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Design, Synthesis and Evaluation of Novel Ellipticine Derivatives and Analogues as Anti-Cancer Agents

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Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

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

Department of Chemistry

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December 2011

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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:_____

Abstract

This thesis describes work carried out on the synthesis of novel 5- and 11-substituted ellipticines and derivatives of the ellipticine analogues, isoellipticine and deazaellipticine, followed by investigation of their potential as anti-cancer agents.

Preparation of the key 5- and 11-substituted ellipticine targets involved the development of regiospecific, sequential alkylation reactions with alkenyllithium and Grignard reagents. Investigation of these novel reactions resulted in a new route towards 5-substituted ellipticines *via* Grignard reaction with vinylmagnesium bromide. These novel 5-vinylellipticine derivatives were further functionalised in an ozonolysis reaction, followed by oxidation to give a range of novel 5-substituted ellipticines. Less success was encountered in the 11-substituted ellipticine series, however preparation of these derivatives using a previously published route was accomplished, and the resulting 11-formylellipticine was further derivatised to give a panel of novel 9- and 11-substituted ellipticines, incorporating amide, carboxylate, imine and amine functionality.

The successful route towards 5-substituted ellipticines was applied to the preparation of a range of novel 11-substituted isoellipticines and 6-substituted deazaellipticines, the first time substantial synthesis has been undertaken with these analogues. In addition to this, the first preparation of isoellipticinium salts is described, and a panel of novel isoellipticinium, 7-formylisoellipticinium and 7-hydroxyisoellipticinium salts were synthesised in good yields.

Biological evaluation of a panel of 43 novel ellipticine, isoellipticine and deazaellipticine derivatives was accomplished with a topoisomerase II decatenation assay and submission to the NCI 60-cell line screen. Four novel isoellipticine topoisomerase II inhibitors were identified from the decatenation assay, with strong activity at 10 μM. In addition to this, NCI screening identified five highly cytotoxic ellipticine and isoellipticine compounds with remarkable selectivity profiles for different cancer types. These novel lead compounds represent new templates for further research and synthesis.

Abbreviations

A	Adenine
ADP	Adenosine diphosphate
AML	Acute myeloid leukemia
Ar	Aryl
ATP	Adenosine triphosphate
aq.	Aqueous
BBB	Blood brain barrier
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
b.p.	Boiling point
br d	Broad doublet
br s	Broad singlet
Bz	Benzoyl
C	Cytosine
Cbz	Carboxybenzyl
CD	Circular dichroism
CDI	Carbonyldiimidazole
CNS	Central nervous system
COSY	Correlation spectroscopy
CR	Complete response
CYP	Cytochrome P450
d	Doublet
DCM	Dichloromethane
dd	Doublet of doublets
ddd	Doublet of doublets of doublets
DDQ	2,3-Dichloro-5,6-dicyano-*p*-benzoquinone
DEPT	Distortionless enhancement of polarisation transfer
DEA	*N,N*-Diethylaniline
DIBAL	Diisobutylaluminium hydride
dil.	Dilute
DMAP	*N,N*-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
DMSO-d_6	Deuterated dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dt	Doublet of triplets
DTP	Developmental Therapeutics Program
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
eq.	Equivalents
ESI	Electrospray ionisation

Et	Ethyl
EtOAc	Ethyl acetate
EtOH	Ethanol
G	Guanine
g	Gram
Glu	Glutamic acid
h	Hour(s)
HETCOR	Heteronuclear correlation
HMBC	Heteronuclear multiple-bond correlation
HMPA	Hexamethylenetetramine
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HRP	Horse radish peroxidase
HSQC	Heteronuclear single quantum correlation
Hz	Hertz
IC_{50}	50% Inhibition concentration
ID_{50}	50% Infectious dose
ILS%	Increase in mean life span
i.p.	Intra peritoneal
i-Pr	Isopropyl
IR	Infrared
i.v.	Intravenous
J	Coupling constant
kb	1000 DNA base pairs
kDNA	kinetoplast DNA
LC_{50}	50% Lethal concentration
LD	Linear dichroism
LDA	Lithium diisopropylamide
Lit.	Literature
m	Multiplet
MCD	Mast cell disorder (aka. mastocytosis)
m-CPBA	*meta*-Chloroperbenzoic acid
Me	Methyl
MeOH	Methanol
mg	milligram
µg	microgram
MHz	Megahertz
min	minutes
mL	millilitre
µM	micromolar
mmol	millimole
mol	mole
m.p.	melting point

MPO	Myeloperoxidase
mRNA	messenger RNA
Ms	Mesylate
mw	microwave
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
n-BuLi	*n*-Butyllithium
NBS	*N*-Bromosuccinimide
NCI	National Cancer Institute
nM	nanomolar
n-Pr	*n*-Propyl
NSCLC	Non-small cell lung cancer
NMR	Nuclear magnetic resonance
OAc	Acetate
PDGFR	Platelet-derived growth factor receptor
Ph	Phenyl
PMB	*para*-Methoxy benzyl
PR	Partial response
psi	pounds per square inch
p-TSA	*para*-Toluenesulfonic acid
q	Quartet
r.t.	room temperature
RTK	Receptor tyrosine kinase
s	Singlet
sat.	Saturated (solution)
SCF	Stem cell factor (aka. Steel factor, mast cell growth factor)
Superhydride	Lithium triethylborohydride
T	Thymine
t	Triplet
TBAHS	Tetrabutylammonium hydrogensulfate
t-BuLi	*tert*-Butyllithium
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TFA	Trifluoroacetic acid
TGI	Total Growth Inhibition
TLC	Thin layer chromatography
TMS	Trimethylsilane
Topo II	Topoisomerase II
Tris	Tris(hydroxymethyl)aminomethane
Ts	*para*-Toluenesulfonyl
UV	Ultraviolet
VEGF	Vascular endothelial growth factor
VIP	Vasoactive intestinal peptide

1 Biological Introduction

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1.1 Cancer

Cancer may be defined as a set of diseases characterised by unregulated cell growth leading to invasion of surrounding tissues and spread (metastasis) to other parts of the body.[1] Although known to the early Egyptians, advances in modern medicine and the subsequent increase in life expectancy have resulted in cancer becoming one of the leading causes of death today. In 2008, the World Health Organisation found that 13.3% of deaths worldwide were caused by malignant neoplasms, second only to cardiovascular disease at 30.5%.[2]

The core feature of cancer is uncontrolled cell proliferation, however this does not necessarily mean faster proliferation. While some cancers do proliferate faster than normal cells, others, such as breast cancer, proliferate slowly. Other key features of cancer cells are extended life and a higher degree of autonomy compared to normal cells. The most common terminology used in relation to cancer are the terms benign and malignant - a benign tumour is characterised by increased cell mass, with clearly defined boundaries and is generally easily treated. On the other hand, a malignant neoplasm or tumour is defined by invasion of surrounding tissues and metastasis to remote tissue. Specific cancers are described by their cell of origin and the tissue in which they arise (Table 1.1).[1]

Epithelium – carcinoma	Glandular	e.g. prostate: adenocarcinoma
	Squamous	e.g. cervix: carcinoma
Mesenchyme – sarcoma	Smooth muscle	Leiomyosarcoma
	Bone	Osteosarcoma
Nervous system	Eye	Retinoblastoma
	Astrocytes	Astrocytoma
White blood cells – leukaemia (chronic or acute)	Myeloid cells	Myelocytic leukaemia
	Lymphocytes	Lymphocytic leukaemia
	Lymphoma	Solid tumour derived from B or T lymphocytes

Table 1.1: Classification of common cancer types[1]

Carcinogenesis, the multi-step process by which a cancer is formed, involves accumulation of sucessive mutations in oncogenes and suppressor genes that deregulate the cell cycle, e.g. point mutations, translocation, deletion and amplification.[3] Once a cancer is formed, such changes can continue to accumulate due to deregulation of cell processes, often leading to increased difficulty in treatment.

It is difficult to identify exact causes for specific cancers, however definitive environmental and genetic risk factors can be identified, e.g. smoking is a major risk factor for lung cancer. Diet, increasing age and family history of cancer have all been identified as risk factors which increase the chance of developing cancer. In addition to this, cancers have different incidences

in different populations e.g. high rates of stomach cancers in Japan, melanomas in Australia and liver cancers in China.

The treatment of cancer is based on the removal or killing (or both) of cancer cells whilst minimising unwanted side effects. This may be achieved by surgery, radiotherapy or chemotherapy, alone or more commonly, in combination. The majority of chemotherapeutic agents act by preventing mitosis or DNA synthesis. Anticancer drugs can be classified according to their mechanism of action, such as DNA-interactive agents, antimetabolites, anti-tubulin agents, molecular targeting agents, hormones, monoclonal antibodies and other biological agents. Some of the most common anticancer drugs are summarised by mechanism of action in Table 1.2 (information collected from several sources).[1,4,5]

DNA Interacting Agents	
Alkylating agents	Alkylation of DNA bases in either the minor or major grooves - dacarbazine, procarbazine and temozolomide
Cross-linking agents	Binding to DNA resulting in intra-strand or inter-strand cross-linking - Platinum complexes e.g. cisplatin, carboplatin, oxaliplatin - Nitrogen mustards e.g., cyclophosphamide, ifosfamide
Intercalating agents	Binding between base pairs - Anthracyclines e.g. doxorubicin, epirubicin - Mitoxantrone and actinomycin-D.
Topoisomerase Inhibitors	- Topoisomerase I: camptothecins - Topoisomerase II: anthracyclines, etoposide
DNA cleaving agents	Cause strand scission at the binding site. - Bleomycin
Antimetabolites	
Pyrimidine analogues	5-Fluorouracil
Purine analogues	Mercaptopurine
Inhibitors	Methotrexate: inhibits DHF reductase
Antitublin Agents	
Taxols	Docetaxel, paclitaxel
Vinca alkaloids	Vinblastine, vincristine, vinorelbine
Tyrosine Kinase Inhibitors	
Small molecule	- Imatinib (Gleevec): inhibits ABL, c-Kit kinase, PDGFR - Gefitinib (Iressa): inhibits EGFR
Monoclonal antibody	Trastuzumab: inhibits EGFR2, HER2, neu
Angiogenesis/Metastasis Inhibitors	
Monoclonal antibody	Bevacizumab (Avastin): targets VEGF

Table 1.2: Common anticancer drugs and their mechanisms of action[1,4,5]

The development and effectiveness of chemotherapeutic treatments is generally hampered by the high toxicity of most anticancer drugs and the non-selectivity of their action. This has lead to a continual search for new anticancer agents, especially amongst the vast array of natural products.

1.2 Ellipticine

The natural product ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) **1** was first isolated from the leaf material of *Ochrosia elliptica* Labill by Goodwin *et al.* in 1959.[6] This small tropical evergreen tree belonging to the Apocynaceae family also contained several other alkaloids, including 9-methoxyellipticine. Ellipticine has since been isolated from several other plants of the Apocynaceae family *(Ochrosia vieillardii, Ochrosia acuminate and Ochrosia moorei)*[7–9] and from *Strychnos dinkagei* of the Loganiaceae family.[10,11]

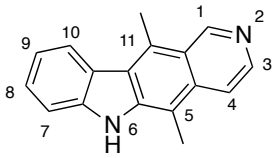


Figure 1.1: Ellipticine ***1***

Ellipticine and its derivatives were found to exhibit potent anticancer activity and have been subject to much study over the last fifty years. Woodward *et al.* reported the first synthesis of ellipticine in 1959 and a vast array of synthetic strategies has been published since (these are discussed in chapter 2).[12] Parallel to this, research into the biological activity of ellipticine, in particular its interaction with DNA, has elucidated much of its mechanism of action.

1.2.1 Reviews

The most extensive reviews of the biological activity of ellipticine are those of Christian Auclair (1987) and Garbett & Graves (2004).[13,14] Harding and Grummitt also published a mini-review of 9-hydroxyellipticine and its derivatives in 2003.[15] The main mechanisms discussed in these reviews are intercalation with DNA, topoisomerase II inhibition and bio-oxidation, however recent research has highlighted some significant new mechanisms of action.

1.3 Mechanisms of Action

The mechanisms of action of ellipticine are considered here in three sections: interaction with DNA, kinase inhibition and other mechanisms. Of these, interactions with DNA are well established, and include intercalation, topoisomerase II inhibition, bio-oxidation and adduct formation. Inhibition of c-Kit kinase and AKT have been reported in the last five to six years and open up the vast area of kinase inhibition for investigation. Finally, one of the most interesting developments in the area is the rescue of mutant p53 function by ellipticine, which has come to light in the last decade.

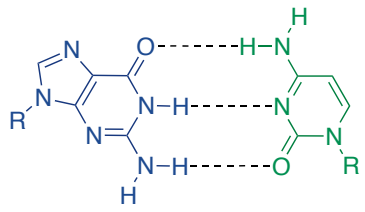
1.3.1 Interaction with DNA

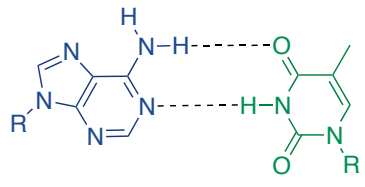
Ellipticine has been shown to interact with DNA in several ways, including non-covalent interactions such as intercalation and topoisomerase II inhibition, along with covalent adduct formation *via* bio-oxidation.

1.3.1.1 Intercalation

Intercalation occurs when a planar aromatic or heteroaromatic ring system inserts between base pairs of DNA and distorts its structure. Once bound, the drug can inhibit enzymes involved in replication and transcription processes, e.g. topoisomerase II. Intercalation was first described in 1961 by Lerman[16] for the synthetic acridine dye proflavine, used in world war II to dress wounds due to it's powerful antiseptic properties. It is now understood to be the major DNA binding mode of any planar polyaromatic ligand.

Generally, a planar intercalating drug is held by stacking (van der Waals) interactions with the base pairs of DNA above and below (Figure 1.2). In addition to this, some intercalating drugs also contain cationic groups, which can interact with the phosphate group of the backbone.[17]





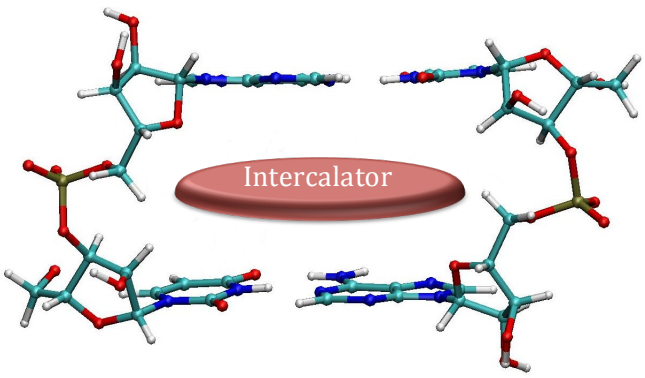


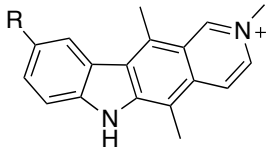
Figure 1.2: DNA base pairs (purines in blue and pyrimidines in green) and intercalation model

When a drug intercalates with DNA, the base pair separation at that site is immediately increased from its normal value of 3.3 Å and the overall winding angle of the DNA double helix is altered, usually referred to as the unwinding angle.

In 1975, Kohn *et al.* measured the UV absorption spectrum of ellipticine in the presence of calf-thymus DNA.[18] The spectrum exhibited hypochromic and bathochromic changes indicating that ellipticine was binding to DNA *via* intercalation. An unwinding angle of 7.9º was reported, however, this was based on a value of 12º for ethidium, which was later found to be much higher (26º).

Four years later, Jain *et al.* obtained an x-ray crystal structure of ellipticine complexed with a self-complementary dinucleoside monophosphate.[19] Ellipticine intercalated parallel to the base pairs of the CpG – GpC dinucleoside with 6.7 Å separation between the pairs and an unwinding angle of 10 – 12 º. While the crystal data was useful, it was questionable whether a simple dinucleoside could be representative of the real DNA binding mode.

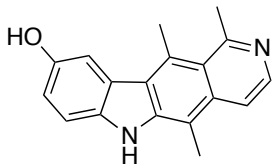
Dodin *et al.* re-estimated the pKa of ellipticine at 7.4 and carried out similar studies to Kohn[18] but at pH 5 and pH 9.[20] The ellipticinium cation bound slightly stronger to calf – thymus DNA than ellipticine, with affinity constants of 8.3×10^5 M^{-1} and 3.3×10^5 M^{-1} at pH 5 & 9 respectively. The group also used fluorescence spectroscopy in deuterated buffer to study interactions of *N*-methylellipticinium **2** and 9-hydroxy-*N*-methylellipticinium **3** with DNA (Figure 1.3). *N*-Methylellipticinium **2** showed no preference for intercalation sites, while **3** was found to interact with a site containing at least one G-C base pair, inferring that the 9-hydroxy group confers G-C preference.



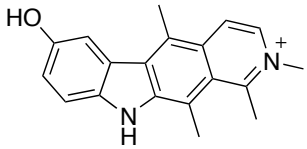
2 R=H
3 R=OH

Figure 1.3

Monnot *et al.* utilised UV absorption and circular dichroism (CD) experiments to study 9-hydroxy-1-methylellipticine **4** and 1,2-dimethyl-7-hydroxyisoellipticinium **5** (Figure 1.4) with poly d(A-T).poly d(A-T) as a DNA model.[21]



4



5

Figure 1.4

Circular dichroism (CD) is the difference in absorbance of left and right circularly polarized light. The ellipticines lack chirality, but optical activity can be conferred by intercalation in the asymmetric environment of DNA, while unbound molecules remain optically silent. Thus, CD can help distinguish between free molecules in solution and those bound to DNA. At a low drug – DNA ratio 9-hydroxy-1-methylellipticine **4** showed an intercalatory binding mode (Figure 1.5, mode A), while at high drug – DNA ratio an outside binding mode was also observed (mode B). In contrast, only one binding mode was seen for 1,2-dimethyl-7-hydroxyisoellipticinium **5**, self-stacked molecules interacted at the surface of DNA in an outside binding mode (mode C).

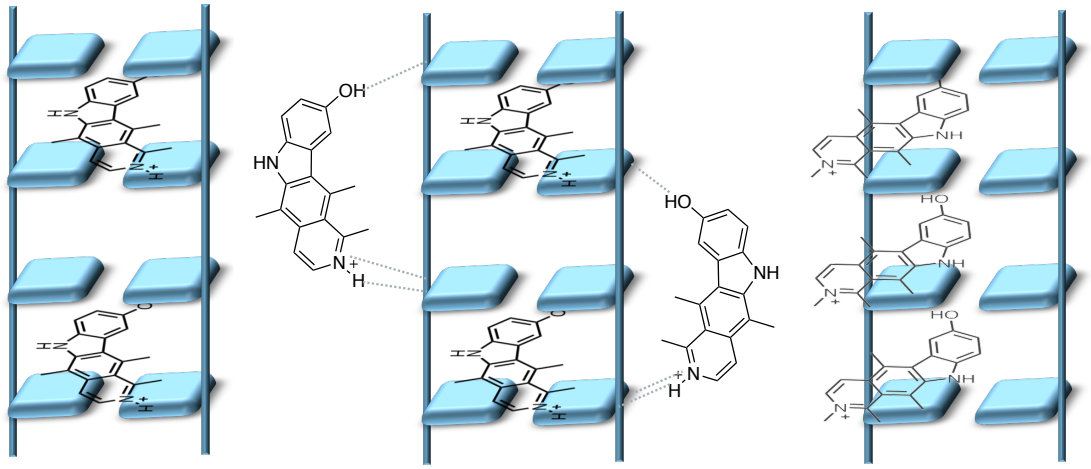


Figure 1.5: Binding modes of Ellipticine derivatives (adapted from Monnot et al.)[21]

Similar results were reported by Dodin *et al.* from their temperature – jump experiments with ellipticine and several derivatives.[22] While ellipticine itself only exhibited intercalation, derivatives such as *N*-methylellipticinium **2** and 9-hydroxy-*N*-methylellipticinium **3** (Figure 1.3) showed both intercalation and outside binding modes.

In 1996, Elcock *et al.* published molecular dynamics simulations investigating intercalation of 9-hydroxyellipticine **6** (Figure 1.6) with the DNA oligonucleotide $d(ATATATATATAT)_2$.[23] Four simulations were carried out, with different initial orientations of the drug. All the simulations converged in placing the pyridine ring in the minor groove, however only two of the four converged overall on the same binding site. In contrast to previous publications, it was concluded that a perpendicular orientation of 9-hydroxyellipticine to the axis of the base pairs was most likely. However, the same group later published CD calculations suggesting that intercalation of 9-hydroxyellipticine **6** occurs with the 9-hydroxy group in the minor groove and the pyridine ring in the major groove (i.e. still perpendicular to base pairs but opposite direction).[24]

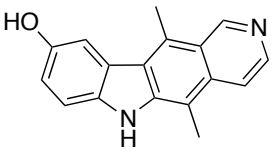


Figure 1.6: 9-Hydroxyellipticine ***6***

In 2005, Canals *et al.* published a crystal structure determination of ellipticine in complex with a six base-pair oligionucleotide – d(CGATCG)$_2$, with 1.5 Å resolution.[25] This showed ellipticine aligned with the major axis, parallel to the hydrogen bonds of the base pairs (Figure 1.7). The d(CGATCG)$_2$ hexanucleotide is intercalated by two ellipticine molecules, with the pyridine nitrogen facing the major groove in both cases. A preference for G-C base pairs is evident, since the AT-TA site is empty. Overall, there was good correlation with the earlier dinucleotide structure published by Jain *et al.*[19] (Figure 1.7 – part c). In Canals crystal structure an unwinding angle of 14º with respect to standard B-DNA was observed for each intercalation site and both sites were separated by 6.9 Å (compared to 3.3 Å for normal base pairs).

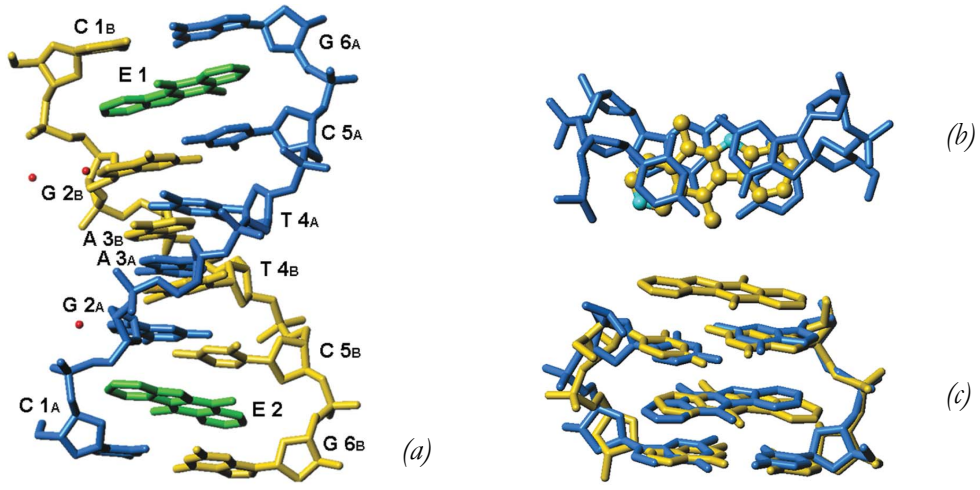


Figure 1.7: (a) The bis-intercalated d(CGATCG)$_2$ hexanucleotide. (b) Projection down the helix axis of a d(CpG)-d(GpC) dinucleotide with the sandwiched ligand. (c) Superimposition of the structure from Jain et al. (1979) in yellow and the equivalent part of Canals' structure in blue.[25]

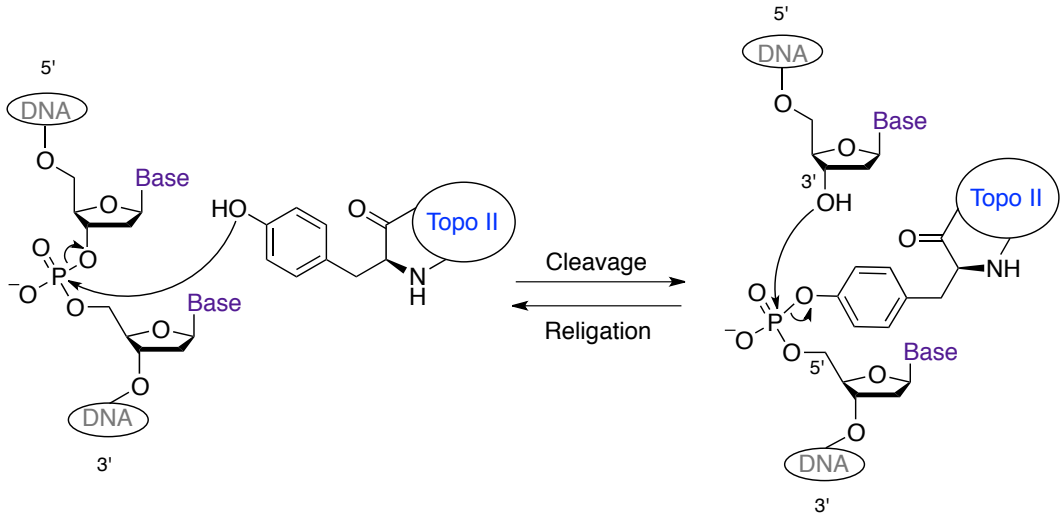
In recent years, various molecular modelling studies concerned with intercalation of ellipticine and its derivatives have been published.[26–31] These were mainly concerned with comparing the usefulness of different force fields and the optimum modelling parameters for the ellipticine series, however, little new data on the binding mode has been reported.

In summary, considerable research has been carried out in the area of ellipticine intercalation with DNA. Recent work by Canals *et al.* has established an intercalation model with ellipticine parallel to the axis of the base pairs with the pyridine nitrogen facing the major groove.[25] While this seems to be a definitive binding mode for ellipticine, derivatives of ellipticine may intercalate in different orientations or possibly not at all.

1.3.1.2 Topoisomerase II inhibition

DNA topoisomerases are enzymes that regulate the three-dimensional topology of DNA. Identical loops of DNA with different numbers of twists are topoisomers, i.e. molecules with the same chemical formula but different topologies, interconversion of these topoisomers requires breaking of DNA strands.[3] Topoisomerase I produces single strand breaks in DNA allowing the release of superhelical tension, while topoisomerase II produces double strand breaks, allowing separate strands of DNA to pass over each other and untangle. These processes are crucial for replication and transcription.

Topoisomerase II is a homodimeric enzyme which requires ATP to function. In the initial strand cleavage step, tyrosine residues of both monomers attack a phosphodiester bond in either strand to give covalent 5' phosphotyrosyl linkages (Scheme 1.1[3]). The religation step is simply the reverse reaction.



Scheme 1.1: Transesterification reactions involved in topoisomerase II activity.[3]
(Reaction of one monomer shown)

The two breaks in the DNA strand are not directly opposite each other, but are separated by four base pairs, in this way a space is created through which another region of intact DNA can pass. Figure 1.8[32] shows a schematic of the catalytic cycle of topoisomerase II, with cleavage of the G-segment of DNA and the T-segment passing through the complex, followed by religation (see over).

Levels of topoisomerase I and II are different in different cell types. A study by Holden *et al.* in 1990 measured topoisomerase II activity in normal and neoplastic tissues.[33] The highest levels in normal tissues were found in the spleen and thymus. In neoplastic tissue, the highest levels were found in those that are clinically most aggressive and proliferative, such as breast carcinoma and lymphomas. These measurements serve to explain the selectivity exhibited by topoisomerase II inhibitors and validate topoisomerase II as a key target for cancer chemotherapy.

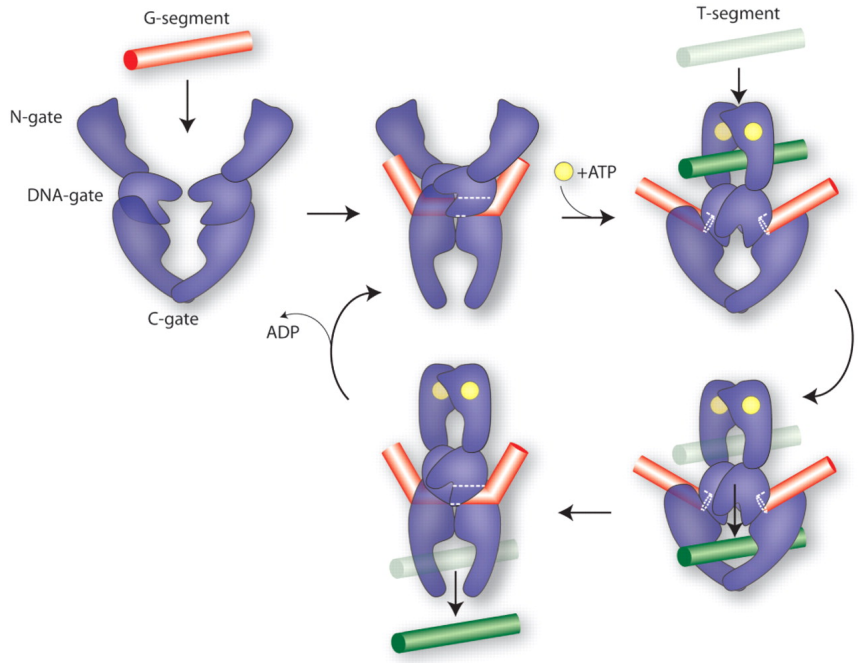


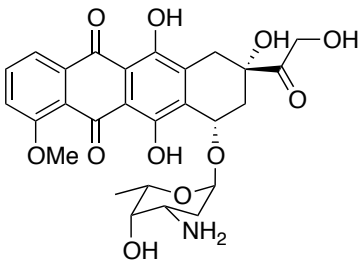
Figure 1.8: Catalytic cycle of topoisomerase II.[32] *Topo II binds to a segment of DNA (G-segment, red bar), ATP (yellow circle) binds to the N-terminal domains, promoting the capture of a second DNA duplex, the T-segment (green bar) and dimerization of the ATPase domains (N-gate). Closure of the N-gate stimulates cleavage of G-segment DNA, coupled with opening of the DNA gate to allow passage of the T-segment through this double-stranded break. The G-segment is religated and the T-segment exits* via *the C-gate.*

In the 1960s and 70s it was recognised that intercalating drugs produced single and double strand breaks in DNA, however the mechanism of this process was unknown. In 1978, Ross *et al.*[34] reported that DNA strand breaks produced by ellipticine were intimately associated with a protein, but it wasn't until the early 1980s that a series of studies on doxorubicin and ellipticine identified the protein involved as topoisomerase II.[35–38]

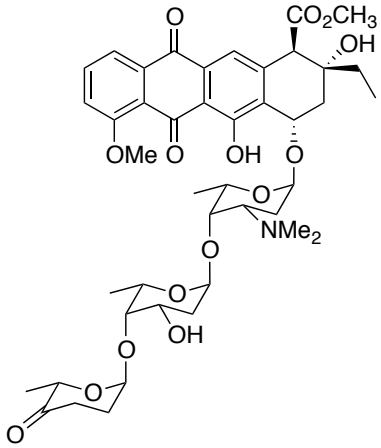
Later, Corbett and Osheroff proposed that topoisomerase II inhibitors fall into two mechanistic classes: (1) those that inhibit enzyme-mediated religation by stabilizing the cleavable complex and (2) those that increase the forward rate of enzyme-mediated DNA cleavage.[39] The first class are also known as topo II poisons since they subvert the activity of topo II to produce permanent double strand breaks in DNA e.g. etoposide and doxorubicin (Figure 1.9). The second class are known as catalytic inhibitors as they act on other steps of the catalytic cycle e.g. aclarubicin and sobuzoxane.[3] Interestingly, there is no correlation between the binding mode (intercalative or outside binding) of a drug and its mechanistic class (poison or inhibitor).[39]

Topo II Poisons

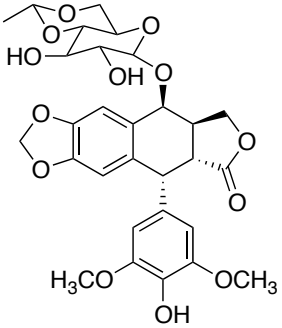
Topo II Catalytic Inhibitors



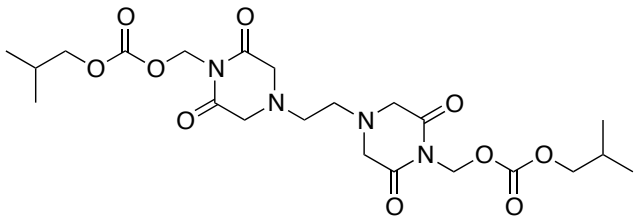
Intercalating – Doxorubicin



Intercalating – Aclarubicin



Non-intercalating – Etoposide



Non-intercalating – Sobuzoxane

Figure 1.9: Examples of Topoisomerase II poisons and inhibitors

Ross and Bradley were the first researchers to associate ellipticine induced DNA strand breaks with topoisomerase II inhibition.[40] Ellipticine was found to induce strand breaks in DNA from L1210 cells, in concentrations of 1.25 – 5.0 μg/mL and the authors to proposed the strand breaks were due to topoisomerase II activity.

In 1984, Tewey *et al.* demonstrated that ellipticine **1** and 9-hydroxy-*N*-methylellipticinium **3** produced reversible protein-linked DNA strand breaks *in vitro*.[41] 9-Hydroxy-*N*-methylellipticinium **3** was found to be the more effective of the two compounds, and it was suggested that ellipticines exerted this action *via* stabilisation of the DNA-topo II cleavable complex. Charcosset *et al.* isolated a Chinese hamster lung cancer cell line resistant to 9-hydroxyellipticine **6** and topoisomerase II inhibitors (amsacrine and etoposide).[42] This cell line was found to exhibit 3.5-fold lower topo II activity than the parent cell line (DC-3F), suggesting that 9-hydroxyellipticine resistance was due to lower levels of topoisomerase II.

Froelich-Ammon *et al.* published a major study of ellipticine-topoisomerase activity in 1995, identifying topoisomerase II as the primary cellular target of the drug.[43] This was determined by employing yeast cells with a temperature sensitive strain of topo II (at 25 °C enzyme activity is 100% and at 30 °C activity decreased to 10%). At 25 °C, 90% of the cell culture was

killed by a 200 μg dose of ellipticine, while at 30 ºC, no cell death was observed at any concentration of ellipticine used.

In a second experiment, a 10 μg dose of ellipticine induced a 6-fold increase in topoisomerase II-mediated strand breakage, but the same concentration of ellipticine had no effect on the rate of religation. Thus, ellipticine may be considered to be a catalytic inhibitor of topo II as opposed to a topo II poison, i.e. ellipticine acts primarily by increasing the forward rate of cleavage of DNA.

Fluorescence spectroscopy was then used to study interaction of ellipticine with DNA, topo II and the DNA-topo II complex. Three possible mechanisms for complex formation were identified: (1) the drug binds only to pre-formed DNA-topo II complex, (2) the drug becomes part of the ternary complex primarily though interactions with DNA, and (3) the drug becomes part of the complex though interactions with the enzyme. It was established that protonated ellipticine binds to DNA, whereas deprotonated ellipticine binds to topoisomerase II and is also the major species present in the ternary complex. Thus the enzyme determines the protonation state of ellipticine in the ternary complex. Finally the authors proposed a binding model (Figure 1.10) in which the topoisomerase II-ellipticine-DNA ternary complex (complex c) is formed *via* the prior association of the drug with either DNA (complex a) or the enzyme (complex b). Formation of the ternary complex results in increased levels of covalent topoisomerase II-DNA cleavage complex (d).

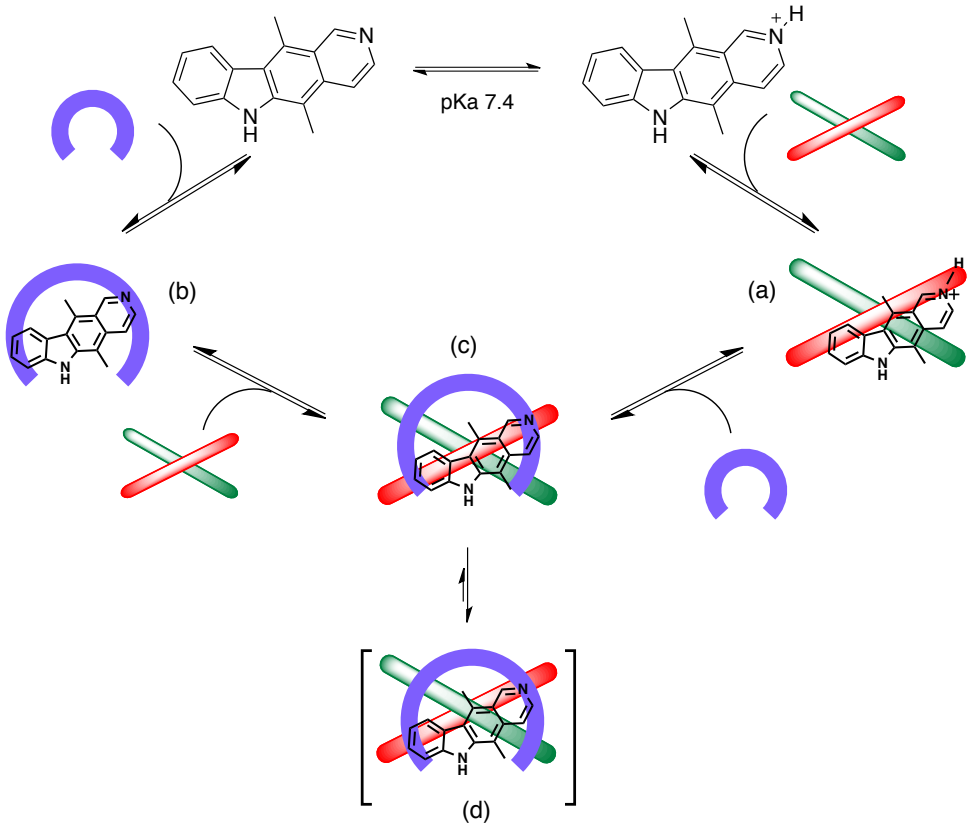
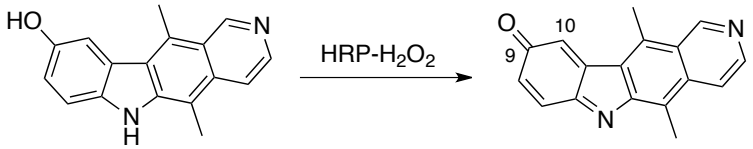


Figure 1.10: Model of ellipticine binding to DNA (G segment, red), topoisomerase II (purple arc) and the topoisomerase II-DNA complex (adapted from Froelich-Ammon, 1995).[43]

1.3.1.3 Bio-oxidation and adduct formation

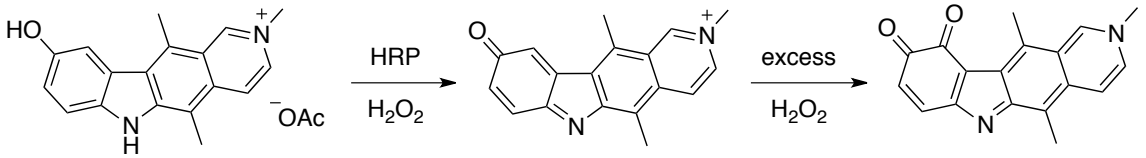
The bio-oxidation pathway was originally proposed by Auclair and Paoletti, who postulated that ellipticine could serve as a substrate for peroxidises *in vivo*.[44] They employed a horse radish peroxidise (HRP) – hydrogen peroxide oxidizing system as a model of bio-oxidation and studied the ability of various ellipticines to undergo oxidation. Under these conditions, 9-hydroxyellipticine **6** was oxidised to the quinone imine **7** (Scheme 1.2). The quinone imine **7** was shown to be a strong electrophile, oxidizing NADH to NAD^+. It is also highly susceptible to nucleophilic attack at the C-10 position, and bound irreversibly to bovine serum albumin.[44]



6 **7**

Scheme 1.2

The group continued their investigation by studying the bio-oxidation of 9-hydroxy-*N*-methylellipticinium acetate **8** under the same conditions.[45] The expected quinone imine **9** was formed with one equivalent of hydrogen peroxide, however in the presence of excess peroxide, further oxidation occurred to yield the *o*-quinone **10** (Scheme 1.3).



8 **9** **10**

Scheme 1.3

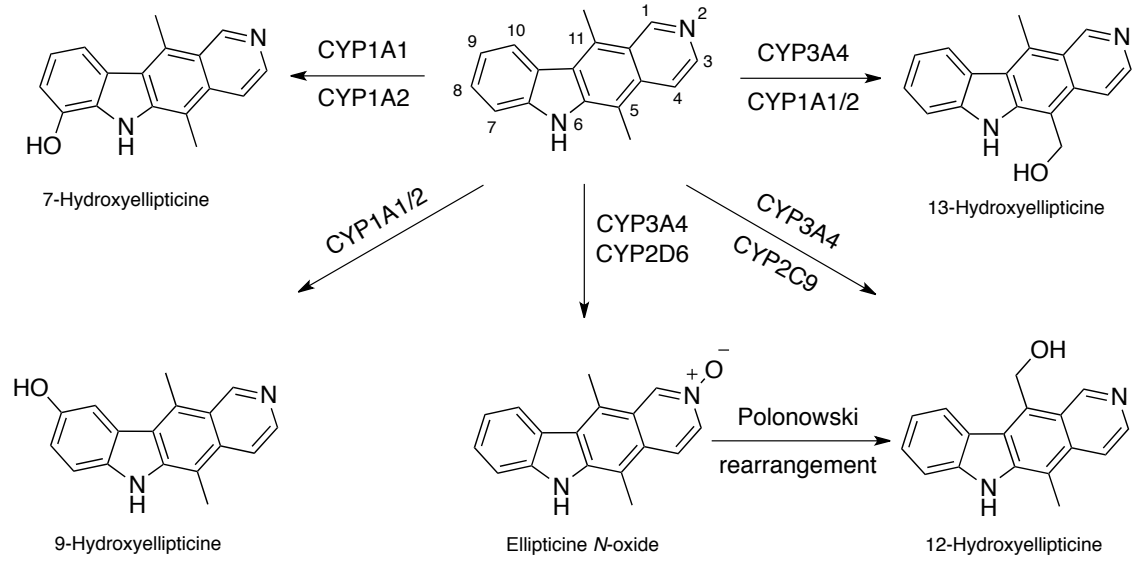
In vitro testing of these products against murine leukaemia L1210 showed that the quinone imine **9** was highly cytotoxic, with an ID_{50} value of 0.05 μM, while the *o*-quinone **10** was much less active at 9.6 μM. The quinone imine **9** was subsequently shown to bind covalently to DNA, giving a DNA-ellipticinium adduct.[46] The fluorescence properties of the adduct indicated binding at C-10 of the quinone imine with a primary amine of DNA.

In the last decade, Stiborová *et al.* have extensively studied the bio-oxidation and adduct formation pathways of ellipticine *in vitro* and *in vivo*. The group began by investigating the potential of ellipticine to form DNA adducts after activation by cytochrome P450 (CYP).[47] Cytochrome P450 enzymes were chosen based on the observation that several tumour types specifically targeted by ellipticine (e.g. breast cancer and renal cell cancer) overexpress CYPs. Rat, rabbit and human microsomes, in the presence of NADPH, were effective in activating ellipticine towards forming DNA adducts. Two adducts were formed with calf thymus DNA,

one major and one minor. A ^{32}P-postlabelling assay confirmed that the adducts were covalent in nature. The minor adduct was formed independently of enzymatic activation (no change was observed on increasing the CYP concentration), whereas the major adduct was formed on catalysis by CYP.

The group then investigated adduct formation in intact cells, using V79 cells expressing CYP, and based on HPLC profiles, the same two adducts were formed.[48] In an effort to identify the site of adduct formation on DNA, a variety of different polydeoxyribonucleotides were used in binding experiments with CYP and ellipticine.[49] Only ^{32}P-labelled adducts derived from deoxyguanosine were found, indicating that guanine is the site of adduct formation with the activated ellipticine species.

The next step was to identify the metabolites formed by human cytochrome P450 enzymes.[50] This was accomplished by isolation and HPLC separation of ellipticine metabolites generated by hepatic microsomes from eight human donors. Five metabolites were assigned based on co-chromatography with synthetic standards, NMR experiments and mass spectrometry (Scheme 1.4). 13-Hydroxyellipticine and the ellipticine *N*-oxide were identified as the metabolites responsible for the two DNA adducts previously identified.



Scheme 1.4: Metabolism of ellipticine by human cytochrome P450 enzymes.[51]

The group turned their attention to the peroxidase-mediated oxidation of ellipticine and performed experiments with peroxidases (human myeloperoxidase, human and ovine cyclooxygenases, bovine lactoperoxidase and horseradish peroxidase).[52] Two metabolites were isolated and identified, an ellipticine dimer and ellipticine *N*-oxide (Figure 1.11). Four DNA adducts were formed, two major and two minor. The two major adducts corresponded to those previously identified, derived from 13-hydroxyellipticine and ellipticine *N*-oxide. The same adducts were formed in leukaemia HL-60 and CCRF-CEM cells and a good correlation between cytotoxicity and the levels of DNA adducts was observed.[53] In particular HL-60 is

known to express high levels of myeloperoxidase (MPO), which could account for the low IC_{50} value of 0.64 μM.

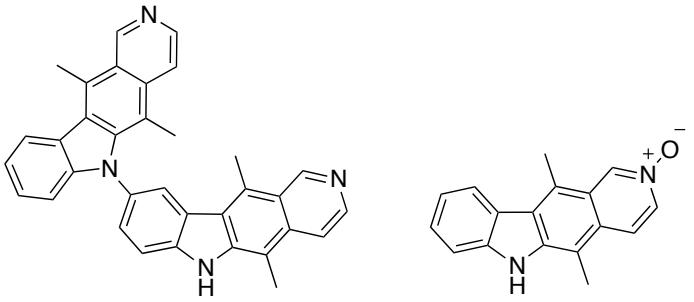


Figure 1.11

In 2007, Stiborová *et al.* published an investigation into the formation and persistence of DNA adducts in rats and found the highest levels of adducts in the liver (402 adducts/10^8 nucleotides after 2 days and 3.6 adducts/10^8 nucleotides after 32 weeks), followed by the kidney, lung, spleen, heart and brain.[54] Once again the two major adducts were those previously reported. During this study it was also found that ellipticine induced CYP1A1 and CYP1A2 activity in the liver, lungs and kidneys of rats.[55]

In their recent work Kotrobova *et al.* found that cytochrome b_5 alters the ratio of ellipticine metabolites formed by CYP1A1 and CYP1A2, favouring the formation of 12-hydroxyellipticine and 13-hydroxyellipticine metabolites implicated in adduct formation.[56]

The key on-going question in this area is whether adduct formation is responsible for the cytotoxicity of ellipticines. A recent publication by Stiborová *et al.* sought to evaluate the contribution of DNA adduct formation to cytotoxicity and found plausible correlation in six out of seven cancer cell lines tested.[57] However, further work is required in this area in order to conclusively understand this mechanism and its overall contribution to ellipticine cytotoxicity.

1.3.2 Kinase Inhibition

In the last decade, ellipticine interactions with several enzymes have been studied, including inhibition of c-Kit kinase and AKT.

1.3.2.1 c-Kit Kinase

In 2005, ellipticine and its derivatives were shown to exhibit c-Kit kinase inhibition.[58] c-Kit kinase is a type III receptor tyrosine kinase (RTK), a class of enzymes which regulate signalling pathways that control cell growth and proliferation. c-Kit plays a key role in mast

cell survival, differentiation, maturation and function.[59] It is expressed by and critical for the development and growth of mast cells, melanocytes, hematopoetic stem cells and the interstitial cells of Cajal.[60] Mutations in the gene encoding for c-Kit kinase are associated with some highly malignant cancers and therefore both wild-type and mutated c-Kit are viable drug targets in anticancer chemotherapy.[61]

In general, protein kinases regulate the biological activity of other proteins by phosphorylating specific amino acids, with ATP as the source of phosphate.[3] To date, over 500 protein kinases have been identified, the majority of which are tyrosine kinases, that is, kinases which phosphorylate the phenolic hydroxyl group of tyrosine (Figure 1.12).

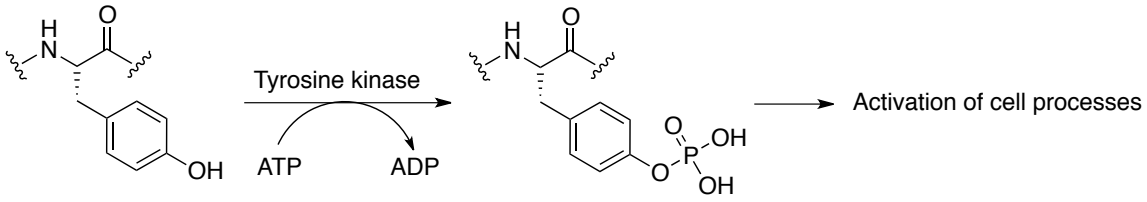


Figure 1.12: Phosphorylation by tyrosine kinases.[3]

Many tyrosine kinases are cytoplasmic, while others traverse the cell membrane and are known as receptor tyrosine kinases (RTKs). c-Kit kinase is one such transmembrane receptor, and it's extracellular ligand is stem cell factor (SCF, also known as steel factor and mast cell growth factor).[62] The c-Kit structure consists of an extracellular ligand binding domain (comprised of five immunoglobulin-like domains), a transmembrane domain, an inhibitory juxtamembrane helix and a cytoplasmic kinase domain which functions as the enzymatic pocket for tyrosine phosphorylation.

Structural studies have elucidated the basis for c-Kit activation, *via* SCF binding and dimerisation.[62] Binding of stem cell factor (SCF) in the extracellular domain induces dimerisation and activation. This results in auto-phosphorylation of specific tyrosine residues in the intra-cellular region which serve as docking sites of signal transduction molecules.[63]

1.3.2.2 Mutations in c-Kit

The c-Kit oncogene may undergo several mutations which then code for mutated c-Kit kinase, such mutations are associated with several highly malignant cancers.[64,65] Longley *et al.* proposed classification of activating mutations into two groups, enzymatic pocket mutations and regulatory mutations.[62] The first group consists of mutations found only in the intracellular enzymatic pocket, while the second group affect other portions of the molecule which regulate kinase activity. Figure 1.13[62] shows a schematic diagram of a c-Kit monomer with specific mutations indicated opposite the corresponding regions.

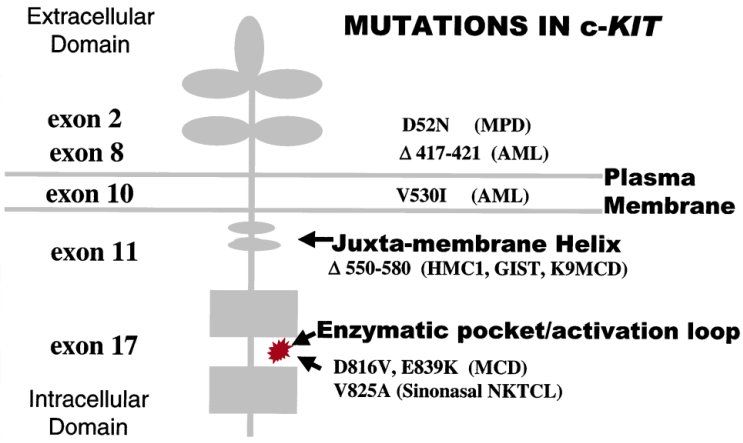


Figure 1.13: Mutations in c-Kit[62]

Longley *et al.* predicted that c-Kit with regulatory type mutations would pose little problem to wild type c-Kit kinase inhibitors. This has been shown in the case of gastrointestinal stromal tumours (GIST), where up to 90% of patients exhibit c-Kit mutation in the juxtamembrane region and the wild type c-Kit inhibitor imatinib (STI571) **11** has been used as a successful treatment (Figure 1.14).[66]

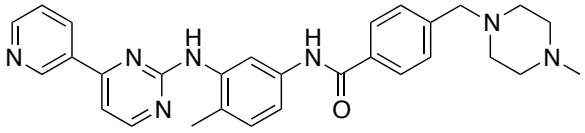


Figure 1.14: Imatinib ***11***

In contrast to this, wild type c-Kit inhibitors are ineffective at inhibiting c-Kit kinase with enzymatic pocket mutations.[67] The most common enzymatic pocket type mutation is the D816V point mutation (replacement of aspartic acid by valine at point 816). Residue 816 is thought to contribute to the structure of the activation loop by forming a hydrogen bond with N819. The D816V mutation disrupts this bond and thus destabilises the inactive conformation of the kinase domain. This results in a shift in the conformational equilibrium of c-Kit towards the active conformation, leading to ligand independent activation and auto-phosphorylation of c-Kit.[58] This explains the inactivity of imatinib against D816V mutated c-Kit, as it has been shown to bind only to the inactive conformation.[68]

The D816V mutation is associated with germ cell tumours, adult mastocytosis and a small proportion of atypical paediatric mastocytosis.[69,70] Mastocytosis is a disease characterised by pathological accumulation and activation of mast cells in tissues such as skin, bone marrow, liver, spleen and lymph nodes.[71] Adult onset mastocytosis usually displays bone marrow involvement at the time of diagnosis and follows a progressive course with poor prognosis.[61] The D816V mutation is detectable in over 70% of patients with systematic mastocytosis in bone marrow cells. Treatment is currently very limited and fails to produce remission in most patients.[61] This has attracted significant interest in developing new c-Kit inhibitors which are active against the D816V mutation[72] and resulted in the development of Dasatinib **12** (BMS-354825), which was approved by the FDA in 2006.[73]

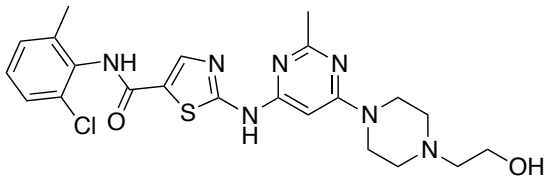


Figure 1.15: Dasatinib ***12***

1.3.2.3 Ellipticine and c-Kit kinase inhibition

In 2005, Vendome *et al.* reported that several ellipticine derivatives exhibited c-Kit kinase inhibition.[58] A range of derivatives significantly inhibited both wild type and D816V mutated c-Kit kinase (Table 1.3), with Imatinib **11** as a reference compund.

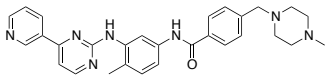
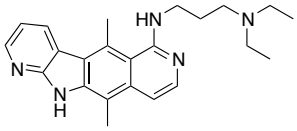
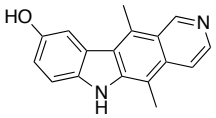
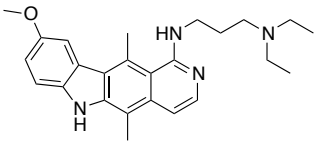
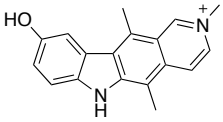
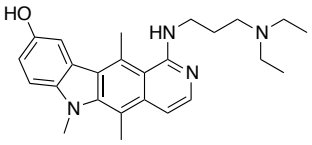
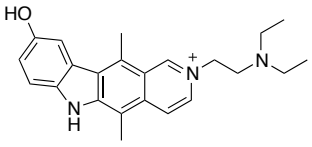
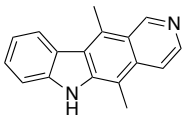
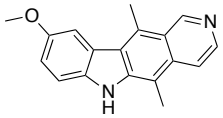
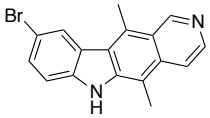
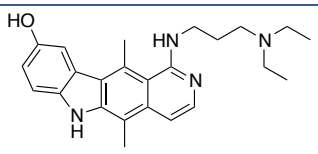
Compound	IC_{50} WT µM	IC_{50} D816V µM	Compound	IC_{50} WT µM	IC_{50} D816V µM
 **11**	0.1	10	 **13**	2.3	1.9
 **6**	0.4	0.4	 **14c**	2.5	2.0
 **3**	0.4	0.4	 **15**	2.5	3.6
 **16**	0.45	0.3	 **1**	>10	>10
 **17**	0.8	0.6	 **18**	>10	>10
 **14f**	1.4	1.2			

Table 1.3: Inhibition of wild type and D816V mutated c-Kit kinase by ellipticine derivatives.[58]

9-Hydroxyellipticine **6** and 9-hydroxy-*N*-methylellipticinium **3**, were the most active of the series, with equal inhibition of wild type and D816V mutated c-Kit. These were closely followed by the *N*-alkylamino-9-hydroxyellipticinium **16** which showed a slight preference for D816V mutated c-Kit. Of the rest of the series, compounds which were unsubstituted at C-9 were generally inactive and those with a C-9 methoxy group were only moderately active. Salt formation at the N-2 position did not seem to affect the c-Kit inhibitory activity, suggesting that the N-2 position is not directly involved in binding to c-Kit. Finally, addition of bulky alkylamino side chains at C-1 was significantly unfavourable for activity.

The possibility of a covalent linkage between the ellipticine derivatives and c-Kit kinase was investigated by incubating c-Kit with 9-hydroxy-*N*-methylellipticinium **3** and then washing the complex. The activity of the enzyme was not affected after washing, indicating a non-covalent mode of binding.

Molecular mechanics simulations were employed in order to identify possible binding sites, with docking sudies carried out using the GOLD program. It was found that all the active compounds docked similarly in part of the ATP binding pocket, which is made favourable by three hydrogen bonding interactions. The docked postion of the most active compunds is shown in Figure 1.16, along with hydrogen bonding interactions for 9-hydroxyellipticine **6**.[58]

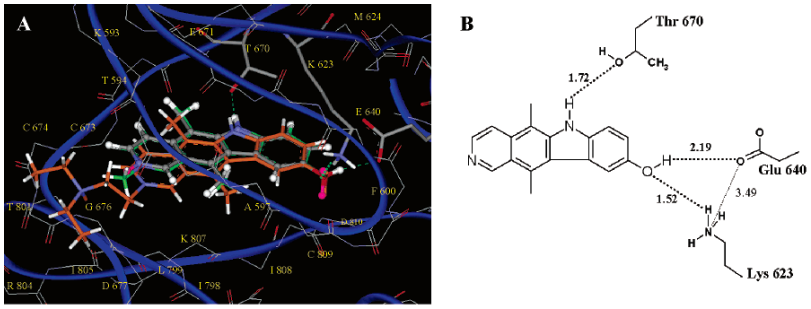


*Figure 1.16: (A) Detail of the docked position of the most active compounds (9-hydroxyellipticine **6** is shown in gray, 9-hydroxy-*N*-methylellipticinium **3** in green, and *N*-alkylamino-9-hydroxyellipticinium **16** in orange). Conserved interactions are represented by dashed lines. (B) Map of the main interactions for 9-hydroxyellipticine **6**.*[58]

1.3.2.4 AKT Inhibition

In 2004, Jin *et al.* reported the inhibition of AKT by 9-methoxy-*N*-methylellipticinium acetate **19**.[74] AKT is a serine/threonine kinase (i.e. it phosphorylates the hydroxyl group of serine and threonine residues of other proteins), which is activated in response to growth factors or cytokines by a mechanism involving PI3-K (phosphoinoside 3-kinase).[75,76] It provides a survival signal that protects cells from apoptosis induced by various stresses. AKT is currently

known to phosphorylate Bad, glycogen synthase kinase-3 (GSK-3), forkhead transcription factor (FKHR) and caspase-9, and thus inactivate their apoptotic functions.[74]

In 40 – 50% of endometrial cancers, AKT is overexpressed due to mutation of tumour suppressor PTEN (phosphatase and tensin homolog). 9-Methoxy-*N*-methylellipticinium acetate **19** (Figure 1.17) was tested against four endometrial cancer cell lines in a 12 μM or 24 μM single dose. Inhibition of AKT kinase activity and apoptosis was observed in the two cell lines with high AKT levels (RL95-2 and Ishikawa cells) but not in the cell lines with normal AKT activity. Further investigation suggested that inhibition was at AKT level and not upstream kinases that phosphorylate AKT.

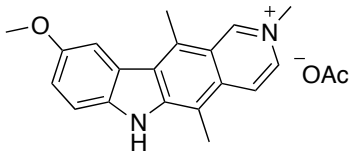


*Figure 1.17: 9-Methoxy-*N*-methylellipticinium acetate* ***19***

Later, Tang *et al.* found that the same compound (9-methoxy-*N*-methylellipticinium acetate **19**) inhibited AKT kinase activity in ovarian cancer cell lines with overactivation of AKT.[77] In this case, 9-methoxy-*N*-methylellipticinium acetate **19** appeared to prevent phosphorylation of AKT at Ser473, so it is possible that inhibition of an upstream kinase such as PDK2 may be involved. Overall the compound selectively induced apoptosis in ovarian cancer cell lines with overactivation of AKT but had minimal effect on normal cells.

In 2009, Fang *et al.* reported that growth of non-small cell lung cancer (NSCLC) epithelial cells A549 was inhibited by ellipticine **1**.[78] They proposed that ellipticine induced cytotoxicity by modulating the signalling pathways and subcellular redistribution of AKT and p53.

1.3.3 Other

1.3.3.1 Interaction with p53 tumour suppressor

The protein p53 is a transcription factor that binds to specific DNA sequences thereby controlling the transcription of genetic information from DNA to mRNA.[3] It is known as the 'guardian of the genome' as it is the key transcription factor which orchestrates coordinated changes in proliferation and apoptosis. On activation, it increases transcription of genes involved in inhibition of DNA replication e.g. p21 and Gadd45, but has opposing effects on BAX and Bcl2 which help regulate apoptosis.[1] In a general sense, p53 can help to promote the repair and survival of damaged cells, or it can promote the permanent removal of damaged cells though apoptosis. In the model of Vousden and Prives (Figure 1.18), p53 activity induced by low-stress elicits the protector responses that support cell survival and promote the repair of genotoxic damage.[79] Sustained stress or irreparable damage, on the other hand,

induces the killer functions of p53 to activate cell death or senescence. Notably, the protector functions of p53 can contribute to tumor development if not properly regulated (red, dashed arrow).

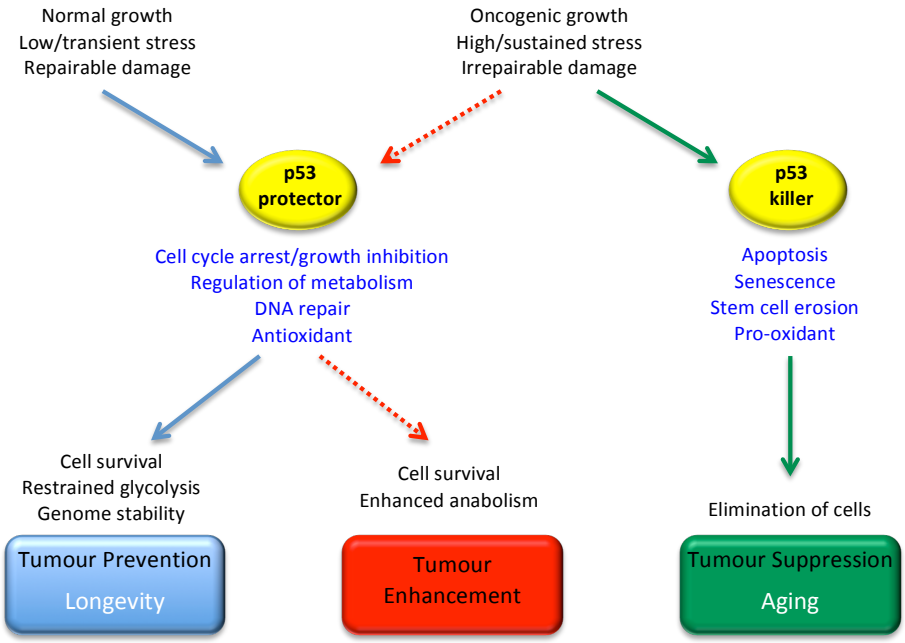


Figure 1.18: Dual mechanisms of p53 function (adapted from Vousden and Prives).[79]

In more specific terms, damage to DNA activates p53, resulting in inhibition of proliferation *via* Gadd45, p21 and PCNA; along with activation of DNA repair pathways (Figure 1.19). Stimulation of BAX and inhibition of Bcl2 results in increased apoptosis.[1]

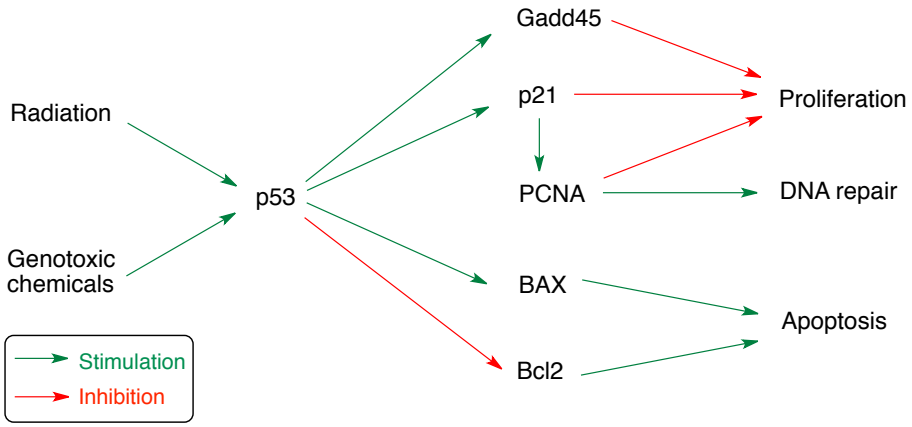


Figure 1.19: Response of p53 to DNA damage.[1]

In the last twenty years, the pivotal role of p53 in cancer has been elucidated. The gene TP53, encoding p53 protein, is mutated or deleted in approximately 55% of human cancers, mainly by point mutations in the core DNA binding domain.[80,81] As a result of this, there has been increasing interest in molecules that interact with p53, especially mutated p53. In 1998, Shi *et al.*[82] performed cluster analysis of 112 ellipticines from the NCI 60-cell line screen and found that the ellipticinium salts, but not the ellipticines, were more potent on average against p53

mutant cell lines over wild type p53 lines. This analysis opened up a new area of ellipticine research in the last decade.

Sugikawa *et al.* found that 9-hydroxyellipticine **6** induced apoptosis in the G1 phase of the cell cycle of mutant p53 cells.[83] They suggested that this activity may be *via* restoration of wild type p53 function. Mizumoto *et al.* then examined the impact of 9-hydroxyellipticine **6** on the cellular responses to various antineoplastic agents in pancreatic cancer cells. Exposure of mutant p53 expressing cells to 1 μM of 9-hydroxyellipticine resulted in restoration to wild type p53 activity without producing apoptosis. This pre-treatment with 9-hydroxyellipticine sensitised cells to treatment with cisplatin and mitomycin C, but not 5-fluorouracil, etoposide or vincristine. The effect was limited to cells expressing mutant p53.

In 2003, Peng *et al.* definitively showed that ellipticine and several derivatives can activate the transcription function of p53, increasing the function of some mutant p53 types by 5-6 fold.[84] Ellipticine derivatives induced cell cycle arrest in wild type p53 cells, with accumulation at the G2/M phase. This activity was attributed to intercalation and topoisomerase II inhibition (i.e. activity independent of p53 activation), whereas in cells with mutant p53, treatment with ellipticine resulted in apoptosis. In mutant p53-transfected H1299 cells, ellipticine induced MDM2 and p21 expression indicating activation of p53 function, at an optimal dose of 8 μM. Since mutant p53 has an altered conformation that can be detected by immunoprecipitation, it was shown that treatment with ellipticine induced a shift of mutant p53 conformation towards that of wild type p53, thus restoring function.

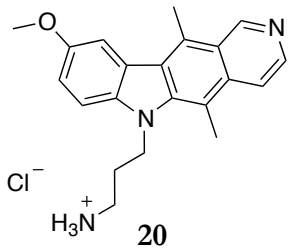
Kuo *et al.* studied the molecular mechanism of ellipticine activity in human breast MCF-7 cancer cells and showed that induction of p53, Fas/Fas ligand death receptor activation and the mitochondrial proapoptotic pathway are all involved in ellipticine action on these cells.[85] An IC_{50} value of 1.52 μM was recorded, with cell cycle arrest at the G2/M phase. Upregulation of p53, KIPI/p27 was observed, along with increased expression of the Fas ligands, mFasL and sFasL. In the mitochondrial proapoptotic pathway, increased expression of BAX and decreased Bcl2 and Bcl-X_L induced the release of cytochrome C from mitochondria to cytoplasm, which then activated caspase-9.

The same group carried out a similar study on ellipticine action in hepatocellular carcinoma (HCC) cells.[86] On treatment with ellipticine (IC_{50} = 4.1 μM), p53 levels increased in a dose dependent manner and reached maximum levels at 12 hours. Fas receptor and Fas ligand expression increased after 3 hours and the activity of caspase-8, a major downstream caspase of the Fas/Fas ligand system was increased after 6 hours. Increased levels of the Bcl2 family proteins involved in the proapoptotic mitochondrial pathway were also observed, along with decreased mitochondrial membrane potential, which resulted in release of cytochrome C to the cytoplasm.

In 2008, Xu *et al.* developed a high-content screen for compounds which increase localisation of p53 to the nucleus of cytoplasm.[87] Ellipticine was among several hits resulting from this

screen and was subjected to further testing. In HCT116 colon cancer cells expressing wild type p53, ellipticine was found to increase overall levels of p53 and induce localisation in the nucleus, resulting in increased p21 expression. This result was in contrast to previous work, which reported that ellipticine had no effect of cells expressing wild type p53.[83,84] It was also shown that p53 localisation was unrelated to ellipticine action on DNA or topoisomerase II, since p53 localisation in the nucleus was observed within one hour, whereas DNA damage was not observed until 16 hours after treatment.

Lu *et al.* identified the ellipticine derivative, 3-(9-methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-6-yl)propan-1-aminium chloride **20** as a potential lead compound for p53 activation and studied its effects on three cancer cell lines.[88] HCT116, a wild type p53 line; SW620 with mutant p53 and HCT116 $p53^{-/-}$, a p53 deficient cell line were treated with **20**, with GI_{50} values between 0.5 and 1 μM. Induction of p53 activity was observed in all three cell lines, but was highest (7.5 fold activity) in HCT116 and much less in SW620 (1.5 fold). Two p53 target proteins, p21 and DR5 were also upregulated in all three cell lines. Another transcription factor p73, with similar function to p53, was also found to be critical in the anti-tumour effects of compound **20**.



20

*Figure 1.20: 3-(9-Methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-6-yl)propan-1-aminium chloride*

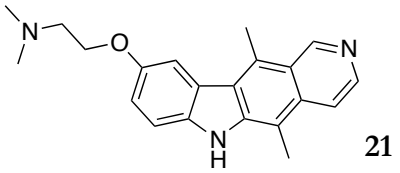
Recent studies have focused on combination treatments of ellipticine with exisitng well known anit-cancer agents. Huang *et al.* found that combined treatment of DLD1 cancer cells with 5-fluorouracil and ellipticine resulted in increased cell death compared to treatment with either agent alone.[89] Wang *et al.* recently reported that ellipticine alone did not alter p53 expression levels in mutant p53 Raji cells.[90] However, in chemo-resistant mutant p53 Ramos cells, ellipticine treatment sensitised cells towards doxorubicine-induced apoptosis.

1.3.3.2 Mitochondrial damage

Ellipticine interactions with mitochondria have been briefly mentioned in section 1.3.3.1, Kuo *et al.* found that the proapoptotic mitochondrial pathway was involved in the cytotoxicity of ellipticine towards human breast MCF-7 cancer cells and hepatocellular carcinoma (HCC) cells.[85,86] Schwaller *et al.* specifically investigated ellipticine induced uncoupling of mitochondrial oxidative phosphorylation.[91] They found that ellipticine had a potent effect on mitochondria and proposed a cyclic efflux/reuptake mechanism for ellipticines: (i)

electrophoretical accumulation of the protonated form of ellipticine in the negatively charged mitochondrial matrix; (ii) deprotonation at the matrix interface; (iii) diffusion outwards; (iv) re-protonation at the external surface. Thus, the protonatable ellipticines induced depolarisation of the mitochondrial inner membrane much more efficiently than ellipticinium cations (where no cyclic mechanism is possible).

Tian *et al.* used the COMPARE algorithm to correlate high expression of CKS1B mRNA (a high-risk molecular marker in multiple myeloma) in the NCI 60-cell line panel with GI_{50} values and identified the 9-(*N,N*-dimethyl ethanamine)-oxyellipticine derivative **21** as a lead compound.[92]



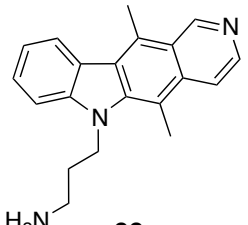
21

*Figure 1.21: 9-(*N,N*-dimethyl ethanamine)-oxyellipticine derivative*

Ellipticine derivative **21** was found to rapidy accumulate in mitochondria and immediately initiate loss of mitochondrial membrane potential, release of cytochrome C and formation of large vacuoles. Cells treated with **21** produced the lowest ATP levels of all the compounds tested, suggesting uncoupling of mitochondrial oxidative phosphorylation, which would thus block energy production in the cell. Although CKS1B gene expression was the initial seed of the investigation, gene expression profiling results showed no significant change in CKS1B expression.

1.3.3.3 Induction of endoplasmic reticulum stress

Hägg *et al.* investigated the activity of the 6-propanamine ellipticine derivative **22** (Figure 1.22) and found that it induced rapid apoptosis in MDA-MB-231 breast cancer cells.[93] In particular, 6-propanamine ellipticine was found to induce endoplasmic reticulum stress, as evidenced by increased expression of the endoplasmic reticulum chaperone GRP78, followed by release of cytochrome C from mitochondria and caspase cleavage of CK18. On the basis of this, the authors proposed that apoptotic signalling is triggered by endoplasmic reticulum stress, which contributes to the cytotoxicity of 6-propanamine ellipticine **22**, but the extent of this contribution is uncertain.



22

Figure 1.22: 6-Propanamine ellipticine

1.3.4 Summary

Since its discovery, several key mechanisms of action have been found to contribute to ellipticine anticancer activity. These include DNA intercalation and topoisomerase II inhibition, two closely related mechanisms which have been well established in the literature. More recent investigations have shown that ellipticines induce multifaceted biological responses, including interaction with kinases, p53 tumour supressor, mitochondria and endoplasmic reticulum. The relative contributions of these interactions are difficult to establish, and further research in the area is required, however it seems clear that the ellipticine family of compounds are truly multi-modal anticancer agents.

1.4 Design of ellipticine anticancer agents

While ellipticine and 9-hydroxyellipticine exhibit potent anticancer activity, properties such as low solubility and bioavailability have impeded their use in a clinical setting. To this end, a range of ellipticine derivatives, hybrids and conjugates have been synthesised and evaluated for potential improvement in cytotoxicity.

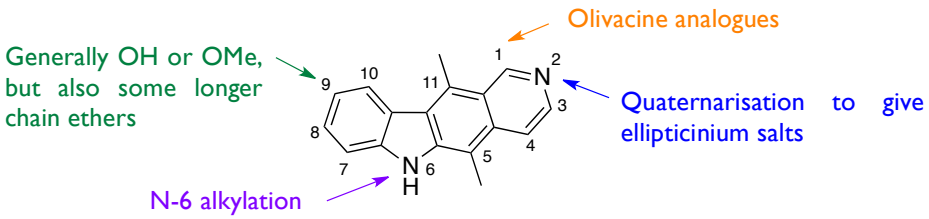


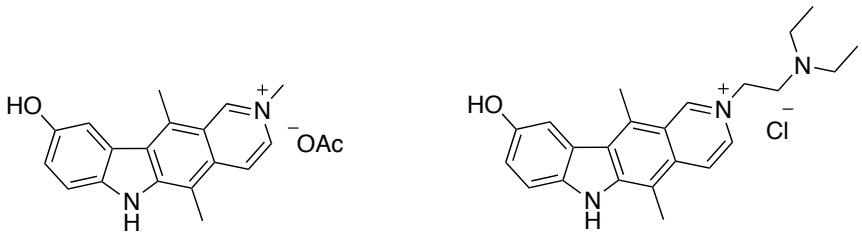
Figure 1.23: Ellipticine numbering system and main areas of derivatisation.

1.4.1 Ellipticine derivatives

In 1976, Le Pecq *et al.* carried out a detailed study on the antitumour activity of 9-hydroxyellipticine **6** against L1210 mouse leukaemia.[94] This *in vivo* mouse study demonstrated the overall potency of 9-hydroxyellipticine against intra peritoneal (i.p.) implanted L1210 leukaemia. When administered by i.p. injection, 9-hydroxyellipticine killed >99% of cells, however a levelling off in the antitumour activity – dose relationship was observed at higher doses, indicating that a small number of cells were resistant to the treatment. This was extensively investigated and concluded to lack of tumour penetration by the drug.

An early lead compound in ellipticine chemotherapy was *N*-methyl-9-hydroxyellipticinium acetate **3**, known as Celiptium® (Figure 1.24 below). This compound entered clinical trials in 1977 but did not progress beyond phase II.[95–97] Nevertheless, the Celiptium was highly active and had good solubility compared to ellipticine, characteristics which researchers aimed to incorporate into new ellipticine derivatives.

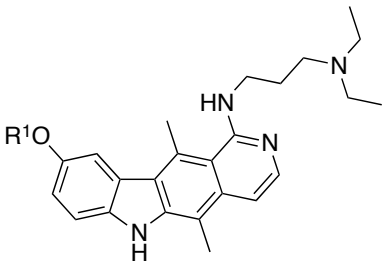
Auclair *et al.* prepared 2-(diethylamino-2-ethyl)-9-hydroxyellipticinium chloride (Datelliptium) **23** and compared its activity with *N*-methyl-9-hydroxyellipticinium acetate **3** (Figure 1.24).[98] Both drugs were found to have similar DNA binding constants, with K_{app} of 1.2×10^6 M^{-1} for *N*-methyl-9-hydroxyellipticinium acetate **3** and 1.04×10^6 M^{-1} for 2-(diethylamino-2-ethyl)-9-hydroxyellipticinium chloride **23**. However, there was a significant difference in the unwinding angles – 21.6° and 10.0° for **3** and **23** respectively. Both compounds were tested against L1210 cells and **23** was found to be more cytotoxic, with ID_{50} = 0.076 μM compared to **3** (ID_{50} = 0.134 μM). This superiority in cytotoxicity was seen across a range of cancer cell lines including P388 leukaemia, B16 melanoma, M5076 reticulosarcoma and colon 38 adenocarcinoma. The authors proposed that the increased cytotoxicity of 2-(diethylamino-2-ethyl)-9-hydroxyellipticinium chloride **23** may be due to an increase in diffusion across the cell membrane and more favourable bio-distribution compared to *N*-methyl-9-hydroxyellipticinium acetate **3**.



3 **23**

*Figure 1.24: Celiptium **3** and Datelliptium **23***

Bisagni *et al.* prepared analogous 1-aminoellipticines **14** in 1979 (synthesis in chapter 2, section 2.6.1, also used in c-Kit kinase inhibition testing, section 1.3.2.3).[99] In later publications, the biological activity of these compounds was reported along with 7-aza and 9-azaellipticine analogues.[100,101] The two most active compounds from the entire series were 1-[3-(diethylamino) propylamino]ellipticines **14c** and **14f**. These derivatives had good DNA binding constants and excellent ID_{50} values (Figure 1.25 and Table 1.4). On the basis of this, a range of different 1-aminoellipticines was prepared, some of these displayed similar cytotoxicities to **14c** and **14f** (e.g. the N-6 methylated analogues), however no further dramatic improvement was seen.[101]

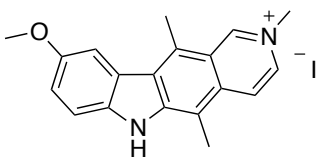


	R^1	K_{app} ($\times 10^{-7}$ M^{-1})	ID_{50} (μM)
14c	CH_3	3.15	0.3
14f	H	2.0	0.03

*Figure 1.25 and Table 1.4: DNA binding constants and ID_{50} values for 1-[3-(diethylamino) propylamino]ellipticines **14c** and **14f** [99]*

NCI screening of a 9-methoxy analogue of Celiptium, *N*-methyl-9-methoxyellipticinium iodide **24** showed a remarkable and unexpected selectivity towards CNS cancer cell lines

(Figure 1.26). This result was particularly surprising given that Celiptium itself displayed no such selectivity. Acton *et al.* from the NCI later published screening results of a large panel of ellipticinium salts which were analysed for CNS cancer cell line selectivity (Table 1.5).[102] It was proposed that C-9 substitution was key in the CNS selectivity of ellipticinium salts, however the C-9 unsubstituted salts (Table 1.5, entries 4 & 5) displayed equal selectivity to *N*-methyl-9-methoxyellipticinium iodide **24** (entry 1). In addition, moderate selectivity was seen with 9-methyl and 9-chloro ellipticinium salts (entries 6 & 7). Only the 9-hydroxyellipticinium salts showed no selectivity (entries 2, 8, & 9). The counter ion had little effect on specificity (entries 1, 3, 4, & 5). Selectivity was lost or considerably diminished by quaternization with bulky groups or alkylation of the indole nitrogen with a bulkier group than methyl (results not shown). Also, the parent ellipticine compounds (R^1=H, OMe, OH, Me, Cl) showed no specificity for CNS cell lines.



24

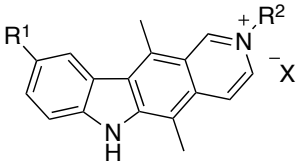


Figure 1.26: N-*Methyl-9-methoxyellipticinium iodide* ***24*** *and general structure of ellipticinium salts.*

Entry	R^1	R^2	X	TGI (μM) Full panel (A)	TGI (μM) CNS panel (B)	Ratio A/B
1	OMe	Me	I	11	1.1	9.8
2	OH	Me	OAc	78	58	1.3
3	OMe	Me	OAc	18	2.1	8.7
4	H	Me	I	12	1.4	8.9
5	H	Me	OSO_2Me	11	1.0	11
6	Me	Me	OAc	21	6.9	3.0
7	Cl	Me	OAc	22	4.7	4.8
8	OH	$CH_2CH_2NEt_2$	Cl	34	16	1.7
9	OH	$CH_2CH_2NC_6H_{10}$	OAc	34	20	1.7

TGI: Total Growth Inhibition

Table 1.5: CNS selectivity of ellipticinium salts[102]

In the same year, Anderson *et al.* synthesised a panel of 9-substituted *N*-methylellipticinium salts and carried out the same NCI analysis as Acton *et al.* (Table 1.6).[103] In general, a decrease in selectivity for CNS cell lines was observed with increasing size of the C-9 substituent. Exceptions to this are the trifluoroethyl ether compounds (entries 6 & 7) which show minimal growth inhibition overall but moderate inhibition of CNS cell lines. Once again the parent 9-substituted ellipticines showed no specificity in growth inhibition or cytotoxicity. The only difference from the study by Acton *et al.* is the selectivity of 9-methoxy-*N*-methylellipticinium

acetate, which displayed much higher selectivity in Anderson's study despite using the same cell lines for measurement (entry 1, Table 1.6 versus entry 3, Table 1.5).

Entry	R^1	R^2	X	TGI (μM) Full panel (A)	TGI (μM) CNS panel (B)	Ratio A/B
1	OMe	Me	OAc	21.4	0.63	33.9
2	OCH_2CH_3	Me	I	16.6	1.74	9.5
3	OCH_2CH_3	Me	OAc	25.7	3.63	7.08
4	$OCH(CH_3)_2$	Me	I	17.8	2.63	6.5
5	$OCH(CH_3)_2$	Me	OAc	25.8	12.4	2.1
6	OCH_2CF_3	Me	I	29.5	3.24	9.11
7	OCH_2CF_3	Me	OAc	>72	5.25	13.7
8	OPh	Me	I	37.6	18.2	2.1
9	OPh	Me	OAc	36.6	11.9	3.1

Table 1.6: CNS Selectivity of 9-substituted ellipticinium salts[103]

Shortly after this, Vistica *et al.* published a specific investigation into the CNS selectivity of *N*-methyl-9-methoxyellipticinium acetate (entry 1, Table 1.6) and found that its selective cytotoxicity was due in part to its preferential transport and accumulation in sensitive cell lines.[104]

The evidence of CNS selectivity of ellipticinium salts prompted further investigation in this area. Jurayj *et al.* proposed that while ellipticinium salts may have high selectivity for CNS cancers *in vitro*, *in vivo* the ability of a charged ion to cross the blood-brain barrier (BBB) or enter the blood-cerebrospinal fluid (BCF) was relatively low.[105] In an effort to overcome this Jurayj *et al.* prepared the neutral 2-alkyl-1,2-dihydroellipticines **25** (Figure 1.27), which would theoretically cross the blood-brain barrier and then undergo oxidation back to the corresponding ellipticinium salt. The ellipticinium salts were prepared and then reduced to the 1,2-dihydroellipticines **25** (preparation in chapter 2, section 2.6.2).

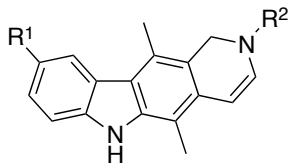
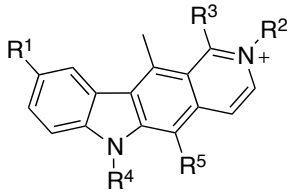


*Figure 1.27: 1,2-Dihydroellipticines **25***

A panel of 2-alkyl-1,2-dihydroellipticines was prepared, unfortunately some of these were unstable in solution and converted back to the ellipticines over a short time. Those that were stable showed little growth inhibition or cytotoxicity in the NCI 60 cell line screen.

Most studies of ellipticine derivatives have sought to determine the cytotoxicity of derivatives towards specific cancer cell lines, however Fossé *et al.* specifically studied topoisomerase II mediated DNA cleavage by a range of ellipticine derivatives, providing an interesting insight into the structure-activity relationship for topo II inhibition.[106] The relative frequency of cleavage of a ^{32}P labelled pBr322 DNA fragment in the presence of calf thymus topoisomerase II and ellipticine derivatives was studied using agarose gel electrophoresis (Table 1.7). Of the ellipticines tested (entries 1 – 4), 9-hydroxyellipticine and 9-aminoellipticine were most active (entries 2 & 4). The *N*-methylellipticiniums generally showed higher activity than the ellipticines (entries 5 – 10), with consistently superior activity for the 9-hydroxy derivatives. The *N*-2-diethylaminoethyl chain conferred slightly reduced activity relative to the *N*-methyl salts (entries 11& 12 versus 5 & 6). In a surprising result, replacement of the C-5 methyl group with an ethyl group significantly increased the cleavage frequency and this was the most potent topoisomerase II inhibitor of the series.



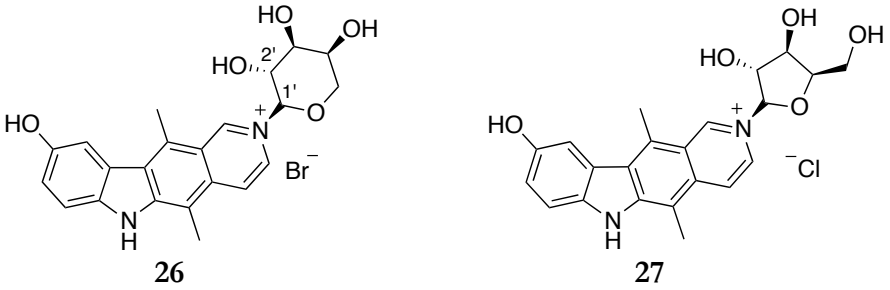
Entry	R^1	R^2	R^3	R^4	R^5	Conc. (μM)	Relative Frequency*
1	H	-	H	H	Me	4	1.6
2	OH	-	H	H	Me	4	4.0
3	OCH_3	-	H	H	Me	4	0.8
4	NH_2	-	H	H	Me	4	2.4
5	H	Me	H	H	Me	2	1.9
6	OH	Me	H	H	Me	2	4.7
7	H	Me	Me	H	Me	2	1.3
8	OH	Me	Me	H	Me	2	6.2
9	H	Me	H	Me	Me	1	1.8
10	OH	Me	H	Me	Me	1	3.3
11	H	$CH_2CH_2NEt_2$	H	H	Me	2	1.6
12	OH	$CH_2CH_2NEt_2$	H	H	Me	2	3.6
13	OH	Me	H	H	Et	2	8.2

*Cumulative frequencies of cleavage pBR322 DNA relative to frequency of cleavage in the absence of drug.

Table 1.7: Topoisomerase II inhibition of ellipticine derivatives[106]

Honda *et al.* prepared *N*-glycosides of ellipticine in order to improve water solubility and selectivity towards tumour cells (see section 2.6.2 for synthesis).[107,108] An extensive panel of ellipticine glycosides was prepared and tested against intra peritoneal (i.p.) implanted L1210

leukaemia in mice with i.p. administration over 5 days. Twenty six of the forty nine glycosides tested were curative, i.e. the mice tested survived for 80 days or longer. Of these 26 compounds, 5 were selected for further testing against a range of cancer cell lines and two highly active compounds emerged, the L-arabinopyranoside **26** and D-xylofuranoside **27** (Figure 1.28).



26 **27**

Figure 1.28: N-*2-Ellipticine glycosides*

L-Arabinopyranoside **26** was curative against L1210 leukaemia in 2 of 6 mice, when administered intravenously for five days, with ILS% (increase in mean life span) of 344%. With P388 leukaemia, both L-arabinopyranoside **26** and D-xylofuranoside **27** were curative (2/6 and 2/6, ILS% = 394 and 417 respectively). Both compounds were much more active than ellipticine and celiptium, and in comparison with doxorubicin, the glycosides had better activity in L1210, P388 and colon 38 cell lines.

Grummitt *et al.* investigated the introduction of a sugar moiety at C-9 of the two most active *N*-2 ellipticinium salts, 9-hydroxy-*N*-methylellipticinium acetate **8** and *N*-2-L-arabinose-9-hydroxyellipticinium bromide **26**.[109] They postulated that such compounds could act as DNA threading agents, with intercalation *via* the ellipticine core, and the *N*-2 and C-9 substituents acting as major and minor groove binders. The ellipticine *O*-glycoside **28** and *bis*(glycoside) **29** were prepared in low yields due to difficulties with purification (Figure 1.29). Interaction of these derivatives with calf thymus DNA was studied using flow linear dichroism spectroscopy. UV and LD spectra indicated that the derivatives **28** and **29** intercalate with DNA, and in the case of *bis*(glycoside) **29**, this would only be possible if the molecule threads through the DNA with a sugar moiety in the major and minor grooves. No other biological data was reported for these compounds, presumably due to the minute quantities obtained from the synthesis.

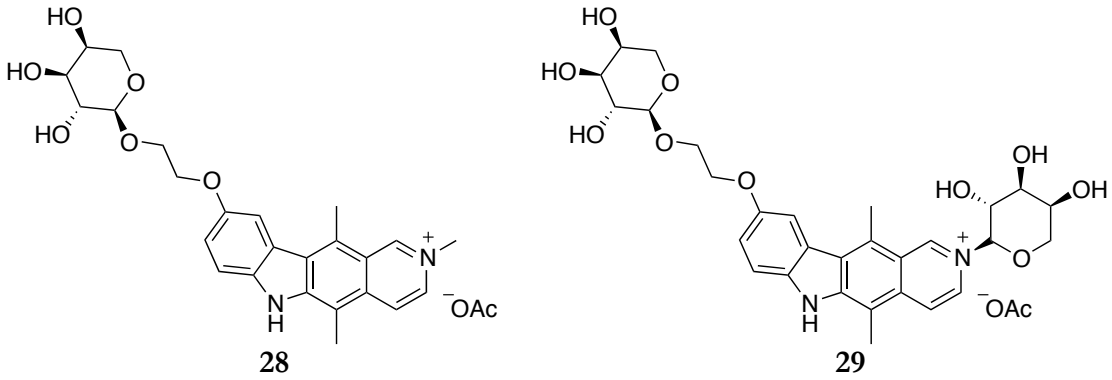


Figure 1.29

1.4.2 Hybrids and Conjugates

In recent decades, a variety of ellipticine hybrids and conjugates have been prepared, opening up new avenues for improvement of anticancer activity. Such molecules have been designed with various aims e.g. increasing overall cytotoxicity, targeted delivery of ellipticine to cancer cells, or improving solubility and bioavailabity of the drug. In this section a hybrid is defined as a compound made up of chemical molecules with different biological properties e.g. an intercalator and a minor groove binder. When one of the elements in a hybrid is a biomolecule e.g. a protein or a nucleic acid, it is referred to as a conjugate.

In 1991, Ding *et al.* prepared a panel of water soluble cationic metalloporphyrin-ellipticine hybrids based on mimicking the effects of the highly successful DNA cleaving agent bleomycin.[110] The porphyrins had either one or two ellipticine molecules attached *via* a range of different side chains. The metals were also varied to include manganese, iron and zinc. Overall, the manganese derivatives were most active, with moderate activity for iron and complete inactivity with zinc. The most active hybrid contained a pentyl carboxamide linker with manganese chelated to the porphyrin (Figure 1.30). This compound had a high affinity for DNA (K_{app} 2.0 × 10^8 M^{-1}) and an IC_{50} value of 0.8 μM against murine leukaemia L1210 cells.

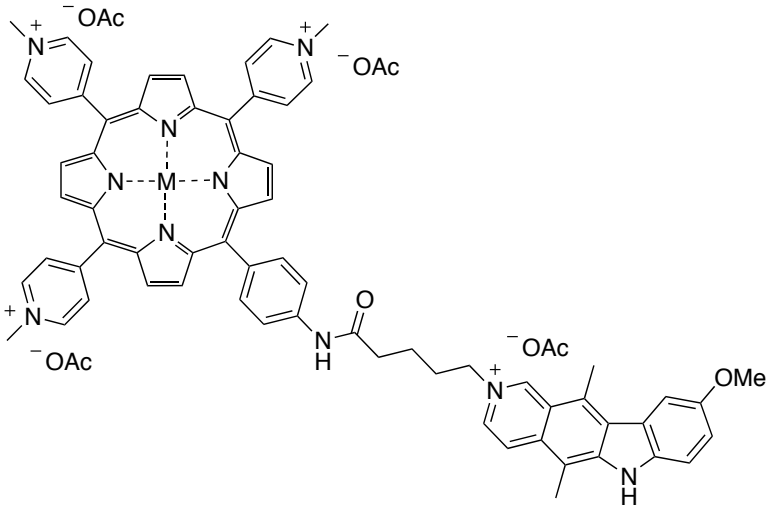


Figure 1.30: Metalloporphyrin-Ellipticine hybrid

Bailly *et al.* designed a distamycin-ellipticine hybrid as a bidentate DNA binding ligand, combining the powerful minor groove binding properties of distamycin and the intercalation ability of ellipticine.[111] The first generation hybrid (Figure 1.31, R=CHO) was relatively unsuccessful, with lower affinity for DNA than either ellipticine or distamycin alone. The authors concluded that some mutual interference was occurring and carried out molecular modelling studies, which indicated that an extra positive charge on the distamycin moiety could overcome the problem.[112] This was achieved by adding a basic side chain to the molecule (R=$COCH_2CH_2NH_3^+$) to give a biscationic hybrid which was tested using circular and linear dichroism.

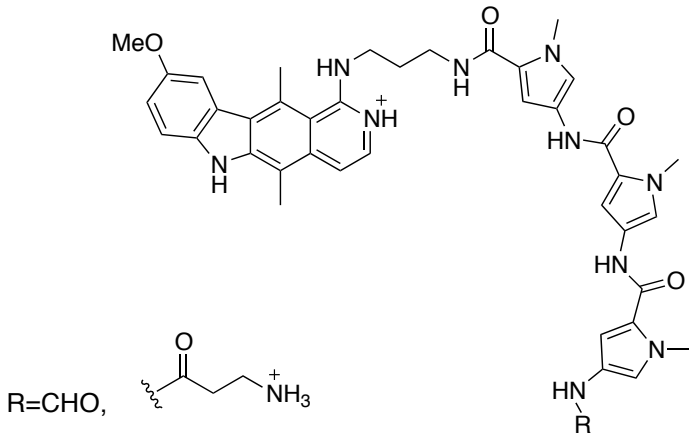


Figure 1.31: Distamycin-ellipticine hybrid

This second generation compound was found to be selective for AT rich DNA sequences (in correlation with the parent distamycin moiety) and engaged in bidentate binding to AT sequences but not to GC sequences. In DNase I footprinting, the concentrations required to detect a clear footprint at AT sites with the 2nd generation hybrid were 4 to 10-fold lower than those required to produce comparable DNase I footprints with distamycin alone.

Routier *et al.* prepared the ellipticine-salen copper hybrid **30** and found that the ellipticine intercalation geometry was preserved and the salen copper complex allowed cleavage of DNA *via* oxygen radicals (Figure 1.32).[113] Incubation of supercoiled plasmid DNA with 20 μM of the hybrid for 2 hours resulted in complete conversion to nicked form II DNA, whereas under the same conditions the salen copper moiety alone only converted ~50% to form II.

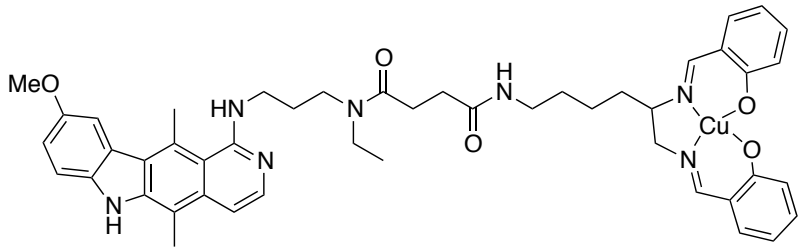
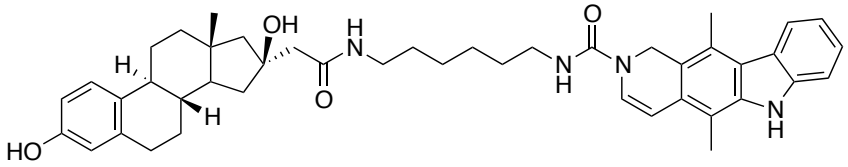
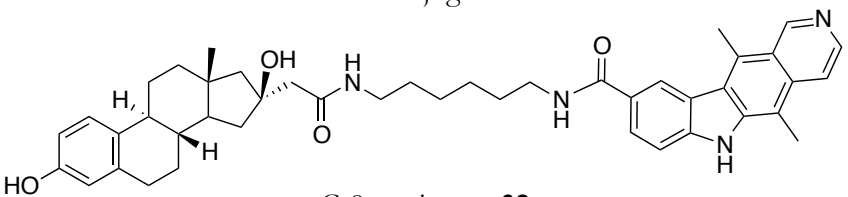


Figure 1.32: Ellipticine-Salen Copper hybrid ***30***

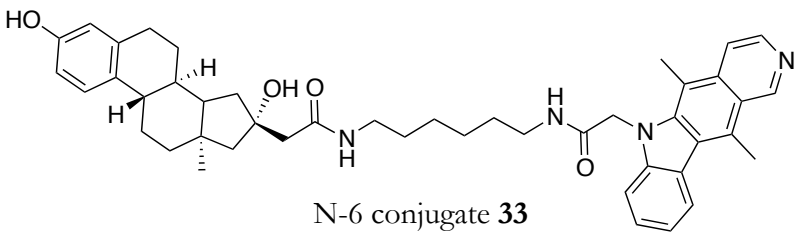
The first ellipticine bioconjugates were prepared by Devraj *et al.* in 1996.[114] Three ellipticine-estradiol conjugates were prepared in order to target the cytotoxicity of ellipticine to estrogen-receptor positive cells (synthesis described in chapter 2, section 2.6). The molecules were linked *via* a 1,6-hexane diamide chain from the 17α position of estradiol to C-9, N-2 and N-6 of ellipticine (Figure 1.32). The conjugates were tested for their abilities to bind to estrogen receptors, topoisomerase II inhibition and cytotoxicities against various cancer cell lines. The *N*-2 dihydroellipticine conjugate **31** showed moderate inhibition of topo II (IC_{50} 24.1 μM) and was also cytotoxic (GI_{50} 1 – 10 μM), whereas the C-9 and N-6 conjugates were inactive. Disappointingly, none of the three compounds displayed any selectivity for estrogen-receptor positive cells, and displayed weak binding affinities compared to estradiol.



N-2 conjugate **31**



C-9 conjugate **32**



N-6 conjugate **33**

Figure 1.33: Ellipticine-estradiol conjugates

In 1998, Czerwinski *et al.* synthesised an ellipticine-heptagastrin conjugate, with the aim of targeting the cytotoxic action of ellipticine towards specific cancers.[115] Heptagastrin is the C-terminal heptapeptide of gastrin, the cell surface receptors of which are overexpressed in several gastrointestinal cancers. The resulting conjugate showed remarkable selectivity for gastrin receptor positive cells, with toxicities in the nanomolar range but was much less toxic to receptor negative cells. In fact, the ellipticine-heptagastrin conjugate bound to the gastrin receptors at levels close to the natural hormone (K_D=1 nM). Ellipticine alone showed equal cytotoxicity to receptor positive and negative cell lines, and was comparable with that of the conjugate in the receptor positive cells *in vitro*. However, *in vivo* experiments showed that the conjugate was concentrated in cytotoxic concentrations in receptor positive cells, unlike free ellipticine, which failed to affect tumours at an equimolar dose.

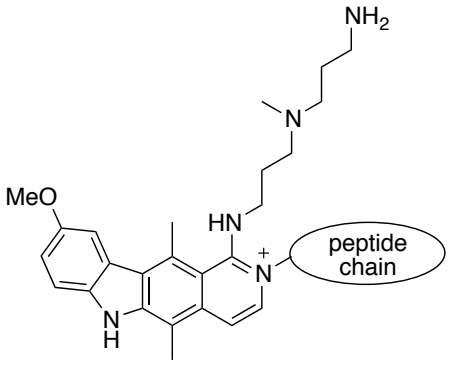


Figure 1.34: Ellipticine-Heptagastrin conjugate

Moody *et al.* later utilised the same synthetic method to prepare ellipticine – VIP conjugates, where VIP is vasoactive intestinal peptide, a 28 amino acid peptide with receptor overexpression in breast cancer cell lines.[116,117] Two ellipticine – VIP conjugates were found to inhibit VIP binding to MCF-7 breast cancer cells with IC_{50} values of 0.2 and 1 μM. A 1 μM dose of either of the conjugates resulted in decreased MCF-7 colony formation and decreased cell viability. Similar results were found with the lung cancer cell line H1299, with identical IC_{50} values of 0.2 and 1 μM for inhibition of VIP binding.

In a recent publication, Sedlacek *et al.* reported preliminary work on a polymer-conjugated ellipticine auger electron emitter.[118] Auger electron emitters are radionuclides such as ^{111}In, ^{125}I and ^{99m}Tc, which emit low energy ionizing radiation called Auger electrons. This radiation is biologically potent within a very short range in living tissue and thus requires targeting and delivery to the cell nucleus of tumour tissue. The polymer-conjugated ellipticine derivatives were designed to target tumour tissue in three ways: first, based on the polymer used and the EPR (enhanced permeation and retention) effect of solid tumours, the derivative should preferentially accumulate in tumour cells, next, pH controlled release of the radiolabelled ellipticine into the cell, and thirdly, delivery of the auger electron emitter to the nucleus *via* ellipticine intercalation with DNA. Results showed that the 9-^{125}iodoellipticinium polymer was stable at pH 7.4 and released the free 9-^{125}iodoellipticinium at pH 5 (solid tumours are generally acidic). The drug was found to accumulate in cell nuclei using confocal microscopy,

and intercalate with DNA (ethidium displacement assay). In addition to this the antiproliferative properties of the ellipticine derivative were retained, with IC_{50} values of 0.27 – 8.8 μM against Raji, EL-4 and 4T1 cell lines. Disappointingly, the Auger electron effect was not achieved in this model, and work is on-going to improve the specific radioactivity of the ellipticine derivative.

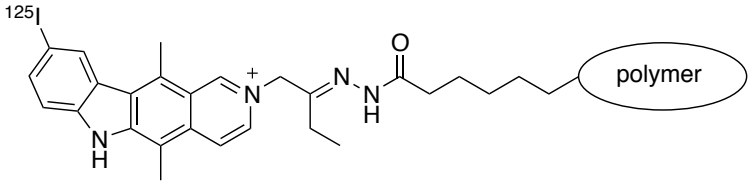
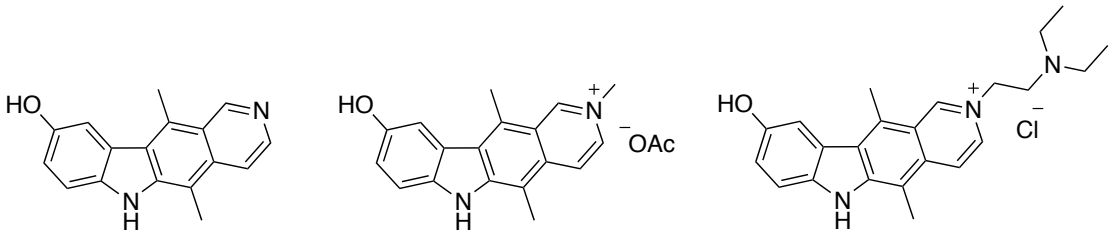


Figure 1.35: Polymer-conjugated Ellipticine auger electron emitter

A broad range of ellipticine hybrids and conjugates have been prepared over the last twenty years, and while none of these have yet progressed to clinical trials, it seems that this area is key to the future development of chemotherapeutic ellipticine derivatives.

1.5 Clinical use of ellipticine derivatives

An early clinical trial of 9-hydroxyellipticine **6** was terminated due to solubility problems (Figure 1.36), however, in 1994 it was used against metastatic breast cancer in combination with etoposide, methotrexate and chlorambucil, producing 48% partial response (PR) and 1.8% complete response (CR). [95, 119]



9-Hydroxyellipticine **6** Celiptium® **8** Datelliptium **23**

Figure 1.36: Ellipticine derivatives used in clinical trials

Clinical trials of 9-hydroxy-*N*-methylellipticinium acetate (Celiptium®) **8** began in 1977 and no favourable therapeutic response was observed in patients with advanced stages of gastric adenocarcinoma, lung carcinoma, metastatic soft tissue carcinoma, lymphoma or ovarian cancer. When administered to patients suffering from advanced metastatic breast cancer (refractory to all other treatment), 9-hydroxy-*N*-methylellipticinium acetate **8** induced objective remissions in 25% of patients, lasting up to 18 months.[95] The dosage was 80 – 100 mg/m^2/week, *via* 1 hour i.v. infusion, for 4 weeks. The treatment was particularly

effective in patients with oesteolytic breast cancer metastasis. 9-Hydroxy-*N*-methylellipticinium acetate **8** also gave 1 CR, 2 PR and 7 stabilisations out of 22 metastatic renal cancers.[119] In another phase II study of 9-hydroxy-*N*-methylellipticinium acetate **8** (100 mg/m^2/week) in advanced breast cancer, an objective response rate (CR & PR) of 19% was observed out of 79 patients (30% in soft tissue metastases).[96]

The clinical success of Celiptium® prompted extensive studies into the synthesis of ellipticinium analogues and several of these were brought forward for clinical trials. In 1992 2-(diethylamino-2-ethyl)-9-hydroxyellipticinium chloride (Datelliptium) **23** was found to be active in previously treated metastatic breast cancer and devoid of the toxicities reported with 9-hydroxy-*N*-methylellipticinium acetate **8**. Dosage was 150 mg/m^2/day i.v. for 5 days, every 3 weeks, and the responses were 1 CR, 4 PR in 30 patients.[119] The toxicity of datelliptium was mainly hepatic, and it induced rare and mild leukopenia and severe fatigue.

1.6 References

1. R. King, *Cancer biology*, Longman, Harlow Essex England, 1996.
2. http://www.who.int/gho/mortality_burden_disease/causes_death_2008/en/index.html, 30/9/2011.
3. C. Avendaño, *Medicinal chemistry of anticancer drugs*, Elsevier, Amsterdam ;;Boston, 1st edn., 2008.
4. S. Nussbaumer, P. Bonnabry, J.-L. Veuthey, and S. Fleury-Souverain, *Talanta*, 2011, **85**, 2265-2289.
5. D. Abraham, *Burger's medicinal chemistry, drug discovery and development.*, Wiley, Hoboken N.J., 7th edn., 2010.
6. S. Goodwin, A. F. Smith, and E. C. Horning, *J. Am. Chem. Soc.*, 1959, **81**, 1903-8.
7. J. Gruneton and A. Cave, *Phytochemistry*, 1972, **11**, 846-7.
8. A. A. Salim, M. J. Garson, and D. J. Craik, *J. Nat. Prod.*, 2004, **67**, 1719-21.
9. A. R. Carroll, R. Addepalli, G. Fechner, J. Smith, G. P. Guymer, P. I. Forster, and R. J. Quinn, *J. Nat. Prod.*, 2008, **71**, 1063-1065.
10. S. Michel, F. Tillequin, M. Koch, and A. Ake, *J. Nat. Prod.*, 1982, **45**, 489-94.
11. S. Michel, F. Tillequin, and M. Koch, *J. Chem. Soc., Chem. Comm.*, 1987, 229.
12. R. B. Woodward, G. A. Iacobucci, and F. A. Hochstein, *J. Am. Chem. Soc.*, 1959, **81**, 4434-5.
13. C. Auclair, *Archiv. Biochem. Biophys.*, 1987, **259**, 1 -14.
14. N. C. Garbett and D. E. Graves, *Curr. Med. Chem.: Anti-Cancer Agents*, 2004, **4**, 149-172.
15. M. M. Harding and A. R. Grummitt, *Mini Reviews Med. Chem.*, 2003, **3**, 67-76.
16. L. S. Lerman, *J. Mol. Biol.*, 1961, **3**, 18-IN14.
17. G. Patrick, *An introduction to medicinal chemistry*, Oxford University Press, Oxford, 4th edn., 2009.
18. K. W. Kohn, M. J. Waring, D. Glaubiger, and C. A. Friedman, *Cancer Res.*, 1975, **35**, 71-6.
19. S. C. Jain, K. K. Bhandary, and H. M. Sobell, *J. Mol. Biol.*, 1979, **135**, 813-40.
20. G. Dodin, M. A. Schwaller, J. Aubard, and C. Paoletti, *Eur. J. Biochem.*, 1988, **176**, 371-6.
21. M. Monnot, O. Mauffret, V. Simon, E. Lescot, B. Psaume, J. M. Saucier, M. Charra, J. Belehradek, and S. Fermandjian, *J. Biol. Chem.*, 1991, **266**, 1820-9.
22. M. A. Schwaller, J. Aubard, and G. Dodin, *Anti-Cancer Drug Des.*, 1990, **5**, 77-87.
23. A. H. Elcock, A. Rodger, and W. G. Richards, *Biopolymers*, 1996, **39**, 309-326.
24. M. A. Ismail, K. J. Sanders, G. C. Fennell, H. C. Latham, P. Wormell, and A. Rodger, *Biopolymers*, 1998, **46**, 127-143.
25. A. Canals, M. Purciolas, J. Aymamí, and M. Coll, *Acta Cryst. Section D Biol. Crystallography*, 2005, **61**, 1009-1012.
26. D. Řeha, M. Kabeláč, F. Ryjáček, J. Šponer, J. E. Šponer, M. Elstner, S. Suhai, and P. Hobza, *J. Am. Chem. Soc.*, 2003, **125**, 5581-5581.
27. M. Dracinsky and O. Castano, *Phys. Chem. Chem. Phys.*, 2004, **6**, 1799-1805.
28. P. A. Holt, J. B. Chaires, and J. O. Trent, *J. Chem. Information and Modeling*, 2008, **48**, 1602-1615.
29. C. G. Ricci and P. A. Netz, *J. Chem. Information and Modeling*, 2009, **49**, 1925-1935.
30. S. Li, V. R. Cooper, T. Thonhauser, B. I. Lundqvist, and D. C. Langreth, *J. Phys. Chem. B*, 2009, **113**, 11166-11172.
31. M. Kolář, T. Kubař, and P. Hobza, *J. Phys. Chem. B*, 2010, **114**, 13446-13454.
32. T. R. L. Collins, G. G. Hammes, and T.-shih Hsieh, *Nucleic Acids Res.*, 2009, **37**, 712 -720.
33. J. A. Holden, D. H. Rolfson, and C. T. Wittwer, *Biochemistry*, 1990, **29**, 2127-2134.
34. W. E. Ross, D. Glaubiger, and K. W. Kohn, *Biochim. Biophys. Acta, Nucleic Acids Protein Synth.*, 1978, **519**, 23-30.
35. K. Tewey, T. Rowe, L. Yang, B. Halligan, and L. Liu, *Science*, 1984, **226**, 466-468.
36. L. Zwelling, S. Michaels, D. Kerrigan, Y. Pommier, and K. Kohn, *Biochem. Pharmacol.*, 1982, **31**, 3261-3267.

37. E. M. Nelson, *Proc. Nat. Acad. Sci.*, 1984, **81**, 1361-1365.
38. T. C. Rowe, G. L. Chen, Y. H. Hsiang, and L. F. Liu, *Cancer Res.*, 1986, **46**, 2021-6.
39. A. Corbett and N. Osheroff, *Chem. Res. Toxicol.*, 1993, **6**, 585-597.
40. W. Ross, *Biochim. Biophys. Acta, Nucleic Acids Protein Synth.*, 1981, **654**, 129-134.
41. K. M. Tewey, G. L. Chen, E. M. Nelson, and L. F. Liu, *J. Biol. Chem.*, 1984, **259**, 9182-7.
42. J. Charcosset, J. Saucier, and A. Jacqueminsablon, *Biochem. Pharmacol.*, 1988, **37**, 2145-2149.
43. S. J. Froelich-Ammon, M. W. Patchan, N. Osheroff, and R. . Thompson, *J. Biol. Chem.*, 1995, **270**, 1498 - 1504.
44. C. Auclair and C. Paoletti, *J. Med. Chem.*, 1981, **24**, 289.
45. J. Bernadou, G. Meunier, C. Paoletti, and B. Meunier, *J. Med. Chem.*, 1983, **26**, 574-579.
46. C. Auclair, B. Dugue, B. Meunier, and C. Paoletti, *Biochemistry*, 1986, **25**, 1240-1245.
47. M. Stiborová, C. A. Bieler, M. Wiessler, and E. Frei, *Biochem. Pharmacol.*, 2001, **62**, 1675-1684.
48. E. Frei, C. A. Bieler, V. M. Arlt, M. Wiessler, and M. Stiborova, *Biochem. Pharmacol.*, 2002, **64**, 289-295.
49. M. Stiborová, A. Breuer, D. Aimová, M. Stiborová‐Rupertová, M. Wiessler, and E. Frei, *Int. J. Cancer*, 2003, **107**, 885-890.
50. M. Stiborová, J. Sejbal, L. Bořek-Dohalská, D. Aimová, J. Poljaková, K. Forsterová, M. Rupertová, J. Wiesner, J. Hudeček, M. Wiessler, and E. Frei, *Cancer Res.*, 2004, **64**, 8374 -8380.
51. M. Stiborová, M. Rupertová, and E. Frei, *Biochim. Biophys. Acta - Proteins & Proteomics*, 2011, **1814**, 175-185.
52. M. Stiborova, J. Poljakova, H. Ryslava, M. Dracinsky, T. Eckschlager, and E. Frei, *Int. J. Cancer*, 2006, **120**, 243-251.
53. J. Poljakova, E. Frei, J. Gomez, D. Aimova, T. Eckschlager, J. Hrabeta, and M. Stiborova, *Cancer Lett.*, 2007, **252**, 270-279.
54. M. Stiborová, M. Rupertová, D. Aimová, H. Ryšlavá, and E. Frei, *Toxicology*, 2007, **236**, 50-60.
55. D. Aimova, L. Svobodova, V. Kotrbova, B. Mrazova, P. Hodek, J. Hudecek, R. Vaclavikova, E. Frei, and M. Stiborova, *Drug Metabolism and Disposition*, 2007, **35**, 1926-1934.
56. V. Kotrbová, B. Mrázová, M. Moserová, V. Martínek, P. Hodek, J. Hudeček, E. Frei, and M. Stiborová, *Biochem. Pharmacol.*, 2011, **82**, 669-680.
57. M. Stiborová, J. Poljaková, E. Martínková, L. Bořek-Dohalská, T. Eckschlager, R. Kizek, and E. Frei, *Interdisciplinary Toxicology*, 2011, **4**, 98-105.
58. J. Vendome, S. Letard, F. Martin, F. Svinarchuk, P. Dubreuil, C. Auclair, and M. Le Bret, *J. Med. Chem.*, 2005, **48**, 6194-6201.
59. T. Tsujimura, K. Hashimoto, H. Kitayama, H. Ikeda, H. Sugahara, I. Matsumura, T. Kaisho, N. Terada, Y. Kitamura, and Y. Kanakura, *Blood*, 1999, **93**, 1319-1329.
60. J. D. Huizinga, L. Thuneberg, M. Kluppel, J. Malysz, H. B. Mikkelsen, and A. Bernstein, *Nature*, 1995, **373**, 347-9.
61. C. Akin, *J. Molecular Diagnostics*, 2006, **8**, 412 - 419.
62. B. J. Longley, M. J. Reguera, and Y. Ma, *Leukemia Res.*, 2001, **25**, 571-576.
63. L. Rönnstrand, *Cellular and Molecular Life Sci.*, 2004, **61**, 2535-2548.
64. C. D. Mol, K. B. Lim, V. Sridhar, H. Zou, E. Y. T. Chein, B. C. Sang, J. Nowakowski, D. B. Kassel, C. N. Cronin, and D. E. McRee, *J. Biol. Chem.*, 2003, **278**, 31461 - 31464.
65. F. Macdonald and C. H. J. Ford, *Mol. Biol. Cancer*, Bios Scientific Publishers, 1997.
66. D. M. Ross and T. P. Hughes, *Br. J. Cancer*, 2004, **90**, 12 -19.
67. Y. Ma, S. Zeng, D. Metcalfe, C. Akin, S. Dimitrijevic, J. Butterfield, G. McMahon, and B. J. Longley, *Blood*, 2002, **99**, 1741 - 1744.
68. C. D. Mol, D. R. Dougan, T. R. Schneider, R. J. Skene, M. L. Kraus, D. N. Scheibe, G. P. Snell, H. Zou, B. C. Sang, and K. P. Wilson, *J. Biol. Chem.*, 2004, **279**, 31655 - 31663.
69. A. T. Liao, M. B. Chien, N. Shenoy, D. B. Mendel, G. McMahon, J. M. Cherrington, and C. A. London, *Blood*, 2002, **100**, 585 - 593.

70. T. Tsujimura, T. Furitsu, M. Morimoto, K. Isozaki, S. Nomura, Y. Matsuzawa, Y. Kitamura, and Y. Kanakura, *Blood*, 1994, **83**, 2619 - 2626.
71. C. Akin and D. Metcalfe, *Annu. Rev. Med.*, 2004, **55**, 419 - 432.
72. A. Fernandez, A. Sanguino, Z. Peng, A. Crespo, E. Ozturk, X. Zhang, S. Wang, W. Bornmann, and G. Lopez-Berestein, *Cancer Res.*, 2007, **67**, 4028 - 4033.
73. N. Shah, F. Lee, R. Luo, Y. Jiang, M. Donker, and C. Akin, *Blood*, 2006, **108**, 286 - 291.
74. X. Jin, D. R. Gossett, S. Wang, D. Yang, Y. Cao, J. Chen, R. Guo, R. K. Reynolds, and J. Lin, *Br. J. Cancer*, 2004, **91**, 1808-1812.
75. T. F. Franke, D. R. Kaplan, and L. C. Cantley, *Cell*, 1997, **88**, 435-437.
76. G. Kulik, A. Klippel, and M. J. Weber, *Mol. Cell. Biol.*, 1997, **17**, 1595-1606.
77. H.-J. Tang, X. Jin, S. Wang, D. Yang, Y. Cao, J. Chen, D. R. Gossett, and J. Lin, *Gynecologic Oncol.*, 2006, **100**, 308-317.
78. K. Fang, S.-P. Chen, C.-W. Lin, W.-C. Cheng, and H.-T. Huang, *Lung Cancer*, 2009, **63**, 227-34.
79. K. H. Vousden and C. Prives, *Cell*, 2009, **137**, 413-431.
80. M. Hollstein, K. Rice, M. S. Greenblatt, T. Soussi, R. Fuchs, T. Sorlie, E. Hovig, B. Smith-Sorensen, R. Montesano, and C. C. Harris, *Nucleic Acids Res.*, 1994, **22**, 3551-5.
81. H. B. Newton, *Exp. Rev. Anticancer Therapy*, 2005, **5**, 177-191.
82. L. M. Shi, T. G. Myers, Y. Fan, P. M. O'connor, K. D. Paull, S. H. Friend, and J. N. Weinstein, *Mol. Pharmacol.*, 1998, **53**, 241-251.
83. E. Sugikawa, T. Hosoi, N. Yazaki, M. Gamanuma, N. Nakanishi, and M. Ohashi, *Anticancer Res.*, 1999, **19**, 3099-3108.
84. Y. Peng, C. Li, L. Chen, S. Sebti, and J. Chen, *Oncogene*, 2003, **22**, 4478-4487.
85. P.-L. Kuo, Y.-L. Hsu, C.-H. Chang, and C.-C. Lin, *Cancer Lett.*, 2005, **223**, 293-301.
86. Y.-C. Kuo, P.-L. Kuo, Y.-L. Hsu, C.-Y. Cho, and C.-C. Lin, *Life Sci.*, 2006, **78**, 2550-2557.
87. G. W. Xu, I. A. Mawji, C. J. Macrae, C. A. Koch, A. Datti, J. L. Wrana, J. W. Dennis, and A. D. Schimmer, *Apoptosis*, 2008, **13**, 413-422.
88. C. Lu, W. Wang, and W. S. El-Deiry, *Cancer Biol. Ther.*, 2008, **7**, 2039-2046.
89. C. Huang, X. M. Zhang, R. T. Tavaluc, L. S. Hart, D. T. Dicker, W. Wang, and W. S. El-Deiry, *Cancer Biol. Ther.*, 2009, **8**, 2185-2192.
90. F. Wang, J. Liu, D. Robbins, K. Morris, A. Sit, Y.-Y. Liu, and Y. Zhao, *Apoptosis*, 2010, **16**, 301-310.
91. M.-A. Schwaller, B. Allard, E. Lescot, and F. Moreau, *J. Biol. Chem.*, 1995, **270**, 22709-13.
92. E. Tian, T. H. Landowski, O. W. Stephens, S. Yaccoby, B. Barlogie, and J. D. Shaughnessy, *Molecular Cancer Therapeutics*, 2008, **7**, 500 -509.
93. M. Hägg, M. Berndtsson, A. Mandic, R. Zhou, M. C. Shoshan, and S. Linder, *Molecular Cancer Therapeutics*, 2004, **3**, 489 -497.
94. J. B. L. Pecq, C. Gosse, Nguyen-Dat-Xuong, S. Cros, C. Paoletti, B. Raynal, and J. Morizet, *Cancer Res.*, 1976, **36**, 3067 -3076.
95. C. Paoletti, P. Le, N. Dat-Xuong, P. Juret, H. Garnier, J. L. Amiel, and J. Rouesse, *Recent Results Cancer Res.*, 1980, **74**, 107-23.
96. J. G. Rouesse, C. T. Le, P. Caille, J. M. Mondesir, H. Sancho-Garnier, F. May-Levin, M. Spielmann, J. R. De, and J. L. Amiel, *Cancer Treat. Rep.*, 1985, **69**, 707-8.
97. J. Rouëssé, M. Spielmann, F. Turpin, T. Le Chevalier, M. Azab, and J. M. Mondésir, *Eur. J. Cancer*, 1993, **29**, 856-859.
98. C. Auclair, A. Pierre, E. Voisin, O. Pepin, S. Cros, C. Colas, J. M. Saucier, B. Verschuere, P. Gros, and C. Paoletti, *Cancer Res.*, 1987, **47**, 6254-61.
99. E. Bisagni, C. Ducrocq, J. M. Lhoste, C. Rivalle, and A. Civier, *J. Chem. Soc. Perkin I*, 1979, 1706 - 1711.
100. C. Ducrocq, F. Wendling, M. Tourbez-Perrin, C. Rivalle, P. Tambourin, F. Pochon, E. Bisagni, and J. C. Chermann, *J. Med. Chem.*, 1980, **23**, 1212-6.

101. C. Rivalle, F. Wendling, P. Tambourin, J. M. Lhoste, E. Bisagni, and J. C. Chermann, *J. Med. Chem.*, 1983, **26**, 181-185.
102. E. M. Acton, V. L. Narayanan, P. A. Risbood, R. H. Shoemaker, D. T. Vistica, and M. R. Boyd, *J. Med. Chem.*, 1994, **37**, 2185-2189.
103. W. K. Anderson, A. Gopalsamy, and P. S. Reddy, *J. Med. Chem.*, 1994, **37**, 1955-63.
104. D. T. Vistica, S. Kenney, M. L. Hursey, and M. R. Boyd, *Biochem. Biophys. Res. Comm.*, 1994, **200**, 1762-1768.
105. J. Jurayj, R. D. Haugwitz, R. K. Varma, K. D. Paull, J. F. Barrett, and M. Cushman, *J. Med. Chem.*, 1994, **37**, 2190-2197.
106. P. Fossé, B. René, M. Charra, C. Paoletti, and J. M. Saucier, *Mol. Pharmacol.*, 1992, **42**, 590 -595.
107. T. Honda, M. Inoue, M. Kato, K. Shima, and T. Shimamoto, *Chem. Pharm. Bull.*, 1987, **35**, 3975-8.
108. T. Honda, M. Kato, M. Inoue, T. Shimamoto, K. Shima, T. Nakanishi, T. Yoshida, and T. Noguchi, *J. Med. Chem.*, 1988, **31**, 1295-1305.
109. A. R. Grummitt, M. M. Harding, P. I. Anderberg, and A. Rodger, *Eur. J. Org. Chem.*, 2003, **2003**, 63-71.
110. L. Ding, G. Etemad-Moghadam, S. Cros, C. Auclair, and B. Meunier, *J. Med. Chem.*, 1991, **34**, 900-906.
111. C. Bourdouxhe, P. Colson, C. Houssier, J. S. Sun, T. Montenay-Garestier, C. Helene, C. Rivalle, E. Bisagni, and M. J. Waring, *Biochemistry*, 1992, **31**, 12385-12396.
112. C. Bailly, C. Michaux, P. Colson, C. Houssier, J.-S. Sun, T. Garestier, C. Helene, J.-P. Henichart, and C. Rivalle, *Biochemistry*, 1994, **33**, 15348-15364.
113. S. Routier, J.-L. Bernier, J.-P. Catteau, P. Colson, C. Houssier, C. Rivalle, E. Bisagni, and C. Bailly, *Bioconjugate Chem.*, 1997, **8**, 789-792.
114. R. Devraj, J. F. Barrett, J. A. Fernandez, J. A. Katzenellenbogen, and M. Cushman, *J. Med. Chem.*, 1996, **39**, 3367-3374.
115. G. Czerwinski, N. I. Tarasova, and C. J. Michejda, *Proc. Nat. Acad. Sci.*, 1998, **95**, 11520-11525.
116. T. W. Moody, G. Czerwinski, N. . Tarasova, and C. . Michejda, *Life Sci.*, 2002, **71**, 1005-1014.
117. T. W. Moody, G. Czerwinski, N. I. Tarasova, D. L. Moody, and C. J. Michejda, *Regulatory Peptides*, 2004, **123**, 187-192.
118. O. Sedlacek, M. Hruby, M. Studenovsky, J. Kucka, D. Vetvicka, L. Kovar, B. Rihova, and K. Ulbrich, *Bioconjugate Chem.*, 2011, **22**, 1194-1201.
119. M. Ohashi and T. Oki, *Expert Opinion on Therapeutic Patents*, 1996, **6**, 1285-1294.


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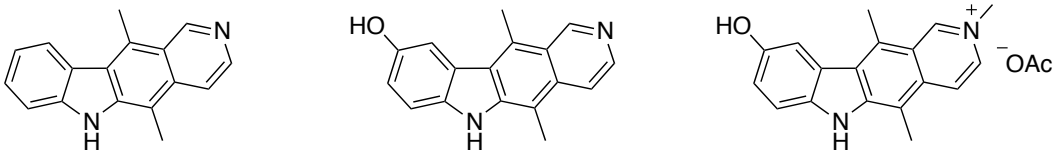
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3 Aims and Objectives

3.1 Overview

As outlined in the introductory chapters, the natural product ellipticine **1** has been the source of a vast body of research in anticancer chemotherapy. Various derivatives of ellipticine have been the subject of clinical trials, but have unfortunately not progressed past phase II.[1–3] Most derivatisation work has focused on the A-ring of ellipticine, particularly C-9 substitution e.g. 9-hydroxyellipticine **6**, and D-ring quaternary salt formation at *N*-2 e.g. 9-hydroxy-*N*-methylellipticinium acetate **8** (Figure 3.1). These modifications resulted in increased anticancer activity and improved water solubility, as shown by the CLogP values below.



Ellipticine **1** CLogP: 4.37

9-Hydroxyellipticine **6** CLogP: 3.70

Celiptium® **8** CLogP: −1.06

Figure 3.1

Previous work within this research group focused on derivatisation of *N*-6, C-9 and *N*-2 of ellipticine.[4] In particular, a large panel of novel ellipticinium salts was prepared, introducing a range of functionality in the salt side chain and also varying the side chain length. Several of these derivatives were found to exhibit strong inhibition of topoisomerase II activity and good overall cytotoxicity profiles.

Looking at the body of research overall, it is clear to see that substitution at the methyl groups of ellipticine (C-5 and C-11) has not been extensively studied, nor have the ellipticine analogues, isoellipticine and deazaellipticine been thoroughly investigated.

In addition to this, molecular modelling work carried out in collaboration with the Tyndall National Institute at the outset of this project focused on the inhibition of c-Kit kinase by ellipticine derivatives and the binding mode of ellipticine derivatives in the c-Kit active site (see appendices for full publication).[5] Extensive molecular dynamics simulations of five different binding modes for 9-hydroxyellipticine **6** suggested a different binding mode to that proposed by Vendome *et al.* in 2005 (see Ch 1, section 1.3.2.3 for detail).[6] In this new binding mode 9-hydroxyellipticine **6** was protonated at *N*-2, and based on pKa calculations, this seems likely to be the case *in vivo*. The key hydrogen bonding interactions are C(9)OH – Glu671 and *N*(2)H – Glu640, with the C-11 methyl group facing the protein backbone and the C-5 methyl group exposed to solvent (Figure 3.2).

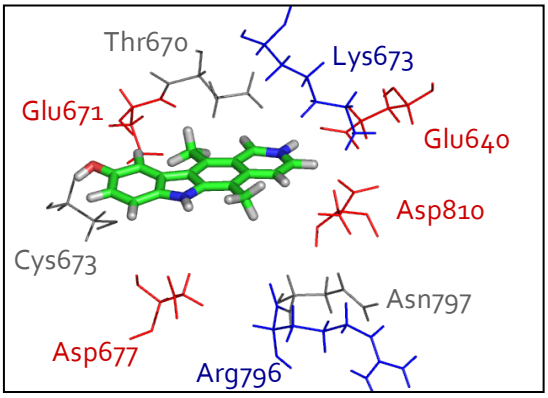


Figure 3.2: Proposed binding mode of protonated 9-Hydroxyellipticine **6** *in the c-Kit kinase active site, determined from Molecular Dynamics simulations.*[5]

In order to obtain definitive conformation of the binding mode of ellipticine derivatives in the c-Kit active site, synthesis of several ellipticine derivatives was required which would subsequently be studied for their c-Kit kinase inhibitory activity. Key derivatives required for testing included a range of C-11 and C-5 derivatives to probe the space and orientation within the active site, along with ellipticine analogues to assess the contribution of the pyridine nitrogen to the overall binding affinity.

3.1.1 Design of targets

In this work we seek to explore new molecular space around the ellipticine pharmacophore by undertaking the synthesis of a range of novel 5- and 11-substituted ellipticines (while maintaining the biologically important C-9 substituents). In particular, we were interested in the introduction of carbonyl and amide functionality at the C-5 and C-11 positions as new hydrogen bonding motifs in the ellipticine series (Figure 3.3). Amide formation would also allow for direct modulation of the substituent size depending on the amine used.

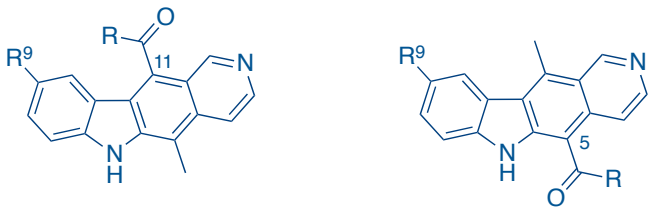


Figure 3.3: Ellipticine derivatives targeted for synthesis and biological evaluation

It was envisaged that successful derivatisation reactions in the ellipticine series would be applied to the preparation of novel isoellipticine and deazaellipticine derivatives (Figure 3.4), as models to probe the role and importance of the pyridine nitrogen. In addition to this, due to the interesting biological activity exhibited by novel ellipticinium salts previously prepared in the group, the synthesis of an analogous panel of isoellipticinium salts was also proposed.

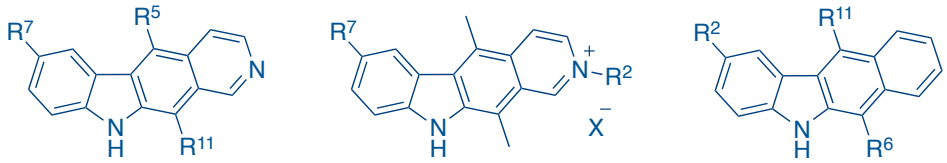


Figure 3.4: Isoellipticine and Deazaellipticine derivatives targeted for synthesis

3.2 Synthesis of Ellipticine Derivatives and Analogues

After reviewing the synthetic routes towards ellipticine in the literature, Sauliner and Gribble's highly versatile synthesis was selected (see Ch 2, section 2.3.2 for detail).[7] The attraction in the route lay in the C-2 coupling reaction between an indole and a pyridine anhydride, which may be varied in order to prepare deazaellipticine and isoellipticine. In addition to this, the ketolactam indolo[1,2-*b*][2,7]naphthyridine-5,12-dione **89** has been shown to readily react with a range of alkyllithium reagents in a regioselective and sequential manner to give 5- and 11-substituted ellipticines.

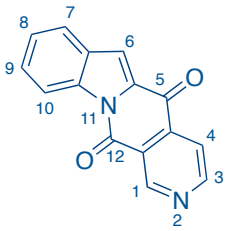


*Figure 3.5: Indolo[1,2-*b*][2,7]naphthyridine-5,12-dione **89***

In this work we proposed to expand the utility of the ketolactam **89** by investigating its reaction with alkenyllithium reagents in order to introduce C-5 and C-11 functionality. Another key aim was to investigate the reaction of indolo[1,2-*b*][2,7]naphthyridine-5,12-dione **89** with Grignard reagents, specifically vinylmagnesium bromide. If successful, replacement of alkyllithiums with Grignard reagents in this route would significantly increase its attractiveness, with milder conditions and a simpler procedure.

Successful derivatisation reactions in the ellipticine series would be applied to the preparation of novel isoellipticine and deazaellipticine derivatives.

3.3 Evaluation of Biological Activity

It was envisaged that evaluation of the anticancer activity of a range of novel ellipticines, isoellipticines and deazaellipticines would be achieved *via* several methods. These would include determination of specific anti-cancer properties such as inhibition of topoisomerase II activity (using a topoisomerase II decatenation assay), along with c-Kit kinase inhibition – as

part of a study to investigate the binding mode of ellipticine derivatives in the c-Kit active site and inhibition of other kinases (e.g. AKT).

Together with these specific measurements, an overall measure of the cytotoxicity of novel ellipticine derivatives and analogues was envisaged by submission to the NCI 60-cell line screen. This service is provided by the Developmental Therapeutics Program (DTP) at the US National Cancer Institute and involves submission of novel compounds for evaluation of activity against 60 different human tumour cell lines, representing leukaemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. This provides a highly useful overall picture of the cytotoxic activity of a compound and also highlights any specificity of the drug for certain cancer cell lines.

3.4 References

1. C. Paoletti, P. Le, N. Dat-Xuong, P. Juret, H. Garnier, J. L. Amiel, and J. Rouesse, *Recent Results Cancer Res.*, 1980, **74**, 107-23.
2. J. G. Rouesse, C. T. Le, P. Caille, J. M. Mondesir, H. Sancho-Garnier, F. May-Levin, M. Spielmann, J. R. De, and J. L. Amiel, *Cancer Treat. Rep.*, 1985, **69**, 707-8.
3. M. Ohashi and T. Oki, *Exp. Opinion Ther. Patents*, 1996, **6**, 1285-1294.
4. F. M. Deane, PhD Thesis, University College Cork, 2010.
5. D. Thompson, C. Miller, and F. O. McCarthy, *Biochemistry*, 2008, **47**, 10333 - 10344.
6. J. Vendome, S. Letard, F. Martin, F. Svinarchuk, P. Dubreuil, C. Auclair, and M. Le Bret, *J. Med. Chem.*, 2005, **48**, 6194-6201.
7. G. W. Gribble, M. G. Saulnier, J. A. Obaza-Nutaitis, and D. M. Ketcha, *J. Org. Chem.*, 1992, **57**, 5891-9.

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