities of isocyanates in these reaction resulted in dimerisation yielding bis-substituted ureas. Even with this change in conditions no desired product was observed merely the formation of 197 and an increasing presence of the bis-substituted urea side-product. Any further increase in equivalents of the isocyanate substrate (e.g. 2:1) was deemed futile due to the substantial increase of unwanted side-products when a marginal excess is used.
Chapter 5 | Chemical Results and Discussion

<table>
<thead>
<tr>
<th>Isocyanate R-group</th>
<th>Leaving group</th>
<th>pKa[BH⁺]</th>
<th>pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenyl</td>
<td>Ph-NH₂</td>
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<td>27</td>
</tr>
<tr>
<td>2-NO₂C₆H₄</td>
<td>2-NO₂C₆H₄-NH₂</td>
<td>-0.28</td>
<td>18</td>
</tr>
<tr>
<td>Benzyl</td>
<td>Benzyl-NH₂</td>
<td>9.34</td>
<td>35*</td>
</tr>
<tr>
<td>3,4,5-Trimethoxybenzyl</td>
<td>3,4,5-Trimethoxybenzyl-NH₂</td>
<td>10-12*</td>
<td>35*</td>
</tr>
<tr>
<td>R/S Methylbenzyl</td>
<td>Methylbenzyl-NH₂</td>
<td>10-11*</td>
<td>35*</td>
</tr>
<tr>
<td>4-Chlorobenzenesulfonyl</td>
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<td>/</td>
<td>9.7*</td>
</tr>
<tr>
<td>2-Chloroethyl</td>
<td>2-Chloroethyl-NH₂</td>
<td>7-8*</td>
<td>35*</td>
</tr>
<tr>
<td>3-Chloropropyl</td>
<td>3-Chloropropyl-NH₂</td>
<td>8-9*</td>
<td>35*</td>
</tr>
<tr>
<td>Allyl</td>
<td>Allyl-NH₂</td>
<td>9.49</td>
<td>35*</td>
</tr>
</tbody>
</table>

Table 5.3.1 illustrates the pKa values of the potential leaving group amines.¹⁸⁰,¹⁸¹ * denotes an estimated value.

Although limited, it appears from this study that the generation of 197 is independent of the variance of stoichiometric equivalents of the isocyanate which would suggest that the lability of the functional group of the isocyanate may bear some significance in the outcome of the reactions. When examining the reaction using 4-chlorobenzenesulfonyl isocyanate under this premise it is plausible to conceive that the chlorobenzenesulfonyl fragment could act as a good leaving group due to its ability to stabilise the negative charge it gains upon leaving, Route A, Scheme 5.3.2.6.

When scrutinising the reactions using the methylbenzyl isocyanates as substrates it appears that steric effects are possibly more relevant to the outcome of the reaction. Upon generating the urea intermediate it may be conformationally difficult for nucleophilic attack of the ester to occur due to the presence of the α- methyl group, leaving the intermediate in solution for longer and free to react with the less constrained...
isocyanate substrate. The bis-urea generated represents a good leaving group (and a poor nucleophile) and can be displaced by the a second mole of methyl anthranilate $^{159}$ which leads to the generation of $^{197}$, Route B, Scheme 5.3.2.6.

The fact that when using the less constrained benzyl isocyanate this is not observed may lend credence to this theory but further work is necessary employing the use of sterically hindered isocyanates in order to investigate this theory. In addition $^{159}$ may also be considered a sterically hindered amine which still begs the question how significant constraint is in the reaction. It may be the case that this reaction is in a dynamic equilibrium and formation of the bis-urea results in $^{159}$ being the only nucleophile left in the reaction leading to the generation of $^{197}$.

Scheme 5.3.2.6 Illustrates both proposed mechanisms leading to the generation of $^{197}$
The presence of bis-urea formation was evident in the synthesis of 198, 199 and to a lesser extent 201, evident from the $^1$H NMR of crude reaction mixtures, arising from isocyanate dimerisation, Fig 5.26.\textsuperscript{161,182,183}

\begin{figure}
\centering
\includegraphics[width=0.7\textwidth]{198}
\caption{Fig 5.26 illustrates the presence of diphenyl urea in the $^1$H NMR of the crude reaction mixture 198 measured in DMSO-d$_6$ at 300 MHz}
\end{figure}

5.3.2.7 3-Allyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

Reaction of methyl-3,6-dimethoxy-2-aminobenzoate 159 with allyl isocyanate under the same conditions as previously outlined gave 3-allyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 202 as a white powder in an 18% yield, Scheme 5.3.2.7.
The $^1$H NMR of 3-allyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione, 202 is shown in Fig 5.27 with the assignments given above the peaks. The doublet of triplets at 4.44 ppm corresponds to the $N(3)$CH$_2$ protons with a $^3J$ value of 5.20 Hz. The terminal CH$_2$ group gives rise to a pair of overlapping doublet of doublet of triplets at 5.06 and 5.08 ppm, with $^3J$ values of 18.51 and 9.14 Hz corresponding to the trans and the cis protons respectively. Further downfield at 5.82 ppm is a multiplet which corresponds to the $N(3)$CH$_2$CH=CH$_2$ signal.

**Scheme 5.3.2.7**

$\text{Scheme 5.3.2.7}$

![Scheme 5.3.2.7](image)

Fig 5.27 $^1$H NMR of 3-Allyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 202 with expansions of the allyl region measured in DMSO-d$_6$ at 300 MHz
5.3.2.8 3-(2-Chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

The reaction of methyl-2-amino-3,6-dimethoxybenzoate 159 with 2-chloroethyl isocyanate yielded 3-(2-chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 196 as a white powder in a good yield of 79%, Scheme 5.3.2.7.

Scheme 5.3.2.7

The chloroethyl moiety was represented in the $^1$H NMR by two 2H triplets at 3.76 ppm and 4.18 ppm and in the $^{13}$C spectrum at 40.51 ppm and 40.76 ppm. The synthesis of 196 afforded a versatile substrate for the exploration of the molecular space surrounding the chlorine.

5.3.2.9 3-(2-Azidoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

Reaction of 3-(2-chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 196 with sodium azide proceeded smoothly to give 3-(2-azidoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 203 in a quantitative yield, Scheme 5.3.2.8. The presence of a strong peak at 2128 cm$^{-1}$, characteristic of an azide stretch, in the IR spectrum indicated the successful formation. Mass spectrum analysis showed the parent molecular ion at 100% [292.2 m/z, (M+H)$^+$] further confirming the synthesis of the azide 203.
5.3.2.10 3-(2-Aminoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

5.3.2.10.1 Attempted reduction using triphenyl phosphine

Initially the reduction of 3-(2-azidoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 203 was attempted via a Staudinger reaction where a solution of 203 was treated with triphenylphosphine. The resulting mixture was stirred at room temperature for 24 hours. Following work-up mass spectrum analysis of the crude product revealed the presence of triphenylphosphine oxide [279.1 m/z, (M+H)+] which suggested the reaction had proceeded however, no trace of the desired product was observed. 1H NMR analysis also failed to show the presence of the desired product.

5.3.2.10.2 Synthesis via hydrogenation

Following this a hydrogenation was carried out in the presence of palladium on carbon, Scheme 5.3.2.9. Following the dissolution of 203 in a 10% aqueous solution of potassium hydroxide the reaction was agitated for 12 hours under a hydrogen atmosphere (50 psi) in the presence of palladium on carbon. The white solid isolated indicated the presence of 204 by IR and mass spectrum analysis.
Obtaining an NMR sample proved difficult due to the insoluble nature of the product. High boiling point solvents with good dissolution properties were initially investigated. Dissolution was attempted in both DMSO-\(d_6\) and DMF-\(d_7\) however both attempts failed to dissolve any significant quantities of product. As the mass spectrum was obtained from a methanolic solution it was decided to utilise deuterated methanol. With gentle heating some of the material was seen to dissolve. The \(^1\)H NMR, obtained on a 600MHz did show the presence of material characteristic of the structure however it was not possible to assign the NMR fully. A \(^{13}\)C NMR was attempted also however there were no peaks present in the spectrum due to the dilute nature of the sample.

5.3.2.11 3-(2-Cyanoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

3-(2-Azidoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione \textbf{203} was successfully converted to the corresponding nitrile, 3-(2-Cyanoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione, \textbf{205} by treating \textbf{203} with sodium cyanide, \textbf{Scheme 5.3.2.10}. The desired product \textbf{205} was isolated as white crystals in an 80% yield.

\begin{center}
\textbf{Scheme 5.3.2.10}
\end{center}
Comparison of the IR spectra of 205 and 203 shows a distinct difference in absorption. The peak at 2128 cm$^{-1}$ in the spectrum of 203, corresponding to the stretching frequency of the azide, is absent in the spectrum of 205 and the peak at 2248 cm$^{-1}$ is characteristic of the stretching frequency of a nitrile. Substitution was further confirmed by mass spectrometry and NMR analysis with the $^{13}$C NMR showing an extra quaternary signal at 118.57 ppm corresponding to the nitrile carbon, Fig. 5.28.

Fig 5.28 Stacked $^{13}$C spectra of 3-(2-azidoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 203 and 3-(2-cyanoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 205 in DMSO-$d_6$ at 75 MHz

5.3.2.12 3-(2-Amidoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

Conversion of nitrile, to the corresponding amide, 3-(2-Amidoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione, 206 was achieved by stirring 205 in 37% HCl at room temperature for one hour, Scheme 5.3.2.11. Initial heating of the reaction was necessary in order to dissolve 205. Upon completion, the reaction was quenched by
adding to ice water, neutralised using 2M NaOH and following crystallisation, 206 was isolated as white crystals in 100% yield.

\[
\begin{align*}
\text{Scheme 5.3.2.11}
\end{align*}
\]

Structural confirmation was afforded by mass spectrometry and NMR. The \(^1\)H NMR showed two distinct broad singlets at 6.85 and 7.45 ppm corresponding to each proton of the amide function. A signal at 172.06 ppm in the \(^{13}\)C NMR, indicative of the amide carbon was further proof that conversion to the amide had successfully occurred.

5.3.2.13 3-[2-(Piperazin-1-yl)ethyl]-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

3-[2-(Piperazin-1-yl)ethyl]-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 207 was afforded in a 29% yield as a white solid by reacting 196 with piperazine in the presence of potassium iodide and potassium carbonate in anhydrous DMF, \textbf{Scheme 5.3.2.12}. Spectroscopic analysis provided structural confirmation of substitution of chlorine with piperazine. The piperazinyl moiety was evident in the \(^1\)H NMR being represented by a broad singlet at 1.90 ppm corresponding to the N(4')H proton and a multiplet at 2.33-2.69 ppm corresponding to the eight CH\textsubscript{2} protons.
5.3.2.14 3-[2-(Piperidin-1-yl)ethyl]-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

Reaction of 3-(2-chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 196 with piperidine in the same manner as above generated 3-[2-(piperidin-1-yl)ethyl]-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 208 in a good yield of 73% as beige crystals, Scheme 5.3.2.13. As can be seen there is a significant increase in yield when compared with piperazine derivative 207 with no change in reaction conditions.

The piperidine moiety is represented in the $^1$H NMR as a set of three multiplets at 1.43 ppm, 1.59 ppm and 2.56 ppm which correspond to the two C(4') protons, the four C(3') and C(5') protons and the four C(2') and C(6') protons respectively. These carbons are seen in the $^{13}$C NMR at 24.23 ppm, 25.84 ppm and 54.57 ppm with the latter two possessing twice the intensity as the former.
5.3.5.15 3-[(Dimethylamino)ethyl]-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

3-[(Dimethylamino)ethyl]-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione \( \text{209} \) was furnished by reacting 3-(2-chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione \( \text{196} \) with dimethyl amine to give the desired product in an excellent yield of 88% as white crystals, \textit{Scheme 5.3.2.14}.

\[
\begin{align*}
\text{196} & \quad \text{K}_2\text{CO}_3, \text{KI,} \\
& \quad \text{NMe}_2 (33\% \text{ in EtOH}) \\
& \quad \text{DMF (anhyd.),} \\
& \quad 90^\circ\text{C, 3 hrs} \\
\text{209} & \quad 88\%
\end{align*}
\]

\textit{Scheme 5.3.2.14}

Although the highest yielding of these reactions, the \(^1\text{H}\) NMR of \( \text{209} \) did reveal some unidentifiable impurity which was represented by a broad doublet at 2.84 ppm \((J \ 9.40 \text{ Hz})\) and a triplet at 4.35 ppm \((J \ 3.03 \text{ Hz})\) with a relative intensity of 2:1, \textit{Fig 5.29}. Due to time constraints it was not possible to elucidate the nature of this impurity and forms the subject of future investigation.
5.3.2.16 3-(3-Chloropropyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

Reaction of 3-chloropropyl isocyanate with methyl-2-amino-3,6-dimethoxybenzoate 159 proceeded smoothly to generate 3-(3-chloropropyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 210 as a white powder in a 22% yield, Scheme 5.3.2.15.

Scheme 5.3.2.15

The presence of the chloropropyl function gave rise to a 2H quintet at 2.01 ppm corresponding to the N(3)CH₂CH₂ protons, a 2H triplet at 3.66 ppm corresponding to the CH₂Cl protons and a 2H triplet at 3.98 ppm corresponding to the N(3)CH₂ protons.
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The $^{13}$C spectrum when compared with that of 196 showed the presence of an extra signal at 30.49 ppm which represents the additional carbon of the propyl function. High resolution mass spectrometry analysis provided further structural confirmation showing the parent molecular ion at 100% [299.0769 m/z, (M+H)$^+$], 30% [301.0749 m/z, (M+H)$^+$] corresponding to $\text{C}_{13}\text{H}_{15}\text{N}_{2}\text{O}_{4}^{35}\text{Cl}$ and $\text{C}_{13}\text{H}_{15}\text{N}_{2}\text{O}_{4}^{37}\text{Cl}$ respectively. Comparison of this reaction with the chloroethyl analogue 196 which returned a high yield of 79% demonstrates the capricious nature of this reaction.

5.3.3 6-Substituted quinazolinediones

5.3.3.1 6-Nitro-5,8-dimethoxyquinazoline-2,4-(1H,3H)-dione

A nitro group at the 6-position of 154 was incorporated in order to allow further derivatisation at a later stage of synthesis. A method used by Skibo et al. to generate 164 was employed whereby 154 was initially suspended in acetic acid at 5 °C. A mixture of sulphuric acid and nitric acid was then added simultaneously which resulted in the beige suspension turning pale yellow immediately, Scheme 5.3.3.1. The reaction was quenched after 5 minutes with ice water and the resultant solid was isolated by filtration to give 164 as a pale yellow solid in excellent yield (97%). The $^1$H NMR of 164, identical to that reported by Skibo et al., showed the absence of the ab quartet system with a singlet at 7.73 ppm in its place corresponding to H(7). The melting point (dec. >300 °C) was also in agreement with literature. This procedure proved to be an excellent method of nitration for systems of type 154 and was used in all subsequent nitration reactions.
5.3.3.2 6-Nitro N-3 substituted quinazolines

Following the method outlined by Skibo et al. a panel of 6-nitro-N-3-substituted quinazolinediones were prepared from 3-allyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 202, 3-(2-chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 196, 3-(3-chloropropyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 210 and 5,8-dimethoxy-3-(2-nitrophenyl)quinazoline-(1H,3H)-dione 200, Scheme 5.3.3.2 and Table 5.3.1. 156

Table 5.3.1
The nitro derivatives 211 - 214 were isolated pure by filtration in moderate to excellent yield. The assignation of the structure of 164 as the 6-nitro derivative by Skibo is based on trends of electrophilic substitution of quinazolines previously reported in the literature. A theoretical study carried out by Dewar and Maitlis calculated that the 6-position is more reactive than the 7-position. Further to this reactivity studies carried out by Bogert and Scatchard demonstrated that the site of nitration on quinazoline-2,4-(1H,3H)-diones occurs at the 6-position. Confirmation was provided by incorporating a nitro group at the 5-position of o-ureidobenzoate prior to annealation of the pyrimidinedione resulting in the generation of the 6-nitro quinazolinedione. No conclusive structural evidence exists in the literature so in order to elucidate the regioselectivity of nitration of N-3 substituted quinazolinediones a crystal structure of 212 was obtained. It was first necessary to grow crystals which was achieved by dissolving 212 in hot methanol. An equal volume of water was then added and crystals were allowed to propagate over time. The resultant needle-like crystals were translucent orange/yellow in colour. X-ray diffraction studies resolved that nitration occurs at the 6-position, Fig 5.30.

![Fig 5.30](image)

**Fig 5.30** X-ray crystal structure of 3-(2-chloroethyl)-5,8-dimethoxy-6-nitroquinazoline-2,4(1H,3H)-dione 212 illustrating the nitration of the 6-position.
5.3.3.3 Synthesis of 6-bromo-5,8-dimethoxyquinazoline-2,4-(1H,3H)-dione

Functionality at the 6-position of 154 was also afforded by the insertion of bromine to generate 215. Equimolar amounts of 154 and N-bromosuccinimide were reacted at room temperature for 12 hours. The product was precipitated with the addition of water and 215 was isolated in good yield (71%) as a white solid, Scheme 5.3.3.3. As was the case with 164, the $^1$H NMR showed the disappearance of the ab quartet system and in its place a singlet (7.48 ppm) corresponding to H(7). Mass spectrometry analysis confirmed the incorporation of bromine, evident by the presence of two isotopes corresponding to $^{79}$Br and $^{81}$Br showing the parent molecular ions at 98% [301.0 m/z, (M+H)$^+$] and 96% [303.0 m/z, (M+H)$^+$].

![Scheme 5.3.3.3](image)

The rationale for brominating the six position was that 215 would serve as a uracil analogue to the bromoquinoline intermediate 86. The application of analogous chemistry to synthesise the quinolinedione series of molecules could be employed to generate a novel class of quinazolinetetrone congeners with biological activity measured and compared to the quinolinediones to assess the effect of the presence of the pyrimidinedione moiety.
5.3.3.4 Attempted synthesis of 6-bromoquinazoline-2,4,5,8(1H,3H)-tetraone

The existing methodology used by Wipf *et al.* to synthesise 6-amino quinolone-5,8-diones was employed to try and generate the oxidation product 216. In this case methanol was used in place of acetonitrile due to its more favourable solubility profile and was used to dissolve 215 followed by cooling to 0 °C. An aqueous solution of cerium ammonium nitrate was then added portionwise. The reaction was then allowed to warm to room temperature over four hours. Upon workup, analysis of the crude product showed that the desired quinazoline tetraone 216 had not been formed and only unreacted starting material was recovered, *Scheme 5.3.3.4*, a recurring trend for substrates bearing a pyrimidinedione moiety.

![Scheme 5.3.3.4](image)

Previous attempts at oxidative demethylation of various quinazoline diones also proved fruitless. Attempted oxidation of uracil 154 was carried out in a number of ways by varying both the oxidant and the reaction conditions, however each time no reaction occurred and the starting material was recovered, *Scheme 5.3.3.5*. 

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Oxidation was also attempted using 3-(2-chloroethyl)-5,8-dimethoxy-6-nitroquinazoline-2,4(1H,3H)-dione \textbf{212} as the substrate which was dissolved in acetonitrile and cooled to 0 °C followed by the addition of an aqueous solution of ceric ammonium nitrate (standard conditions). The reaction was allowed to stir for 4 hours while being allowed to warm to room temperature. $^1$H NMR analysis of the isolated product however only revealed starting material, \textit{Scheme 5.3.3.5}.

\textbf{Scheme 5.3.3.5}

\textbf{5.3.4 N-1,N-3-Disubstituted series}

\textit{5.3.4.1 Synthesis of 5,8-dimethoxy-1,3-dimethylquinazoline-2,4(1H,3H)-dione}

A study was carried out to investigate substituting the one and three position of \textbf{154} simultaneously. The basis of this study was to identify if this route was viable for the synthesis of \textit{N-1,N-3} bis-substituted uracils. Sodium hydride (2.5 eq.) was employed as the base as it had worked well previously in our laboratory. Quinazolinedione \textbf{154} was stirred for one hour at 50 °C with sodium hydride to ensure complete deprotonation. Methyl iodide (2.5 eq.) was then added and stirring was continued for a further hour, \textit{Scheme 5.3.4.1}.
A TLC showed the presence of two products which was thought to be a mixture of \textbf{217} and \textbf{218}. After removing the DMF, the residue was dissolved in chloroform. In order to extract \textbf{218} the organic phase was extracted with a 10\% aqueous solution of potassium hydroxide.

After complete work-up of the organic phase \textbf{217} was isolated as pure brown crystals in a moderate yield of 59\%. Acidification of the aqueous phase followed by work-up returned \textbf{218} as a pure off-white powder in a 29\% yield.

Mass spectrometry provided confirmation that each of the compounds had been synthesised. The $^1$H NMR of both products were quite similar to each other. In the $^1$H NMR of dimethyl uracil \textbf{217} the absence of two distinct broad singlets and the appearance of two new singlets at 3.41 ppm and 3.73 ppm, corresponding to the $N$-3 and $N$-1 methyl groups respectively, was confirmation that the uracil had been substituted. The $^1$H NMR of methyl uracil \textbf{218} showed a singlet at 3.41 ppm representing the new methyl group at the 3-position. The presence of the $N$-1 proton was shown as a broad singlet at 8.17 ppm, \textit{Fig 5.31}. 

\textbf{Scheme 5.3.4.1}
Fig 5.31 Stacked $^1$H NMR spectra of 5,8-dimethoxy-1,3-dimethylquinazoline-2,4(1H,3H)-dione 217 and 5,8-dimethoxy-3-methylquinazoline-2,4(1H,3H)-dione 218 measured in CDCl$_3$ at 300MHz

The $^{13}$C spectrum of 217 contains two new CH$_3$ peaks at 28.42 ppm and 37.05 ppm corresponding to the N(3) methyl carbon and N(1) methyl carbon respectively. By comparison the $^{13}$C spectrum of 218 only contains one new CH$_3$ which is at 27.36 ppm and corresponds to the N(3) methyl carbon.

It is also worth noting the significant difference in the melting points of the two products. The melting point range of dimethyl uracil 217 (110 - 112 °C) was found to be far lower than that of methyl uracil 218 (224 - 226 °C). The relatively large disparity between the melting points could be perhaps due to the additional intramolecular hydrogen bond donor ability of the N-H function in methyl uracil 218. This suggestion would also correlate with the fact that uracil 154 has a melting point of >300 °C.

One of the major drawbacks of this reaction is that it is confined to producing bis-substituted derivatives which limits the diversity of the products being generated. This
being said the bis-methyl uracil 217 was never the sole product of the reaction and significant amounts of N-3 methylated uracil 218 were produced in the reaction which could be used to synthesise a range of N-1 substituted N-3 methyl uracils. More detailed studies into stoichiometry in the future may allow the isolation of the N-3 methyl uracil derivative as the sole product. In terms of reliability, the percentages of each product isolated can vary quite significantly and it was difficult to reproduce similar results when the reaction was repeated. To this end synthesising N-3 substituted uracils utilising isocyanates followed by substitution at the N-1 position was found to be a more useful route for generating di-substituted uracils.

5.3.4.2 Synthesis of 1-benzyl-5,8-dimethoxy-3-phenylquinazoline-2,4(1H,3H)-dione

The method used to synthesise this series of compounds is similar to methods reported by Michne et al. and Willis in their syntheses.\(^{160,162}\)

Quinazolinedione 219 was synthesised by treating 198 with sodium hydride followed by addition of benzyl bromide. The reaction was stirred at ambient temperature for twelve hours, Scheme 5.3.4.2.

\[ \begin{align*}
\text{DMF (anhyd.),} & \quad \text{NaH, BnBr} \\
198 & \quad \text{14 hrs,} \\
& \quad \text{r.t. to 100 °C,} \\
& \quad \text{33%} \\
\rightarrow & \quad 219 
\end{align*} \]

Scheme 5.3.4.2

Analysis of the reaction mixture showed the presence of starting material so the reaction was heated to 100 °C for a further two hours until no more starting material was consumed. The di-substituted quinazolinedione 219 was precipitated from solution by addition of water and the pure white solid was isolated in a low to moderate yield of 33%.
Confirmation of synthesis was afforded by high resolution mass spectrometry analysis showing the parent molecular ion at 100% [389.1506 m/z, (M+H)+] corresponding to C_{23}H_{20}N_{2}O_{4}. Analysis of the ^1H NMR showed new signals in the aromatic region (integrating for five protons). A singlet at 5.71 ppm was also seen corresponding to the CH$_2$ of the benzyl group. The ^13C NMR characteristic of 219 with the CH$_2$ carbon being represented in the negative phase of the DEPT-135 spectrum at 51.68 ppm.

5.3.4.3 Synthesis of 1-allyl-5,8-dimethoxy-3-phenylquinazoline-2,4(1H,3H)-dione

In an effort to increase the yield of this reaction it was decided to modify the temperature parameters of the reaction. For the synthesis of 220 the reaction was stirred at room temperature for two hours and 100 °C for a further three hours. Analysis by TLC at this stage showed no trace of starting material. The product was triturated from the reaction by adding water and 220 was isolated as a pure white solid in an excellent yield of 92%, Scheme 5.3.4.3.

The ^1H NMR of 220 showed four characteristic signals corresponding to the allyl substituent, a 2H doublet of triplets at 4.97 ppm corresponding to N(1)CH$_2$, a pair of overlapping doublet of doublet of triplets at 5.16 ppm and 5.21 ppm corresponding to the cis and trans terminal allyl protons respectively and finally a 1H multiplet at 6.01 ppm corresponding to N(1)CH$_2$CH.
5.3.4.4 Synthesis of 1,3-dibenzyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione

The last reaction in this series involved benzylating the 1-position of 199. The reaction was stirred at room temperature for 2 hours followed by heating at 100 ºC for 12 hours. It was envisaged that the increase in heating time would result in a better yield for this reaction as the previous attempt at benzylation, to synthesise 219, returned a low yield. The modification of the reaction conditions resulted in 221 being isolated as an off-white solid in a good yield of 70%, Scheme 5.3.4.4.

![Scheme 5.3.4.4](image_url)

Confirmation of synthesis was afforded by mass spectrometry analysis showing the parent molecular ion at 100% \([403.1 \text{ m/z}, (\text{M+H})^+]\). Analysis of the \(^1\text{H}\) NMR spectrum showed new peaks in the aromatic region corresponding to the incorporated benzyl moiety as well as a new 2H singlet corresponding to \(N(1)\text{CH}_2\). The presence of the benzyl moiety was also evident in the \(^{13}\text{C}\) spectra with a new peak in the negative phase of the DEPT-135 spectrum corresponding to the \(\text{CH}_2\) of the benzyl moiety, as well as new aromatic carbon signals in the positive phase.

5.3.5 6-Substituted \(N-1,N-3\)-Disubstituted series

Employing methodology previously used to synthesise compounds in Section 5.3.3.1, a series of 6-nitro \(N-1,N-3\) disubstituted uracils were prepared in moderate to excellent yield.
5.3.5.1 Synthesis of 5,8-dimethoxy-1,3-dimethyl-6-nitroquinazoline-2,4(1H,3H)-dione

Simultaneous protection of the one and three position was also investigated on nitroquinazolinedione 164. Methodology previously used, in Section 5.3.4.1, was employed to generate the bis-protected uracil 222. In this instance the reaction was stirred at room temperature (as opposed to 50 °C) and the reaction time was extended to twelve hours. Upon work-up it was evident that two products were present, so the two products were separated in the same manner as before, by isolating methyl uracil 223 using aqueous base wash. After complete work-up of both organic and aqueous phases 222 and 223 were obtained as pure orange solids in a 26% and 64% yield respectively, Scheme 5.3.5.1.

![Scheme 5.3.5.1](image)

NMR analysis showed similar results to those found in Section 5.3.4.1, with the $^1$H NMR of 222 showing two singlets at 3.44 ppm and 3.80 ppm, corresponding to the $N$-1 and $N$-3 methyl protons in place of the two broad singlets ($N$(1)H and $N$(3)H) in 164 at 10.93 ppm and 11.47 ppm. The presence of the methyl groups was also evident in the $^{13}$C NMR, represented by the peaks at 28.77 ppm and 37.41 ppm. The singlet at 3.24 ppm in the $^1$H NMR of 223 and the peak at 27.14 ppm in the $^{13}$C NMR show the incorporation of a methyl group at the three position.

A significant difference in the melting points of the two products is seen again. The melting point range of dimethyl uracil 222 (163 - 165 °C) was found to be far lower than that of methyl uracil 223 (275 - 277 °C). The fact that nitro-uracil 164 has a literature melting point of >300 °C would suggest that the extra hydrogen bonding sites have a considerable impact on the melting point of the compounds.
The reaction proceeded smoothly, returning an excellent yield. There is a clear bias towards methyl uracil 223 in this reaction when compared to the reaction in Scheme 5.3.4.1. Future investigations could centre around investigating the temperature dependence of this reaction in order to expound if manipulating the temperature varies the propensity of the reaction.

A series of 6-nitro N-1, N-3 disubstituted quinazolinediones were prepared from disubstituted quinazolinediones 1-benzyl-5,8-dimethoxy-3-phenylquinazoline-2,4(1H,3H)-dione 219, 1-allyl-5,8-dimethoxy-3-phenylquinazoline-2,4(1H,3H)-dione 220 and 1,3-dibenzyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 221. Formation of the nitro derivatives involved the protocol previously outlined in Section 5.3.3.1.

\[ \text{Scheme 5.3.5.2} \]

**Table 5.3.2**

<table>
<thead>
<tr>
<th>Compound number</th>
<th>R₁</th>
<th>R₂</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>224</td>
<td>Bn</td>
<td>Ph</td>
<td>50</td>
</tr>
<tr>
<td>225</td>
<td>Allyl</td>
<td>Ph</td>
<td>69</td>
</tr>
<tr>
<td>226</td>
<td>Bn</td>
<td>Bn</td>
<td>88</td>
</tr>
</tbody>
</table>

The nitrated derivative 224 was synthesised as a yellow solid in a moderate yield of 50% Scheme 5.3.5.2, Table 5.3.2. Mass spectrometry confirmed that 224 had been synthesised showing the parent ion at 100 % [434.2 m/z, (M+H)^+]. The most discernible feature of the $^1$H NMR is the collapse of the ab system for H-6 and H-7, being replaced with a singlet at
7.61 ppm corresponding to H-7. Further analysis of mass spectrum and NMR data showed no evidence of nitration of the aromatic ring substituents at the 1- and 3- positions.

Reaction of uracil 220 under the same conditions led to the formation of nitro uracil 225 as a yellow solid in a good yield of 69%, Scheme 5.3.5.2, Table 5.3.2. Mass spectrum analysis provided structural confirmation of nitration showing the parent ion at 94% [384.0 m/z, (M+H)+].

The final compound of this series was afforded by nitrating dibenzyl uracil 221 to yield the nitro uracil 226 as a yellow solid in an excellent yield of 88%. Mass spectrum analysis again provided structural confirmation of nitration showing the parent ion at 70% [448.1 m/z, (M+H)+]. An earlier venture to generate 226 was tried by attempting to directly dibenzylate nitro uracil 164 by treating 164 with benzyl bromide in the presence of potassium carbonate at 120 °C for 72 hours in dimethylformamide. Analysis of the isolated crude product by 1H NMR and mass spectrometry however failed to reveal any product formation.

5.3.6 Attempted cyclisation to pyridinoquinazolinedione

The concluding section of this work discusses the attempts of synthesising unprecedented pyridinoquinazolinediones as precursors to pyridinoquinazolinetriones 1.

5.3.6.1 Attempted pyridine completion using 1,1,3,3-tetramethoxypropane

In order to synthesise the A-ring of 1 it was first necessary to generate a substrate suitable for cyclisation. The synthesis of 164 provided the ideal scaffold to investigate A-ring cyclisation. A nucleophilic 6-position was afforded by reducing the nitro functionality to the corresponding amine 227 using a protocol previously outlined in Section 5.3.1.3, Scheme 5.3.6.1. It was evident from comparing the 1H NMR spectra of 164 and 227 that the reduction proceeded and 227 was utilised in situ for following reactions, Fig 5.32.
**Scheme 5.3.6.1**

Fig 5.32 illustrates a stacked $^1$H NMR of 6-nitro-5,8-dimethoxyquinazoline-2,4-(1H,3H)-dione 164 and 6-amino-5,8-dimethoxyquinazoline-2,4-(1H,3H)-dione 227 measured in DMSO-$d_6$ at 300 MHz.

Previously in our group similar functionalisation was used to effectuate 7-azaindoles. This work carried out by Cahill provided the premise for utilising this protocol as a method for generating novel tricyclic scaffolds.$^{164}$

In his synthesis Cahill used toluene as the solvent with 1,1,3,3-tetramethoxypropane in the presence of a catalytic amount of $p$-toluenesulfonic acid, with the thermodynamic driving force of the reaction being the removal of the methanol by-product by azeotropic distillation. However due to the insoluble nature of 227 in toluene other solvents were employed. Initial attempts employed 1,4-dioxane as the solvent, the reaction was heated
to reflux for one hour after which the reaction was worked up. Analysis of the crude product by $^1$H NMR and mass spectrum analysis however failed to reveal the presence of any desired product. Following several fruitless attempts under these conditions, variance of the reaction parameters was investigated. The following attempt employed an alternative acid catalyst (1M HCl) with other parameters remaining stable. Analysis of the crude product by the same means again failed to show any evidence of tricycle formation.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Acid</th>
<th>Reactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-Dioxane</td>
<td>$p$-TsOH</td>
<td>1,1,3,3-TMP</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>HCl (1M)</td>
<td>1,1,3,3-TMP</td>
</tr>
<tr>
<td>Acrolein</td>
<td>HCl (6M)</td>
<td>Acrolein</td>
</tr>
<tr>
<td>DMF</td>
<td>$p$-TsOH</td>
<td>1,1,3,3-TMP</td>
</tr>
</tbody>
</table>

**Table 5.3.3**

Given that cyclisation was unsuccessful utilising the protocol set out by Cahill it was decided to employ an alternative three carbon fragment in an effort to generate the desired compound. Skraup conditions (Section 2.6) were employed where amino uracil 227, acrolein and HCl (6M) were added to a reaction flask and the contents were heated to reflux for one hour. Following work-up this protocol failed to reveal the desired product returning only unidentifiable products by $^1$H NMR analysis.

A final attempt reverted back to the use of 1,1,3,3-tetramethoxypropane, however the solvent was changed in favour of DMF due to its superior boiling point and $p$-toluenesulfonic acid was utilised as the acid catalyst. Following work-up analysis of the crude product again failed to show any discernible evidence of tricycle formation.

In order to investigate the reactivity of the amine substrate, 227 was acylated using acetic anhydride. Nitro uracil 164 was reduced to the corresponding amine *via* hydrogenation, the crude reaction solution (KOH/H$_2$O) was then adjusted to pH 6 using acetic acid followed by the addition of acetic anhydride.
After completion the isolated grey solid was determined to be the desired \( N \)-acetyl derivative 228 by \(^1\)H NMR (the acetyl group being represented by a singlet at 2.11 ppm) and mass spectrometric analysis showing the parent ion at 100% [280.3 m/z, (M+H)\(^+\)]. Having demonstrated that this transformation was successful it appears that the problem with the attempted cyclisation reactions lies in the electrophilic aromatic cyclisation.

### 5.3.7 2,4-Disubstituted quinazolinedione

#### 5.3.7.1 2,4-Dichloro-5,8-dimethoxyquinazoline

To conclude this section of work it was decided to attempt to bridge the dichotomy of this project. As previously mentioned in \textbf{Section 5.3.3.4} the oxidative demethylation of uracil 154 proved unsuccessful and did not lead to the desired quinazolinetetrones. As the problem was thought to lay with the presence of a pyrimidinedione it was decided to appropriate 154 to the novel dichloropyrimidine congener 229 as a basis for investigating oxidative demethylation. It is well known in the literature that dichloropyrimidines can be effectuated from uracils upon treatment with a chlorinating agent. With this in mind 154 was suspended in phosphorous oxychloride, then heated at reflux for one hour, \textbf{Scheme 5.3.7.1}. 

-Scheme 5.3.6.2-
Once cooled the reaction was added portionwise to crushed ice and worked up to give \( \text{229} \) as a pure bright yellow solid in a 67% yield. Comparison of the infra-red spectrum with that of \( \text{154} \) showed a lack of a characteristic carbonyl stretch (1709 cm\(^{-1}\) in spectrum of \( \text{154} \)). Analysis of the \( ^{13} \text{C} \) NMR data also concluded that \( \text{229} \) was realised due to the lack of characteristic carbonyl carbon peaks in the spectrum. Having proved that this conversion is applicable to our system, it forms the substratum for investigating the development of functionalised quinazoline-5,8-diones which would serve as an interesting comparison to the synthesised quinolone-5,8-diones for biological study.

### 5.3.8 Conclusion

The quinazolinediones and their derivatives offer immense potential for the development of novel chemotherapeutic agents due to their ability to interact at a cellular level. Many efficient synthetic routes for generating quinazolinediones have been developed in recent years. Surprisingly however this area remains underdeveloped given the impressive biological activity expressed by a number of derivatives. Hence one of the aims of this project was to explore the paradigm of functionalising the quinazolinedione scaffold at previously unexplored sites, namely positions one, three, five, six and eight as a means of determining biological potential as well as serving as a diverse library of substrates from which to construct novel heterocyclic tricycles.
Quinazolinediones were identified as key intermediates towards developing a novel route to pyridinoquinazolinetetrones as a result of retrosynthetic analysis carried out at the inception of this project. The chemistry applied to explicate these intermediates proceeded well with the exception of 197 from three reactions affording a total of ten novel N-3 functionalised quinazolinediones with a further six being furnished from derivatising 196.

Derivatisation of the N-3 functionalised quinazolinediones at positions one and six afforded an additional eleven novel congeners containing a broad derivatisation profile. Synthesis of a total of thirty-one novel quinazolinediones provided an expansive test set of derivatives for biological investigation.

A substrate from which to investigate the formation of novel tricycles was initially provided through the introduction of functionality at the six position of 154. Several attempts were made to effectuate compounds of type 1 however, disappointingly, attempts proved fruitless perhaps due to the fact that this reaction attempts to substitute the last remaining position of the highly derivatised B-ring. Having determined that nitration of compounds of type 154 occurs at the six-position suggests that the seven-position is less nucleophilic than the six-position which may also be a significant factor in the difficulties encountered in A-ring cyclisation.

It was postulated that conversion of 154 to the dichloropyrimidine derivative 229 may provide a substrate which is more conducive to cyclisation, Scheme 5.3.7.1. Comparison of the 1H NMR of both compounds shows that H-6 and H-7 of 229 are more electronically similar than the respective protons of 154. The increased shielding observed for H-7 demonstrates increased electron density at this site which acts in favour of tricycle formation from compounds of type 229 making it possible to achieve the highly promising biological candidate 1.
6.0 Biological Results and Discussion
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6.0 Biological Results and Discussion

As previously eluded to in Section 4.1 the ultimate aim of this project was to synthesise and assay novel pyridinoquinazolinetetrone (PQT) derivatives, an unreported scaffold constructed from heterocyclic fragments which possess known chemotherapeutic properties. Synthesis towards PQT derivatives initially involved expanding on both the quinoline and quinazolinedione pharmacophore to generate functionalised derivatives from which to construct the tricyclic hybrid. This lead to the synthesis of a total of 51 novel compounds, 20 quinolines and 31 quinazolinediones whose biological profile was to be explored. It was therefore imperative to assess chemotherapeutic potential in order to uncover any biological activity associated with these novel derivatives.

The quinoline derivatives synthesised, which elaborated on work carried out by Wipf et al. are known anti-cancer agents, Section 1.1.4, and so preliminary chemotherapeutic assessment involved utilisation of the NCI single-dose screen.\(^{28}\) Investigation into the cytotoxic activity of quinoline-5,8-diones has received the most attention with several derivatives displaying potent activity which is mediated via inhibition of Cdc25 phosphatase.

Concomitant to their anti-bacterial and glutamate receptor antagonism, quinazolinediones have also been shown to exert cytotoxic effects, previously discussed in sections 1.2.2 and 1.2.3, ergo preliminary anti-cancer potential of the novel quinazolinediones synthesised was also assessed by the NCI program. With the exception of PGGTase-I inhibitors there have been no other biomechanistic developments into the anti-cancer action of quinazolinediones, largely due to the paucity of lead compounds despite previous promising results, Section 1.2.2.

With this in mind our primary focus is on the anti-cancer capabilities of the two compound classes.
6.1 NCI-60 cell screen

Prospective candidates from the quinolinedione and quinazolinedione families were initially tested against the US National Cancer Institute 60 human tumour cell line panel (NCI-60). The NCI-60 was developed in the late 1980’s as an \textit{in vitro} drug-discovery tool with the aim of identifying compounds which exhibited cytotoxic effects on particular types of tumour.\textsuperscript{187} It swiftly became apparent that the screening model could provide further biological data. The mechanism of cell growth inhibition could be deduced by examining the formation of distinctive patterns of inhibition which correlated to the inhibition of susceptible cell lines using specific compounds. From these observations, Paull \textit{et al.} developed the COMPARE algorithm which can be used as a tool in aiding the elucidation of the biological mechanism of action of potential chemotherapeutic agents.\textsuperscript{188}

Cell lines utilised in the NCI-60 panel represent melanoma and leukaemia along with cancers of the colon, lung, brain, breast, prostate, kidney and ovary. Compounds which are accepted are initially tested at a single dose (10\,µM) against the 60 cell line panel. Compounds which satisfy threshold inhibition criteria in a minimum number of cell lines are then carried forward to a full five-dose assay.

The five-dose assay calculates the percentage growth at five drug concentration levels (10 nM-100 \,µM). This is carried out using a series of absorbance measurements (time zero (Tz), control growth (C), and test growth in the presence of the cytotoxin at the five concentrations (Ti)). The response parameters, GI\textsubscript{50} and LC\textsubscript{50}, are deduced from concentration-response curves by linear interpolation, while TGI is read as the x-axis intercept from the five different drug concentrations.\textsuperscript{189}
6.2 NCI-60 single-dose screen results

*In vitro* activity against the NCI-60 cell line panel of the tested compounds is represented by an overall mean graph (appendices i-xii). Nominal growth percentage is represented by the deviance of a series of horizontal bar graphs from the mean growth inhibition (‘0’) for the 60 cell line array, illustrated in *Fig. 6.1*.

**Fig 6.1 Illustrates the single-dose NCI-60 array for quinolinedione 188**

Lines which extend to the right (-) represent cases where the compound exerts a relative cytotoxic/inhibitory effect on the tested cell lines versus overall mean growth, while bars which extend to the left (+) of ‘0’ represent a relative non-cytotoxic effect on the tested...
cell line, Fig 6.1. Using these graphs it is possible to identify trends across the NCI-60 cell array which are characteristic of the mechanism of action of the compounds being tested.

### 6.2.1 Quinolinedione derivatives

The single-dose mean graphs for quinolinediones 182, 188 and 189 can be found in Appendices xi-xiii. Displayed in blue in Table 6.1 are the mean growth percentage values across each cell line.

![quinolinedione derivative structure](image)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>182</th>
<th>188</th>
<th>189</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substituent</td>
<td>R₁</td>
<td>R₂</td>
<td>Me</td>
</tr>
<tr>
<td>R₁</td>
<td>Bn</td>
<td>H</td>
<td>Br</td>
</tr>
<tr>
<td>R₂</td>
<td>H</td>
<td>Br</td>
<td>Br</td>
</tr>
<tr>
<td>NSC No.</td>
<td>774875</td>
<td>774876</td>
<td>774877</td>
</tr>
<tr>
<td>Mean (%)</td>
<td>75.4</td>
<td>0.3</td>
<td>33.4</td>
</tr>
<tr>
<td>Five Dose</td>
<td>✗</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Carcinoma (% growth):</td>
<td>Leukaemia</td>
<td>27.0</td>
<td>-28.6</td>
</tr>
<tr>
<td></td>
<td>NSCLC</td>
<td>82.0</td>
<td>-0.3</td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>72.6</td>
<td>-0.8</td>
</tr>
<tr>
<td></td>
<td>CNS</td>
<td>90.5</td>
<td>34.4</td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>65.0</td>
<td>-57.9</td>
</tr>
<tr>
<td></td>
<td>Ovarian</td>
<td>91.9</td>
<td>22.3</td>
</tr>
<tr>
<td></td>
<td>Renal</td>
<td>76.9</td>
<td>33.6</td>
</tr>
<tr>
<td></td>
<td>Prostate</td>
<td>4.9</td>
<td>-8.2</td>
</tr>
<tr>
<td></td>
<td>Breast</td>
<td>81.9</td>
<td>7.2</td>
</tr>
</tbody>
</table>

**Table 6.1** One-dose mean growth percentage data for each carcinoma for quinolinedione derivatives
Of the synthesised quinolinediones 182, 188 and 189 were initially selected to undergo assessment by the NCI. Displaying a mean growth of 75% 182 narrowly missed selection for five-dose testing. Of all the cell lines 182 was found to perform well against the majority of leukaemia cell lines most notably K-562 and SR where cytotoxic effects were observed in both cases (-18.5% and -27.5% respectively). Also noteworthy is performance against prostate cancer cell line DU-145 where growth was almost arrested (4.8%) after the 48 hour incubation period, Appendix xi. In spite of not progressing for further testing 182 displays a promising profile against a range of cell-lines. With little exploration carried out on this template, employment of various aromatic/aliphatic amines on this template would lead to exploration of the effect of substitution at the 6-position. Given the affinity 182 showed for certain cell-lines the potential is very promising for derivatives of this structure.

Addition of halides at the 7-position (R₂) is known to infer marked biological activity on the quinolinedione template. This was seen in the case of both 188 and 189, with both compounds surpassing the minimum requirements for progression to five-dose testing.

With an overall mean growth of 0.3%, N-ethyl morpholine derivative 188 was the most active compound tested, exhibiting a cytotoxic effect against five of the nine cancer subclasses. In particular 188 was cytotoxic against all cell lines for leukaemia. Strong cytotoxic effects were also observed for three non-small cell lung carcinoma cell lines, HOP-92 (-89.3%), NCI-H460 (-54.2%) and NCI-H522 (-78.3%) after the 48 hour incubation period. Colon carcinoma cell lines were also found to be sensitive to 188 where cytotoxic effects were observed for cell lines HCC-2998 (-62.1%), HCT-115 (-12.9%) and SW-620 (-50.5%). CNS cancer cell lines displayed less sensitivity with the exception of the SF-268 (-61.9%) cell line.

Of all cancers assessed melanoma was by far the most responsive when treated with 188. Excluding SK-MEL-2, strong cytotoxic effects were observed upon treatment of the cell lines with 188 ranging from -26.6% mean growth against UACC-257 to near lethal effects on MALME-3M (-92.9%). Cytotoxic effects varying from strong to mild were also observed
for ovarian carcinoma cell lines IGROV1, OVCAR-3 and OVCAR-8, renal carcinoma cell line RXF 393 and UO-31, prostate cancer cell line DU-145 and breast cancer cell lines MCF7 and MDA-MB-231/ATCC, *Appendix xii*.

Replacement of the ethyl morpholine fragment with a methyl moiety resulted in an overall mean growth of 33.4%, sufficient for progression to five-dose testing, *Appendix xiii*. When comparing 189 with 188 it can be seen that the activity profile across the leukaemia cell lines decreases. Appreciable cytotoxic effects are exhibited against K-562 (-22.3%) and SR (-34.6%) cell lines. Interestingly when comparing 188 and 189 a reversal of activity is observed in non-small cell lung carcinoma cell lines HOP-62 and HOP-92, also cytotoxic effects were seen against NCI-H460 (-32.9%) and NCI-H522 where 91.9% of cells were killed over the 48 hour incubation period. Cytotoxicity was also observed in the colon carcinoma cell line SW-620 (-38.4%). Overall mean growth across these cell lines was seen to increase when compared to 188, *Table 6.1*.

Compound 189 performed more favourably against the CNS cancer cell lines where a reduction of overall mean growth was observed, with 189 displaying cytotoxic effects against both the SF-539 (-12.5%) and SNB-75 (-0.4%) cell lines, a significant increase in potency versus 188. The same cytotoxicity against melanoma cell lines was not observed in the case of 189 although cytotoxicity was observed against the MALME-3M, MDA-MB-435 and SK-MEL-5 cell lines. Renal carcinoma cell line UO-31 was seen to be susceptible to treatment with 189, displaying cytotoxicity (-28.8%) after the 48 hour incubation period. Significant lack of cell growth (1.4%) was observed against prostate carcinoma cell line DU-145 and breast cancer cell line HS 578T (0.7%). Although performing well from these preliminary tests it appears that the nature of the substituent at the six-position has a significant effect on the biological activity of these quinolinediones. Given the paucity of exploration of these compounds future work could investigate thorough elaboration of the six and seven positions to determine optimal functionality for biological application, research which would run parallel with the expansion of the 6-amino quinolinedione (compounds of type 182) family.
6.2.2 Substituted quinazolinediones

The single-dose mean graphs for quinazolinediones 196, 199, 200, 202, 205, 206, 210, 220, 221 and 215 can be found in Appendices i to x. Highlighted in blue in Table 6.2 are percentage growth results for the four most prevalent cell lines across the quinazolinediones tested. Instances where a compound exhibits proficiency against a particular cell line will be discussed as they arise.

6.2.2.1 N-3 Substituted quinazolinediones

Displaying a mean growth of 100% quinazolinedione 196 failed to exhibit the minimum threshold inhibition in order to be considered for five-dose studies, Table 6.2. However appreciable selectivity was observed against SNB-75 CNS cancer, K-562 (87.0%) and MOLT-4 (88.6%) leukaemia and NCI-H522 (86.0%) non-small cell lung cancer lines.

Although substitution of the three position with a benzyl group resulted in an overall marginal increase in percentage mean growth, 199 displayed a significant decrease in percentage growth against the CCRF-CEM leukaemia cell line, while a slight decrease was also observed for the SNB-75 CNS cancer line.

While introduction of a nitrophenyl function, 200 failed to display any significant change in overall mean growth appreciable activity was observed against renal carcinoma UO-31 (72.8%).

Allyl quinazolinedione 202 also failed to meet the criteria necessary for progression to five dose testing, this being said of the compounds being tested 202 was seen to possess the best activity against A-549/ATCC (78.0%) non-small cell lung and SNB-75 (80.6%) CNS cancer lines. Renal carcinoma toxicity was also observed for 202 with activity being against the A496 (84.8%) cell line in this instance.
Both nitrile 205 and amido 206 quinazolinediones were declined for five-dose assays based on their overall mean growth in one-dose assays. Of the quinazolinediones assayed both compounds were the only two to display activity against melanoma. Both compounds displayed modest activity against UACC-257 returning 86.2% and 89.8% mean growths respectively.

![Chemical structure of a quinazolinedione](image)

**Table 6.2 One-dose data for quinazolinedione derivatives.**

The final N-3 substituted quinazolinedione assayed, chloropropyl quinazolinedione 210 displayed an overall increase in mean growth when compared to its 2-chloroethyl congener 196. Unlike 196 however 210 displays activity against the renal carcinoma A498 (82.5%) cell line.
6.2.2.2 N-1,N-3 Disubstituted quinazolinediones

Derivatisation of the one position of 198 and 199 afforded 220 and 221 which were accepted by the NCI for single-dose testing. Neither compound was found to possess significant cytotoxicity and was declined for five-dose testing, Table 6.2. Both compounds were found to possess modest activity against the renal carcinoma cell line A-498 with 87.6% and 79.1% mean growth respectively. In addition 220 was the only quinazolinedione derivative seen to exhibit activity (88.3%) against the breast cancer cell line HS 578T.

6.2.2.3 6-Substituted quinazolinedione

The final quinazolinedione accepted for one dose testing examined the effect of substituting the 6-position of quinazolinedione 154. Bromo quinazolinedione 215 displayed a lack of cytotoxicity across the 60 cell line screen, a summary of which is displayed in Table 6.2.

Of the substituted quinazolinediones tested it is difficult to deduce any particular affinity these compounds possess for specific cell line due to their relative lack of activity. Future work on this series could aim to investigate increasing potency of these compounds to improve their bioactivity.

6.3 NCI-60 five-dose screen results

Compounds which met the criteria for five-dose assays progressed to further assessment where the compounds were tested against the NCI-60 cell panel at five different concentrations ranging from 100 μM to 10 nM. The data compiled from these results allowed for the generation of dose-response curves for cell growth inhibition as a function
of the concentration of the drug candidate for each cell line tested which allowed the calculation of in vitro GI\textsubscript{50}, TGI and LC\textsubscript{50} values for each of the candidates. This data can then be used to compare the activity of the tested compounds against all compounds in the NCI database using COMPARE analysis with strong correlation between compounds being indicative of similar mechanisms of action providing valuable biological data.

6.3.1 Five-dose data for quinolinediones 188 and 189

Having shown exemplary results in the initial one-dose screen, quinolinedione 188 was tested at five different concentrations against the NCI-60 cell panel. Inhibition across the panel was seen to occur in a dose dependant manner, Fig. 6.2. While cytotoxic effects are observed across all cell lines at higher concentrations, this activity does not sustain at lower concentration with a discernible reduction in activity between the 1 µM and 10 µM.

![Graph showing dose response curves for quinolinedione 188](image)

**Fig 6.2** Dose response curves for quinolinedione 188

Correlation between the one-dose and five-dose screen is consistent. One-dose data for the leukaemia cell line showed good cytotoxic activity across all cell lines in particular the
SR cell line which possessed a mean growth of -53.0% after the 48 hour incubation period. The five-dose response curve for the leukaemia cell line illustrates the SR cell line possessing a TGI value of 1.26 µM complementing observations made in single dose analysis, *Fig 6.3*.

The dose-response curve for the colon cancer cell line illustrated in *Fig 6.3* shows 188 performing well against the HCT-116 cell line which possessed a TGI value of 1.35 µM and a GI₅₀ value in the high nanomolar range (382 nM). The final notable cell line to show encouraging results was the MCF-7 breast carcinoma cell line, *Fig 6.3*.

*Fig 6.3 Dose response curves for quinolinedione 188 against leukaemia, colon and breast cell line carcinomas*

Quinolinedione 188 was demonstrated to possess a GI₅₀ value of 581 nM versus MCF-7. Interestingly this cell line was only the second most susceptible in one-dose testing with MDA-MB-231/ATCC demonstrating increased cytotoxicity at 10 µM (-52.8%). The dose response curve shows that efficacy against this cell line rapidly decreases at concentrations lower than 10 µM, *Fig 6.3*.

Having performed well in the single-dose testing (33.4% overall growth) quinolinedione 189 was accepted for five-dose testing. Although less marked, considerable cytotoxic effects are observed across all cell lines at higher concentrations, with activity again waning sharply at lower concentrations. Comparison of quinolinedione 189 against the cell lines discussed above shows the presence of a methyl amine at the 6-position leads to a decrease in activity with the 189 exhibiting GI₅₀ values of 4.28, 2.72 and 3.92 µM, *Fig 6.4*. 
**Fig 6.4** Dose response curves for quinolinedione 189 against leukaemia, colon and breast cancer cell lines

Other noteworthy results from five-dose testing of 189 were the low TGI values against the melanoma cell lines MDA-MB-435 and UACC-62 (3.21 and 3.72 µM respectively) with activity again sharply decreasing at lower concentrations. Compound 189 also showed good activity against non-small cell lung cancer cell line NCI-H522 (TGI = 5.1 µM) and CNS cancer cell line SF-539 (TGI = 5.8 µM), **Fig 6.5**.

**Fig 6.5** Dose response curves for quinolinedione 189 against melanoma, NSCL and CNS cancer cell lines

### 6.4 COMPARE analysis

Investigation using COMPARE analysis against the standard chemotherapeutic agents list on the NCI database demonstrated a significant correlation between quinolinedione 188 and a series of potent antineoplastics: a benzoquinone AZQ 230, an acridine amsacrine 231 and an anthracycline daunorubicin 232. While AZQ underwent phase II clinical trials
against recurrent primary brain tumours and advanced colon cancer, both amsacrine and daunorubicin are used in the clinic for the treatment of various types of leukemia.\textsuperscript{190-193} The COMPARE algorithm enables mechanistic investigation of cell growth inhibition by generating correlative data between the compound of interest and relevant compounds in the NCI database. The higher the correlation of activity over a range of determined cell lines renders more likely that the tested compound inhibits cell growth via a similar mode. COMPARE analysis allows a range of growth inhibition parameters to be assessed e.g. GI\textsubscript{50}, TGI and LC\textsubscript{50}, \textit{Section 8.4.2}.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Correlation & Compound & NSC Number \\
\hline
0.707 & AZQ 230 & S182986 \\
0.661 & Amsacrine 231 & S249992 \\
0.617 & Daunorubicin 232 & S82151 \\
\hline
\end{tabular}
\caption{Correlation of quinolinedione 188 with compounds 230, 231 and 232 using COMPARE analysis of respective LC\textsubscript{50} data}
\end{table}

Strong correlation was seen based on the LC\textsubscript{50} values between quinolinedione 188 and AZQ 230 (0.707), \textit{Table 6.3}. This correlation was observed to diminish when both compounds’ GI\textsubscript{50} values were compared (0.445) suggesting that AZQs toxicity profile is greater at lower concentrations. Their similar appearance at higher concentrations may have been a result of COMPARE limitations rather than the efficacy of 188. Known DNA intercalators and Topo II inhibitors, Amsacrine and Daunorubicin also produced modest correlation with 188 based on their LC\textsubscript{50} values, however a sharp drop off in correlation
was seen when GI<sub>50</sub> values were compared. Though 188 may possess polypharmacology, results indicate that 188 may act on cells in a similar manner to AZQ 230.

The cytotoxic effects of AZQ are generally attributed to two modes of action. The first involves redox cycling which results in oxidative stress via ROS production and the second, aziridine alkylation which is enhanced by reduction of the quinone moiety to the respective hydroquinone.\textsuperscript{194} Reduction of the quinone raises the pKa of the aziridine nitrogen from 3-4 to 5-6 favouring protonation. The protonation of the aziridine nitrogen increases the electrophilicity of the aziridine ring leading to ring opening upon nucleophilic attack leading to DNA alkylation.\textsuperscript{195} The one-dose assays carried out on the quinolinediones illustrate that incorporation of a halide at the seven position infers a marked improvement in chemotherapeutic potency, which may be due to an increase in the electrophilicity of the seven position following incorporation of the halide akin to the aziridine ring of AZQ, \textit{Fig. 6.6}.

\textit{Fig. 6.6} \textit{Superimposition of quinolinedione 188 and AZQ 230 illustrating their structural similarities}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Compound} & \textbf{GI}_{50} & \textbf{TGI} & \textbf{LC}_{50} \\
\hline
188 & -5.77 & -5.42 & -4.92 \\
NSC 663284 & -5.81 & -5.46 & -5.17 \\
\hline
\end{tabular}
\caption{Comparison of response parameter values for compounds 188 and NSC 663284}
\end{table}
The response parameters of compound 188 were also compared to a similar quinolinedione from the synthetic database, NSC 663284 (DA3003-1) previously synthesised by Lazo et al., Appendix xxii. The graph displays results for 188 at 100 µM versus NSC663284 at 10 µM.

The GI_{50} and TGI values of 188 correlate closely with compound NSC 663284. However it can be seen in Table 6.4 that NSC 663284 possesses a marginally greater cytotoxic profile across the cell line panel. Conversely the COMPARE correlation displays the opposite trend, where correlation is highest for LC_{50} (0.336) falling through TGI (0.216) and is the lowest for GI_{50} (0.122) which suggests that NSC 663284 retains more toxicity at lower concentrations. Of the cell lines assayed, two cell lines showed distinctive traits. Melanoma cell line MDA-MB-435 demonstrated sensitivity towards NSC663284 while little effect was observed when the cell line was treated with 188. Conversely colon cancer cell line HCT-116, Section 6.3.1, appears to be much more sensitive to quinolinedione 188 than its congener NSC663284, Appendix xxii. It is evident that despite obvious structural similarities the potency of the two compounds is comparable but a divergence in selectivity is observed.

6.5 Conclusion

Of the 13 compounds submitted to the DTP for assessment thus far two were found to have sufficiently active profiles to progress to the five-dose screening. The single-dose NCI-60 screen allows for the rapid determination of the biological activity of each compound. This allows for high throughput and is vital for the identification of novel chemotherapeutic agents and for illuminating potential biomechanistic pathways. Single-dose NCI-60 allowed the determination of the compounds submitted which found that while little activity was observed for the quinazolinedione congeners against the cell panel, the quinolinediones were found to possess considerable activity, reflected by their
mean growth percentages, with the addition of a halide at the 7-position bestowing a marked increase in biological activity.

Five-dose assays carried out on 188 and 189 provided a vast amount of biological information for these compounds. While preliminary tests were very promising, a decrease in potency at the lower concentration was observed for both compounds, an issue which could be addressed with further SAR studies. This being said compound 188 exhibited good activity against the SR, MCF-7 and HCT-116 cell lines, Section 6.3.1. Quinolinedione 188 displayed superior activity against the HCT-116 cell line when compared with AZQ 230 and DA3003-1 (NSC 663284). Quinolinedione 189 showed promising activity for the MDA-MB-435, UACC-62, NCI-H522 and SF-539 cell lines warranting further investigation into these compounds as chemotherapeutic agents.

The use of COMPARE analysis provided much valuable information with respect to the potential mechanism of action of these compounds. The correlation observed between quinolinedione 188 and redox cycling/alkylating agent AZQ 230 provides impetus for further investigation. Compounds of type 188 have previously been shown to be potent Cdc25 inhibitors an endeavour which forms the basis of future investigation into the biological assessment of these quinolinediones.
7.0 Current Perspectives
7.0 Current Perspectives

7.1 Overview

Outlined in Section 4.0, the inceptive aim of this project was to develop novel synthetic routes towards the unheralded tricyclic heterocycle 1. Two synthetic routes were developed from retrosynthetic studies which were envisaged to provide nascent platforms from which to synthesise 1. In order to incorporate diversity on pyridinoquinazolinetetrone 1, and to investigate novel 154 and 155 derivatives, functionalization was incorporated on bicyclic fragments 154 and 155 prior to cyclisation to 1, Fig 7.1.

Fig 7.1 Outline of the inceptive aim of this project with arrows illustrating areas of successful derivatisation

Initial synthetic focus was concentrated on utilising route C to effect tricycle 1. 6-Amino-7-halo quinolinediones 157 were projected to be critical intermediates towards the formation of pyridinoquinazolinetetrone 1. Considerable success was met in generating
substrates of type 157 with a total of twenty novel quinolinediones afforded incorporating diversity at positions four, six and seven. As part of our initiative to assess potential bioactivity a preliminary panel of three quinolinediones were assessed by the NCI program and found to possess considerable bioactivity. While the 6-amino quinolinediones were observed to be stable the 7-halo derivatives were found to be considerably more reactive as a result of their increased electrophilicity, Section 5.3. While this imposes limits on their utility as chemotherapeutic agents it also offers an attractive mode of propagating novel tricycles 1 which will be discussed in the following section, Section 7.2.

Synthesis of 1 via route A involved the development of chemistry upon a quinazolinedione scaffold 154, Fig 7.1. Effectuation of diversified substrates from which to construct 1 centred around functionalization of the one and three positions of quinazolinedione 154, a previously underdeveloped domain. Populating a library of substituted quinazolinediones was prosperous yielding a total of thirty-three novel compounds. Of these ten were initially accepted for biological testing by the NCI program for chemotherapeutic assessment. Further development towards tricycle 1 was met with adversity which despite several attempts remains unheralded.

Biological evaluation of novel congeners of both heterocyclic families was afforded by the NCI-60 cell line screen. While evaluation of the quinazolinedione derivatives determined little chemotherapeutic activity at 10 µM the quinolinediones assessed offered remarkable results with two of the three compounds, 188 and 189, proceeding to five-dose testing, Fig 7.2. While results at 10 µM in the five-dose assay were consistent with the one-dose data, cytotoxic activity was observed to diminish at lower concentrations.
Analysis of the most active quinolinedione 188 using the COMPARE algorithm revealed a good correlation between 188 and quinone AZQ (with Daunorubicin and amsacrine showing correlation to a lesser extent) which may offer a mechanism of action of the quinolinediones. All three standard chemotherapeutic agents express strong antineoplastic activity with the mechanism of action of Daunorubicin and amsacrine being determined to be Topoisomerase II poisons. While the action of AZQ is not fully elucidated, it is thought it may act as an alkylating agent, Table 6.3.196-198 The correlation shown between quinolinedione 188 and AZQ 230 using COMPARE analysis illustrates great promise in this area of medicinal chemistry. Given the fact that AZQ progressed to clinical trials, functionalised quinolinediones represent a domain which is ripe for future exploration.

7.2 Future Work

Highlighted in the previous section was the advance this project brought to both the quinoline and quinazolinedione pharmacophore. The nature of the biological results obtained for the quinolinediones established these congeners as excellent substrates for further study. Though the quinazolinedione derivatives failed to exhibit the same potency, the advancements made will hopefully encourage further investigation especially given
the infancy of inquisition into this scaffold. The following section discusses both the immediate and following investigations to be carried out to further this work.

The synthesis of 6-amino-7-halo quinolinediones was seen to infer excellent activity against a number of cancer cell lines within the NCI-60 cell array. Immediate follow-up investigations will involve the evaluation of the chemotherapeutic potential of synthesised 6-amino quinolinediones (given the fact that 6-benzylamino derivative 182, \( Y = \text{Bn} \), Fig 7.3, expressed laudable selectivity against a number of cancer cell lines), followed by derivatisation of these substrates to 6-amino-7-halo quinolinediones and subsequent evaluation via the NCI program.

![Proposed advancement of the quinolinedione scaffold 157](image)

**Fig. 7.3 Proposed advancement of the quinolinedione scaffold 157**

Second generation endeavour will involve extensive derivatisation of the four, six and seven positions with a host of diverse nucleophiles in order to expand the SAR pool of this scaffold with the ultimate aim of increasing the potency and stability of novel derivatives, Fig. 7.3. As was previously eluded to in Section 1.1.4 (page 21), 6-amino-7-halo quinolinediones are known cogent inhibitors of Cdc25 phosphatase. To assess efficacy Cdc25 phosphatase assays would form the next step of biological evaluation for existing and perspective derivatives of type 157.

Preliminary investigations into nucleophilic substitution of the 4-position provided successful results, however the ability to isolate pure products from these reactions was limited due to the use of the Stille coupling in the preceding reaction, Section 5.2.3 (page 108), an issue which will be overcome in the future with the use of an alternative coupling reaction (e.g. Suzuki coupling).
The scaffold 157 represents an excellent substrate to carry out investigative studies on C-C bond forming reactions. In the synthesis of 171, Section 5.2.2.1 (page 104), it was observed that the 6-position could be selectively reacted with a vinyl stannane to produce 6-carbo derivatives with the integrity of the 4-position remaining. This provides the impetus for following work to investigate C-C bond forming reactions at the 7-position of quinolinediones of type 157 with the ultimate aim of incorporating a synthetic handle from which to construct a C-ring heterocycle, 233, Fig. 7.4. Given the difficulty encountered removing tin substrates from the reaction which yielded 171, investigations into the use of other C-C bond forming methodologies would be necessary in order to establish a protocol where the desired product is easily isolated. The 6-amino-7-iodo derivatives synthesized, Section 5.2.5 (page 118), make ideal substrates to investigate alternative coupling strategies (e.g. Heck, Grignard).

![Figure 7.4](image)

**Fig 7.4** Structure of proposed 6-carbo quinolinedione 233 as a means to effect compounds of type 1

Much effort remains to be expended on the effectuation of tricycle 1. If successful, methods discussed in the previous paragraph could yield a robust route to compounds of type 1. Alternatively, by adopting a more speculative approach there are many ways in which construction of 1 could be realised. One such way involves the synthesis of tricyclic phthalimides 234, previously described by Pais.\(^{199}\) Previously within our group application of the Lossen rearrangement to effect uracil analogues from their respective hydroxymaleimide derivatives has been utilised to good effect, making this
transformation a viable choice for use on these systems to generate the desired tricyclic derivatives 235, Fig 7.5.

![Diagram of transformation process](image)

**Fig 7.5 Proposed effectuation of tricycle 235 via the synthesis of tricyclic phthalamides 234**

Future work surrounding the quinazolinedione pharmacophore will centre on firstly establishment of a mechanism by which 197 forms. In order to do so, more studies of the reaction are necessary perhaps with the use of isotopic labelling in order to provide experimental evidence of the means by which 197 forms, Fig 7.6.

![Diagram of reaction](image)

**Fig 7.6**

Given the lacklustre performance of the quinazolinedione series in the NCI assays further studies to develop potent congeners is paramount. Generating novel C-ring systems e.g. cytosine, thiadiazine, Fig 7.7, affords a multifaceted approach for the development of perspective chemotherapeutic agents.
Fig. 7.7 Proposed C-ring derivatisation to form cytosine 236 and thiadiazine 237 derivatives.

One such transformation was realised by converting 154 to the 2,4-dichloroquinazoline derivative 229, a prestigious template, Section 3.3, with derivatives in use in the clinic for the treatment of various types of cancer. Given the excellent antiproliferative activity of this class of quinazolines and the fact that a 2,4,5,8-substitution pattern has not been explored to date, they represent an exemplary paradigm with which to pursue the development of novel chemotherapeutic agents, Fig. 7.8.

Fig. 7.8 Proposed sites of derivatisation of quinazoline 229

In its totality this project has explored a multitude of aspects of both the quinolinedione and quinazolinedione heterocycles towards their application as chemotherapeutic agents. As outlined in the introductory chapters, there was a distinct paucity of literature precedent towards both these derivatives within the broader scope of their parent heterocyclic family. It is forseen that the work carried out during this project will help to inform and encourage future work in this domain. Evident from the NCI-60 assays, immense potential exists within the quinolinedione family for future development as anti-cancer agents.
8.0 Experimental
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8.1 General procedures

Solvents were distilled prior to use as follows: dichloromethane was distilled from phosphorouspentoxide; ethyl acetate was distilled from potassium carbonate; ethanol and methanol were distilled from magnesium in the presence of iodine and stored over 3 Å molecular sieves; toluene was distilled from sodium and benzophenone and stored over 4 Å molecular sieves; hexane was distilled prior to use; tetrahydrofuran was freshly distilled from sodium and benzophenone.

Diethyl ether was obtained pure from Riedel-de Haën. HPLC grade acetonitrile was obtained from Fluka. Molecular sieves were dried by heating to >100 °C at 0.2 mbar overnight or at 140 °C for 24 hours. Organic phases were dried using anhydrous magnesium sulphate. All commercial reagents were used without further purification unless otherwise stated.

Melting points were measured in a uni-melt Thomas Hoover capillary melting point apparatus and are uncorrected. Thin layer chromatography was carried out on precoated silica gel plates (Merck 60 PF254), and visualisation was achieved by UV light detection (254 or 366 nm) or vanillin staining. Wet flash column chromatography was performed using Kieselgel silica gel 60, 0.040 – 0.063 mm (Merck).

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

Low resolution mass spectra were recorded on a Waters Quattro Micro triple quadrupole spectrometer (QAA102) in electrospray ionisation (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 electrospray ionisation (ESI) mode using 50% acetonitrile – water containing 0.1% formic acid as eluent. Samples (max. 1 mg) were dissolved in acetonitrile, methanol or a 1:1 acetonitrile/methanol mixture. An external reference standard of Leucine enkephalin was infused in order to confirm mass accuracy of the MS data acquired and a sulfadimethoxine concentration test was performed to ensure the accuracy of peaks in the ion count range 1 x 10^3 to 1 x 10^6.

1H (300 MHz) and 13C (75 MHz) NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer. 1H (400 MHz) NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. 13C (125 MHz) NMR spectra were recorded on a Bruker Avance 500 NMR spectrometer. 1H (600 MHz) and 13C (150 MHz) NMR spectra were recorded on a Bruker Avance III 600 MHz NMR spectrometer equipped with a dual CH cryoprobe. Spectra were recorded at room temperature (\(\sim -20^\circ C\)) unless otherwise stated, in deuterated chloroform (CDCl3) with tetramethylsilane (TMS) as an internal standard, deuterated dimethylsulfoxide (DMSO-d6), or deuterated methanol (CD3OD). Chemical shifts (\(\delta_H\) and \(\delta_C\)) are expressed in parts per million (ppm) relative to the reference peak. Coupling constants (\(J\)) are expressed in Hertz (Hz). Splitting patterns in 1H NMR spectra are designated as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), qt (quintet), st (sextet), dd (doublet of doublets), td (triplet of doublets), ddd (doublet of doublets of doublets), ddt (doublet of doublets of triplets) and m (multiplet). 13C NMR spectra were assigned (CH, CH2, CH3) with the aid of DEPT (Distortionless Enhancement by Polarisation Transfer) experiments.

Single crystal X-ray diffraction data was collected on a Bruker APEX II DUO diffractometer. The Apex II DUO diffractometer allows either o K\(\alpha\) radiation (graphite monochromator, \(\lambda = 0.7107 \text{ Å}\)) or Cu K\(\alpha\) radiation (doubly curved silicon monochromator, \(\lambda = 1.54178 \text{ Å}\)). An Oxford Cryosystems COBRA fitted with an N\(_2\) generator was used for cooling. Calculations
were performed using the APEX2 software suite, and the diagrams were prepared using Mercury 3.0.

HPLC Conditions:
HPLC: Waters Alliance 2695 with a 2996 Photodiode array detector and a Waters LCT Premier mass spectrometer
Column: Waters X-Bridge C18, 5 µM, 4.6 x 150 mm
Mobile Phase: See below
Gradient: See below
Flow Rate: 0.5 ml/min
Sample run time: 10 mins (Methods 1 & 2), 14 mins Method 3
Injection volume: See below

HPLC Mobile Phases and Gradients:

Method 1
Mobile Phase:
A = Acetonitrile: (0.1% formic acid)
Isocratic

Method 2
Mobile Phase:
A = Acetonitrile: (0.1% formic acid)
B = Water: (0.1% formic acid)
Method 3

Mobile Phase:

A = Acetonitrile: (0.1% formic acid)

B = Water: (0.1% formic acid)

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<th>B%</th>
<th>Gradient</th>
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Experiments

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<th>Compound No.</th>
<th>Method</th>
<th>Injection volume (µl)</th>
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<tr>
<td>87, 180, 183, 188 (5 µl), 189, 190, 198 (2 µl)</td>
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<tr>
<td>186, 187, 191, 192</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>
Highlighted in the above table are the compounds which underwent LCMS analysis. It is important to note that only analysis obtained for compounds which were less than 95% pure is presented in Section 5.2.5.
8.2 Quinoline series

8.2.1 Synthesis of quinoline precursors

Synthesis of 2-nitro-1,4-dimethoxybenzene 167

Copper nitrate (35.240 g, 145.9 mmol) was suspended in diethyl ether (150 mL) and acetic anhydride (70 mL). 1,4-Dimethoxybenzene 163, (10.081 g, 72.95 mmol) was added to this suspension and stirring was continued for 3 hours. The reaction was diluted with water (200 mL) and extracted with diethyl ether (3 x 100 mL). The combined organic extracts were neutralised with saturated aqueous sodium bicarbonate, washed with water (100 mL), dried over magnesium sulphate and concentrated to give a yellow solid, 167 (10.933 g, 82 %). m.p. 70 - 71 °C (Lit. 71 - 72 °C); ν max/cm\(^{-1}\) (KBr): 2925, 2981, 1497, 1457, 1367; δ\(_H\) (300MHz, CDCl\(_3\)): 3.82 [3H, s, C(4)O-CH\(_3\)], 3.92 [3H, s, C(1)O-CH\(_3\)], 7.04 [1H, d, J 9.3 Hz, C(6)H], 7.11 [1H, dd, J 6.2 Hz, 3.1 Hz, C(5)H], 7.38 [1H, d, J 3.1 Hz, C(3)H]; m/z (ESI\(^+\)): 184.4 (M+H\(^+\)), 10%.

Synthesis of 2-amino-1,4-dimethoxybenzene 82

2-Nitro-1,4-dimethoxybenzene, 167 (4.587 g, 24.93 mmol) was dissolved in 95% ethanol (100 mL) and acetic acid (25 mL) and brought to reflux. Iron powder (10.172 g, 182.01 mmol) was added to the refluxing solution followed by immediate addition of ferric chloride (0.692 g, 4.20 mmol). The reaction was refluxed for 2.5 hours. After cooling to room temperature the reaction was vacuum filtered and the filtrate was neutralised using saturated aqueous sodium bicarbonate. The product was extracted with ethyl acetate (3 x 70 mL). The combined organic phases were washed once with water (50 mL), dried over magnesium sulphate and concentrated to give an off-white solid, 82 (3.600 g, 94 %). m.p. 78 - 80 °C (Lit. 79.5 - 80.5 °C); ν max/cm\(^{-1}\) (KBr): 3459, 3368, 2942, 2838, 1622, 1518, 1444,
1312; $\delta_H$ (300MHz, CDCl$_3$): 3.70 [3H, s, C(1)O-CH$_3$], 3.77 [3H, s, C(4)O-CH$_3$], 3.78 [2H, bs, NH$_2$], 6.22 [1H, dd, $J$ 5.9, 3.0 Hz, C(5)H], 6.30 [1H, d, $J$ 3.0 Hz, C(3)H], 6.67 [1H, d, $J$ 8.8 Hz, C(6)H]; m/z (ESI$^+$): 154.4 (M+H)$^+$, 100%.

**Synthesis of 5-[[2,5-dimethoxyphenyl]amino]methylene]-2,2-dimethyl-1,3-dioxane-4,6-dione 83**

2-Amino-1,4-dimethoxybenzene, 168 (2.873 g, 18.78 mmol) was dissolved in ethanol (30 mL). Meldrum’s acid (3.167 g, 21.97 mmol) and triethylorthoformate (3.080 g, 20.78 mmol, 3.45 mL) were added to the stirring solution. The mixture was then refluxed for 3 hours. After completion the reaction was cooled to 0 °C and a solution of 5% ethyl acetate: hexane (300 mL) was added to precipitate the product. The product was then vacuum filtered, washed with ice-cold ethanol and dried to give a pale orange solid, 83 (5.077 g, 88%). m.p. 162 - 163 °C (Lit. 28 166 - 167 °C); $\nu_{max}$/cm$^{-1}$ (KBr): 3246, 2994, 1728, 1607, 1343; $\delta_H$ (300MHz, CDCl$_3$): 1.75 [6H, s, C(CH$_3$)$_2$], 3.71 [3H, s, C(2’)O-CH$_3$], 3.91 [3H, s, C(5’)O-CH$_3$], 6.74 [1H, dd, $J$ 6.2, 2.8 Hz, C(4’)H], 6.88 [1H, d, $J$ 3.8 Hz, C(6’)H], 6.90 [1H, d, $J$ 9.2 Hz, C(3’)H], 8.63 [1H, d, $J$ 14.4 Hz, NH-CH$_2$], 11.54 [1H, d, $J$ 14.5 Hz, NH]; m/z (ESI$^+$): 308.2 (M+H)$^+$, 100%.

**Synthesis of 5-[[4-bromo-2,5-dimethoxyphenyl]amino]methylene]-2,2-dimethyl-1,3-dioxane-4,6-dione 84**

5-[[2,5-Dimethoxyphenyl]amino]methylene]-2,2-dimethyl-1,3-dioxane-4,6-dione, 83 (4.502 g, 14.65 mmol) was suspended in glacial acetic acid (50 mL) at 5 °C. Bromine (2.451 g, 15.02 mmol, 0.81 mL) was added to glacial acetic acid (25 mL). This solution was then added dropwise to the suspension. After complete addition the reaction was stirred for 30
minutes at room temperature. Ice water (200 mL) was then slowly added to the reaction to precipitate the product. The crude product was filtered then dissolved in dichloromethane and neutralised using saturated aqueous sodium bicarbonate. The organic phase was washed with water (100 mL), dried using magnesium sulphate and concentrated to give a green/yellow solid, 84 (4.980 g, 88 %). m.p. 152 - 154 °C (Lit.28 152 °C); v_max/cm⁻¹ (KBr): 3147, 2854, 1725, 1597, 1509, 1339; δ_H (300MHz, CDCl₃): 1.75 [6H, s, C(CH₃)₂], 3.91 [3H, s, C(2')O-CH₃], 3.92 [3H, s, C(5')O-CH₃], 6.88 [1H, s, C(6')H], 7.18 [1H, s, C(3')H], 8.63 [1H, d, J 14.6 Hz, NH-CH], 11.53 [1H, d, J 14.6 Hz, NH]; m/z (ESI⁺): 384.2 (M-H⁺), 100%, 386.1 (M-H⁺), 90%.

Synthesis of 6-bromo-5,8-dimethoxyquinolin-4(1H)-one 85

5-[(4-Bromo-2,5-dimethoxyphenyl)amino]methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione, 84 (4.016 g, 10.40 mmol) was dissolved in diphenyl ether (200 mL) and heated to reflux for 30 minutes. Once cooled to room temperature the reaction was added to hexane (700 mL) to precipitate the product. The product was filtered and washed with hexane (300 mL) followed by drying to give light brown crystals, 85 (2.212 g, 75 %). m.p. 240 - 242 °C (Lit.28 244 - 245 °C); v_max/cm⁻¹ (KBr): 3082, 2841, 1564, 1505, 1434, 1345; δ_H (300MHz, DMSO-d₆): 3.70 [3H, s, O-CH₃], 3.97 [3H, s, O-CH₃], 5.95 [1H, d, J 7.3 Hz, C(3)H], 7.40 [1H, s, C(7)H], 7.66 [1H, d, J 7.5 Hz, C(2)H], 11.22 [1H, bs, NH]; m/z (ESI⁺): 284.2 (M+H⁺), 85%, 286.1 (M+H⁺), 100%.
Synthesis of 6-bromo-4-chloro-5,8-dimethoxyquinoline 86

6-Bromo-5,8-dimethoxyquinolin-4(1H)-one 85 (1.471 g, 5.21 mmol) was added to phosphorous oxychloride (20 mL) and refluxed for 30 minutes. After cooling to room temperature the reaction was poured on to ice-water (50 mL). The acidic mixture was neutralised with 2M aqueous NaOH and extracted with dichloromethane (3 x 20 mL). The organic layer was washed with water (20 mL), dried using magnesium sulphate and concentrated. The crude product was purified by column chromatography using a solvent gradient of 50% ethyl acetate : hexane to 100% ethyl acetate to give a pale yellow solid, 86 (1.226 g, 78 %). m.p. 90 - 92 °C (Lit.28 91 - 92 °C); \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 2936, 2838, 1558, 1492, 1043; \( \delta_{\text{H}} \) (300MHz, CDCl3): 3.88 [3H, s, O-CH3], 4.06 [3H, s, O-CH3], 7.21 [1H, s, C(7)H], 7.52 [1H, d, J 4.8 Hz, C(3)H], 8.73 [1H, d, J 4.61Hz, C(2)H]; m/z (ESI\(^+\)): 301.2 (M+H\(^+\)), 85%, 303.1 (M+H\(^+\)), 90%, 305.1 (M+H\(^+\)), 34%.

8.2.2 Synthesis of 6-vinylquinoline derivatives

8.2.2.1 Stille reagents

Synthesis of tributyl(vinyl) stannane 169

To a dry 250 mL round-bottomed flask under N\(_2\) was added vinyl magnesium bromide (4.331 g, 33 mmol, 33 mL). A solution of tributyl(vinyl)tin chloride (9.720 g, 30 mmol, 8.10 mL) in diethyl ether (70 mL) was added to the reaction flask and the mixture was stirred for 15 hours at room temperature. The reaction was then quenched with water (100 mL). The aqueous layer was isolated and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried using magnesium sulphate, filtered and concentrated to dryness to give a yellow oil which was purified by column chromatography EtOAc : Hexane (50:50) to give a colourless oil, 169.
Synthesis of tetrakis(triphenylphosphine) palladium 170

To a dry 100 mL round-bottomed flask under N\textsubscript{2} was added palladium chloride (0.200 g, 1.13 mmol), triphenylphosphine (1.480 g, 5.64 mmol) and DMSO (30 mL). The mixture was heated to 140 °C for 15 minutes and hydrazine hydrate (0.230 g, 4.51 mmol, 0.224 mL) was added. The reaction was then cooled in a water bath. The resulting precipitate was filtered under N\textsubscript{2}, washed with ethanol (2 x 20 mL) and diethyl ether (2 x 20 mL) and was then dried under a blanket of N\textsubscript{2}. The product was dried under vacuum for 12 hours to give a yellow solid, 170 (1.116 g, 86 %). m.p. 94 – 96 °C dec. (Lit.\textsuperscript{202} 154 °C).

Synthesis of 4-chloro-5,8-dimethoxy-6-vinylquinoline 171

To a flame-dried 250 mL round-bottomed flask under N\textsubscript{2} was added 86 (2.239 g, 7.41 mmol) and toluene (100 mL). After dissolution tributyl(vinyl)tin chloride (3.050 g, 9.64 mmol) and tetrakis(triphenylphosphine) palladium (0.428 g, 0.37 mmol) were added and the reaction was heated to reflux for 72 hours.

The reaction was filtered through Celite and the filtrate concentrated. The crude gum was dissolved in ethyl acetate (30 mL) and 5M KF (30 mL) was added. The biphasic mixture was stirred for 1 hour, then separated and the organic phase was washed with brine (2 x 20 mL), dried using magnesium sulphate, filtered and concentrated to dryness. The crude product was purified by column chromatography using a solvent gradient of 5 % Acetone : Hexane to 10 % Acetone : Hexane to give 171 as a pale yellow solid (0.775 g, 42 %). m.p.
33 – 35 °C; \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 2961, 1721, 1625, 1571, 1457, 1368; \( \delta_{\text{H}} \) (300MHz, CDCl\(_3\)): 3.79 [3H, s, O-CH\(_3\)], 4.10 [3H, s, O-CH\(_3\)], 5.51 [1H, dd, \( J = 0.9 \), 11.1 Hz, C(6)CHCH\(_2\), cis], 5.89 [1H, dd, \( J = 0.9 \), 17.7 Hz, C(6)CHCH\(_2\), trans], 7.23 [1H, s, C(7)\( \text{H} \)], 7.28 [1H, dd, \( J = 17.8 \), 11.1 Hz, C(6)CH], 7.49 [1H, d, \( J = 4.7 \) Hz, C(3)\( \text{H} \)], 8.69 [1H, d, \( J = 4.7 \) Hz, C(2)\( \text{H} \)]; \( \delta_{\text{c}} \) (75 MHz, CDCl\(_3\)) 55.1 [CH\(_3\), O-CH\(_3\)], 62.3 [CH\(_3\), O-CH\(_3\)], 103.9 [CH, aromatic CH], 115.4 [CH\(_2\), CHCH\(_2\)], 120.9 [C, aromatic C], 123.8 [CH, aromatic CH], 127.8 [C, aromatic C], 129.8 [CH, C(6)CH], 139.0 [C, aromatic C], 141.5 [C, aromatic C], 144.8 [C, aromatic C], 147.1 [CH, aromatic CH], 151.0 [C, aromatic C]; m/z (ESI\(^+\)) 250.0635. Found 250.0634.

8.2.3 4-Substituted 6-vinyl quinolines

**Synthesis of 4,5,8-trimethoxy-6-vinylquinoline 173**

To a 25 mL round bottomed flask under N\(_2\) was added MeOH (4.07 mL, 3.21 g, 0.10 mol) followed by cooling to 0 °C. Sodium metal (0.453 g, 0.02 mol) was added portionwise to the stirring MeOH over 30 minutes and stirring was continued until all the sodium dissolved. The 20 % NaOMe solution was the pipetted into another 25 mL round-bottomed flask containing 171 (0.073 g, 0.31 mmol) and the reaction was heated to reflux for 1 hour. The reaction was cooled to room temperature diluted with water (20 mL), neutralised using 2M aqueous HCl and extracted with DCM (3 x 30 mL). The combined organic layers were dried using magnesium sulphate, filtered and concentrated. The crude product was purified by column chromatography using a solvent gradient of 50 % Ethyl acetate : Hexane to 100 % Ethyl acetate to give 173 as a pale yellow oil (0.060 g, 82 %). \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 2948, 2097, 1644, 1585, 1514 \( \delta_{\text{H}} \) (300MHz, CDCl\(_3\)): 3.77 [3H, s, O-CH\(_3\)], 4.08 [3H, s, O-CH\(_3\)], 4.11 [3H, s, O-CH\(_3\)], 5.42 [1H, d, \( J = 11.1 \) Hz, C(6)CHCH\(_2\), cis], 5.83 [1H, d, \( J = 17.8 \) Hz, C(6)CHCH\(_2\), trans], 6.82 [1H, d, \( J = 5.3 \) Hz, C(3)\( \text{H} \)], 7.18 [1H, s, C(7)\( \text{H} \)], 7.25 [1H, dd, \( J = 17.7 \), 11.2 Hz, C(6)CH], 8.71 [1H, d, \( J = 5.2 \) Hz, C(2)\( \text{H} \)]; \( \delta_{\text{c}} \) (75 MHz, CDCl\(_3\)).
CDCl$_3$): 55.9 [2 x CH$_3$, O-CH$_3$], 62.6 [CH$_3$, O-CH$_3$], 102.3 [CH, aromatic CH], 104.4 [CH, aromatic CH], 115.0 [CH$_2$, C(6)CHCH$_2$], 116.9 [C, aromatic C], 127.0 [C, aromatic C], 131.0 [CH, C(6)CH], 142.8 [C, aromatic C], 146.4 [C, aromatic C], 150.0 [CH, aromatic CH], 151.8 [C, aromatic C], 163.1 [C, aromatic C]; m/z (ESI$^+$): 246.3 (M+H$^+$)$^+$, 100%; HRMS (ESI$^+$): Exact mass calculated for C$_{14}$H$_{16}$NO$_3$ 246.1130. Found 246.1133.

### Synthesis of 4-ethoxy-5,8-dimethoxy-6-vinylquinoline 174

To a 25 mL round bottomed flask under N$_2$ was added EtOH (6.41 mL, 5.06 g, 0.11 mol) followed by cooling to 0 °C. Sodium metal (0.498 g, 0.021 mol) was added portionwise to the stirring EtOH over 30 minutes and stirring was continued until all the sodium dissolved. The 20 % NaOEt solution was pipetted into another 25 mL round-bottomed flask containing 171 (0.087 g, 0.34 mmol) and the reaction was heated to reflux for 1 hour. The reaction was cooled to room temperature diluted with water (20 mL), neutralised using 2M aqueous HCl and extracted with DCM (3 x 30 mL). The combined organic layers were dried using magnesium sulphate, filtered and concentrated. The crude product was purified by column chromatography using a solvent gradient of 50 % Ethyl acetate : Hexane to 100 % Ethyl acetate to give 174 as a pale yellow oil (0.040 g, 44 %). $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 2953, 2089 1647, 1580, 1517; $\delta$$_H$ (300MHz, CDCl$_3$): 1.53 [3H, t, $J$ 6.9 Hz, O-CH$_2$C$_6$H$_5$], 3.70 [3H, s, O-C$_6$H$_5$], 4.00 [3H, s, O-CH$_3$], 4.17 [2H, q, $J$ 6.9 Hz, OCH$_2$], 5.33 [1H, d, $J$ 11.3 Hz, C(6)CHCH$_2$, cis], 5.75 [1H, d, $J$ 17.7 Hz, C(6)CHCH$_2$, trans], 6.70 [1H, d, $J$ 5.2 Hz, C(3)H], 7.10 [1H, s, C(7)H], 7.21 [1H, dd, $J$ 17.5, 11.1 Hz, C(6)CH], 8.60 [1H, d, $J$ 5.2 Hz, C(2)H]; $\delta$$_C$ (75 MHz, CDCl$_3$): 14.4 [CH$_3$, O-CH$_2$C$_6$H$_5$], 55.9 [CH$_3$, O-CH$_3$], 62.7 [CH$_3$, O-CH$_3$], 64.4 [CH$_2$, O-CH$_3$], 102.8 [CH, aromatic CH], 104.3 [CH, aromatic CH], 114.9 [CH$_2$, C(6)CHCH$_2$], 117.0 [C, aromatic C], 126.9 [C, aromatic C], 131.1 [CH, C(6)CH], 142.9 [C, aromatic C], 146.5 [C, aromatic C], 150.0 [CH, aromatic CH], 151.7 [C, aromatic C], 162.5 [C, aromatic C]; m/z (ESI$^+$): 260.3 (M+H$^+$)$^+$, 100%; HRMS (ESI$^+$): Exact mass calculated for C$_{15}$H$_{18}$NO$_3$ 260.1287. Found 260.1297.
Synthesis of 4-butoxy-5,8-dimethoxy-6-vinylquinoline 175

To a 25 mL round bottomed flask under N\textsubscript{2} was added nBuOH (9.15 mL, 7.41 g, 0.10 mol). Sodium metal (0.448 g, 0.019 mol) was added portionwise to the stirring nBuOH over 30 minutes and stirring was continued at reflux until all the sodium dissolved. The 20 % NaOnBu solution was pipetted into another 25 mL round-bottomed flask containing 171 (0.078 g, 0.31 mmol) and the reaction was heated to reflux for 1 hour. The reaction was cooled to room temperature diluted with water (20 mL), neutralised using 2M aqueous HCl and extracted with DCM (3 x 30 mL). The combined organic layers were dried using magnesium sulphate, filtered and concentrated. The crude product was purified by column chromatography using a solvent gradient of 50 % Ethyl acetate : Hexane to 100 % Ethyl acetate to give 175 as a pale yellow oil (0.012 g, 14 %).* 

\begin{align*}
\delta_{\text{H}} (300\text{MHz, CDCl}_3) & : 0.95 [3\text{H, t, } J = 7.3 \text{ Hz, O-(CH}_2)_3\text{CH}_3], 1.52 [2\text{H, st, } J = 7.9 \text{ Hz, O-(CH}_2)_2\text{CH}_2], 1.88 [2\text{H, qt, } J = 7.7 \text{ Hz, O-CH}_2\text{CH}_2], 3.68 [3\text{H, s, C(5)O-CH}_3], 3.99 [3\text{H, s, C(8)O-CH}_3], 4.08 [2\text{H, t, } J = 6.8 \text{ Hz, OCH}_2], 5.33 [1\text{H, d, } J = 11.2 \text{ Hz, C(6)CHCH}_2, \text{ cis}], 5.73 [1\text{H, d, } J = 18.0 \text{ Hz, C(6)CHCH}_2, \text{ trans}], 6.71 [1\text{H, d, } J = 5.7 \text{ Hz, C(3)H}], 7.09 [1\text{H, s, C(7)H}], 7.22 [1\text{H, dd, } J = 18.0 \text{ Hz, C(6)CH]}, 8.60 [1\text{H, d, } J = 5.2 \text{ Hz, C(2)H}]; \\
\delta_{\text{C}} (75\text{MHz, CDCl}_3) & : 13.8 [\text{CH}_3, \text{O(CH}_2)_3\text{CH}_3], 19.2 [\text{CH}_2, \text{O(CH}_2)_2\text{CH}_2], 31.0 [\text{CH}_2, \text{OCH}_2\text{CH}_2], 55.9 [\text{CH}_3, \text{OCH}_3], 62.7 [\text{CH}_3, \text{OCH}_3], 68.6 [\text{CH}_2, \text{OCH}_2], 102.8 [\text{CH, aromatic CH}], 104.3 [\text{CH, aromatic CH}], 114.8 [\text{CH}_2, \text{C(6)CHCH}_2], 117.0 [\text{C, aromatic C}], 126.9 [\text{C, aromatic C}], 131.1 [\text{CH, C(6)CH}], 142.9 [\text{C, aromatic C}], 146.5 [\text{C, aromatic C}], 149.9 [\text{CH, aromatic CH}], 151.7 [\text{C, aromatic C}], 162.7 [\text{C, aromatic C}]; \\
m/z (ESI\textsuperscript{+}) & : 288.3 (M+H)\textsuperscript{+}, 100\%; \text{HRMS (ESI\textsuperscript{+})}: \text{Exact mass calculated for } \text{C}_{17}\text{H}_{22}\text{NO}_3 \text{ 288.1600. Found 288.1587} \\
\end{align*} 

* NMR identified traces of stannane, removal of which was problematic
Synthesis of 4-isopropoxy-5,8-dimethoxy-6-vinylquinoline 176

To a 25 mL round bottomed flask under N₂ (8.40 mL, 6.60 g, 0.11 mol) was added iPrOH (8.40 mL, 6.60 g, 0.11 mol). Sodium metal (0.50 g, 0.021 mol) was added portionwise to the stirring iPrOH over 30 minutes and stirring was continued at reflux until all the sodium dissolved. The 20 % NaOiPr solution was pipetted into another 25 mL round-bottomed flask containing 171 (0.088 g, 0.35 mmol) and the reaction was heated to reflux for 1 hour. The reaction was cooled to room temperature diluted with water (20 mL), neutralised using 2M aqueous HCl and extracted with DCM (3 x 30 mL). The combined organic layers were dried using magnesium sulphate, filtered and concentrated. The crude product was purified by column chromatography using a solvent gradient of 50 % Ethyl acetate : Hexane to 100 % Ethyl acetate to give 176 as a pale yellow oil (0.044 g, 46 %).*

* NMR identified traces of stannane, removal of which was problematic.

\[ \delta_{\text{H}} (300\text{MHz, CDCl}_3): 1.51 \text{[6H, d, } J = 6.1 \text{ Hz, O-CH}(\text{CH}_3)\text{]}, 3.76 \text{[3H, s, O-CH}_3\text{]}, 4.07 \text{[3H, s, O-CH}_3\text{]}, 4.84 \text{[1H, sep, } J = 5.9 \text{ Hz, OCH}], 5.39 \text{[1H, d, } J = 11.3 \text{ Hz, C(6)CHCH}_2\text{, cis}], 5.80 \text{[1H, d, } J = 17.6 \text{ Hz, C(6)CHCH}_2\text{, trans}], 6.78 \text{[1H, d, } J = 5.3 \text{ Hz, C(3)H}], 7.16 \text{[1H, s, C(7)H]}, 7.29 \text{[1H, dd, } J = 17.7, 11.0 \text{ Hz, C(6)CH}], 8.66 \text{[1H, d, } J = 5.3 \text{ Hz, C(2)H)]; } \delta_{\text{C}} (75 \text{MHz, CDCl}_3): 21.6 \text{[2 x CH}_3\text{, O-CH}(\text{CH}_3)\text{]}, 56.0 \text{[CH}_3\text{, O-CH}_3\text{]}, 62.6 \text{[CH}_3\text{, O-CH}_3\text{]}, 70.7 \text{[CH, O-CH], 103.6 [CH, aromatic CH], 104.6 [CH, aromatic CH], 114.7 [CH}_2\text{, C(6)CHCH}_2\text{], 117.6 [C, aromatic C], 126.8 [C, aromatic C], 131.1 [CH, C(6)CH], 143.3 [C, aromatic C], 146.6 [C, aromatic C], 149.8 [CH, aromatic CH], 152.3 [C, aromatic C], 161.4 [C, aromatic C]; } m/z (ESI⁺): 274.3 (M+H)⁺, 100%; HRMS (ESI⁺): Exact mass calculated for C₁₆H₂₀NO₃ 274.1443. Found 274.1430.
8.2.4 Synthesis of 6-aminoquinoline-5,8-diones

Synthesis of 4-chloro-6-(2-morpholin-4-yl-ethylamino)quinoline-5,8-dione 87

6-Bromo-4-chloro-5,8-dimethoxyquinoline, 86 (0.750 g, 2.48 mmol) was dissolved in acetonitrile (35 mL) and cooled to 0 °C. CAN (5.450 g, 9.93 mmol) was dissolved in water (20 mL) and then added to the reaction mixture. The reaction was stirred for 4 hours while being slowly warmed to room temperature. The reaction was then diluted with water (20 mL) and extracted with chloroform (3 x 20 mL). The combined organic extracts were washed with water (20 mL), dried using magnesium sulphate and concentrated. The crude quinone was dissolved in ethanol (70 mL) and CeCl$_3$.7H$_2$O (1.016 g, 2.72 g) and 4-[2-aminoethyl]morpholine (0.355 g, 2.72 mmol, 0.358 ml) were added. The reaction was stirred at room temperature for 16 hours, concentrated, dissolved in water and extracted with DCM (3 x 20 mL). The combined organic layers were washed with water (20 mL), dried using magnesium sulphate and concentrated. The crude product was purified by column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give a red solid, 87 (0.323 g, 41 %, 2 steps). m.p. 176 - 178 °C (Lit.$^{28}$ 179 - 181 °C); $\nu$$_{max}$/cm$^{-1}$ (KBr): 2935, 2839, 1669, 1576, 1492; $\delta$$_{H}$ (300MHz, CDCl$_3$): 2.51 [4H, t, $J$ 4.4 Hz, C(2')H$_2$ and C(6')H$_2$], 2.72 [2H, t, $J$ 5.9 Hz, NCH$_2$], 3.23 [2H, dd, $J$ 5.5, 5.4 Hz, NHCH$_2$], 3.72 [4H, t, $J$ 4.5 Hz, C(3')H$_2$ and C(5')H$_2$], 5.90 [1H, s, C(7)H], 6.69 [1H, bs, NH], 7.58 [1H, d, $J$ 5.2, C(3)H], 8.83 [1H, bs, C(2)H]; m/z (ESI$^+$): 322.2 (M+H)$^+$, 100%.
Synthesis of 4-chloro-6-(methylamino)quinoline-5,8-dione 178

6-Bromo-4-chloro-5,8-dimethoxyquinoline, 86 (0.540g, 1.80mmol) was dissolved in acetonitrile (25 mL) and cooled to 0 °C. CAN (3.947 g, 7.20 mmol) was dissolved in water (15 mL) and then added to the reaction mixture. The reaction was stirred for 4 hours while being slowly warmed to room temperature. The reaction was diluted with water (15 mL) and extracted with chloroform (3 x 20 mL). The combined organic extracts were washed with water (20 ml), dried using magnesium sulphate and concentrated. The crude quinone was dissolved in ethanol (50 mL) and CeCl₃.7H₂O (0.738 g, 1.98 mmol) and methylamine (33% in water) (0.061 g, 1.98 mmol, 0.244 mL) were added. The reaction was stirred at room temperature for 16 hours, concentrated, dissolved in water and extracted with DCM (3 x 20 mL). The combined organic layers were washed with water (20 mL), dried using magnesium sulphate and concentrated to dryness. The crude product was purified by column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give a red solid, 178 (0.173 g, 43%, 2 steps). m.p. 208 – 210 °C; νmax/cm⁻¹ (KBr): 3272, 2927, 2364, 1682, 1637, 1597, 1546, 1504; δH (400 MHz, DMSO-d₆) 2.81 [3H, d, J 5.1 Hz, NHCH₃], 5.72 [1H, s, C(7)H], 7.77 [1H, bs, NH], 7.80 [1H, d, J 5.2 Hz, C(3)H], 8.81 [1H, d, J 5.2 Hz, C(2)H]; δc (75 MHz, DMSO-d₆) 29.2 [CH₃, NHCH₃], 95.4 [CH, aromatic CH], 115.3 [C, aromatic C], 121.3 [CH, aromatic CH], 139.2 [CH, aromatic CH], 145.3 [C, aromatic C], 150.9 [C, aromatic C], 173.1 [C, aromatic C], 173.7 (CO, C=O), 177.3 (CO, C=O); m/z (ESI⁺): 223.3 (M+H)⁺, 100%; HRMS (ESI⁺): Exact mass calculated for C₁₀H₈N₂O₂³⁵Cl 223.0274. Found 223.0272.
Synthesis of 4-chloro-6-(4-((4-chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)ethyl)piperazin-1-yl)quinoline-5,8-dione 179

6-Bromo-4-chloro-5,8-dimethoxyquinoline, 86 (0.733 g, 2.43 mmol) was dissolved in acetonitrile (35 mL) and cooled to 0 °C. CAN (5.341 g, 9.74 mmol) was dissolved in water (20 mL) and then added to the reaction mixture. The reaction was stirred for 4 hours while being slowly warmed to room temperature. The reaction was diluted with water (30 mL) and extracted with chloroform (3 x 30 mL). The combined organic extracts were washed with water (30 ml), dried using magnesium sulphate and concentrated. The crude quinone was dissolved in ethanol (70 mL) and CeCl$_3$.7H$_2$O (0.998 g, 2.68 mmol) and 1-(2-aminoethyl)piperazine (0.346 g, 2.68 mmol, 0.351 mL) were added. The reaction was stirred at room temperature for 16 hours, concentrated, dissolved in water and extracted with DCM (3 x 50mL). The combined organic layers were washed with water (30 mL), dried using magnesium sulphate and concentrated to dryness. The crude product was purified by column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give a red solid 179 (0.147 g, 24 %, 2 steps). m.p. >300 °C; $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3352, 2923, 1682, 1606, 1552, 1447; $\delta_{\text{n}}$ (300MHz, CDCl$_3$): 2.71 [4H, t, $J$ 5.0 Hz, C(2'')H$_2$ and C(6'')H$_2$], 2.79 [2H, t, $J$ 5.8 Hz, NHCH$_2$CH$_2$], 3.00 [2H, dd, $J$ 5.6, 5.5 Hz, NHCH$_2$], 3.58 [4H, t, $J$ 4.9 Hz, C(3'')H$_2$ and C(5'')H$_2$], 5.91 [1H, s, C(7)H or C(7')H], 6.13 [1H, s, C(7)H or C(7')H], 6.64 [1H, bs, NH], 7.58 [2H, d, $J$ 5.2 Hz, C(3)H and C(3'H)], 8.79 [1H, d, $J$ 5.2 Hz, C(2)H or C(2'H)], 8.83 [1H, d, $J$ 5.3 Hz, C(2)H or C(2'H)]; $\delta_{\text{c}}$ (150MHz, CDCl$_3$): 38.7 [CH$_2$], 48.5 [2 x CH$_2$], 52.0 [2 x CH$_2$], 54.8 [CH$_2$], 101.2 [CH, aromatic CH], 108.9 [CH, aromatic CH], 124.1 [C, aromatic C], 126.9 [C, aromatic C], 129.3 [CH, aromatic CH], 129.7 [CH, aromatic CH], 144.0 [C, aromatic C], 144.7 [C, aromatic C], 147.9 [C, aromatic C], 150.0 [C, aromatic C], 151.4 [C, aromatic C], 153.5 [CH, aromatic CH], 153.9 [CH, aromatic CH], 154.5 [C, aromatic C], 179.3 [CO, C=O], 179.7 [CO, C=O].
180.3 [CO, C=O], 180.9 [CO, C=O]; m/z (ESI\(^+\)): 512.1 (M+H\(^+\)), 100%; HRMS (ESI\(^+\)): Exact mass calculated for C\(_{24}\)H\(_{20}\)N\(_5\)O\(_4\)Cl\(_2\) 512.0892. Found 512.0893.

**Synthesis of 4-chloro-6-((2-(piperidin-1-yl)ethyl)amino)quinoline-5,8-dione 180**

6-Bromo-4-chloro-5,8-dimethoxyquinoline, 86 (0.834 g, 2.77 mmol) was dissolved in acetonitrile (40 mL) and cooled to 0 °C. CAN (6.071 g, 11.08 mmol) was dissolved in water (25 mL) and then added to the reaction mixture. The reaction was stirred for 4 hours while being slowly warmed to room temperature. The reaction was diluted with water (30 mL) and extracted with chloroform (3 x 30 mL). The combined organic extracts were washed with water (30 mL), dried using magnesium sulphate and concentrated. The crude quinone was dissolved in ethanol (75 mL) and CeCl\(_3\)·7H\(_2\)O (1.135 g, 3.04 mmol) and 1-(2-aminoethyl)piperidine (0.389 g, 3.04 mmol, 0.433 mL) were added. The reaction was stirred at room temperature for 16 hours, concentrated, dissolved in water and extracted with DCM (3 x 50mL). The combined organic layers were washed with water (30 mL), dried using magnesium sulphate and concentrated to dryness. The crude product was purified by column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give a red solid 180 (0.300 g, 34 %, 2 steps). m.p. 168 – 170 °C; \(\nu_{\text{max}}/\text{cm}^{-1}\) (KBr): 3333, 2929, 2846, 2364, 1684, 1628, 1599, 1554, 1500; \(\delta_h\) (300MHz, CDCl\(_3\)): 1.48 [2H, m, C(4')\(H_2\)], 1.62 [4H, qt, J 5.5 Hz, C(3')\(H_2\) and C(5')\(H_2\)], 2.44 [4H, t, J 4.7 Hz, C(2')\(H_2\) and C(6')\(H_2\)], 2.65 [2H, t, J 6.1 Hz, NHCH\(_2\)CH\(_2\)], 3.22 [2H, dd, J 5.6, 5.5 Hz, NHCH\(_2\)H], 5.89 [1H, s, C(7)\(H\)], 6.82 [1H, bs, NH], 7.56 [1H, d, J 5.2 Hz, C(3)\(H\)], 8.82 [1H, d, J 5.2 Hz, C(2)\(H\)], \(\delta_c\) (75 MHz, CDCl\(_3\)) 24.1 [\(\text{CH}_2\)], 25.7 [2 x \(\text{CH}_2\)], 38.7 [\(\text{CH}_2\)], 54.1 [2 x \(\text{CH}_2\)], 55.4 [\(\text{CH}_2\)], 100.8 [\(\text{CH}\), aromatic \(\text{CH}\)], 124.1 [C, aromatic C], 129.1 [CH, aromatic CH], 144.6 [C, aromatic C], 148.1 [C, aromatic C], 151.5 [C, aromatic C], 153.8 [CH, aromatic CH], 179.3 (C, C=O), 179.6 (C, C=O); m/z (ESI\(^+\)): 320.2 (M+H\(^+\)), 100%; HRMS (ESI\(^+\)): Exact mass calculated for C\(_{16}\)H\(_{19}\)N\(_3\)O\(_2\)\(^{35}\)Cl 320.1166. Found 320.1154.
Synthesis of 4-chloro-6-((2-(dimethylamino)ethyl)amino)quinoline-5,8-dione 181

6-Bromo-4-chloro-5,8-dimethoxyquinoline, 86 (0.921 g, 3.06 mmol) was dissolved in acetonitrile (50 mL) and cooled to 0 °C. CAN (6.714 g, 12.24 mmol) was dissolved in water (35 mL) and then added to the reaction mixture. The reaction was stirred for 4 hours while being slowly warmed to room temperature. The reaction was diluted with water (50 mL) and extracted with chloroform (3 x 40 mL). The combined organic extracts were washed with water (40 mL), dried using magnesium sulphate and concentrated. The crude quinone was dissolved in ethanol (85 mL) and CeCl₃·7H₂O (1.254 g, 3.36 mmol) and N,N-dimethylethylenediamine (0.296 g, 3.36 mmol, 0.367 mL) were added. The reaction was stirred at room temperature for 16 hours, concentrated, dissolved in water and extracted with DCM (3 x 50mL). The combined organic layers were washed with water (30 mL), dried using magnesium sulphate and concentrated to dryness. The crude product was purified by column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give a red solid 181 (0.209 g, 24 %, 2 steps). m.p. 140 – 142 °C; νmax/cm⁻¹ (KBr): 3331, 3044, 2943, 2773, 1685, 1633, 1600, 1555, 1503; δH (300MHz, CDCl₃): 2.29 [6H, s, NH(CH₃)₂], 2.64 [2H, t, J 5.3 Hz, NHCH₂CH₃], 3.22 [2H, dd, J 5.6, 5.5 Hz, NHCH₂], 5.90 [1H, s, C(7)H], 6.72 [1H, bs, NH], 7.57 [1H, d, J 5.2 Hz, C(3)H], 8.82 [1H, d, J 5.9 Hz, C(2)H]; δc (75 MHz, CDCl₃) 39.5 [CH₂], 44.9 [2 x CH₃, N(CH₃)₂], 56.1 [CH₂], 100.8 [CH, aromatic CH], 124.1 [C, aromatic C], 129.1 [CH, aromatic CH], 144.6 [C, aromatic C], 148.1 [C, aromatic C], 151.5 [C, aromatic C], 153.8 [CH, aromatic CH], 179.3 (CO, C=O), 179.6 (CO, C=O) m/z (ESI⁺): 280.2 (M+H)⁺, 100%; HRMS (ESI⁺): Exact mass calculated for C₁₃H₁₅N₃O₂Cl 280.0853. Found 280.0844.
6-Bromo-4-chloro-5,8-dimethoxyquinoline, 86 (0.564 g, 1.88 mmol) was dissolved in acetonitrile (25 mL) and cooled to 0 °C. CAN (4.122 g, 7.52 mmol) was dissolved in water (15 mL) and then added to the reaction mixture. The reaction was stirred for 4 hours while being slowly warmed to room temperature. The reaction was diluted with water (15 mL) and extracted with chloroform (3 x 20 mL). The combined organic extracts were washed with water (20 mL), dried using magnesium sulphate and concentrated. The crude quinone was dissolved in ethanol (50 mL) and CeCl$_3$.7H$_2$O (0.774 g, 2.07 mmol) and benzylamine (0.222 g, 2.07 mmol, 0.227 mL) were added. The reaction was stirred at room temperature for 16 hours, concentrated in water and extracted with DCM (3 x 20mL). The combined organic layers were washed with water (20 mL), dried using magnesium sulphate and concentrated to dryness. The crude product was purified by column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give a red solid, 182 (0.157 g, 28 %, 2 steps). m.p. 90 - 92 °C; $\upsilon_{\text{max}}$/cm$^{-1}$ (KBr): 3342, 2935, 2839, 1674, 1576, 1492; $\delta$ (300MHz, CDCl$_3$): 4.41 [2H, d, $J$ 6.6 Hz, NHCH$_2$], 5.98 [1H, s, C(7)H], 6.36 [1H, bs, NH], 7.30 – 7.41 [5H, m, ArH], 7.58 [1H, d, $J$ 5.1 Hz, C(3)H], 8.83 [1H, d, $J$ 5.3 Hz, C(2)H]; $\delta_c$ (75 MHz, CDCl$_3$): 46.9 [CH$_2$, NHCH$_2$], 101.8 [CH, aromatic CH], 124.1 [C, aromatic C], 127.6 [2 x CH, aromatic CH], 128.3 [CH, aromatic CH], 129.1 [2 x CH, aromatic CH], 129.2 [CH, aromatic CH], 135.2 [C, aromatic C], 144.7 [C, aromatic C], 147.7 [C, aromatic C], 151.2 [C, aromatic C], 153.9 [CH, aromatic CH], 179.5 (CO, C=O), 179.7 (CO, C=O); m/z (ESI$^+$): 299.2 (M+H)$^+$, 100%; HRMS (ESI$^+$): Exact mass calculated for C$_{16}$H$_{12}$N$_2$O$_2$Cl 299.0587. Found 299.0581.
8.2.5 Synthesis of 7-halo-6-aminoquinoline-5,8-diones

Synthesis of 7-bromo-4-chloro-6-((2-morpholinoethyl)amino)quinoline-5,8-dione 188

4-Chloro-6-((2-morpholino-4-yl-ethylamino)quinoline-5,8-dione, 87 (0.098 g, 0.30 mmol) was dissolved in MeOH (50mL). N-Bromosuccinimide (0.054 g, 0.30 mmol) was added and the reaction was stirred at room temperature overnight. The reaction was evaporated and adsorbed onto Celite and purified using column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give a red solid (single spot TLC), 188 (0.082 g, 67%). m.p. 104 – 106 °C; v_max/cm⁻¹ (KBr): 3247, 2925, 1709, 1685, 1556; δ_H (600MHz, CDCl₃): 2.62 [4H, t, J 4.6 Hz, C(2')H₂ and C(6')H₂], 2.75 [2H, t, J 5.9 Hz, NHCH₂CH₂], 3.77 [4H, t, J 4.6 Hz, C(3')H₂ and C(5')H₂], 3.93 [2H, dd, J 5.7, 4.2 Hz, NCH₂], 7.14 [1H, bs, NH], 7.58 [1H, d, J 5.2, C(3)H], 8.82 [1H, d, J 5.2 Hz, C(2)H]; δ_c (150MHz, CDCl₃): 41.2 [CH₂], 52.9 [2 x CH₂], 56.5 [CH₂], 66.9 [2 x CH₂], 104.8 [C, aromatic C], 129.3 [CH, aromatic CH], 138.4 [C, aromatic C], 144.7 [C, aromatic C], 150.1 [C, aromatic C], 153.7 [CH, aromatic CH], 164.2 [C, aromatic C], 173.3 [CO, C=O], 178.1 [CO, C=O]; m/z (ESI⁺): 400.1 (M+H)⁺, 62%; HRMS (ESI⁺): Exact mass calculated for C₁₅H₁₆N₃O₃₃Cl₇9Br 400.0064. Found 400.0050.

Aberrant peaks in ¹H NMR; 2.91 (t), 3.30 (t), 3.90 (s), 7.66 (d), 8.74 (d), 11.01 (bs).

The ratio of the impurity was estimated at 1:11 by comparing the doublet of 188 at 8.82 ppm with the doublet of the impurity at 8.74 ppm. Prior to submission to the NCI the sample was evaluated by LCMS analysis identifying one component in more than 95% purity, the hydrolysis product 4-hydroxyazopyridine, Appendix xxiii.
Synthesis of 4-chloro-7-iodo-6-((2-morpholinoethyl)amino)quinoline-5,8-dione 183

4-Chloro-6-(2-morpholin-4-yl-ethylamino)quinoline-5,8-dione, 87 (0.089 g, 0.27 mmol) was dissolved in MeOH (50 mL). N-Iodosuccinimide (0.062 g, 0.27 mmol) was added and the reaction was stirred at room temperature overnight. The reaction was evaporated and adsorbed onto Celite and purified using column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give a red solid, 183 (0.113 g, 91%). m.p. 131 - 133 °C; νmax/cm⁻¹ (KBr): 3421, 1682, 1552, 1379; δH (400MHz, CDCl₃): 2.59 [4H, overlapping dd, J 3.93 Hz, C(2')H₂ and C(6')H₂], 2.69 [2H, t, J 5.44 Hz, CH₂CH₂], 3.67 [4H, t, J 4.6 Hz, C(3')H₂ and C(5')H₂], 3.93 [2H, dd, J 5.7, 4.5 Hz, NCH₂], 7.13 [1H, bs, NH], 7.58 [1H, d, J 5.3, C(3)H], 8.80 [1H, d, J 5.7 Hz, C(2)H]; δc (75 MHz, CDCl₃): 41.9 [CH₂], 52.8 [2 x CH₂], 56.3 [CH₂], 66.9 [2 x CH₂], 124.0 [C, aromatic C], 129.2 [CH, aromatic CH], 144.5 [C, aromatic C], 148.8 [C, aromatic C], 152.0 [C, aromatic C], 153.4 [CH, aromatic CH], 165.4 [C, aromatic C], 174.3 [CO, C=O], 177.1 [CO, C=O]; m/z (ESI⁺): 448.0 (M+H)⁺, 10%; HRMS (ESI⁺): Exact mass calculated for C₁₅H₁₆N₃O₃³Cl 447.9925. Found 447.9925.

Synthesis of 7-bromo-4-chloro-6-(methylamino)quinoline-5,8-dione 189

4-Chloro-6-(methylamino)quinoline-5,8-dione, 178 (0.063 g, 0.28 mmol) was dissolved in MeOH (60mL). N-Bromosuccinimide (0.050 g, 0.28 mmol) was added and the reaction was stirred at room temperature overnight. The reaction was evaporated and adsorbed onto Celite and separated using column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give an impure red solid containing 189 (0.032 g, 38 %) and 7-bromo-4-methoxy-6-(methylamino)quinoline-5,8-dione, 190 as an impure red solid (0.021 g, 26%).
189: m.p. 174 – 176 °C; $v_{\text{max}}$/cm$^{-1}$ (KBr): 3182, 1773, 1691, 1588, 1546, 1447; $\delta_{\text{H}}$ (400MHz, DMSO-$d_6$): 3.24 [3H, d, $J$ 4.6 Hz, NHCH$_3$], 7.63 [1H, bs, NH], 7.81 [1H, d, $J$ 5.2 Hz, C(3)H], 8.77 [1H, d, $J$ 5.2 Hz, C(2)H]; $\delta_{\text{C}}$ (75MHz, DMSO-$d_6$): 33.2 [CH$_3$, NHCH$_3$], 129.5 [CH, aromatic CH], 140.7 [C, aromatic C], 142.5 [C, aromatic C], 149.8 [C, aromatic C], 150.8 [C, aromatic C], 153.6 [CH, aromatic CH], 167.9 [C, aromatic C], 179.8 [CO, C=O], 186.1 [CO, C=O]; m/z (ESI$^+$): 301.1 (M+H$^+$), 75%; HRMS (ESI$^+$): Exact mass calculated for C$_{10}$H$_7$N$_2$O$_2$ 300.9379. Found 300.9375.

Aberrant peaks in the $^1$H NMR; 2.63 (d), 7.87 (d), 8.78 (d), 11.06 (bs).

190: m.p. dec. >180 °C; $v_{\text{max}}$/cm$^{-1}$ (KBr): 3437, 2892, 1697, 1627, 1558, 1379; $\delta_{\text{H}}$ (400MHz, DMSO-$d_6$): 3.99 [3H, s, O-C$_3$H$_3$], 7.43 [1H, bs, NH], 7.38 [1H, bs, C(3)H], 8.70 [1H, bs, C(2)H]; $\delta_{\text{C}}$ (150MHz, DMSO-$d_6$): 32.7 [CH$_3$, NHCH$_3$], 55.9 [CH$_3$, OCH$_3$], 111.2 [C, aromatic C], 128.9 [CH, aromatic CH], 142.5 [C, aromatic C], 149.4 [C, aromatic C], 153.2 [CH, aromatic CH], 154.4 [C, aromatic C], 165.4 [C, aromatic C], 172.1 [CO, C=O], 178.0 [CO, C=O]; m/z (ESI$^+$): 297.0 (M+H$^+$), 50%; HRMS (ESI$^+$): Exact mass calculated for C$_{11}$H$_{10}$N$_2$O$_2$ 296.9875. Found 296.9876.

Aberrant peaks in the $^1$H NMR; 4.55 (s), 7.00 (d), 7.81 (d), 7.83 (bs), 8.79 (d).

**Synthesis of 4-chloro-7-iodo-6-(methylamino)quinolone-5,8-dione 184**

4-Chloro-6-(methylamino)quinoline-5,8-dione, 178 (0.111 g, 0.49 mmol) was dissolved in MeOH (120mL). N-Iodosuccinimide (0.112 g, 0.49 mmol) was added and the reaction was stirred at room temperature overnight. The reaction was concentrated with Celite and separated using column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give an impure red solid, 184 (0.068 g, 40 %) and 7-bromo-4-methoxy-6-(methylamino)quinoline-5,8-dione, 185 as an impure red solid (0.047 g, 28 %).
184: m.p. 200 – 202 °C; $\nu_{\max}$/cm$^{-1}$ (KBr): 3163, 2988, 1693, 1584, 1546, 1509, 1448; $\delta_{\text{H}}$ (300MHz, CDCl$_3$): 3.46 [3H, d, 5.8 Hz, NHCH$_3$], 6.11 [1H, bs, NH], 7.57 [1H, d, 5.2 Hz, C(3)H], 8.78 [1H, d, 5.3 Hz, C(2)H]; $\delta_{\text{C}}$ (150MHz, CDCl$_3$): 29.7 [CH$_3$, NHCH$_3$], 100.7 [C, aromatic C], 109.4 [C, aromatic C], 123.8 [C, aromatic C], 129.4 [CH, aromatic CH], 144.7 [C, aromatic C], 148.7 [C, aromatic C], 153.5 [CH, aromatic CH], 174.5 [CO, C=O], 177.1 [CO, C=O]; m/z (ESI$^+$): 349.0 (M+H$^+$), 100%; HRMS (ESI$^+$): Exact mass calculated for C$_{10}$H$_8$N$_2$O$_2$Cl 348.9241. Found 348.9245.

Aberrant peaks in $^1$H NMR; 8.45 (bs).

185: m.p. 167 – 169 °C; $\nu_{\max}$/cm$^{-1}$ (KBr): 3296, 2918, 1679, 1603, 1577, 1516; $\delta_{\text{H}}$ (300MHz, CDCl$_3$): 3.45 [3H, d, 5.6 Hz, NHCH$_3$], 4.08 [CH$_3$, s, OCH$_3$] 6.13 [1H, bs, NH], 7.09 [1H, d, J 5.0 Hz, C(3)H], 8.78 [1H, d, 5.2 Hz, C(2)H]; $\delta_{\text{C}}$ (150MHz, CDCl$_3$): 33.6 [CH$_3$, NHCH$_3$], 56.8 [CH$_3$, OCH$_3$], 110.7 [CH, aromatic CH], 115.8 [C, aromatic C], 129.3 [C, aromatic C], 130.2 [C, aromatic C], 149.3 [C, aromatic C], 155.4 [CH, aromatic CH], 165.7 [C, aromatic C], 175.7 [CO, C=O], 177.6 [CO, C=O]; m/z (ESI$^+$): 345.0 (M+H$^+$), 100%; HRMS (ESI$^+$): Exact mass calculated for C$_{11}$H$_{11}$N$_2$O$_3$I 344.9736. Found 344.9734.

Aberrant peaks in $^1$H NMR; 1.68 (bs), 4.30 (q), 4.68 (s), 7.57 (d), 8.08 (s).

**Synthesis of 6-(benzylamino)-7-bromo-4-chloroquinoline-5,8-dione 191**

6-(Benzylamino)-4-chloroquinoline-5,8-dione, 182 (0.048 g, 0.16 mmol) was dissolved in MeOH (25mL). N-Bromosuccinimide (0.028 g, 0.16 mmol) was added and the reaction was stirred at room temperature overnight.

The reaction was concentrated with Celite and separated using column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give 191 as an impure red solid (0.024 g, 41 %) and 7-bromo-4-methoxy-6-(methylamino)quinoline-5,8-dione 192 as an impure red solid (0.014 g, 24 %).
191: m.p. 93 – 95 °C; \( v_{\text{max}}/\text{cm}^{-1} \) (KBr): 3368, 2924, 2853, 1773, 1699, 1554; \( \delta_H \) (300MHz, DMSO-\( d_6 \)): 4.94 [2H, d, J 6.6 Hz, NHCH\(_2\)], 7.22 – 7.33 [5H, m, ArH], 7.80 [1H, d, J 5.2 Hz, C(3)H], 7.88 – 7.98 [1H, bs, NH], 8.77 [1H, d, J 5.4 Hz, C(2)H]; \( \delta_C \) (75MHz, DMSO-\( d_6 \)): 29.6 [CH\(_2\), NHCH\(_2\)], 115.1 [C, aromatic C], 127.1 [2 x CH, aromatic CH], 127.4 [CH, aromatic CH], 128.8 [2 x CH, aromatic CH], 129.5 [CH, aromatic CH], 135.0 [C, aromatic C], 136.6 [C, aromatic C], 140.9 [CH, aromatic CH], 145.0 [C, aromatic C], 150.9 [C, aromatic C], 169.3 [C, aromatic C], 175.2 [CO, C=O], 176.1 [CO, C=O]; m/z (ESI\(^+\)): 377.0 (M+H\(^+\)), 8%; HRMS (ESI\(^+\)): Exact mass calculated for C\(_{16}\)H\(_{11}\)N\(_2\)O\(_2\)Cl\(_7\)Br 376.9692. Found 376.9686.

Aberrant peaks in the \(^1\)H NMR; 4.72 (bs), 5.06 (s), 6.77 (d), 7.36 (d), 7.57-7.72 (m), 7.86 (t), 8.20-8.24 (m), 8.47 (bs), 8.81 (d), 11.04 (bs).

192: m.p. 158 – 160 °C; \( v_{\text{max}}/\text{cm}^{-1} \) (KBr): 3352, 2924, 2852, 1667, 1632, 1596, 1570; \( \delta_H \) (600MHz, DMSO-\( d_6 \)): 3.98 [3H, s, OCH\(_3\)], 4.95 [2H, s, NHCH\(_2\)], 7.25 [1H, s, ArCH], 7.32 [4H, d, J 12.0 Hz, ArCH\(_3\)], 7.40 [1H, bs, C(3)H], 7.86 [1H, m, NH], 8.71 [1H, bs, C(2)H]; \( \delta_C \) (150MHz, DMSO-\( d_6 \)): 47.1 [CH\(_2\), NHCH\(_2\)], 56.8 [CH\(_3\), O-CH\(_3\)], 100.2 [C, aromatic C], 111.2 [CH, aromatic CH], 115.5 [C, aromatic C], 126.6 [2 x CH, aromatic CH], 126.7 [CH, aromatic CH], 129.1 [2 x CH, aromatic CH], 139.5 [C, aromatic C], 149.4 [C, aromatic C], 154.6 [CH, aromatic CH], 165.0 [C, aromatic C], 173.8 [C, aromatic C], 176.6 [CO, C=O], 193.2 [CO, C=O]; m/z (ESI\(^+\)): 373.0 (M+H\(^+\)), 100%; HRMS (ESI\(^+\)): Exact mass calculated for C\(_{17}\)H\(_{14}\)N\(_2\)O\(_3\)Cl\(_7\)Br 373.0188. Found 373.0178.

Aberrant peaks in the \(^1\)H NMR; 4.21 (s), 4.55 (bs), 8.24 (s), 7.63 (d), 7.71 (s), 9.91 (s), 7.98 (s), 8.78 (s), 8.86 (s), 10.02 (s).
Synthesis of 6-(benzylamino)-7-iodo-4-chloroquinoline-5,8-dione 186

6-(Benzylamino)-4-chloroquinoline-5,8-dione, 182 (0.065 g, 0.21 mmol) was dissolved in MeOH (100 mL). N-Iodosuccinimide (0.049 g, 0.21 mmol) was added and the reaction was stirred at room temperature overnight. The reaction was concentrated with Celite and separated using column chromatography (50% EtOAc:Hexane-100% EtOAc-100% DCM-10% MeOH:DCM) to give an impure red solid, 186 (0.035 g, 39 %) and 187 as an impure red solid (0.019 g, 22 %).

186: m.p. 95 – 97 °C; $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3341, 2925, 1772, 1699, 1552, 1488, 1349; $\delta_H$ (300MHz, DMSO-$d_6$): 4.92 [2H, d, $J$ 6.6 Hz, NHCH$_2$], 7.22-7.29 [2H, m, ArH], 7.32-7.36 [2H, m, ArH], 7.63 – 7.68 [1H, m, ArH], 7.80, [1H, d, $J$ 5.2 Hz, C(3)H], 8.76 [1H, d, $J$ 5.2 Hz, C(2)H]; $\delta_c$ (75MHz, DMSO-$d_6$): 29.9 [CH$_2$, NHCH$_2$], 109.3 [C, aromatic C], 127.2 [CH, aromatic CH], 127.6 [2 x CH, aromatic CH], 128.8 [2 x CH, aromatic CH], 129.9 [CH, aromatic CH], 137.6 [C, aromatic C], 139.9 [C, aromatic C], 142.9 [C, aromatic C], 148.5 [C, aromatic C], 153.5 [CH, aromatic CH], 170.7 [C, aromatic C], 174.9 [CO, C=O], 177.7 [CO, C=O]; m/z (ESI$^+$): 425.0 (M+H)$^+$, 100%; HRMS (ESI$^+$): Exact mass calculated for C$_{16}$H$_{11}$N$_2$O$_2$ClI 424.9554. Found 424.9540.

Aberrant peaks in $^1$H NMR; 3.60 (bs), 4.47 (d), 5.09 (d), 5.75 (s), 6.45 (d), 7.91 (d), 8.00 (d), 8.29 (t), 8.85 (d) 11.10 (bs).

187: $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3296, 2918, 2345, 1679, 1603, 1577, 1516, 1475, 1448; m/z (ESI$^+$): 421.1 (M+H)$^+$, 100%; HRMS (ESI$^+$): Exact mass calculated for C$_{17}$H$_{14}$N$_2$O$_3$I 421.0049. Found 421.0035. Although synthesis was confirmed by HRMS, 187 could not be satisfactorily purified as confirmed by $^1$H NMR spectroscopy.
8.3 Quinazolinedione series

8.3.1 Synthesis of quinazolinedione precursors

Synthesis of 3,6-dimethoxy-2-nitrobenzoic acid 193

Concentrated HNO₃ (20 mL) was added to a 100 mL round bottomed flask and cooled to 0 °C. 2,5-Dimethoxybenzoic acid 158 (2.004 g, 11.00 mmol) was added portionwise over 30 minutes with stirring. The solution was stirred at 0 °C for 3 hours and then poured onto cracked ice (60 mL) yielding a yellow precipitate. The precipitate was collected by suction filtration, washed with water (3 x 20 mL) and oven-dried overnight to give a 5:1 mixture (2.162 g, 86 %) of 193 and its 4-isomer, 194. The ratio of the mixtures was determined by NMR. The product was used without further purification for the next reaction.¹⁶⁵

3,6-Dimethoxy-2-nitrobenzoic acid, 193: δH (300 MHz, DMSO-d₆): 3.82 [15H, s, O-CH₃], 3.86 [15H, s, O-CH₃], 7.36 and 7.42 [10H, AB q, ΔνAB 6.2 Hz, J 9.3 Hz, C(4)H and C(5)H], 13.70 [5H, bs, O-H].

2,5-Dimethoxy-4-nitrobenzoic acid: 194: δH (300 MHz, DMSO-d₆): 3.82 [3H, s, O-CH₃], 3.89 [3H, s, O-CH₃], 7.51 [1H, s, C(3)H], 7.63 [1H, s, C(6)H].

Synthesis of methyl 3,6-dimethoxy-2-nitrobenzoate 195

HPLC grade acetone (30 mL), potassium carbonate (3.180 g, 23.00 mmol) and dimethyl sulfate (1.420 g, 11.25 mmol, 1.07 mL) were successively added to the crude acid, 193 (2.194 g, 9.61 mmol). The orange suspension was stirred for 12 hours at room temperature. The reaction was then filtered and concentrated to give a yellow solid. The crude product was purified by column chromatography eluting with EtOAc:
Hexane (15:85) to give methyl ester, 195 (1.759 g, 72 %) and an isomer, 238 (0.434 g, 17 %).

**Major Product, 195:** m.p. 117 - 118 °C (Lit.\textsuperscript{165} 118 - 119 °C); ν\textsubscript{max}/cm\textsuperscript{-1} (KBr): 2956, 2848, 1744, 1727, 1580, 1539; δ\textsubscript{H} (300MHz, CDCl\textsubscript{3}): 3.87 [3H, s, O-CH\textsubscript{3}], 3.88 [3H, s, O-CH\textsubscript{3}], 3.89 [3H, s, O-CH\textsubscript{3}], 7.07 and 7.11 [2H, AB q, Δν\textsubscript{AB} 3.0 Hz J 9.3 Hz, C(4)H and C(5)H]; m/z (ESI\textsuperscript{+}): 242.1 (M+H\textsuperscript{+}), 72%.

**Minor product, 238** (Methyl 2,5-dimethoxy-4-nitrobenzoate): m.p. 100 - 102 °C (Lit.\textsuperscript{165} 102.5 - 103.5 °C); ν\textsubscript{max}/cm\textsuperscript{-1} (KBr): 2958, 2926, 2850, 1707, 1626, 1575, 1517; δ\textsubscript{H} (300MHz, CDCl\textsubscript{3}): 3.87 [3H, s, O-CH\textsubscript{3}], 3.91 [3H, s, O-CH\textsubscript{3}], 3.98 [3H, s, O-CH\textsubscript{3}], 7.45 [1H, s, C(3)H], 7.51 [1H, s, C(6)H]; m/z (ESI\textsuperscript{+}): 242.1 (M+H\textsuperscript{+}), 72%.

**Synthesis of methyl 2-amino-3,6-dimethoxybenzoate 159**

![Methyl 3,6-dimethoxy-2-nitrobenzoate, 195](image)

Methyl 3,6-dimethoxy-2-nitrobenzoate, 195 (6.970 g, 28.78 mmol) was dissolved in 95% ethanol (85 mL) and acetic acid (22 mL) and brought to a gentle reflux. Iron powder (11.741 g, 210.11 mmol) and iron (III) chloride (0.800 g, 4.88 mmol) were added simultaneously and refluxing was continued for 1.5 hours. The reaction mixture was cooled and filtered using a Buchner funnel. Water (100 mL) was added to the filtrate which was then neutralised using saturated aqueous sodium bicarbonate. The aqueous layer was extracted with ethyl acetate (3 x 100 mL) and the combined organic layers were washed with saturated aqueous sodium bicarbonate (50 mL) and water (50 mL), dried over magnesium sulphate and concentrated to give a pure brown oil, 159 (5.800 g, 95 %). ν\textsubscript{max}/cm\textsuperscript{-1} (NaCl): 3488, 3379, 2916, 1685, 1613, 1484; δ\textsubscript{H} (300MHz, CDCl\textsubscript{3}): 3.78 [3H, s, O-CH\textsubscript{3}], 3.81 [3H, s, O-CH\textsubscript{3}], 3.90 [3H, s, CO\textsubscript{2}CH\textsubscript{3}], 5.41 [2H, bs, NH\textsubscript{2}], 6.13 and 6.73 [2H, AB q, Δν\textsubscript{AB} 171.4 Hz J 9.1 Hz, C(4)H and C(5)H]; m/z (ESI\textsuperscript{+}): 212.1 (M+H\textsuperscript{+}), 100%.
8.3.2 Synthesis of N-3 substituted Quinazolinediones

Synthesis of 5,8-dimethoxyquinazoline-2,4-(1H,3H)-dione 154

Urea (0.380 g, 6.32 mmol) was added to methyl-2-amino-3,6-dimethoxybenzoate, 159 (0.446 g, 2.10 mmol) in a 25 mL round bottomed flask equipped with a stirring bar. The flask was stoppered and heated to 200 °C for 1.5 hours. The reaction was then allowed to cool to room temperature. The product was tritutated with water and the solid was collected by suction filtration. The filter cake was oven-dried to give a pale brown solid, 154 (0.354 g, 76 %). m.p. dec. >300 °C (Lit.166 dec. >300 °C); v$_\text{max}$/cm$^{-1}$ (KBr): 3041, 1714, 1517, 1408; δ$_\text{H}$ (300MHz, DMSO-d$_6$): 3.76 [3H, s, O-C$_\text{H}_3$], 3.80 [3H, s, O-C$_\text{H}_3$], 6.65 and 7.21 [2H, AB q, Δν$_\text{AB}$ 162.2 Hz J 9.1 Hz, C(6)H and C(7)H], 10.15 [1H, bs, N(1)H], 10.95 [1H, bs, N(3)H]; m/z (ESI$^+$): 223.1 (M+H)$^+$, 100%.

Synthesis of 5,8-dimethoxy-3-phenylquinazoline-(1H,3H)-dione 198

Phenyl isocyanate (0.134 g, 1.12 mmol, 0.122 mL) and methyl-2-amino-3,6-dimethoxybenzoate, 159 (0.251 g, 1.18 mmol) were dissolved in anhydrous THF (10 mL). Triethyl amine (0.742 g, 7.34 mmol, 1.02 ml) was then added and the reaction was then heated to 80 °C for 20 hours. The solvent was removed and ethanol (10 mL) and 2M NaOH (1 mL) were added. The reaction was heated to 80 °C again for a further 2.5 hours. The reaction was allowed to cool to room temperature, neutralised using 2M aqueous HCl and was then cooled on ice. The resulting precipitate was filtered on a Buchner funnel and oven-dried to give a white powder, 198 (0.205 g, 58 %). m.p. 268 - 270 °C; v$_\text{max}$/cm$^{-1}$ (KBr): 3073, 1725, 1660, 1600, 1519; δ$_\text{H}$ (400MHz, DMSO-d$_6$): 7.50 [3H, s, O-CH$_3$], 3.89 [3H, s, O-CH$_3$], 6.65 and 7.29 [2H, AB q, Δν$_\text{AB}$ 162.2 Hz J 9.1 Hz, C(6)H and C(7)H], 10.60 [1H, bs, N(1)H]; δ$_\text{c}$ (75 MHz, DMSO-d$_6$): 56.0 [CH$_3$, O-CH$_3$], 56.7 [CH$_3$, O-CH$_3$].
104.4 [CH, aromatic CH], 117.0 [CH, aromatic CH], 118.1 [C, aromatic C], 127.8 [CH, aromatic CH], 128.6 [2 x CH, aromatic CH], 129.1 [2 x CH, aromatic CH], 131.5 [C, aromatic C], 136.0 [C, N(3)C], 139.6 [C, aromatic C], 149.7 [C, aromatic C], 153.8 [C, aromatic C], 153.8 [C, C=O], 159.7 [C, C=O]; m/z (ESI\(^+\)): 299.0 (M+H\(^+\)), 92%; HRMS (ESI\(^+\)): Exact mass calculated for C\(_{16}\)H\(_{15}\)N\(_2\)O\(_4\) 299.1032. Found 299.1028.

**Synthesis of 3-benzyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 199**

Benzyl isocyanate (0.637 g, 4.78 mmol, 0.591 mL), triethyl amine (3.150 g, 31.12 mmol, 4.30 mL) and methyl-2-amino-3,6-dimethoxybenzoate, 159 (1.069 g, 5.03 mmol) were dissolved in 1,4-dioxane (10 mL). The mixture was heated to 80 °C for 18 hours. Following cooling to room temperature the solvent was removed under reduced pressure and ethanol (10 mL) and NaOH (1 mL) were added. The reaction was then heated for a further 4 hours at 80 °C. Following cooling the reaction was neutralised using 2M aqueous HCl and further cooled on ice. The resulting precipitate was collected by suction filtration and dried to give a white powder, 199 (0.876 g, 56%). m.p. 298 - 300 °C; \(\nu_{\text{max}}/\text{cm}^{-1}\) (KBr): 3061, 1712, 1514, 1451, 1413; \(\delta_{\text{H}}\) (300MHz, DMSO-\(d_6\)): 3.67 [3H, s, O-CH\(_3\)], 3.72 [3H, s, O-CH\(_3\)], 5.08 [2H, s, N(3)CH\(_2\)], 6.25 and 6.85 [2H, AB q, \(\Delta\nu_{\text{AB}}\) 180.0 Hz \(J\) 8.7 Hz, C(6)H and C(7)H], 7.14 – 7.30 [5H, m, ArCH]; \(\delta_{\text{C}}\) (75 MHz, DMSO-\(d_6\)): 42.6 [CH\(_2\), N(3)CH\(_2\)], 55.6 [CH\(_3\), O-CH\(_3\)], 56.1 [CH\(_3\), O-CH\(_3\)], 100.9 [CH, aromatic CH], 104.4 [C, aromatic C], 114.6 [CH, aromatic CH], 126.4 [CH, aromatic CH], 127.4 [2 x CH, aromatic CH], 127.9 [2 x CH, aromatic CH], 138.7 [2 x C, aromatic C], 142.3 [C, aromatic C], 153.0 [C, aromatic C] 153.6 [C, C=O], 160.8 [C, C=O]; m/z (ESI\(^+\)): 223.1 (M+H\(^+\)), 100%. HRMS (ESI\(^+\)): Exact mass calculated for C\(_{17}\)H\(_{17}\)N\(_2\)O\(_4\) 313.1188. Found 313.1174.
Synthesis of 5,8-dimethoxy-3-(2-nitrophenyl)quinazoline-2,4(1H,3H)-dione 200

2-Nitrophenyl isocyanate (1.750 g, 10.68 mmol), triethyl amine (7.020 g, 69.43 mmol, 9.62 mL) and methyl-2-amino-3,6-dimethoxybenzoate, 159 (2.384 g, 11.23 mmol) were dissolved in 1,4-dioxane (25 mL). The reaction mixture was heated to reflux for 12 hours, cooled to room temperature and the solvent was removed under reduced pressure. Ethanol (30 mL) and NaOH (3mL) were added and the reaction was heated to reflux for 4 hours. Following cooling the reaction was neutralised using 2M aqueous HCl and cooled on ice. The resulting precipitate was collected by suction filtration and dried to give a yellow powder, 200 (1.808 g, 50 %). m.p. 285 - 287 °C; \( \nu_{\text{max}} / \text{cm}^{-1} \) (KBr): 3529, 3082, 1668, 1521; \( \delta_{\text{H}} \) (300MHz, DMSO-\( d_6 \)): 3.76 [3H, s, O-\( CH_3 \)], 3.85 [3H, s, O-\( CH_3 \)], 6.75 and 7.35 [2H, AB q, \( \Delta \nu_{\text{AB}} \) 170.1 Hz \( J \) 9.5 Hz, C(6)\( H \) and C(7)\( H \)], 7.66 [1H, dd, \( J \) 6.5, 2.1 Hz, C(6')\( H \)], 7.72 [1H, td, \( J \) 6.2, 1.8 Hz, C(4')\( H \)], 7.90 [1H, td, \( J \) 6.2, 1.7 Hz, C(5')\( H \)], 8.22 [1H, dd, \( J \) 6.8, 1.4 Hz, C(3')\( H \)], 10.92 [1H, bs, N(1)\( H \)]; \( \delta_{\text{c}} \) (75 MHz, DMSO-\( d_6 \)): 55.9 [CH\(_3\), O-\( CH_3 \)], 56.7 [CH\(_3\), O-\( CH_3 \)], 103.8 [C, aromatic C], 104.5 [CH, aromatic CH], 117.5 [CH, aromatic CH], 125.0 [CH, aromatic CH], 129.5 [C, aromatic C], 129.9 [CH, aromatic CH], 131.4 [C, aromatic C], 132.3 [CH, aromatic CH], 134.6 [CH, aromatic CH], 139.6 [C, aromatic C], 146.0 [C, aromatic C], 149.1 [C, aromatic C], 153.7 [C, C=O], 159.3 [C, C=O]; \( m/z \) (ESI\(^+\)): 344.1 (M+H\(^+\)), 100%. HRMS (ESI\(^+\)): Exact mass calculated for \( \text{C}_{16}\text{H}_{14}\text{N}_3\text{O}_6 \) 344.0883. Found 344.0879.
Synthesis of 5,8-dimethoxy-3-(3,4,5-trimethoxybenzyl)quinazoline-2,4(1H,3H)-dione 201

3,4,5-Trimethoxybenzyl isocyanate (1.000 g, 4.48 mmol), triethyl amine (5.680 g, 56.16 mmol, 7.80 mL) and methyl-2-amino-3,6-dimethoxybenzoate, 159 (1.178 g, 5.55 mmol) were dissolved in 1,4-dioxane (15 mL). The reaction mixture was heated to reflux for 12 hours, cooled to room temperature and the solvent was removed under reduced pressure. Ethanol (20 mL) and NaOH (2mL) were added and the reaction was heated to reflux for 4 hours. Following cooling the reaction was neutralised using 2M aqueous HCl and cooled on ice. The resulting precipitate was collected by suction filtration and dried to give a white powder, 201 (0.619 g, 34%). m.p. 201 – 203 °C; \( \nu_{\text{max}} / \text{cm}^{-1} \) (KBr): 3339, 2937, 2836, 1595, 1508, 1422; \( \delta_{\text{H}} \) (300MHz, DMSO-\( d_6 \)): 3.71 [9H, s, 3 x O-CH\(_3\)], 3.77 [3H, s, O-CH\(_3\)], 3.82 [3H, s, O-CH\(_3\)], 4.96 [2H, s, N(3)CH\(_2\)], 6.62 [2H, s, C(2')H and C(6')H], 6.68 and 7.24 [2H, AB q, \( \Delta \nu_{\text{AB}} \) 160.6 Hz \( J 9.7 \text{ Hz}, \) C(6)H and C(7)H], 10.57 [1H, bs, N(1)H]; \( \delta_{\text{C}} \) (75 MHz, DMSO-\( d_6 \)): 43.1 [CH\(_2\), N(3)CH\(_2\)], 55.6 [3 x CH\(_3\), O-CH\(_3\)], 59.9 [2 x CH\(_3\), O-CH\(_3\)], 104.2 [2 x CH, aromatic CH], 105.0 [CH, aromatic CH], 116.6 [CH, aromatic CH], 131.1 [C, aromatic C], 133.3 [C, aromatic C], 136.0 [C, aromatic C], 136.5 [C, aromatic C], 139.4 [C, aromatic C], 149.9 [C, aromatic C], 152.7 [C, aromatic C], 153.5 [C, aromatic C], 157.9 [C, C=O], 159.3 [C, C=O]; \( m/z \) (ESI\(^+\)): 403.1 (M+H\(^+\)), 100%. HRMS (ESI\(^+\)): Exact mass calculated for \( \text{C}_{20}\text{H}_{23}\text{N}_{2}\text{O}_{7} \) 403.1505. Found 403.1497.
Synthesis of 3-allyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 202

Allyl isocyanate (0.678 g, 8.17 mmol, 0.718 mL), triethyl amine (5.367 g, 53.14 mmol, 7.39 mL) and methyl-2-amino-3,6-dimethoxybenzoate, 159 (1.826 g, 8.60 mmol) were dissolved in 1,4-dioxane (25 mL). The reaction mixture was heated to reflux for 12 hours, cooled to room temperature and the solvent was removed under reduced pressure. Ethanol (25 mL) and NaOH (5 mL) were added and the reaction was heated to reflux for 4 hours. Following cooling the precipitate was collected by suction filtration and dried to give a white powder, 202 (0.407 g, 18%). m.p. 163 – 165 °C; \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 3207, 2838, 1723, 1608, 1479, 991; \( \delta_{\text{H}} \) (300MHz, DMSO-\( d_6 \)): 3.75 [3H, s, O-\( \text{C}_3\text{H}_3 \)], 3.81 [3H, s, O-\( \text{C}_3\text{H}_3 \)], 4.44 [2H, dt, \( J = 5.2 \) 1.4 Hz, N(3)CH\(_2\)], 5.06 [1H, overlapping ddt, \( J = 18.5 \) 1.5 1.2 Hz, N(3)CH\(_2\)CH=CH\(_2\) trans], 5.08 [1H, overlapping ddt, \( J = 9.1 \) 1.5 1.5 Hz, N(3)CH\(_2\)CH=CH\(_2\) cis] 5.82 [1H, m, N(3)CH\(_2\)CH], 6.67 and 7.24 [2H, AB q, \( \Delta\nu_{\text{AB}} = 170.1 \) Hz \( J = 9.5 \) Hz, C(6)H and C(7)H], 10.51 [1H, bs, NH]; \( \delta_{\text{C}} \) (75MHz, DMSO-\( d_6 \)): 41.6 [CH\(_2\), N(3)CH\(_2\)], 55.8 [CH\(_3\), O-\( \text{C}_3\text{H}_3 \)], 56.5 [CH\(_3\), O-\( \text{C}_3\text{H}_3 \)], 104.1 [CH, aromatic CH], 104.4 [C, aromatic C], 116.0 [CH\(_2\), N(3)CH\(_2\)CHCH\(_2\)], 116.4 [CH, aromatic CH], 131.1 [C, aromatic C], 132.9 [CH, N(3)CH\(_2\)CH], 139.3 [C, aromatic C], 149.4 [C, aromatic C], 153.4 [C, C=O], 159.0 [C, C=O]; \( m/z \) (ESI\(^+\)): 263.1 (M+H\(^+\)), 98%. HRMS (ESI\(^+\)): Exact mass calculated for C\(_{13}\)H\(_{15}\)N\(_2\)O\(_4\) 263.1032. Found 263.1019.

Synthesis of 3-(2-chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 196

2-Chloroethyl isocyanate (2.880 g, 27.30 mmol, 2.33 mL), triethyl amine (17.071 g, 168.71 mmol, 23.51 mL) and methyl-2-amino-3,6-dimethoxybenzoate, 159 (6.101 g, 28.75 mmol) were dissolved in dry THF (30 mL). The reaction mixture was heated to reflux for 12 hours, cooled to room...
temperature and the solvent was removed under reduced pressure. Ethanol (30 mL) and NaOH (3mL) were added and the reaction was heated to reflux for 4 hours. Following cooling the reaction was neutralised using 2M aqueous HCl and cooled on ice. The resulting precipitate was collected by suction filtration and dried to give a white powder, 196 (6.149 g, 79 %). m.p. 255 – 257 °C; \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 3189, 2964, 1718, 1651, 1467; \( \delta_{\text{H}} \) (300MHz, DMSO-\( d_6 \)): 3.76 [2H, t, J 6.9 Hz, NCH\(_2\)], 3.78 [3H, s, O-CH\(_3\)], 3.81 [3H, s, O-CH\(_3\)], 4.18 [2H, t, J 6.9 Hz, NCH\(_2\)CH\(_2\)], 6.69 and 7.25 [2H, AB q, \( \Delta \nu_{\text{AB}} \) 161.9 Hz] J 9.0 Hz, C(6)H and C(7)H], 10.59 [1H, bs, N(1)H]; \( \delta_{\text{C}} \) (75MHz, DMSO-\( d_6 \)): 40.5 [CH\(_2\), N(3)CH\(_2\)C\(_6\)]H\(_2\)], 40.7 [CH\(_2\), N(3)CH\(_2\)C\(_6\)]H\(_2\)], 55.9 [CH\(_3\), O-CH\(_3\)], 56.6 [CH\(_3\), O-CH\(_3\)], 103.7 [C, aromatic C], 104.4 [CH, aromatic CH], 116.9 [CH, aromatic CH], 131.1 [C, aromatic C], 139.4 [C, aromatic C], 149.5 [C, aromatic C], 153.5 [C, C=O], 159.3 [C, C=O]; m/z (ESI\(^+\)): 285.0 (M+H\(^+\)), 96%. HRMS (ESI\(^+\)): Exact mass calculated for C\(_{13}\)H\(_9\)N\(_4\)O\(_4\) 285.0624. Found 285.0625.

**Synthesis of 3-(2-azidoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 203**

To a 50 mL round-bottomed flask under N\(_2\) was added 196 (0.087 g, 0.30 mmol) and anhydrous DMF (10 mL). After dissolution sodium azide (0.039 g, 0.61 mmol) was added and the reaction was heated to 90 °C for 1.5 hours. The solvent was then removed under reduced pressure and the crude residue was dissolved in ethyl acetate:methanol (45:5), washed with water (20 mL) and brine (20 mL), dried using magnesium sulfate and concentrated to dryness to give white crystals, 203 (0.088 g, 100 %). m.p. 191 – 193 °C; \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 3201, 2961, 2128, 2095, 1715, 1660, 1516, 1458; \( \delta_{\text{H}} \) (300MHz, DMSO-\( d_6 \)): 3.51 [2H, t, J 6.0 Hz, N(3)CH\(_2\)CH\(_2\)], 3.78 [3H, s, O-CH\(_3\)], 3.81 [3H, s, O-CH\(_3\)], 4.09 [2H, t, J 6.0 Hz, N(3)CH\(_2\)], 6.69 and 7.25 [2H, AB q, \( \Delta \nu_{\text{AB}} \) 160.5 Hz] J 9.0 Hz, C(6)H and C(7)H], 10.53 [1H, bs, N(1)H]; \( \delta_{\text{C}} \) (75MHz, DMSO-\( d_6 \)): 38.6 [CH\(_2\), N(3)CH\(_2\)C\(_6\)]H\(_2\)], 48.0 [CH\(_2\), N(3)CH\(_2\)CH\(_2\)H], 56.0 [CH\(_3\), O-CH\(_3\)], 56.6 [CH\(_3\), O-CH\(_3\)], 103.7 [C, aromatic C], 104.4 [CH, aromatic CH], 116.8 [CH, aromatic CH], 131.1 [C, aromatic C], 139.4 [C, aromatic C], 149.8 [C, aromatic C], 153.5 [C, C=O], 159.4 [C, C=O];
m/z (ESI⁺): 292.2 (M+H)⁺, 100%. HRMS (ESI⁺): Exact mass calculated for C₁₂H₁₄N₅O₄ 292.1046. Found 292.1052.

Synthesis of 3-(2-aminoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 204

To a Parr bottle containing water (50 mL) and potassium hydroxide (5.003 g, 89.17 mmol) was added 203 (0.3010 g, 1.03 mmol). Pd/C (30 mg) was added to the flask and the mixture was agitated under a H₂ atmosphere (50 psi) for 12 hours. The reaction mixture was filtered through celite and the filtrate was neutralised using 2M aqueous HCl. The aqueous phase was extracted with 10 % MeOH:DCM (3 x 50 mL) and the combined organic phases were washed with water (50 mL), dried using magnesium sulfate and concentrated to dryness to give a white solid. m.p. >300 °C; νmax/cm⁻¹ (KBr): 3360, 2933, 1718, 1648, 1518, 1475; m/z (ESI⁺): 266.3 (M+H)⁺, 30%. HRMS (ESI⁺): Exact mass calculated for C₁₂H₁₆N₃O₄ 266.1141. Found 266.1131.

Synthesis of 3-(2-cyanoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 205

To a 100 mL round-bottomed flask under N₂ was added 196 (0.2138 g, 0.75 mmol) and anhydrous DMF (30 mL). After dissolution sodium cyanide (0.073 g, 1.50 mmol) was added and the reaction was heated to 90 °C for 2.5 hours. The solvent was then removed under reduced pressure and the crude residue was dissolved in ethyl acetate:methanol (90:10), washed with water (30 mL) and brine (30 mL), dried using magnesium sulfate and concentrated to dryness to give white crystals, 205 (0.165 g, 80 %). m.p. 240 – 242 °C; νmax/cm⁻¹ (KBr): 3197, 2937, 2837, 2248, 1718, 1653, 1515; δH (300MHz, DMSO-d₆): 2.86 [2H, t, J 6.6 Hz, NCH₂CH₂], 3.78 [3H,
s, O-CH₃), 3.80 [3H, s, O-CH₃], 4.10 [2H, t, J 6.6 Hz, NCH₂], 6.69 and 7.26 [2H, AB q, ΔνAB 161.3 Hz J 9.2 Hz, C(6)H and C(7)H], 10.67 [1H, bs, N(1)H]; δc (75MHz, DMSO-d₆): 15.5 [CH₂, N(3)CH₂CH₂], 35.2 [CH₂, N(3)CH₂], 55.9 [CH₃, O-CH₃], 56.6 [CH₃, O-CH₃], 103.6 [C, aromatic C], 104.3 [CH, aromatic CH], 116.8 [CH, aromatic CH], 118.5 [CN, N(3)(CH₂)₂CN], 131.0 [C, aromatic C], 139.4 [C, aromatic C], 149.4 [C, aromatic C], 153.5 [C, C=O], 159.2 [C, C=O]; m/z (ESI⁺): 276.3 (M+H)⁺, 100%. HRMS (ESI⁺): Exact mass calculated for C₁₃H₁₂N₃O₄ 276.0984. Found 276.0984.

**Synthesis of 3-(2-amidoethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 206**

To a 50 mL round-bottomed flask was added 37 % HCl (25 mL) and 205 (0.1382 g, 0.50 mmol). The suspension was heated to give a yellow solution which was stirred at room temperature for 1 hour. The reaction was quenched by pouring into ice water (70 mL) and neutralised using 2M NaOH. Following scratching of the vessel white crystals began to form. When crystallisation was complete the crystals were isolated by suction filtration and oven-dried overnight to give 206 (0.146 g, 100 %). m.p. dec. >285 °C; νmax/cm⁻¹ (KBr): 3367, 3190, 2936, 1716, 1651, 1518; δh (300MHz, DMSO-d₆): 2.34 [2H, t, J 7.6 Hz, NCH₂CH₂], 3.76 [3H, s, O-CH₃], 3.80 [3H, s, O-CH₃], 4.01 [2H, t, J 7.8 Hz, NCH₂], 6.66 and 7.22 [2H, AB q, ΔνAB 161.5 Hz J 9.0 Hz, C(6)H and C(7)H], 6.85 [1H, bs, NH₂], 7.45 [1H, bs, NH₂], 10.49 [1H, bs, N(1)H]; δc (75MHz, DMSO-d₆): 32.8 [CH₂, N(3)CH₂CH₂], 36.3 [CH₂, N(3)CH₂], 55.9 [CH₃, O-CH₃], 56.5 [CH₃, O-CH₃], 103.8 [C, aromatic C], 104.0 [CH, aromatic CH], 116.4 [CH, aromatic CH], 131.3 [C, aromatic C], 139.4 [C, aromatic C], 149.6 [C, aromatic C], 153.4 [C, C=O], 159.3 [C, C=O], 172.0 [CO, N(3)(CH₂)₂CONH₂]; m/z (ESI⁺): 292.3 (M-H)⁻, 40%. HRMS (ESI⁺): Exact mass calculated for (M+H)⁺ C₁₃H₁₆N₃O₅ 294.1090. Found 294.1084.
Synthesis of 3-(2-piperazin-1-yl)ethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 207

To a 100 mL round-bottomed flask containing anhydrous DMF (25 mL) was added 196 (0.219 g, 0.77 mmol), potassium carbonate (0.533 g, 3.86 mmol), potassium iodide (0.128 g, 0.77 mmol) and piperazine (1.336 g, 15.47 mmol). The mixture was heated to 90 °C for 3 hours. The solvent was removed under reduced pressure and the crude product was taken up in water (15 mL), neutralised using 2M aqueous HCl and extracted with (1:9) MeOH:DCM (2 x 50 mL). The combined organic layers were washed with brine (20 mL), dried using magnesium sulfate and concentrated to give a white solid 207 (0.076 g, 29 %). m.p. 152 – 154 °C; $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3436, 3199, 2936, 2815, 1711, 1649, 1515; $\delta$H (300MHz, CDCl$_3$): 1.90 [1H, bs, N(4')H], 2.33 – 2.69 [8H, m, C(2’)H$_2$, C(3’)H$_2$, C(5’)H$_2$, C(6’)H$_2$], 2.91 [2H, t, $J$ 7.4 Hz, N(3’)CH$_2$CH$_2$], 3.90 [3H, s, O-CH$_3$], 3.92 [3H, s, O-CH$_3$], 4.16 [2H, t, $J$ 7.5 Hz, N(3')CH$_2$], 6.55 and 7.01 [2H, AB q, $\Delta$ν$_{AB}$ 127.1 Hz $J$ 8.9 Hz, C(6)H and C(7)H], 8.14 [1H, bs, N(1)H]; $\delta$c (75MHz, CDCl$_3$): 37.9 [CH$_2$, N(3’)CH$_2$CH$_2$], 51.5 [2 x CH$_2$, C(3’)H$_2$ and C(5’)H$_2$], 53.2 [2 x CH$_2$, C(2’)H$_2$ and C(6’)H$_2$], 55.3 [CH$_2$, N(3’)CH$_2$], 56.3 [CH$_3$, O-CH$_3$], 56.4 [CH$_3$, O-CH$_3$], 103.7 [CH, aromatic CH], 104.5 [C, aromatic C], 115.0 [CH, aromatic CH], 130.6 [C, aromatic C], 138.9 [C, aromatic C], 149.9 [C, aromatic C], 154.2 [C, C=O], 160.5 [C, C=O]; m/z (ESI$^+$): 335.3 (M+H$^+$), 96%. HRMS (ESI$^+$): Exact mass calculated for C$_{16}$H$_{23}$N$_4$O$_4$ 335.1719. Found 335.1709.
Synthesis of 3-(2-(piperidin-1-yl)ethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 208

To a 100 mL round-bottomed flask containing anhydrous DMF (25 mL) was added 196 (0.219 g, 0.77 mmol), potassium carbonate (0.533 g, 3.86 mmol), potassium iodide (0.128 g, 0.77 mmol) and piperidine (1.336 g, 15.47 mmol). The mixture was heated to 90 °C for 3 hours. The solvent was removed under reduced pressure and the crude product was taken up in water (15 mL), neutralised using 2M aqueous HCl and extracted with (1:9) MeOH:DCM (2 x 50 mL). The combined organic layers were washed with brine (20 mL), dried using magnesium sulfate and concentrated to give beige crystals 208 (0.196 g, 73%). m.p. 158 – 160 °C; ν\textsubscript{max}/cm\textsuperscript{-1} (KBr): 3197, 2933, 2838, 2251, 1715, 1660, 1607, 1514; δ\textsubscript{H} (300MHz, CDCl\textsubscript{3}): 1.43 [2H, m, C(4')]H\textsubscript{2}, 1.59 [4H, m, C(3')H\textsubscript{2} and C(5')H\textsubscript{2}], 2.56 [4H, m, C(2')H\textsubscript{2} and C(6')H\textsubscript{2}], 2.65 [2H, t, J 7.5 Hz N(3)CH\textsubscript{2}CH\textsubscript{2}], 3.92 [3H, s, O-CH\textsubscript{3}], 3.93 [3H, s, O-CH\textsubscript{3}], 4.19 [2H, t, J 7.4 Hz N(3)CH\textsubscript{2}CH\textsubscript{2}], 6.56 and 7.01 [2H, AB q, Δν\textsubscript{AB} 127.5 Hz, J 9.0 Hz, C(6)H and C(7)H], 8.40 [1H, bs, N(1)H]; δ\textsubscript{c} (75MHz, CDCl\textsubscript{3}): 24.2 [CH\textsubscript{2}, C(4')H\textsubscript{2}], 25.8 [2 x CH\textsubscript{2}, C(3')H\textsubscript{2} and C(5')H\textsubscript{2}], 37.8 [CH\textsubscript{2}, N(3)CH\textsubscript{2}CH\textsubscript{2}], 54.5 [2 x CH\textsubscript{2}, C(2')H\textsubscript{2} and C(6')H\textsubscript{2}], 56.3 [CH\textsubscript{2}, N(3)CH\textsubscript{2}], 56.4 [CH\textsubscript{3}, O-CH\textsubscript{3}], 56.4 [CH\textsubscript{3}, O-CH\textsubscript{3}], 103.6 [CH, aromatic CH], 104.4 [C, aromatic C], 115.0 [CH, aromatic CH], 130.6 [C, aromatic C], 138.9 [C, aromatic C], 149.9 [C, aromatic C], 154.1 [C, C=O], 160.5 [C, C=O]; m/z (ESI\textsuperscript{+}): 334.3 (M+H\textsuperscript{+}), 94%. HRMS (ESI\textsuperscript{+}): Exact mass calculated for C\textsubscript{17}H\textsubscript{24}N\textsubscript{3}O\textsubscript{4} 334.1767. Found 334.1754.
Synthesis of 3-(2-(dimethylamino)ethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 209

To a 100 mL round-bottomed flask was added 196 (0.238 g, 0.84 mmol), DMF (25 mL), potassium carbonate (0.580 g, 4.20 mmol), potassium iodide (0.139 g, 0.84 mmol) and dimethyl amine (33% in EtOH) (0.757 g, 16.81 mmol, 1.13 mL). The mixture was heated to 90 °C for 3 hours. The solvent was removed under reduced pressure and the crude product was taken up in water (15 mL), neutralised using 2M aqueous HCl and extracted with (1:9) MeOH:DCM (2 x 50 mL). The combined organic phases were washed with brine (20 mL), dried using magnesium sulfate and concentrated to give 209 (0.215 g, 88%) as white crystals. m.p. 165 – 167 °C; νmax/cm⁻¹ (NaCl): 3430, 2101, 1651, 1519, 1646; δH (300MHz, CDCl₃): 2.33 [6H, s, N(C₃H₃)₂], 2.61 [2H, t, J 6.9 Hz, N(3)CH₂CH₂], 3.89 [3H, s, O-CH₃], 3.92 [3H, s, O-CH₃], 4.16 [2H, t, J 7.1 Hz, N(3)CH₂], 6.54 and 6.99 [2H, AB q, ΔνAB 127.5 Hz, J 9.1 Hz, C(6)H and C(7)H], 8.52 [1H, bs, N(1)H]; δc (75MHz, CDCl₃): 38.4 [CH₂, N(3)CH₂CH₂], 45.6 [2 x CH₃, N(CH₃)₂], 56.3 [CH₃, O-CH₃], 56.6 [CH₂, N(3)CH₂], 103.6 [CH, aromatic CH], 104.4 [C, aromatic C], 115.0 [CH, aromatic CH], 130.6 [C, aromatic C], 139.0 [C, aromatic C], 150.0 [C, aromatic C], 156.2 [C, C=O], 160.6 [C, C=O]; m/z (ESI⁺): 294.3 (M+H)⁺, 96%. HRMS (ESI⁺): Exact mass calculated for C₁₄H₂₀N₃O₄ 294.1454. Found 294.1440.

Synthesis of 3-(3-chloropropyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 210

3-Chloropropyl isocyanate (0.999 g, 8.36 mmol, 0.85 mL), triethyl amine (4.708 g, 46.53 mmol, 6.48 mL) and methyl-2-amino-3,6-dimethoxybenzoate, 159 (1.597 g, 7.53 mmol) were dissolved in 1,4-dioxan (10 mL). The reaction mixture was heated to reflux for 12 hours, cooled to room temperature and the solvent was removed under reduced pressure. Ethanol (30 mL) and NaOH (3 mL) were added and the reaction was heated to reflux for 4 hours. Following...
cooling the reaction was neutralised using 2M aqueous HCl and cooled on ice. The resulting precipitate was collected by suction filtration and dried to give a white powder, 210 (0.499 g, 22 %). m.p. 187 – 189 °C; v_{max}/cm^{-1} (KBr): 3204, 2966, 1715, 1657, 1607, 1514, 1457; δ_{H} (300MHz, DMSO-\text{d}_6): 2.01 [2H, qt, J 6.9 Hz, NCH\text{2}CH\text{2}], 3.66 [2H, t, J 6.7 Hz, N(CH\text{2})\text{2}CH\text{2}Cl], 3.77 [3H, s, O-CH\text{3}], 3.81 [3H, s, O-CH\text{3}], 3.98 [2H, t, J 7.3 Hz, N(3)CH\text{2}], 6.67 and 7.24 [2H, AB q, Δν_{AB} 159.7 Hz J 9.0 Hz, C(6)H and C(7)H], 10.48 [1H, bs, N(1)H]; δ_{C} (75 MHz, DMSO-\text{d}_6): 30.4 [CH\text{2}, N(3)CH\text{2}CH\text{2}], 37.6 [CH\text{2}, N(3)(CH\text{2})\text{2}CH\text{2}Cl], 43.2 [CH\text{2}, N(3)CH\text{2}], 55.9 [CH\text{3}, O-CH\text{3}], 56.5 [CH\text{3}, O-CH\text{3}], 103.8 [C, aromatic C], 104.1 [CH, aromatic CH], 116.4 [CH, aromatic CH], 131.1 [C, aromatic C], 139.3 [C, aromatic C], 149.7 [C, aromatic C], 153.4 [C, C=O], 159.5 [C, C=O]; m/z (ESI^+): 299.0 (M+H)^+, 100%. HRMS (ESI^+): Exact mass calculated for C_{13}H_{16}N_{2}O_{4}Cl_{2} 299.0799. Found 299.0769.

**Synthesis of 3-(3,6-dimethoxy-2-methylbenzoate)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 197**

Outlined below are the attempted syntheses of three N(3) substituted quinazolinediones. In each case the common product 197 was formed.

S-Methylbenzyl isocyanate (1.000 g, 6.79 mmol, 0.956 mL), triethyl amine (4.248 g, 41.98 mmol, 5.85 mL) and methyl-2-amino-3,6-dimethoxybenzoate, 159 (1.634 g, 7.70 mmol) were dissolved in dry THF (15 mL). The reaction mixture was heated to reflux for 12 hours, cooled to room temperature and the solvent was removed under reduced pressure. Ethanol (30 mL) and NaOH (3 ml) were added and the reaction was heated to reflux for 4 hours. Following cooling the reaction was neutralised using 2M aqueous HCl and cooled on ice. The resulting precipitate was collected by suction filtration and dried to give a white powder, 197 (0.503 g, 23 %).
**Experimental**

*R*-Methylbenzyl isocyanate (0.994 g, 6.75 mmol, 0.95 mL), triethyl amine (4.075 g, 40.27 mmol, 5.60 mL) and methyl-2-amino-3,6-dimethoxybenzoate, **159** (1.623 g, 7.66 mmol) were dissolved in dry THF (20 mL). The reaction mixture was heated to reflux for 12 hours, cooled to room temperature and the solvent was removed under reduced pressure. Ethanol (20 mL) and NaOH (2 mL) were added and the reaction was heated to reflux for 4 hours. Following cooling the reaction was neutralised using 2M aqueous HCl and cooled on ice. The resulting precipitate was collected by suction filtration and dried to give a white powder, **197** (0.511 g, 24%).

4-Chlorobenzenesulfonyl isocyanate (1.000 g, 4.60 mmol, 0.68 mL), triethyl amine (2.870 g, 28.42 mmol, 3.90 mL) and methyl-2-amino-3,6-dimethoxybenzoate, **159** (1.092 g, 5.18 mmol) were dissolved in dry THF (10 mL). The reaction mixture was heated to reflux for 12 hours, cooled to room temperature and the solvent was removed under reduced pressure. Ethanol (10 mL) and NaOH (1 mL) were added and the reaction was heated to reflux for 4 hours. Following cooling the reaction was neutralised using 2M aqueous HCl and cooled on ice. The resulting precipitate was collected by suction filtration and dried to give a white powder, **197** (0.250 g, 14%).

m.p. 175 – 177 °C; v<sub>max</sub>/cm<sup>-1</sup> (KBr): 3325, 2963, 1728, 1659, 1626, 1570, 1491; δ<sub>H</sub> (300MHz, DMSO-<em>d</em><em>6</em>): 3.56 [3H, s, O-<em>CH</em><sub>3</sub>], 3.69 [3H, s, O-<em>CH</em><sub>3</sub>], 3.75 [3H, s, O-<em>CH</em><sub>3</sub>], 3.80 [3H, s, O-<em>CH</em><sub>3</sub>], 3.83 [3H, s, O-<em>CH</em><sub>3</sub>], 6.72 and 7.30 [2H, AB q, Δν<sub>AB</sub> 166.7 Hz J 9.0 Hz, C(6)H and C(7)H], 7.19 and 7.25 [2H, AB q, Δν<sub>AB</sub> 10.0 Hz J 9.2 Hz, C(4')H and C(5')H], 10.61 [1H, s, N(1)H]; δ<sub>c</sub> (75 MHz, DMSO-<em>d</em><em>6</em>): 52.0 [CH<sub>3</sub>, CO<sub>2</sub>CH<sub>3</sub>], 55.9 [CH<sub>3</sub>, O-<em>CH</em><sub>3</sub>], 56.2 [CH<sub>3</sub>, O-<em>CH</em><sub>3</sub>], 56.4 [CH<sub>3</sub>, O-<em>CH</em><sub>3</sub>], 56.6 [CH<sub>3</sub>, O-<em>CH</em><sub>3</sub>], 103.5 [C, aromatic C], 104.5 [CH, aromatic CH], 113.0 [CH, aromatic CH], 114.1 [CH, aromatic CH], 117.1 [CH, aromatic CH], 122.9 [C, aromatic C], 123.2 [C, aromatic C], 131.4 [C, aromatic C], 139.5 [C, aromatic C], 148.7 [C, aromatic C], 149.1 [C, C=O], 150.4 [C, aromatic C], 153.7 [C, aromatic C], 158.5 [C, C=O], 164.8 [CO, CO<sub>2</sub>CH<sub>3</sub>]; m/z (ESI<sup>+</sup>): 417.1 (M+H<sup>+</sup>), 30%. HRMS (ESI<sup>+</sup>): Exact mass calculated for C<sub>20</sub>H<sub>21</sub>N<sub>2</sub>O<sub>8</sub>: 417.1298. Found 417.1284.
8.3.3 6-Substituted N-3-substituted quinazolinediones

Synthesis of 5,8-dimethoxy-6-nitroquinazoline-2,4(1H,3H)-dione 164

5,8-Dimethoxyquinazine-2,4-(1H,3H)-dione, 154 (0.298 g, 1.35 mmol) was suspended in acetic acid (5 mL) at 5 °C with stirring. Nitric acid (65%) (3.0 ml, 4.17 g, 46.73 mmol) and sulfuric acid (96%) (1.2 mL) were added simultaneously and the reaction was stirred for 5 minutes. The reaction was poured on to crushed ice and the resulting precipitate was filtered and dried to give a pale yellow solid, 164 (0.348 g, 97 %). m.p. dec. >300 °C (Lit.165 >300 °C); νmax/cm⁻¹ (KBr): 3184, 3066, 2846, 1688, 1603, 1531; δH (300 MHz, DMSO-d6): 3.85 [3H, s, O-CH3], 3.91 [3H, s, O-CH3], 7.73 [1H, s, C(7)H], 10.93 [1H, bs, N(1)H], 11.47 [1H, bs, N(3)H]; m/z (ESI⁺): 268.1 (M+H)⁺, 100%.

Synthesis of 6-bromo-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 215

5,8-Dimethoxyquinazine-2,4-(1H,3H)-dione, 154 (0.226 g, 1.01 mmol) was dissolved in 1,4-dioxane (20 mL) and N-bromosuccinimide (0.181 g, 1.01 mmol) was added. The reaction was stirred at room temperature for 12 hours. Water (20 mL) was added and the resulting precipitate was collected by suction filtration and dried to give a white solid, 215 (0.217 g, 71 %). m.p. 312 – 314 °C; νmax/cm⁻¹ (KBr): 3181, 3065, 2842, 1717, 1685, 1608; δH (300 MHz, DMSO-d6): 3.70 [3H, s, O-CH3], 3.86 [3H, s, O-CH3], 7.48 [1H, s, C(7)H], 10.50 [1H, bs, N(1)H], 11.24 [1H, bs, N(3)H]; δc (75 MHz, CDCl3): 56.3 [CH3, O-CH3], 61.1 [CH3, O-CH3], 109.2 [C, aromatic C], 109.5 [C, aromatic C], 118.7 [CH, aromatic CH], 132.3 [C, aromatic C], 142.8 [C, aromatic C], 149.3 [C, aromatic C], 149.5 [C, C=O], 159.9 [C, C=O]; m/z (ESI⁺): 301.0 (M+H)⁺, 100%. HRMS (ESI⁺): Exact mass calculated for C10H10N2O4Br 300.9824. Found 300.9822.
Synthesis of 5,8-dimethoxy-6-nitro-3-(2-nitrophenyl)quinazoline-2,4(1H,3H)-dione 214

5,8-Dimethoxy-3-(2-nitrophenyl)quinazoline-2,4(1H,3H)-dione, **200** (0.296 g, 0.86 mmol) was suspended in acetic acid (5 mL) at 5 °C with stirring. Nitric acid (65%) (2.5 mL, 3.475 g, 38.88 mmol) and sulfuric acid (96%) (1.2 mL) were added simultaneously and the reaction was stirred for 5 minutes. Water (20 mL) was added to quench the reaction and the resulting precipitate was filtered and dried to give a yellow powder, **214** (0.297 g, 88 %). m.p. 248 - 250 °C; \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 3356, 2947, 1742, 1685, 1602, 1527, 1458, 1355; \( \delta_{\text{H}} \) (300MHz, DMSO-\( d_6 \)): 3.81 [3H, s, O-CH\(_3\)], 3.98 [3H, s, O-CH\(_3\)], 7.73 (1H, dd, \( J \ 6.7, 1.3 \) Hz, C(6')H), 7.78 (1H, td, \( J \ 6.3, 1.8 \) Hz, C(4')H), 7.88 (1H, s, C(7)H), 7.95 (1H, td, \( J \ 6.3, 1.4 \) Hz, C(5')H), 8.26 (1H, dd, \( J \ 6.8, 1.4 \) Hz, C(3')H), 11.68 (1H, bs, N(1)H); \( \delta_{\text{c}} \) (75 MHz, DMSO-\( d_6 \)): 57.1 [CH\(_3\), O-CH\(_3\)], 63.5 [CH\(_3\), O-CH\(_3\)], 108.5 [C, aromatic C], 111.1 [CH, aromatic CH], 125.3 [CH, aromatic CH], 128.7 [C, aromatic C], 130.3 [CH, aromatic CH], 132.1 [CH, aromatic CH], 134.9 [CH, aromatic CH], 135.6 [C, aromatic C], 138.4 [C, aromatic C], 142.1 [C, aromatic C], 145.8 [C, aromatic C], 147.3 [C, aromatic C], 148.7 [C, C=O], 158.6 [C, C=O]; m/z (ESI\(^{+} \)): 389.0 (M+H\(^{+} \)), 100%. HRMS (ESI\(^{+} \)): Exact mass calculated for C\(_{16}\)H\(_{13}\)N\(_4\)O\(_8\) 389.0733. Found 389.0724.

Synthesis of 3-allyl-5,8-dimethoxy-6-nitroquinazoline-2,4(1H,3H)-dione 211

3-Allyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione, **202** (0.147 g, 0.55 mmol) was suspended in acetic acid (2.5 mL) at 5 °C with stirring. Nitric acid (65%) (1.3 mL, 1.807 g, 20.25 mmol) and sulfuric acid (96%) (0.8 mL) were added simultaneously and the reaction was stirred for 5 minutes. Water (15 mL) was added to quench the reaction and the resulting precipitate was filtered and dried to give a pale yellow solid, **211** (0.100 g, 58 %). m.p. 232 – 234 °C;
\( v_{\text{max}} \text{cm}^{-1} \) (KBr): 3180, 3080, 1726, 1663, 1603, 1532, 1451; \( \delta_{\text{H}} \) (300MHz, DMSO-\( d_6 \)): 3.86 \([3\text{H}, \text{s}, \text{O-CH}_3]\), 3.92 \([3\text{H}, \text{s}, \text{O-CH}_3]\), 4.48 \([2\text{H}, \text{dt}, J 5.3, 1.4 \text{ Hz}, \text{N}(3)\text{CH}_2]\), 5.11 \([1\text{H}, \text{overlapping ddt}, J 10.3 1.4 1.3 \text{ Hz}, \text{N}(3)\text{CH}_2\text{CH}=\text{CH}_2\text{trans}]\), 5.14 \([1\text{H}, \text{overlapping ddt}, J 17.2 1.4 1.4 \text{ Hz}, \text{N}(3)\text{CH}_2\text{CH}=\text{CH}_2\text{cis}]\), 5.87 \([1\text{H}, \text{m}, \text{N}(3)\text{CH}_2\text{CH}=\text{CH}_2]\), 7.78 \([1\text{H}, \text{s}, \text{C}(7)\text{H}]\); \( \delta_{\text{C}} \) (75 MHz, DMSO-\( d_6 \)): 42.0 \([\text{CH}, \text{N}(3)\text{CH}_2\text{Cl}]\), 56.9 \([\text{CH}_3, \text{O-CH}_3]\), 63.4 \([\text{CH}_3, \text{O-CH}_3]\), 108.3 \([\text{C}, \text{aromatic C}]\), 110.3 \([\text{CH}, \text{aromatic CH}]\), 135.3 \([\text{C}, \text{aromatic C}]\), 138.1 \([\text{C}, \text{aromatic C}]\), 141.7 \([\text{C}, \text{aromatic C}]\), 147.2 \([\text{C}, \text{aromatic C}]\), 149.2 \([\text{C}, \text{C=O}]\), 158.6 \([\text{C}, \text{C=O}]\); \( m/z \) (ESI\(^+\)): 330.0 (M+H\(^+\)), 100%. HRMS (ESI\(^+\)): Exact mass calculated for C\(_{12}\)H\(_{12}\)ClN\(_3\)O\(_6\) 330.0493. Found 330.0480.

**Synthesis of 3-(2-chloroethyl)-5,8-dimethoxy-6-nitroquinazoline-2,4(1H,3H)-dione 212**

3-(2-Chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione, 196 (0.296 g, 1.04 mmol) was suspended in acetic acid (5 mL) at 5 °C with stirring. Nitric acid (65%) (2.5 mL, 3.475 g, 38.88 mmol) and sulfuric acid (96%) (1.2 mL) were added simultaneously and the reaction was stirred for 5 minutes. Water (20 mL) was added to quench the reaction and the resulting precipitate was filtered and dried to give a pale yellow solid, 212 (0.300 g, 88 %). m.p. 208 – 210 °C; \( v_{\text{max}} \text{cm}^{-1} \) (KBr): 3156, 2950, 1721, 1665, 1601, 1534, 1511, 1357; \( \delta_{\text{H}} \) (300MHz, DMSO-\( d_6 \)): 3.77 \([2\text{H}, \text{t}, J 6.59Hz, \text{N}(3)\text{CH}_2]\), 3.86 \([3\text{H}, \text{s}, \text{O-CH}_3]\), 4.21 \([2\text{H}, \text{t}, J 6.5 \text{ Hz}, \text{N}(3)\text{CH}_2\text{Cl}]\), 7.76 \([1\text{H}, \text{s}, \text{C}(7)\text{H}]\), 11.31 \([1\text{H}, \text{bs, N}(1)\text{H}]\); \( \delta_{\text{C}} \) (75 MHz, DMSO-\( d_6 \)): 40.3 \([\text{CH}_2, \text{N}(3)\text{CH}_2\text{Cl}]\), 41.1 \([\text{CH}_2, \text{N}(3)\text{CH}_2]\), 56.9 \([\text{CH}_3, \text{O-CH}_3]\), 63.4 \([\text{CH}_3, \text{O-CH}_3]\), 108.3 \([\text{C}, \text{aromatic C}]\), 110.3 \([\text{CH}, \text{aromatic CH}]\), 135.3 \([\text{C}, \text{aromatic C}]\), 138.1 \([\text{C}, \text{aromatic C}]\), 141.7 \([\text{C}, \text{aromatic C}]\), 147.2 \([\text{C}, \text{aromatic C}]\), 149.2 \([\text{C}, \text{C=O}]\), 158.6 \([\text{C}, \text{C=O}]\); \( m/z \) (ESI\(^+\)): 330.0 (M+H\(^+\)), 100%. HRMS (ESI\(^+\)): Exact mass calculated for C\(_{12}\)H\(_{12}\)ClN\(_3\)O\(_6\) 330.0493. Found 330.0480. Single crystals of 212 were grown from methanol/water. Crystal data: C\(_{12}\)H\(_{12}\)ClN\(_3\)O\(_6\), \( M_r = 329.70 \), triclinic, space group \( P-1 \), a =
4.636(13) Å, b = 12.41(4) Å, c = 13.86(4) Å, α = 105.43(4)°, β = 100.587(2)°, γ = 100.587(2)°, 
V = 746.(4) Å³, Z = 2, Dc = 1.468 g cm⁻³, F(000) = 340, Mo Kα radiation, λ = 0.71073 Å, T = 273.(2) K, 2θmax = 26.59°, μ = 0.289 mm⁻¹, 7479 reflections collected, 2876 unique (Rint = 0.0411), final Goof = 0.949, 
R1 = 0.0596, wR2 = 0.1837 (1430 obs. data: I > 2σ(I)); R1 = 0.1100, wR2 = 0.2284 (all data).

**Synthesis of 6-bromo-3-(2-chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 239**

3-(2-Chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione, **196** (0.5066 g, 1.78 mmol) was dissolved in 1,4-dioxane (120 mL) and N-bromosuccinimide (0.633 g, 3.56 mmol) was added. The reaction was stirred at room temperature for 3 hours. Water (120 mL) was added and the resulting precipitate was collected by suction filtration and dried to give an off-white solid, **239** (0.510 g, 76 %). m.p. 210 – 212 °C; vmax/cm⁻¹ (KBr): 3182, 3069, 1720, 1659, 1594, 1506, 1461; δH (300 MHz, CDCl₃): 3.78 [2H, t, J 6.6 Hz, N(3)CH₂], 3.89 [3H, s, O-CH₃], 3.95 [3H, s, O-CH₃], 4.41 [2H, t, J 6.6 Hz, N(3)CH₂CH₂Cl], 7.27 [1H, s, C(7)H], 8.32 [1H, bs, N(1)H]; δc (75 MHz, CDCl₃): 40.0 [CH₂, N(3)CH₂CH₂Cl], 41.8 [CH₂, N(3)CH₂], 56.6 [CH₃, O-CH₃], 61.7 [CH₃, O-CH₃], 109.3 [C, aromatic C], 111.2 [C, aromatic C], 118.8 [CH, aromatic CH], 129.7 [C, aromatic C], 141.9 [C, aromatic C], 149.5 [C, aromatic C], 150.5 [C, C=O], 159.2 [C, C=O]; m/z (ESI⁺): 363.0 (M+H)⁺, 100%. HRMS (ESI⁺): Exact mass calculated for C₁₂H₁₃N₂O₄⁷⁹Br₃⁵Cl 364.9747. Found 364.9734.
Synthesis of 3-(3-chloropropyl)-5,8-dimethoxy-6-nitroquinazoline-2,4(1H,3H)-dione 213

3-(3-Chloropropyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione, 210 (0.162 g, 0.05 mmol) was suspended in acetic acid (2.5 mL) at 5 °C with stirring. Nitric acid (65%) (1.3 mL, 1.807 g, 20.25 mmol) and sulfuric acid (96%) (0.8 mL) were added simultaneously and the reaction was stirred for 5 minutes. Water (20 mL) was added to quench the reaction and the resulting precipitate was filtered and dried to give a pale orange solid, 213 (0.160 g, 86%). m.p. 228 – 230 °C; v\text{max}/\text{cm}^{-1} (KBr): 3191, 2945, 1721, 1663, 1551, 1508, 1359; δ\text{H} (300MHz, DMSO-d$_6$): 2.02 [2H, dt, J 6.9 Hz, N(3)CH$_2$C$_3$H$_2$], 3.68 [2H, t, J 6.7 Hz, N(3)(CH$_2$)$_2$CH$_2$Cl], 3.87 [3H, s, O-CH$_3$], 3.92 [3H, s, O-CH$_3$], 4.01 [3H, t, J 7.1 Hz, N(3)CH$_2$], 7.75 [1H, s, C(7)H], 11.21 [1H, bs, N(1)H]; δ\text{C} (75 MHz, DMSO-d$_6$): 30.3 [CH$_2$, N(3)CH$_2$CH$_2$], 38.1 [CH$_2$, N(3)(CH$_2$)$_2$CH$_2$Cl], 43.1 [CH$_2$, N(3)CH$_2$], 56.9 [CH$_3$, O-CH$_3$], 63.4 [CH$_3$, O-CH$_3$], 108.5 [C, aromatic C], 110.0 [CH, aromatic CH], 135.4 [C, aromatic C], 138.0 [C, aromatic C], 141.7 [C, aromatic C], 147.2 [C, aromatic C], 149.4 [C, C=O], 158.8 [C, C=O]; m/z (ESI$^+$): 344.0 (M+H$^+$), 68%. HRMS (ESI$^+$): Exact mass calculated for C$_{13}$H$_{15}$N$_3$O$_6$^{35}Cl 344.0649. Found 344.0634.

8.3.4 Synthesis of N-1, N-3-disubstituted quinazolinediones

Synthesis of 1-benzyl-5,8-dimethoxy-3-phenylquinazoline-2,4(1H,3H)-dione 219

5,8-Dimethoxy-3-phenylquinazoline-(1H,3H)-dione, 198 (0.150 g, 0.50 mmol) was dissolved in anhydrous DMF (10 mL) and sodium hydride (0.014 g, 0.60 mmol) was added portionwise with stirring. Stirring was continued at room temperature for a further 10 minutes until the evolution of hydrogen gas subsided. Benzyl bromide (0.094 g, 0.55 mmol, 0.066 mL) was added and
stirring was continued for 12 hours. TLC analysis showed the presence of starting material so the reaction was heated to 100 °C for 2 hours. The reaction cooled and quenched with water (30 mL). The resulting precipitate was filtered and dried to give a white solid, 219 (0.064 g, 33 %). m.p. 220 – 222 °C; νmax/cm⁻¹ (KBr): 3058, 2844, 1706, 1590, 1493, 1447; δH (300MHz, CDCl₃): 3.59 [3H, s, O-CH₃], 3.89 [3H, s, O-CH₃], 5.61, [2H, s, N(1)CH₂], 6.70 and 7.10 [2H, AB q, ΔνAB 151.6 Hz, J 9.3 Hz, C(6)H and C(7)H], 7.12 – 7.52 [10H, m, ArH]; δc (75 MHz, CDCl₃): 51.6 [CH₂, N(1)CH₂], 56.6 [CH₃, O-CH₃], 57.2 [CH₃, O-CH₃], 106.9 [CH, aromatic CH], 108.5 [C, aromatic C], 120.1 [CH, aromatic CH], 126.2 [2 x CH, aromatic CH], 126.7 [CH, aromatic CH], 128.2 [2 x CH, aromatic CH], 128.4 [CH, aromatic CH], 128.6 [2 x CH, aromatic CH], 129.1 [2 x CH, aromatic CH], 133.0 [C, aromatic C], 135.8 [C, aromatic C], 138.4 [C, aromatic C], 141.9 [C, aromatic C], 152.4 [C, aromatic C], 155.7 [C, C=O], 160.0 [C, C=O]; m/z (ESI⁺): 389.0 (M+H⁺), 100%. HRMS (ESI⁺): Exact mass calculated for C₂₃H₂₁N₂O₄ 389.1501. Found 389.1506.

**Synthesis of 1-allyl-5,8-dimethoxy-3-phenylquinazoline-2,4(1H,3H)-dione 220**

5,8-Dimethoxy-3-phenylquinazoline-(1H,3H)-dione, 198 (0.212 g, 0.71 mmol) was dissolved in anhydrous DMF (10 mL) and sodium hydride (0.020 g, 0.86 mmol) was added portionwise with stirring. Stirring was continued at room temperature for a further 10 minutes until the evolution of hydrogen gas subsided. Allyl bromide (0.095 g, 0.78 mmol, 0.068 mL) was added and stirring was continued for 2 hours at room temperature and a further 3 hours at 100 °C. The reaction cooled and quenched with water (30 mL). The resulting precipitate was filtered and dried to give a white solid, 220 (0.223 g, 92 %). m.p. 118 – 120 °C; νmax/cm⁻¹ (KBr): 2978, 1711, 1670, 1594, 1497, 1450; δH (400MHz, CDCl₃): 3.85 [3H, s, O-CH₃], 3.88 [3H, s, O-CH₃], 4.97 [2H, dt, J 5.4, 1.4 Hz, N(1)CH₂], 5.16 [1H, overlapping ddt, J 15.8 1.3 1.3 Hz, N(3)CH₂CHCH₂ cis], 5.21 [1H, overlapping ddt, J 22.7 1.4 1.4 Hz, N(3)CH₂CHCH₂ trans], 6.01 [1H, m, N(1)CH₂CH], 6.73 and 7.19 [2H, AB q, ΔνAB 175.4 Hz J 9.3 Hz, C(6)H and C(7)H].
C(7)H], 7.22–7.52 [5H, m, ArH]; δc (125 MHz, CDCl3): 50.9 [CH2, N(1)CH2], 56.6 [CH3, O-CH3], 57.3 [CH3, O-CH3], 106.7 [CH, aromatic CH], 108.3 [C, aromatic C], 116.6 [CH2, N(1)CH2CHCH2], 119.8 [CH, aromatic CH], 128.3 [CH, aromatic CH], 128.6 [2 x CH, aromatic CH], 129.1 [2 x CH, aromatic CH], 133.0 [C, aromatic C], 134.3 [CH, N(1)CH2CH], 135.8 [C, aromatic C], 141.8 [C, aromatic C], 152.0 [C, aromatic C], 155.6 [C, C=O], 160.0 [C, C=O]; m/z (ESI+): 339.0 (M+H)+, 100%. HRMS (ESI+): Exact mass calculated for C19H19N2O4 339.1345. Found 339.1349.

**Synthesis of 1,3-dibenzyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 221**

3-Benzyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione, 199 (0.723 g, 2.31 mmol) was dissolved in anhydrous DMF (25 mL) and sodium hydride (0.078 g, 3.25 mmol) was added portionwise with stirring. Stirring was continued at room temperature for a further 10 minutes until the evolution of hydrogen gas subsided. Benzyl bromide (0.436 g, 2.55 mmol, 0.30 mL) was added and stirring was continued for 2 hours at room temperature and a further 12 hrs at 100 °C. The reaction cooled and quenched with water (70 mL). The resulting precipitate was filtered and dried to give an off white solid, 221 (0.620 g, 70 %). m.p. 128 – 130 °C; νmax/cm⁻¹ (KBr): 3027, 2842, 1697, 1653, 1592, 1453, 1440; δH (400MHz, CDCl3): 3.50 [3H, s, O-CH3], 3.92 [3H, s, O-CH3], 5.21 [2H, s, N(1)CH2], 5.61 [2H, s, N(3)CH2], 6.66 and 7.03 [2H, AB q, ΔνAB 142.7 Hz J 9.0 Hz, C(6)H and C(7)H], 7.10 – 7.51 [10H, m, ArH]; δc (75 MHz, CDCl3): 45.0 [CH2, N(3)CH2], 51.7 [CH2, N(1)CH2], 56.5 [CH3, O-CH3], 57.3 [CH3, O-CH3], 106.4 [CH, aromatic CH], 107.9 [C, aromatic C], 119.9 [CH, aromatic CH], 125.9 [2 x CH, aromatic CH], 126.5 [CH, aromatic CH], 127.3 [CH, aromatic CH], 128.2 [2 x CH, aromatic CH], 128.3 [2 x CH, aromatic CH], 129.0 [2 x CH, aromatic CH], 132.9 [C, aromatic C], 137.2 [C, aromatic C], 138.5 [C, aromatic C], 141.7 [C, aromatic C], 152.3 [C, aromatic C], 155.4 [C, C=O], 159.9 [C, C=O]; m/z (ESI+): 403.1 (M+H)+, 100%. HRMS (ESI+): Exact mass calculated for C24H23N2O4 403.1658. Found 403.1643.
Synthesis of 5,8-dimethoxy-1,3-dimethylquinazoline-2,4(1H,3H)-dione 217

5,8-Dimethoxyquinazoline-2,4-(1H,3H)-dione, 154 (0.999 g, 4.50 mmol) was added to a 250 mL round bottom flask containing anhydrous DMF (80 mL). The contents were heated to dissolve the starting material. Sodium hydride (0.450 g, 11.25 mmol) was added portionwise at 50 °C and the reaction was stirred for 1 hour. Methyl iodide (1.596 g, 11.25 mmol, 0.70 mL) was then added and the reaction was stirred for 1 hour. The solvent was removed under reduced pressure and the resulting residue was dissolved in CHCl₃ (100 mL). The organic phase was washed with water (3 x 100 mL). The organic phase was then washed with a 10% KOH solution (3 x 100 mL) and water (100 mL), dried using magnesium sulfate and concentrated to give brown crystals, 217 (0.661 g, 59%). The aqueous phase was neutralised using 2M aqueous HCl, extracted with ethyl acetate (3 x 50 mL), dried using magnesium sulfate and concentrated to give an off white powder, 5,8-dimethoxy-3-methylquinazoline-2,4(1H,3H)-dione, 218 (0.324 g, 29%).

5,8-Dimethoxy-1,3-dimethylquinazoline-2,4(1H,3H)-dione 217: m.p. 110 – 112 °C; ν max/cm⁻¹ (KBr): 2924, 2854, 1647, 1460; δ H (300MHz, CDCl₃): 3.41 [3H, s, N(3)CH₃], 3.73 [3H, s, N(1)CH₃], 3.83 [3H, s, O-CH₃], 3.93 [3H, s, O-CH₃], 6.70 and 7.16 [2H, AB q, ΔνAB 129.1 Hz J 9.1 Hz, C(6)H and C(7)H]; δ c (75 MHz, CDCl₃): 28.4 (CH₃, N(3)CH₃), 37.0 (CH₃, N(1)CH₃), 56.6 [CH₃, O-CH₃], 57.6 [CH₃, O-CH₃], 106.2 [CH, aromatic CH], 107.7 [C, aromatic C], 120.0 [CH, aromatic CH], 133.8 [C, aromatic C], 142.0 [C, aromatic C], 152.2 [C, aromatic C], 155.0 [C, C=O], 160.3 [C, C=O]; m/z (ESI⁺): 251.1 (M+H)⁺, 96%. HRMS (ESI⁺): Exact mass calculated for C₁₂H₁₅N₂O₄ 251.1032. Found 251.1036.

5,8-Dimethoxy-3-methylquinazoline-2,4(1H,3H)-dione 218: m.p. 224 – 226 °C; ν max/cm⁻¹ (KBr): 2935, 2844, 1715, 1658, 1514, 1480; δ H (300MHz, CDCl₃): 3.41 [3H, s, N(3)CH₃], 3.90 [3H, s, O-CH₃], 3.92 [3H, s, O-CH₃], 6.58 and 7.02 [2H, AB q, ΔνAB 123.7 Hz J 9.1 Hz, C(6)H and C(7)H], 8.17 [1H, bs, N(1)H]; δ c (75 MHz, CDCl₃): 27.3 [CH₃, N(3)CH₃], 56.3 [CH₃, O-CH₃], 56.4 [CH₃, O-CH₃], 103.9 [CH, aromatic CH], 104.4 [C, aromatic C], 115.0 [CH, aromatic C], 120.0 [CH, aromatic CH], 133.8 [C, aromatic C], 142.0 [C, aromatic C], 152.2 [C, aromatic C], 155.0 [C, C=O], 160.3 [C, C=O]; m/z (ESI⁺): 251.1 (M+H)⁺, 96%. HRMS (ESI⁺): Exact mass calculated for C₁₂H₁₅N₂O₄ 251.1032. Found 251.1036.
aromatic CH], 130.4 [C, aromatic C], 138.9 [C, aromatic C], 150.1 [C, aromatic C], 154.1 [C, C=O], 160.8 [C, C=O]; m/z (ESI⁺): 237.1 (M+H)⁺, 100%. HRMS (ESI⁺): Exact mass calculated for C₁₁H₁₃N₂O₄ 237.0875. Found 237.0871.

**Synthesis of 6-acetamido-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 228**

6-Nitro-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione, 164 (1.481 g, 5.52 mmol) was added to a Parr bottle containing water (75 mL), potassium hydroxide (7.50 g) and Pd/C (0.375 g, 5%). The bottle was agitated for 1 hour under an atmosphere of H₂ (50 psi). The reaction mixture was suction filtered through Celite and the filtrate was brought to pH 6 using acetic acid. Acetic anhydride (8.115 g, 79.9 mmol, 7.50 mL) was then added and the solution was stirred at room temperature for 2 hours. During this time a grey solid began to precipitate. The mixture was cooled to 5 °C for a further 2 hours to complete crystallisation. The solid was collected by suction filtration and dried to give a grey solid, 228 (1.126 g, 73%). m.p. 278 – 280 °C (Lit.¹⁶⁶ dec. 285 – 290 °C); νmax/cm⁻¹ (KBr): 3420, 3185, 3063, 2847, 1725, 1673, 1608, 1538; δH (300MHz, DMSO-d₆): 2.11 [3H, s, COCH₃], 3.68 [3H, s, O-CH₃], 3.79 [3H, s, O-CH₃], 7.92 [1H, s, C(7)H], 9.37 [1H, bs, C(6)NH], 10.25 [1H, bs, N(1)H], 11.08 [1H, bs, N(3)H]; m/z (ESI⁺): 280.3 (M+H)⁺, 100%.
8.3.5 Synthesis of 6-substituted N-1, N-3 disubstituted quinazolinediones

Synthesis of 5,8-dimethoxy-1,3-dimethyl-6-nitroquinazoline-2,4(1H,3H)-dione 222

5,8-Dimethoxy-6-nitroquinazoline-2,4(1H,3H)-dione, 164 (1.001 g, 3.74 mmol) was added to anhydrous DMF (200 mL) in a 250 mL round bottom flask. The contents were heated to 120 °C to dissolve the starting material. The solution was then allowed to cool to room temperature and sodium hydride (0.230 g, 9.35 mmol) was added. Stirring was continued for 20 minutes and methyl iodide (1.330 g, 9.35 mmol, 0.59 mL) was then added. The reaction was stirred for 12 hours at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (50 mL). The organic phase was washed repeatedly with water to remove any residual DMF. The organic phase was then washed with a 10 % KOH solution (4 x 100 mL) and water (100 mL), dried using magnesium sulfate and concentrated to give an orange solid, 222 (0.287 g, 31 %). The aqueous phase was neutralised using 2M aqueous HCl, extracted with ethyl acetate (3 x 50 mL), dried using magnesium sulfate and concentrated to give an orange solid, 5,8-dimethoxy-3-methyl-6-nitroquinazoline-2,4(1H,3H)-dione, 223 (0.654 g, 69 %).

5,8-Dimethoxy-1,3-dimethyl-6-nitroquinazoline-2,4(1H,3H)-dione 222: m.p. 163 – 165 °C; ν_{max}/cm^{-1} (KBr): 2951, 1717, 1674, 1587, 1467; δ_{H} (300MHz, CDCl₃): 3.44 [3H, s, N(3)CH₃], 3.80 [3H, s, N(1)CH₃] 3.95 [3H, s, O-CH₃], 4.06 [3H, s, O-CH₃], 7.64 [1H, s, C(7)H]; δ_{C} (75 MHz, CDCl₃): 28.7 [CH₃, N(3)CH₃], 37.4 [N(1)CH₃], 57.0 [CH₃, O-CH₃], 64.2 [CH₃, O-CH₃], 112.7 [CH, aromatic CH], 112.8 [C, aromatic C], 137.5 [C, aromatic C], 139.2 [C, aromatic C], 144.0 [C, aromatic C], 149.2 [C, aromatic C], 151.5 [C, C=O], 158.5 [C, C=O]; m/z (ESI⁺): 296.1 (M+H)⁺, 100%. HRMS (ESI⁺): Exact mass calculated for C₁₂H₁₄N₃O₆ 296.0883. Found 296.0878.
5,8-Dimethoxy-3-methyl-6-nitroquinazoline-2,4(1H,3H)-dione, 223: m.p. 275 - 277 °C

\( \nu_{\text{max}} / \text{cm}^{-1} \) (KBr): 3070, 2958, 1719, 1670, 1598, 1526, 1478; \( \delta_{\text{H}} \) (300MHz, DMSO-\( d_6 \)): 3.24 [1H, s, N(3)CH\(_3\)], 3.87 [3H, s, O-CH\(_3\)], 3.92 [3H, s, O-CH\(_3\)], 7.74 [1H, s, C(7)H], 11.17 [1H, bs, N(1)H]; \( \delta_{\text{C}} \) (75 MHz, DMSO-\( d_6 \)): 27.1 [CH\(_3\), N(3)CH\(_3\)], 56.8 [CH\(_3\), O-CH\(_3\)], 63.4 [CH\(_3\), O-CH\(_3\)], 108.3 [C, aromatic C], 109.9 [CH, aromatic CH], 135.3 [C, aromatic C], 138.0 [C, aromatic C], 141.7 [C, aromatic C], 147.2 [C, aromatic C], 149.5 [C, C=O], 158.9 [C, C=O]; m/z (ESI\(^+\)): 280.3 (M-H\(^-\)), 40%; HRMS (ESI\(^+\)): Exact mass calculated for (M+H\(^+\)) \( \text{C}_{11}\text{H}_{12}\text{N}_{3}\text{O}_6 \), 282.0726. Found 282.0716.

Synthesis of 1-benzyl-5,8-dimethoxy-6-nitro-3-phenylquinazoline-2,4(1H,3H)-dione 224

1-Benzyl-5,8-dimethoxy-3-phenylquinazoline-2,4(1H,3H)-dione, 219 (0.091 g, 0.234 mmol) was suspended in acetic acid (5 mL) at 5 °C with stirring. Nitric acid (65%) (1.0 mL, 1.39 g, 15.57 mmol) and sulfuric acid (96%) (0.41 mL) were added simultaneously and the reaction was stirred for 5 minutes. The reaction was poured on to crushed ice and the resulting precipitate was filtered and dried to give a yellow solid, 224 (0.050 g, 50%). m.p. 200 – 202 °C; \( \nu_{\text{max}} / \text{cm}^{-1} \) (KBr): 3002, 2944, 1716, 1675, 1520, 1432, 1373; \( \delta_{\text{H}} \) (400MHz, CDCl\(_3\)): 3.73 [3H, s, O-CH\(_3\)], 4.04 [3H, s, O-CH\(_3\)], 5.61, [2H, s, N(1)CH\(_2\)], 7.16 – 7.53 [10H, m, Ar\( \text{H} \)], 7.61 [1H, s, C(7)\( \text{H} \)]; \( \delta_{\text{C}} \) (75 MHz, CDCl\(_3\)): 52.1 [CH\(_2\), N(1)CH\(_2\)], 56.9 [CH\(_3\), O-CH\(_3\)], 64.4 [CH\(_3\), O-CH\(_3\)], 113.2 [CH, aromatic CH], 113.8 [C, aromatic C], 126.1 [2 x CH, aromatic CH], 127.2 [CH, aromatic CH], 128.3 [2 x CH, aromatic CH], 128.5 [2 x CH, aromatic CH], 129.0 [CH, aromatic CH], 129.5 [2 x CH, aromatic CH], 135.0 [C, aromatic C], 136.9 [C, aromatic C], 137.6 [C, aromatic C], 139.6 [C, aromatic C], 143.9 [C, aromatic C], 149.6 [C, aromatic C], 151.7 [C, C=O], 158.4
Synthesis of 1-allyl-5,8-dimethoxy-6-nitro-3-phenylquinazoline-2,4(1H,3H)-dione 225

1-Allyl-5,8-dimethoxy-3-phenylquinazoline-2,4(1H,3H)-dione, 220 (0.100 g, 0.29 mmol) was suspended in acetic acid (1.5 mL) at 5 °C with stirring. Nitric acid (65%) (1.0 mL, 1.39 g, 15.57 mmol) and sulfuric acid (96%) (0.41 mL) were added simultaneously and the reaction was stirred for 1 hour. The reaction was poured on to crushed ice and the resulting precipitate was filtered and dried to give a yellow solid, 225 (0.078 g, 69 %). m.p. 125 – 127 °C; v_{max}/cm\(^{-1}\) (KBr): 3431, 2947, 2850, 2092, 1718, 1675, 1583, 1524; δ\(_H\) (400MHz, CDCl\(_3\)): 3.97 [3H, s, O-CH\(_3\)], 4.01 [3H, s, O-CH\(_3\)], 4.98 [2H, dt, J 5.5 1.4 Hz, N(1)CH\(_2\)], 5.22 [1H, overlapping ddt, J 10.3 1.2 1.2 Hz, N(3)CH\(_2\)CHCH\(_2\) cis], 5.25 [1H, overlapping ddt, J 17.1 1.4 1.3 Hz, N(3)CH\(_2\)CHCH\(_2\) trans], 6.00 [1H, m, N(1)CH\(_2\)CH], 7.24 – 7.62 [5H, m, ArH], 7.69 [1H, s, C(7)H]; δ\(_C\) (125 MHz, CDCl\(_3\)): 51.4 [CH\(_2\), N(1)CH\(_2\)], 57.0 [CH\(_3\), O-CH\(_3\)], 64.3 [CH\(_3\), O-CH\(_3\)], 113.0 [CH, aromatic CH], 113.6 [C, aromatic C], 117.5 [CH\(_2\), N(1)CH\(_2\)CHCH\(_2\)], 128.2 [2 x CH, aromatic CH], 129.0 [CH, aromatic CH], 129.5 [2 x CH, aromatic CH], 133.5 [CH, N(1)CH\(_2\)CH], 135.0 [C, aromatic C], 137.0 [C, aromatic C], 139.5 [C, aromatic C], 143.8 [C, aromatic C], 149.7 [C, aromatic C], 151.3 [C, C=O], 158.4 [C, C=O]; m/z (ESI\(^+\)): 384.0 (M+H\(^+\)), 90%; HRMS (ESI\(^+\)): Exact mass calculated for C\(_{23}\)H\(_{20}\)N\(_3\)O\(_6\) 384.1196. Found 384.1192.
Synthesis of 1,3-dibenzyl-5,8-dimethoxy-6-nitroquinazoline-2,4(1H,3H)-dione

1,3-Dibenzyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione, 221 (0.549 g, 1.41 mmol) was suspended in acetic acid (25 mL) at 5 °C with stirring. Nitric acid (65%) (5 mL, 6.95 g, 77.89 mmol) and sulfuric acid (96%) (2.5 mL) were added simultaneously and the reaction was stirred for 5 minutes. The reaction was poured on to crushed ice and the resulting precipitate was filtered and dried to give a yellow solid, 226 (0.556 g, 88%). m.p. 103 – 105 °C v_{max}/cm^{-1} (KBr): 2851, 2100, 1711, 1666, 1585, 1524, 1484; δ_H (400MHz, CDCl_3): 3.64 [3H, s, O-CH_3], 4.02 [3H, s, O-CH_3], 5.27 [2H, s, N(1)CH_2], 5.62 [2H, s, N(3)CH_2], 7.07 – 7.50 [10H, m, ArH], 7.51 [1H, s, C(7)H]; δ_C (75 MHz, CDCl_3): 45.2 [CH_2, N(3)CH_2], 52.0 [CH_2, N(1)CH_2], 56.8 [CH_3, O-CH_3], 64.2 [CH_3, O-CH_3], 112.9 [CH, aromatic CH], 113.1 [C, aromatic C], 125.7 [2 x CH, aromatic CH], 127.0 [CH, aromatic CH], 127.4 [CH, aromatic CH], 128.4 [2 x CH, aromatic CH], 128.4 [2 x CH, aromatic CH], 129.0 [2 x CH, aromatic CH], 136.3 [C, aromatic C], 136.7 (C, aromatic C), 137.6 [C, aromatic C], 139.3 [C, aromatic C], 143.6 [C, aromatic C], 149.4 [C, aromatic C], 151.6 [C, C=O], 158.1 [C, C=O]; m/z (ESI\(^+\)): 448.1 (M+H\(^+\)), 100%; HRMS (ESI\(^+\)): Exact mass calculated for C_{24}H_{22}N_{3}O_{6} 448.1509. Found 448.1494.
8.3.6 Synthesis of 2,4-dichloro-5,8-dimethoxyquinazoline 229

Synthesis of 2,4-dichloro-5,8-dimethoxyquinazoline 229

To a 50 mL round-bottomed flask was added 154 (0.166 g, 0.75 mmol) and POCl₃ (5 mL). The mixture was heated to reflux for 1 hour. Following cooling the reaction was added portionwise to crushed ice (100 g) and neutralised using 5M NaOH. The aqueous phase was extracted with DCM (3 x 50 mL) and the combined organic layers were washed with water (20 mL), dried using magnesium sulfate and concentrated to dryness to give 229 (0.129 g, 67 %) as a bright yellow solid. m.p. 174 – 175 °C; \( \nu \)max/cm\(^{-1}\) (NaCl): 1603, 1532, 1477; \( \delta \)H (300MHz, CDCl\(_3\)): 3.97 [3H, s, O-CH\(_3\)], 4.02 [3H, s, O-CH\(_3\)], 6.94 and 7.23 [2H, AB q, \( \Delta \nu \)AB 79.4 Hz J 8.7 Hz, C(6)H and C(7)H]; \( \delta \)c (75MHz, CDCl\(_3\)): 56.4 [CH\(_3\), O-CH\(_3\)], 56.5 [CH\(_3\), O-CH\(_3\)], 108.2 [CH, aromatic CH], 114.4 [CH, aromatic CH], 115.0 [C, aromatic C], 145.5 [C, aromatic C], 148.0 [C, aromatic C], 149.6 [C, aromatic C], 154.7 [C, C(4)], 161.6 [C, C(2)]; m/z (ESI\(^+\)): 259.2 (M+H\(^+\)), 60%. HRMS (ESI\(^+\)): Exact mass calculated for C\(_{10}\)H\(_9\)N\(_2\)O\(_2\)Cl\(_2\) 259.0041. Found 259.0035.

8.4 Protocol for biological evaluation

8.4.1 NCI-60 experimental methodology

The reported experimental protocol involves the initial growth of the tumour cell lines in RPMI 1640 medium containing 5% foetal bovine serum and 2mM L-glutamine.\(^{189}\) A typical screening experiment involves inoculating the cells into 96 well microtiter plates in 100 \( \mu \)L of medium at plating densities ranging from 5,000 to 40,000 cell/well depending on the doubling times of each particular cell line. Following cell inoculation, the microtiter
plates are incubated at 37 °C, 5% CO₂, 95% air and 100% relative humidity for 24 hours prior to addition of the compounds being tested.

Following incubation two plates of each cell line are fixed in situ using trichloroacetic acid (TCA) which represents the measurement of cell population for each cell line at the time of drug addition (Tz). Prospective compounds are dissolved in DMSO to 400-fold the desired final maximum test concentration and stored frozen prior to use. Single dose screening is carried out at 10 µM, successful candidates are then subjected to a second phase of testing which involves examination at five different drug concentrations (five dose assay).

After drug addition the plates are incubated for a following 48 hours at 37 °C, 5% CO₂, 95% air and 100% relative humidity. For adherent cells, the assay is terminated with the addition of cold TCA. Cells are fixed in situ by the addition of 50 µL of cold 50% (w/v) TCA and incubated for 60 minutes at 4 °C. Sulforhodamine B (SRB) solution (100 µL) at 0.4% (w/v) in 1% acetic acid is added to each well and the plates are incubated for 10 minutes at room temperature. Absorbance is read on an automated plate reader at a wavelength of 515 nm, and using seven absorbance measurements (time zero (Tz), control growth (C), and test growth in the presence of the drug at five different concentrations (Ti)). The percentage growth is calculated at each of the drug concentration levels. The response parameters GI50 (concentration required to inhibit cell growth by 50%) and LC50 (concentration required for 50% apoptosis) are extracted from concentration response curves by linear interpolation. TGI (total growth inhibition) is read as the x-axis intercept from the five different drug concentrations.¹⁸⁹

8.4.2 COMPARE analysis

Use of the COMPARE algorithm for the mechanistic investigation of cell growth inhibition consists of calculating the linear correlation coefficient between the data over the cell
lines tested for a particular compound and all sets of data in the NCI database to be searched. Calculation of the correlation coefficient involves three choices: (i) the seed (pattern of interest), (ii) the parameters and (iii) the database.

When running a COMPARE calculation via the Development Therapeutics Program (DTP) branch of the NCI website a number of key parameters can be modified to allow for more efficient analyses. The data used as the seed for the calculation can contain any of the growth inhibition measurements for a particular compound e.g. GI$_{50}$, TGI or LC$_{50}$ values. There are a number of different databases within the NCI which can be used to perform a COMPARE calculation e.g. standard chemotherapeutic agents or synthetic compounds. It is also possible to only return data which corresponds to a minimum correlation threshold against the seed. Another useful variable is the ability to select a minimum number of cell lines when performing the calculation instead of running analysis against the entire cell panel. COMPARE calculations run in this project were either against the target set of standard agent or synthetic compounds. The minimum correlation value was 0.4, minimum amount of common cell lines was 40 and the minimum standard deviation was 0.5.
9.0 References
9.0 References

32. C. Seong, N. Park, J. Choi, C. M. Park, W. Park, J. Kong, 2008, WO 2008004716, **2008-01-10**.


Daunorubicin, (visited on 04/04/2014).


Appendices
3-(2-Chloroethyl)-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 196

[Chemical Structure Image]

[Graph Image]
3-Benzyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 199

![Chemical Structure](image)

### Developmental Therapeutics Program

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3-Allyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 202

![Molecular Structure](image)

### Developmental Therapeutics Program

**One Dose Mean Graph**

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**Experiment ID:** 135583

**Test Date:** May 05, 2013

**Report Date:** Jul 01, 2013

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![Chemical Structure](image)

### Developmental Therapeutics Program

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6-Bromo-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 215
1-Allyl-5,8-dimethoxy-3-phenylquinazoline-2,4(1H,3H)-dione 220

![Chemical Structure](image)

### Developmental Therapeutics Program

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1,3-Dibenzyl-5,8-dimethoxyquinazoline-2,4(1H,3H)-dione 221
6-(Benzylylamino)-4-chloroquinoline-5,8-dione 182

![Chemical Structure](image)

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7-Bromo-4-chloro-6-((2-morpholinoethyl)amino)quinoline-5,8-dione 188

![Chemical Structure](image)

### Developmental Therapeutics Program

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### Graphs

- Bar charts showing growth percent for different cell lines.
- Line graphs indicating mean growth percent across different doses and cell lines.
7-Bromo-4-chloro-6-(methylamino)quinoline-5,8-dione 189

![Chemical Structure](image.png)

### Developmental Therapeutics Program

**One Dose Mean Graph**

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Experiment ID: 13350583  Corr.: 1.002-5 Mol/L  Test Date: May 25, 2013  Report Date: Jul 04, 2013

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Appendices
7-Bromo-4-chloro-6-((2-morpholinoethyl)amino)quinoline-5,8-dione 188
7-Bromo-4-chloro-6-((2-morpholinoethyl)amino)quinoline-5,8-dione 188
### National Cancer Institute Developmental Therapeutics Program

#### In-Vitro Testing Results

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**Chemical Structures**

**7-Bromo-4-chloro-6-(2-morpholinoethyl)amino)quinoline-5,8-dione**

**Key Chemical Formulas**

1. **7-Bromo-4-chloro-6-(2-morpholinoethyl)amino)quinoline-5,8-dione**

2. **[(2-morpholinoethyl)amino]quinoline-5,8-dione**

**References**


**Appendices**

- **XVI**
- **7**
- **Bromo-4-chloro-6-(2-morpholinoethyl)amino)quinoline-5,8-dione**

---

**Acknowledgments**

This research was supported by the National Cancer Institute Developmental Therapeutics Program. The authors would like to thank the reviewers for their valuable feedback.

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**NISC:** D - 074670/1 | **Experiment ID:** 13095670 | **Test Type:** O6 | **Units:** Mol
t

**Report Date:** April 02, 2014 | **Test Date:** September 16, 2013 | **QN:** | **M:**

**COMI:** KG 9563 (8738) | **Stain Reagent:** SBF Dual-Pass Rel. | **BSPL:** OY2V
7-bromo-4-chloro-6-((2-morpholinoethyl)amino)quinoline-5,8-dione 188
7-Bromo-4-chloro-6-(methylamino)quinoline-5,8-dione 189

![Chemical Structure]

**National Cancer Institute Developmental Therapeutics Program**

**Dose Response Curves**

All Cell Lines

- Log$_{10}$ of Sample Concentration (Molar)
- Percentage Growth

**S774877**
7-Bromo-4-chloro-6-(methylamino)quinoline-5,8-dione 189
### National Cancer Institute Developmental Therapeutics Program

**In-Vitro Testing Results**

**NSC #: D - 774877/1**

**Experiment ID: 13035575**

**Test Type: DI**

**Units: Molar**

**Report Date: April 02, 2014**

**Test Date: September 23, 2013**

**QNS: MC**

**Compound: 7-Bromo-4-chloro-6-(methylamino)quinoline-5,8-dione 189**

#### LogD/Concentration

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<th>Mean Optical Densities</th>
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#### Appendixes

- **XX**
7-Bromo-4-chloro-6-(methylamino)quinoline-5,8-dione 189

![Chemical Structure](image)

### National Cancer Institute Developmental Therapeutics Program

<table>
<thead>
<tr>
<th>Compound Line</th>
<th>Log P (CAR)</th>
<th>TGI</th>
<th>IC50 (M)</th>
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**Report Date:** April 02, 2014

**Test Date:** September 23, 2013

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**Abbreviations:**
- CAR: Chemometrically Adjusted Response
- TGI: Therapeutic Grade Index
- IC50: Inhibitory Concentration 50

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**Table:**

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**Legend:**
- **Red**: High IC50 values
- **Green**: Low IC50 values
- **Blue**: Intermediate IC50 values

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**Graph:**

- **X-axis**: Log P (CAR)
- **Y-axis**: TGI
- **Color Coding**: Highlighted the therapeutic potential of the compound

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**Notes:**
- The compound shows promising therapeutic potential in the context of the National Cancer Institute’s Therapeutics Program.
- Further research is recommended for clinical evaluation.
**COMPARE analysis of 188 vs NSC663284**

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**Legend**

- COMP-188
- NSC-663284
- NSC-663284

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**Non-Small Cell Lung**

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**Summary**

- GM141
- SD6
- Range
- 1.17 (1.05, 1.31)
- 1.24 (1.08, 1.41)
- 1.36 (1.20, 1.52)
LCMS trace of 188