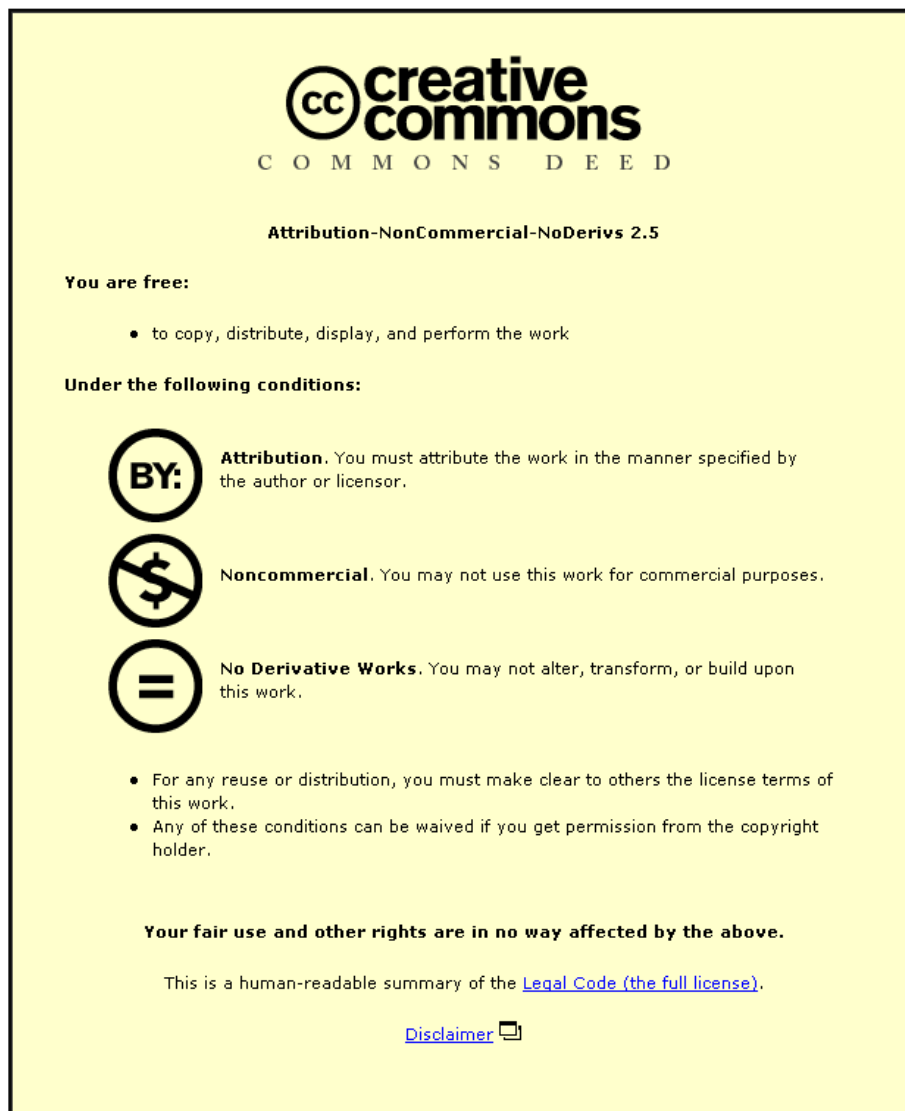


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**The Synthesis of Indole Containing
Anticancer Compounds**

By

Jonathan R. A. Roffey

A Doctoral Thesis

**Submitted in partial fulfilment of the requirements
for the award of**

Doctor of Philosophy of Loughborough University

May 1996

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Abstract

The concept of bioreductive prodrug chemotherapy is introduced in chapter 1. Tumour cell hypoxia is a significant factor in limiting tumour growth control with conventional radiotherapy and some chemotherapeutic agents. Following therapy these cells can repopulate and cause a relapse of the cancer. On the other hand, hypoxia is unique to tumours, and is therefore potentially exploitable. Bioreductive prodrugs are compounds in which an oxygen inhibited redox-based bioactivation step triggers a reaction leading to a lethal intermediate. The concept of bioreductive DNA alkylators and DNA topoisomerase II inhibitors is discussed.

The synthesis of model thiazolylindole compounds based on the natural product BE 10988 are discussed in chapter 2. Two strategies were employed for the construction of the thiazolylindoles: the Hantzsch reaction; and nucleophilic substitution on 2-bromothiazole by an indolyl anion.

The synthesis of thiazolylindolequinone compounds are discussed in chapter 3. The quinone C(5) position of the thiazolylindolequinone analogues was elaborated to provide a series of cyclic and acyclic C(5)-amino derivatives.

Synthetic strategies towards the synthesis of indole-2-carboxylates are discussed in chapter 4. The Moody-Rees and Cadogan-Sundberg reactions were employed to provide a synthesis of the useful highly substituted indole [154].

The Brederik imidazole reaction (*i.e.*, the reaction of an amidine and α -haloketone) is discussed in chapter 5. Application of the Brederik reaction was employed towards the construction of the bisindole imidazole natural compounds, the nortopsentins.

The biological properties of the compounds of the compounds synthesised are discussed in chapter 6. The compounds were tested for DNA topoisomerase II inhibitory activity and cytotoxicity under a hypoxic environment.

Acknowledgements

I wish to thank my supervisor, Professor Christopher J. Moody, for his sound advice, much appreciated knowledge and positive encouragement throughout the course of this work. Much appreciated thanks are also due to the academic staff at Loughborough, in particular Dr. Russ Bowman and Professor Harry Heaney. I would also like to thank Dr. Lawrence Patterson, Pharmacy Department DeMontfort University, for helpful discussions, with regards to topoisomerase II inhibitors. I would also like to thank the Macintosh guru, Colin Goodwin.

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It is impossible to enjoy time spent in a laboratory, unless colleagues provide a relaxed and jovial atmosphere. Those I have had the pleasure to work with I greatly appreciate. It is difficult to list everyone, however, in particular I would like to acknowledge Anne, Liz, Dave, Lou, Andy, Craig, Graham, Justin, Gary, Eddie, John, Mike, Soyfur, Serge and Rich. And to all those organic chemists out there "do your best, don't get stressed".

Sometimes (perhaps the majority of times) extracurricular activities are required, in order to maintain high levels of motivation. I would like to thank many of the above and other friends (namely those from Leicester, Loughborough, Leeds and Worcester) for many an enjoyable session, more often than not, ending in a complete pickled state.

Finally, I would like to thank my parents and brothers for their continued support throughout my education.

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Abbreviations

A	Adenine
AIBN	2, 2'-Azobis-(2-methylpropionitrile)
ATP	Adenosine triphosphate
BD	Bioreductive drug
BI	Benzimidazole
BLM	Bleomycin
Bn	Benzyl
Boc	<i>tert</i> -Butyloxycarbonyl
B5R	NADH : cytochrome <i>b</i> ₅
Bu	Butyl
Bz	Benzoyl
C	Cytosine
CAN	Ceric ammonium nitrate
CSI	Chlorosulfonylisocyanate
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL-H	Diisobutylaluminium hydride
DMAP	4-Dimethylaminopyridine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTD	DT-Diaphorase
Et	Ethyl
G	Guanine
GSH	Glutathione
HMPA	Hexamethylphosphoramide
IR	Infrared
LDA	Lithium diisopropylamide
LR	Lawesson's reagent
Me	Methyl
MMC	Mitomycin C
MO	Molecular orbital
m.p.	Melting point
NAD ⁺ , NADH	Nicotinamide adenine dinucleotide and its reduced form
NADP ⁺ , NADPH	Nicotinamide adenine dinucleotide phosphate and its reduced form
NMR	Nuclear magnetic resonance

Nu	Nucleophile
PBI	pyrrolobenzimidazole
Ph	Phenyl
PPA	Polyphosphoric acid
P450R	NADPH : cytochrome P450
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rt	Room temperature
SDS	Sodium dodecyl sulfate
SEM	(trimethylsilyl)ethoxymethyl
SM	Starting material
T	Thymine
TAF	Tumour angiogenesis factor
TBDMS	<i>tert</i> -Butyldimethylsilyl
THF	Tetrahydrofuran
TLC	Thin later chromatography
topo II	DNA topoisomerase II
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase

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"What have you lost, Mulla ?"

"My key," said Nasrudin.

"Where did you drop it ?"

"At home."

"Then why, for heaven's sake, are you looking for it here ?"

"There is more light here."

a Sufi parable

Chapter 1

Introduction.

1. Introduction

The late John Wayne referred to cancer as "the big C" and indeed it is a big disease, roughly one person in five in the prosperous countries in the world will die from it. In the US the death rate from cancer has increased by 7% between 1975 and 1990, and this number has been adjusted to compensate for changing size and composition of the population with respect to age, so the increase can't be blamed on people dying less often from other diseases.¹ The challenge to find a cure for this disease has not been taken lightly, it has been estimated that since president Richard M. Nixon initiated the US "war on cancer" in 1971 over \$25 billion has been spent on research in the US alone.

1.1. What are cancers ?

Cancers arise when a cell acquires multiple genetic mutations that together cause the cell to escape from normal controls on replication and migration.² As the cell and its offspring multiply uncontrollably, they can invade and damage nearby tissue. Some may also break away and travel to parts of the body where they do not belong, establishing new malignancies (metastases) at different sites.³

The clearest definition of cancer is to describe it as a micro evolutionary process.⁴ The body of an animal can be viewed as a society or ecosystem whose individual members are cells, reproducing by cell division and organised into collaborative assemblies or tissues. In a healthy body, self sacrifice, rather than competition is the rule for almost every class of cell. All somatic cell lineages are committed to die, leaving no progeny but dedicating their existence to support germ cells, which alone have a chance for survival. The cells of multicellular organisms are committed to collaboration. Any mutation that gives rise to non altruistic behaviour by individual members of the co-operative will jeopardise the future of the whole enterprise. Thus mutation, competition and natural selection operating within the population of somatic cells are ingredients for a disaster. Cancer is a disease in which individual cells begin by prospering selfishly at the expense of their neighbours but in the end destroy the whole cellular society and die.

1.1.1. Strategies involved in cancer therapy

A major problem in finding a cure for cancer lies in the very nature of the disease. Cancer is a disease in which an organisms own cells deviate from normality and eventually cause death. The paradox lies in being able to differentiate between "normal" healthy and cancerous cells.

Successful cancer treatment must rely on the exploitation of consistent and significant biological differences between normal and malignant tissues. Three main lines of attack are used in contemporary cancer treatment: surgery; radiotherapy; and chemotherapy.⁴ For cancer treatment to be successful the following criteria must be met:

- i) will the treatment prolong the period of useful life ?
- ii) will the quality of life be acceptable ?
- iii) will the patient be able to work, to care for itself and to be in less pain ?

The success of surgery in treating localised primary tumours depends on differences in topography. The primary tumour is a discrete lesion and, if dissemination has not occurred, then surgery exploits this topographical difference and is successful. Once malignant tissue has appeared beyond the discrete primary tumour, *ie.* after metastasis, surgery, in the long term, fails.

Radiotherapy treatments also primarily exploit differences due to locale. If the tumour mass is localized, with no distant spread and is not located within or close to highly radiosensitive tissues or organs then it may be curable. Intrinsic cellular and tissue differences in radiosensitivity of cells to radiation may also determine response to treatment. The radiosensitivity of cells depends in part on the oxygen tension (pO_2) present in the tissue at the time of irradiation, referred to as the oxygen effect. Cells in air saturated environments are about three times as sensitive to radiotherapy as cells in an oxygen-free environment. Solid tumours that contain high proportions of hypoxic (oxygen deficient, low pO_2) cells are usually resistant to attack by radiotherapy.⁵

Most of the drugs that are used in cancer chemotherapy are antiproliferative agents. DNA replication is a necessary precondition for cell division and it contributes a vulnerable target for the action of cytotoxic drugs intended to block the multiplication of malignant cells. Two main classes of cytotoxic agents will be discussed: the DNA alkylators, drugs that form covalent bonds with DNA; and topoisomerase inhibitors, drugs that effect the action of the topoisomerase enzymes, enzymes which play a vital role in DNA metabolism.

A recurring theme in cancer therapy is the need to differentiate between healthy and diseased cells. Hypoxic cells are a significant factor in limiting tumour growth control with conventional radiotherapy and some chemotherapeutic agents. Following therapy these cells can repopulate and cause a relapse of the cancer. On the other hand, hypoxia is a condition unique to tumours, and is therefore potentially exploitable.

A field of growing importance in cancer treatment is bioreductive prodrug chemotherapy,⁶ whereby the hypoxic chemical environment is used to the advantage of the clinician, in that it provides a means for the chemical activation of an inert prodrug into a cytotoxic species. These bioactivated prodrugs can be used in combination with radio-therapy and conventional chemotherapeutic agents. Before discussing the field of bioreductive prodrugs in more detail a brief description of the events leading to hypoxia will be mentioned.

1.1.3. Vasculisation of tumours and the events leading to hypoxia

Malignant growths are not merely clusters of proliferating cells, often less than half the volume of the tumour consists of cancerous cells.⁷ One to ten percent of the volume is contributed by blood vessels weaving through the tumour mass. The remaining space is filled, primarily by an abundant collagen rich matrix, the interstitium, that surrounds the cancer cells and can separate them from the vasculature. The clinical event that converts a self contained pocket of aberrant cells into a rapidly growing malignancy comes when the tumour becomes vascularised, a process called angiogenesis. This event is triggered when the tumour cells release a substance named tumour angiogenesis factor (TAF). This has the capacity to stimulate nearby blood vessels to send out new capillaries that grow towards the small colony of tumour cells and finally penetrate it. Fresh nutrients pour in and wastes are speedily removed. The vascular system of tumours can be highly disorganised, both in its structure and operation. The capillaries grow in unpredictable directions that can change from day to day. Consequently some areas of the tumour may be well vascularised, whereas others have little or no blood supply. Tumour cells in those blood-starved areas may seem, on inspection to be dead, but they frequently revive if nourishment returns.

From the differences in the vascular supply within the tumour mass two different classes of tumour microenvironment can be described: those cells nearest the blood supply, well oxygenated and well nourished, are the cells most likely to survive and proliferate (oxic cells); and hypoxic cells, in which the cellular oxygen tension is depleted, they are poorly nourished, are bathed in their own excretion products and they have low proliferation rates.⁸ Due to poor nutrition, hypoxic cells are found which supply their energy requirements by anaerobic glycolysis and hydrolysis of ATP. The increased production of protons (by the dissociation of the lactic acid produced) during these metabolic processes causes an acidic microenvironment. The intracellular pH of hypoxic cells is maintained close to neutral by membrane pumps to efflux the protons, thus, resulting in chronic acidification of the extracellular compartment.

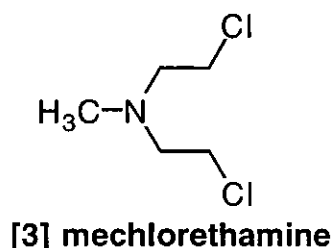
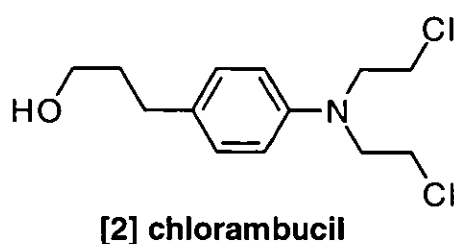
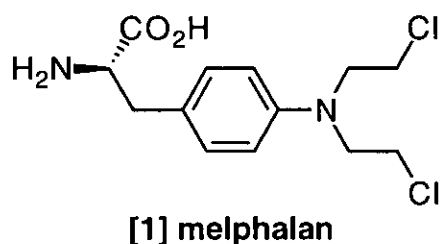
1.2. The DNA Alkylating Agents

The alkylating agents are a class of compounds that are capable of reacting in a manner such that an alkyl group or a substituted alkyl group becomes covalently linked to cellular constituents.⁹ The main property required by alkylating agents is that they proceed *via* an electrophilic intermediate which will then form a covalent bond with nucleophilic groups on cellular constituents such as the nucleic acids, DNA and RNA and proteins.

Two broad classes of DNA alkylating agents can be described: monofunctional alkylating agents; and bifunctional alkylating agents. Monofunctional alkylating agents form a covalent bond with a single strand of DNA. A possible consequence of monofunctional alkylation is the mispairing of bases in DNA. Such mispairing could lead to miscoding and mutation.¹⁰ Bifunctional alkylating agents result in interstrand cross-linking of DNA, this leads to impaired template function for further DNA replication.¹¹ Rapidly proliferating cells are supposed to be more sensitive to DNA cross-linking than normal cells, because they are not able to repair damaged DNA.¹²

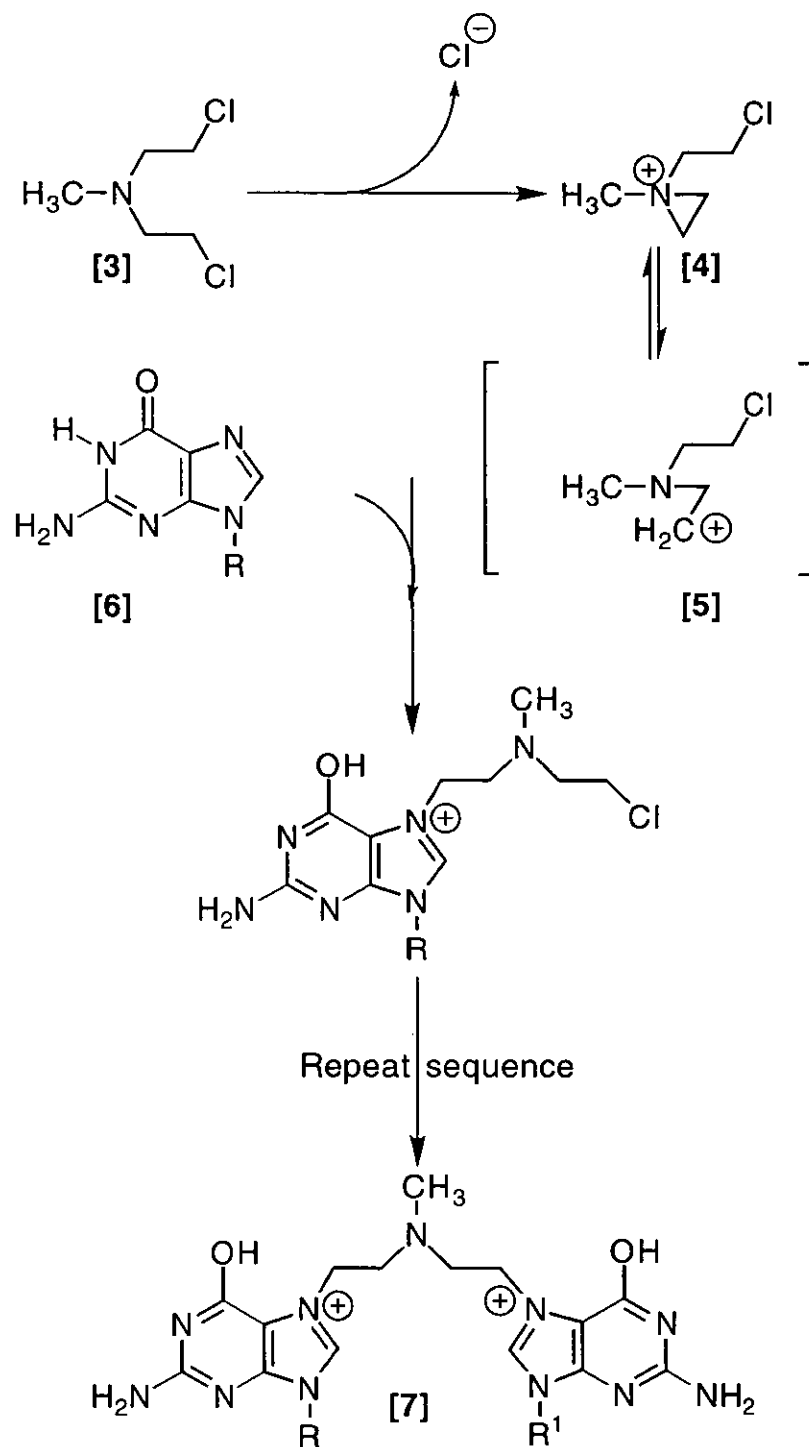
1.2.1. The nitrogen mustards

The first alkylating agents to be used in the clinic were the nitrogen mustards *eg.* melphalan [1] and chlorambucil [2], which were originally designed from the nitrogen mustard mechlorethamine [3], which was synthesised in 1942 and primarily developed as a chemical warfare agent. However, on closer inspection into the toxicity of mechlorethamine and related compounds it was found that the toxicity was most pronounced in rapidly dividing cells. This led to clinical studies throughout the early 1940's into the clinical efficacy of the mustards as antitumour agents, however, because of wartime secrecy surrounding all work on nitrogen mustards, information about the clinical trials was suppressed until 1946.¹³



The proposed mechanism of action of the mustards is shown, **Scheme 1**, one of the chloroethyl side chains undergoes a cyclisation, releasing a chloride ion and forming an aziridinium ion intermediate [4],¹⁴ in some cases the reaction may proceed via the formation of a carbonium ion intermediate [5]. Both the intermediates [4] and [5] are labile towards nucleophilic attack by nucleic acids or groups such as amino, carboxyl, sulfhydryl or imidazole moieties in proteins. One favoured reaction, of major importance to the cytotoxic effect of the mustards, is the formation of a covalent bond between the mustard and the N(7)-nitrogen group of a guanine nucleotide [6]. After forming a covalent bond with one DNA strand, the bifunctional alkylating agents can then undergo a similar cyclisation of the second side chain and form a covalent bond with another nucleophilic group. This second reaction could involve the N(7)-nitrogen of another guanine or some other nucleophilic moiety. This may result in the formation of drug DNA cross-links [7], or in the binding of DNA to a protein.

Some of the problems associated with the clinical use of mechlorethamine [3] are due to its high chemical reactivity. At physiological pH, this compound can rapidly cyclise to react with water or blood and tissue constituents. Replacement of an aromatic ring adjacent to the nitrogen as in melphalan [1] and chlorambucil [2] reduces the nucleophilicity of the nitrogen, this is due to the electron withdrawing effect of the aromatic ring, and so the rate of cyclisation to form the reactive aziridinium ion species is reduced. This decreased reactivity allows time for absorption and wide distribution before extensive alkylation occurs. Thus melphalan [1] and chlorambucil [2] can be given orally, whereas mechlorethamine [3] can only be given intravenously.

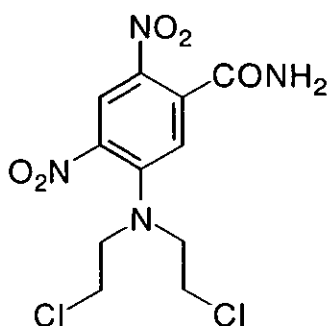


Scheme 1: Proposed mechanism of action of the nitrogen mustard mechloroethamine

A number of modified mustards have been synthesised in an attempt to obtain an anticancer drug that will preferentially localise in a particular tissue. Melphalan [1] is an example of such a compound. Since phenylalanine is a precursor of melanin, it was postulated that a phenylalanine mustard might preferentially accumulate in melanomas and thereby produce a selective effect.¹⁵

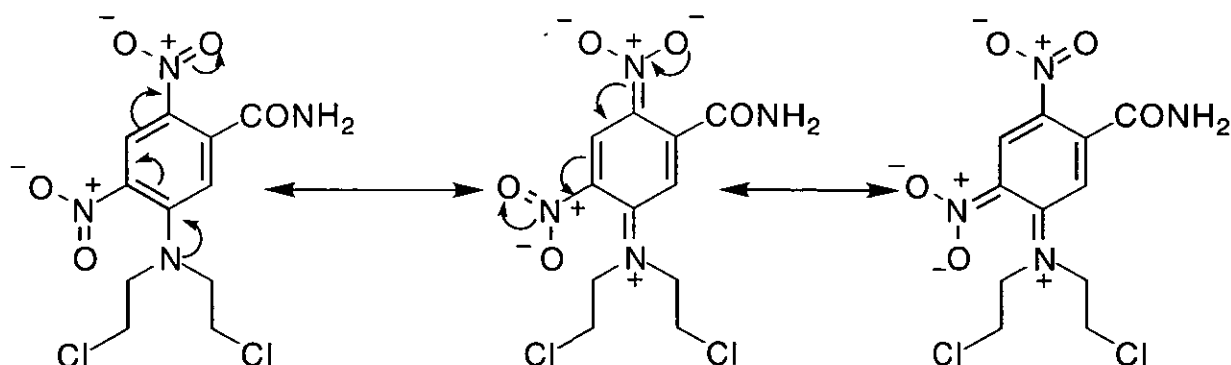
1.2.2. Bioreductive alkylating agents

Denny *et al.* have designed a series of nitrogen mustards which are specifically activated in hypoxic environments.¹⁶ As mentioned previously in this chapter, solid tumours are difficult to eradicate by conventional techniques because of tumour hypoxia. The dinitrobenzamide mustard [8] (NSC 646392) is a poor alkylating agent due to conjugation of the lone pair of electrons of the nitrogen mustard with the two nitro groups on the aromatic ring, **Scheme 2**.



[8] NSC 646392

Thus, electron delocalisation reduces the formation of the reactive aziridinium ion intermediate to such an extent that the mustard can be considered unreactive. Cellular enzymatic reduction of one or both nitro groups will therefore increase the nucleophilicity on the nitrogen and hence increase the formation of the reactive aziridinium ion intermediate. However, enzymatic reductions of nitro groups are normally oxygen inhibited and so only in hypoxic cells (low pO_2) will there be a build up of the reactive species. In preliminary studies NSC 646392 [8] displayed hypoxic selectivity ratios of 10 to 60 fold *in vitro* cytotoxic assays using a range of mammalian cell lines, while separate DNA elution studies showed that more soluble analogues based on [8] were activated under hypoxia to form DNA-crosslinking agents.



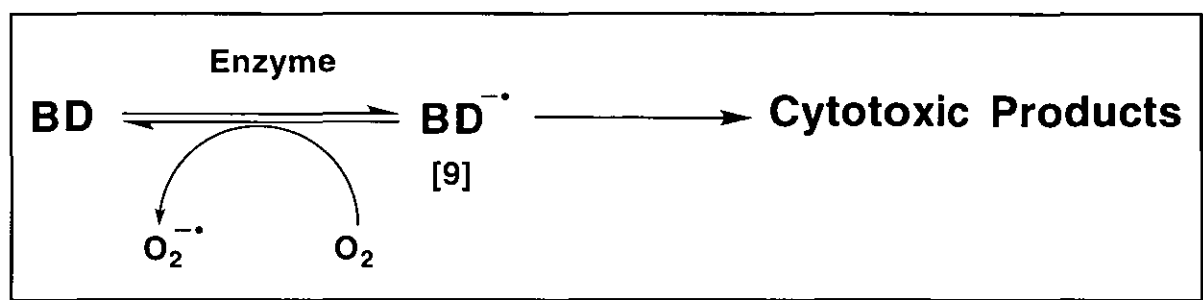
Scheme 2: Conjugation of the lone pair of electrons with the nitro groups, greatly reduces the nucleophilicity of the mustard nitrogen

Bioactivated prodrug therapy offers a unique opportunity for rational drug design within the strict criteria dictated by the biological environment. To fully exploit the small proportion of fully hypoxic cells present in most solid tumours, bioreductive drugs (BD) must be engineered to do three things:

- i) distribute efficiently to the hypoxic regions in tumours;
- ii) undergo selective (oxygen inhibited) metabolism in these hypoxic regions;
- iii) be thus converted to very toxic species.

Denny has defined BD nicely as being comprised of three domains, namely trigger and effector units joined by a linker mechanism.¹⁷ While it is not always possible to separate these functions clearly, this concept allows independent consideration of the types of structures most suitable for each process, and criteria which they must meet.

The trigger is where selectivity resides. For drugs designed to be reduced by the "background" of reductase enzymes in cells, the reduction potentials need to be within a narrow range, to ensure appropriate rates of both the forward-reduction and the back-oxidation of the resulting one-electron species by oxygen in oxygenated cells, **Scheme 3**.

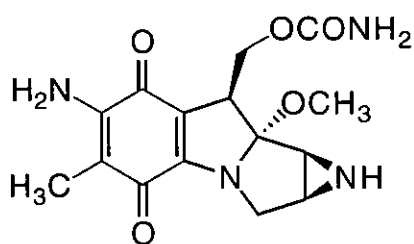


Scheme 3: One electron reduction of a BD to the corresponding radical anion, and hence to a cytotoxic species

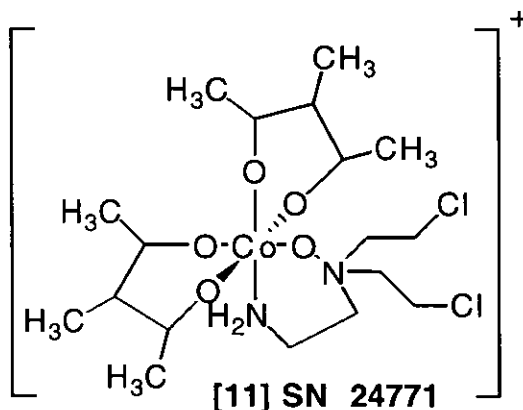
The bioreductive drug (BD) is reduced by a one electron process to the radical anion [9]. Any residual oxygen within the cell will reoxidise [9] back to the original drug and form the reactive oxygen species (ROS) superoxide ($\text{O}_2^{\cdot-}$). Oxygen readily accepts electrons from other molecules to form oxygen-derived free radicals. It is this property that makes it toxic to cells. Many intracellular reactions, including respiration, reduce oxygen to superoxide or hydrogen peroxide. These molecules are only moderately reactive with other biological molecules, but they can form a hydroxyl radical ($\cdot\text{OH}$), which is highly reactive and might be directly responsible for most of the oxidative damage from ROS in biological systems. Aerobic cells are endowed with extensive antioxidant defence mechanisms to counteract the damaging effects

of ROS, and can be viewed as being under a continual state of "oxidative siege", their survival depending on a balance between ROS and antioxidants.

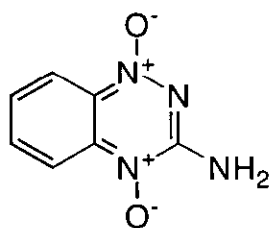
Moieties which have proved useful as triggers for BD include: nitroaromatics [8],¹⁶ quinones [10],¹⁸ transition metal complexes [11],¹⁹ aromatic [12]²⁰ and aliphatic *N*-oxides [13]²¹, shown below.



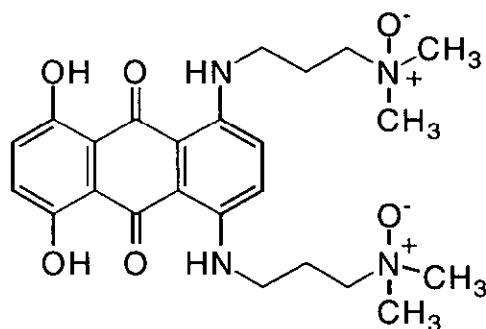
[10] MMC



[11] SN 24771



[12] SR 4233 Tirapazamine



[13] AQ4N

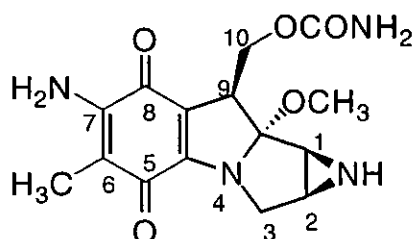
The effector, when released or activated must be much more toxic than both the parent prodrug and any ROS generated by futile cycling in oxygenated cells. It should be effective against cells in a variety of pH environments and proliferative states and particularly against non-cycling cells. A further advantage is an ability (defined by half life and reversible binding to cell components) to back-diffuse a limited distance from the hypoxic cells in order to kill surrounding oxygenated tumour cells. Moieties which have proved useful as effectors for BD include: the nitrogen mustards [8] and [11], quinone methides [10], DNA cleaving agents such as free radical species [12] and topoisomerase II inhibitors [13].

The linker mechanism is harder to define physically, and consists largely of mechanistic concepts. One widely exploited is activation of an attached mustard by electron release following reduction of a nitro aromatic, *eg.* the dinitrobenzamide mustard [8]. Also the

indolequinone anticancer compounds, of which mitomycin C (MMC) [10] is a archetypical example, can be categorised in terms of the linker mechanism. Reduction of the quinone moiety results in the formation of a intermediate species, in which the indole nitrogen is no longer in conjugation with the quinone, this leads to formation of a highly electrophilic quinone methide intermediate which can alkylate cellular constituents. The indolequinone moiety, as in MMC [10], has proved to be an attractive generic scaffold for the synthesis of many novel bioreductive prodrugs.

1.2.3. The mitomycins

The mitomycins were first discovered in 1956 by workers at Kyowa Hakko Kogyo Co., a Tokyo drug company, among fermentation products of *Streptomyces caespitosus*.²² Commercial production of large batches of MMC by fermentation of *Streptomyces caespitosus* has allowed its development as a clinical anticancer agent. Despite its toxicity, this chemotherapeutic agent was introduced in Japan (1960) and later in the US (1974) for treatment of several types of tumours. A compromise between potency and toxicity established MMC [10] as the best candidate within the naturally occurring mitomycins for clinical use.²³ MMC has proven to be an effective drug for stomach, breast, and colon cancers and to a lesser extent for pancreatic and lung cancers.



Szybalski and Iyer first proposed that MMC acts as an alkylating agent that is unmasked following reductive activation.²⁴ Two latent functionalities, the carbamoyl group and the aziridine, under appropriate circumstances are transformed into electrophilic species, a process termed bioelectrophilicity. Biochemical investigations have demonstrated that upon reductive activation MMC first reacts at C(1) and then at C(10) with the nucleophilic 2-amino residue in deoxyguanosines located on complementary DNA strands, thus allowing the formation of a bis-DNA cross-linked adduct.^{25,26} It has been suggested that the formation of intrahelical interstrand DNA-MMC adducts inhibits DNA replication, resulting in cessation of cell division. The proportion of cross-linked DNA is only about 10% of the mono-alkylation, suggesting that there might be a minor pathway with different factors controlling the product outcome.²⁷

Enzymatic reduction of a quinone moiety can proceed either by a one electron reduction pathway or a two electron reduction, **Figure 1**. The hypothetical quinone [A] can be reduced

by a one electron reduction to the semiquinone radical anion [B], for example by an enzyme such as NADPH: cytochrome P450 reductase, the radical anion [B] can also be further reduced to the hydroquinone [C]. In the presence of oxygen the radical anion [B] will be oxidised to the quinone [A]. However, the quinone [A] can also be reduced by an obligate two electron reduction, for example by NAD(P)H: quinone oxidoreductase (DT-diaphorase), to the hydroquinone [C]. Danishefsky and Ciufolini have shown that treatment the hydroquinone form of MMC with oxygen results in the immediate regeneration of MMC.²⁸ The ease of bioreduction of a given quinone containing drug will depend on: (i) the ability of the drug to act as a substrate for the enzyme(s); and (ii) the expression levels of these enzymes in a particular cell type.

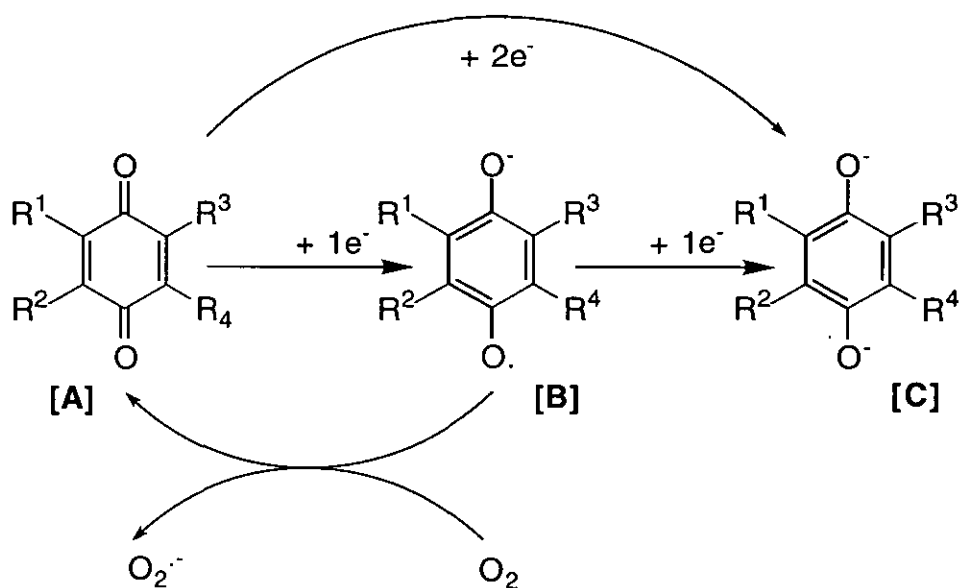


Figure 1: Reduction of a quinone moiety to either a semiquinone radical anion or a hydroquinone species

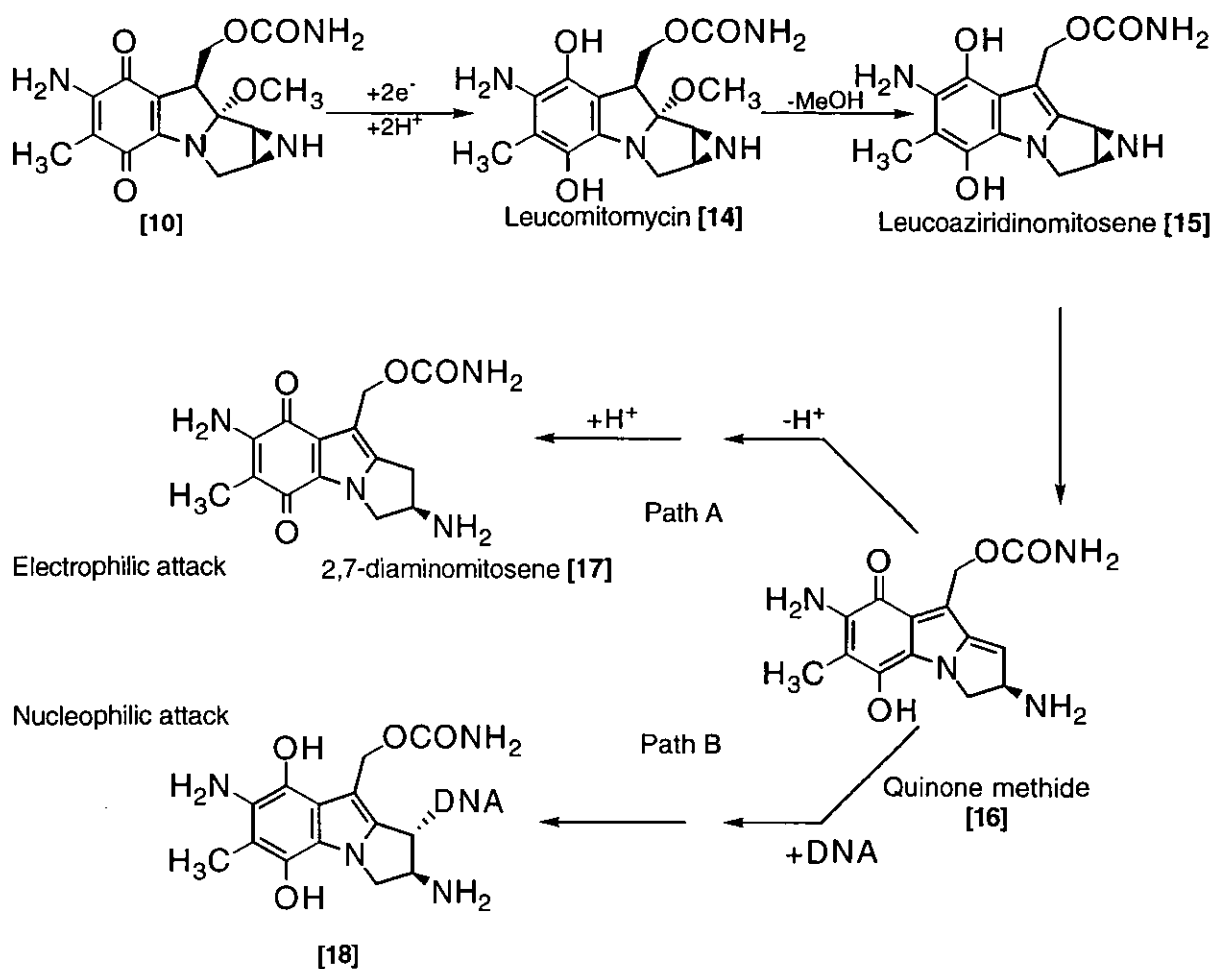
MMC mechanism of reaction with DNA remains fertile ground for investigation. Attempts to model the biological reductive process in the absence of DNA have provided information concerning this mechanistic scenario. The focus for debate surrounds the initial reductive activation step. The reduction can proceed either by an initial one electron reduction, thus forming a mitosene semiquinone radical anion or by a two electron reduction process to provide a mitosene hydroquinone.

It has been proposed that a two electron reduction of MMC to a hydroquinone [14] (leucomitomycin C) would lead to localization of electron density on nitrogen, triggering the loss of methanol and resulting in increased electrophilicity of centres C(1) and C(10), **Scheme 4**.²⁴ This mechanism explains mitomycin's requirement for reductive alkylation and its DNA cross-linking. The lone pair of electrons, on the indoline nitrogen, are no longer in conjugation with the quinone moiety and are therefore "unleashed", such that β -elimination of the methoxy

substituent is facilitated by neighbouring-group participation, resulting in the indole species leucoaziridinomitosenone [15], the thermodynamic driving force being the resonance stabilisation energy of the aromatic indole nucleus. Release of the high ring strain energy in the aziridine ring, thus provides the quinone methide [16]. The quinone methide can then undergo electrophilic attack by a proton to form 2,7-diaminomitosene [17], path A. Thus resulting in a species unable to covalently bond with DNA at the C(1) position. Conversely, nucleophilic attack by a species such as the 2-amino residue in deoxyguanosine affords the C(1) drug-DNA adduct [18], path B. Ross *et al.* have demonstrated the formation of 2,7-diaminomitosene [17] as the principal metabolite after administration of MMC to HT-29 human colon carcinoma cells and rat hepatic DT-diaphorase.²⁹

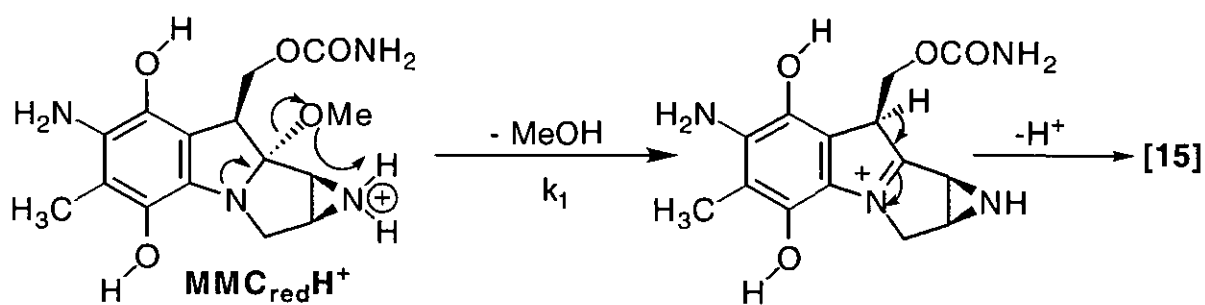
Schiltz and Kohn have shown that leucoaziridinomitosenone [15] in water in the absence of an external nucleophile undergoes principally electrophilic substitution at C(1), to provide 2,7-diaminomitosene [17], either at pH 5.5 or 8.5.³⁰ However, in the presence of aniline, as an external nucleophile, C(1) nucleophilic adducts predominated at pH 5.5 (path B), while at pH 8.5 the C(1) electrophilic adduct [17] predominated (path A). The sensitivity of reductively activated MMC transformations to both pH and added nucleophile suggested that in MMC *in vivo* processes DNA bonding will not efficiently proceed unless select interactions between the activated drug and the DNA surface exist, thereby allowing proper alignment of the activated drug in the minor groove. Under conditions where this does not occur, self-destruction of the activated drug by primary C(1) electrophilic transformations proceed to yield 2,7-diaminomitosene [17]. This process prevents indiscriminate drug-DNA bonding and is expected to contribute to the overall DNA sequence selectivity observed in MMC-DNA transformations.

MMC, unlike many naturally occurring anticancer drugs, is a small, compact compound. Despite what seems to be a structural deficit MMC preferentially bonds to specific DNA sequences.³¹ It has been demonstrated that *in vitro* MMC mono-alkylation transformations proceed only at guanine (G*) sites, with 5'CG* dinucleotide sequences being modified at appreciably higher levels than 5'AG*, 5'GG*, and 5'TG* sequences and that *in vitro* and *in vivo* MMC DNA bis-alkylation, interstrand cross-linking processes occur within complementary 5'CG* sequences rather than at 5'G*C sequences.³² Kohn *et al.* have shown that MMC monoalkylation reactions at 5'CG*.5'CG sequences are facilitated, in part, by the formation of a hydrogen bond between the carbonyl oxygen of the carbamoyl group of the activated mitomycin species and the N(2)-amino proton on the guanine (G) of the nonbonding strand.³³ This arrangement is expected to promote monoalkylation of DNA by positioning the activated drug near a guanine (G*) present on the bonding strand.



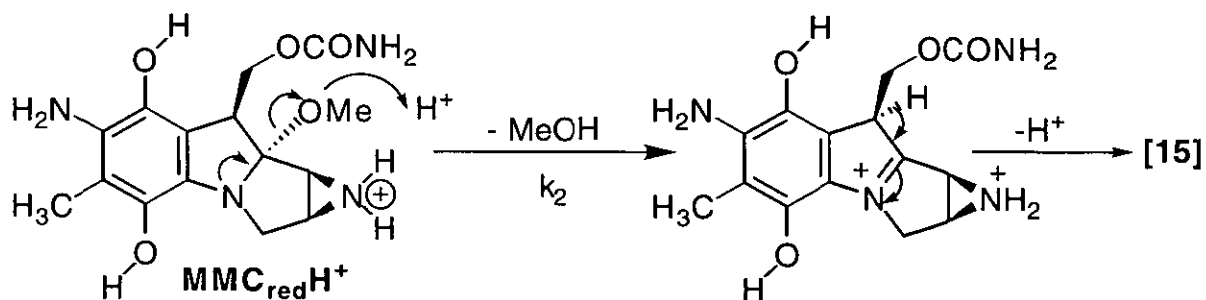
Scheme 4

β -Elimination of the methoxy substituent to form leucoaziridinomitosenone [15] has been shown to be enhanced under acid catalysis (*i.e.*, methanol is a better leaving group than methoxide). There are two mechanisms for methoxy elimination: firstly, specific acid-catalysed elimination assisted by the protonated aziridine; and secondly, non-specific elimination.³⁴ The pK_a for the aziridine functionality in leucomitomycin C [14] is 9.1, therefore, at physiological pH the aziridine functionality will exist excessively in the protonated form. The protonated aziridino nitrogen can act as an internal general acid, thus facilitating the specific elimination of methanol (rate constant k_1), **Scheme 5**.



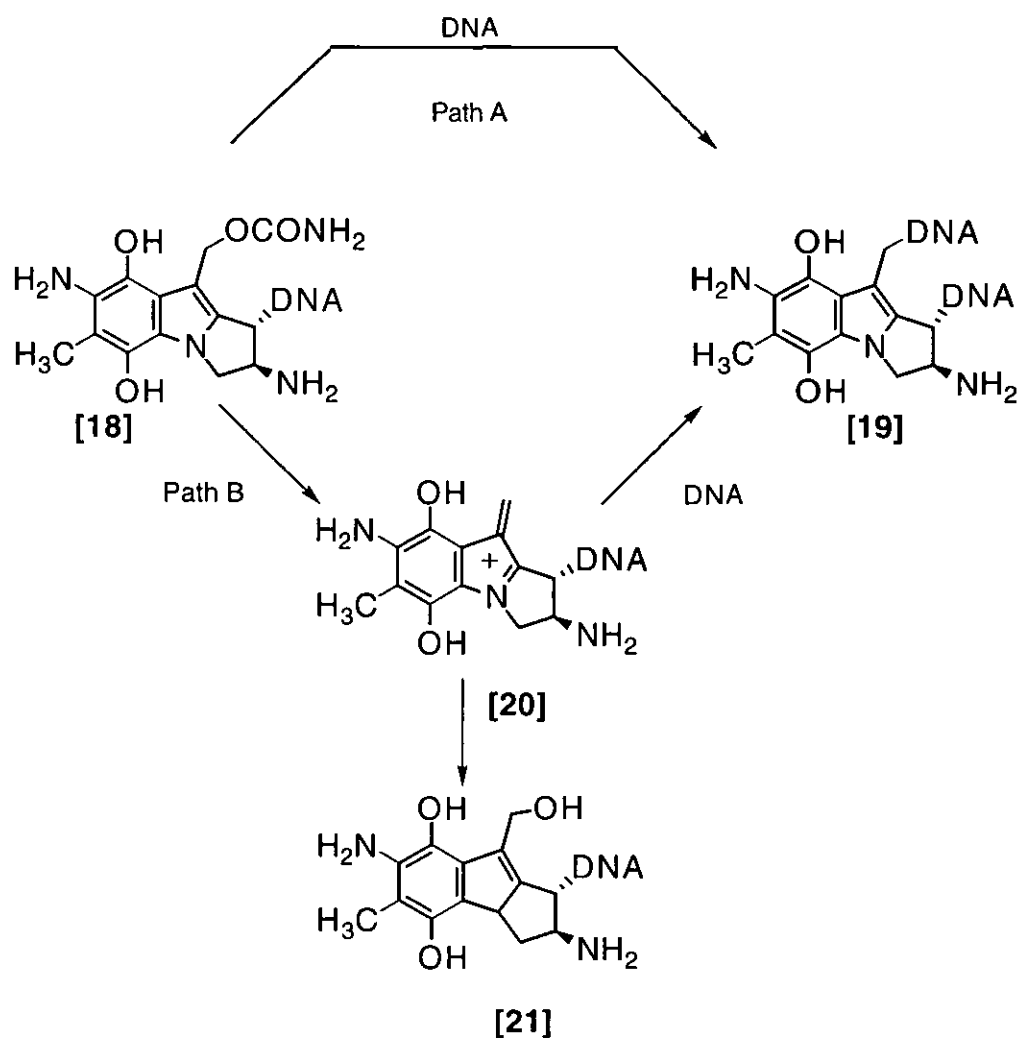
Scheme 5

The protonated leucomitomycin species can also facilitate the loss of methanol by a spontaneous elimination process (rate constant k_2), **Scheme 6**. Skibo and Boruah reported the rate constant for the specific acid-catalysed elimination of methanol (k_1) to be $1.5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and for spontaneous elimination of methanol (k_2) to be $0.015 \text{ M}^{-1} \text{ s}^{-1}$.³⁴ The authors also reported a 100,000-fold increase in the rate constant (k_1) upon reduction of MMC to leucomitomycin C [14].



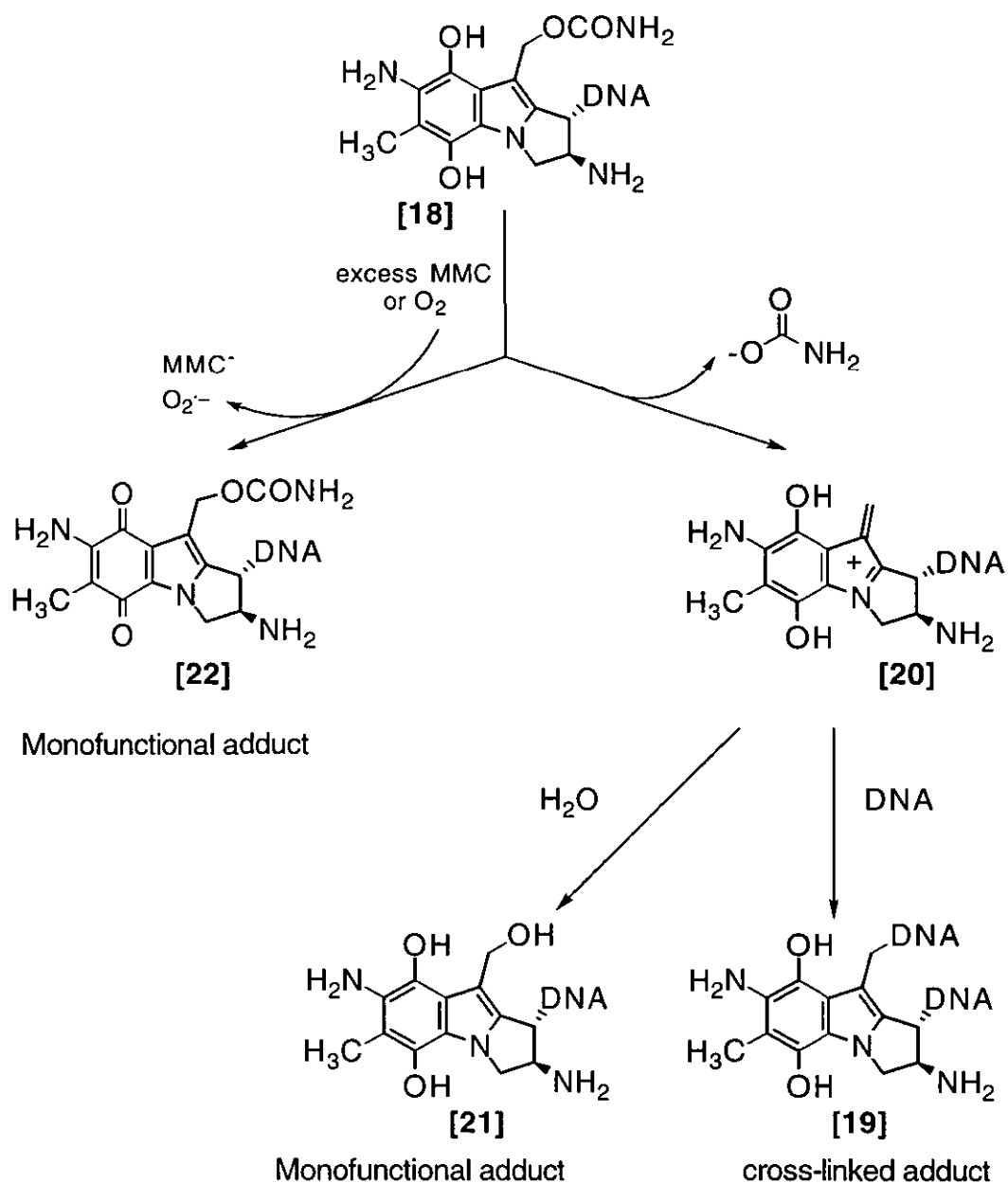
Scheme 6

MMC can also be rendered electrophilic at the C(10) position, in the case of the the C(1) drug-DNA adduct [18], a bis-DNA adduct will be formed [19], **Scheme 7**. Several mechanisms have been proposed for mitomycin C(10) transformations. In 1981 Moore and Czerniak suggested that C(10) nucleophilic processes occurred by an $\text{S}_{\text{N}}2$ pathway, path A,³⁵ while in 1987, Kohn and Zein proposed that C(10) nucleophilic reactions could proceed *via* an iminium ion [20], which can either undergo nucleophilic attack by DNA to provide the bis-DNA adduct [19] or by water to form the mono-DNA adduct [21].³⁶



Scheme 7

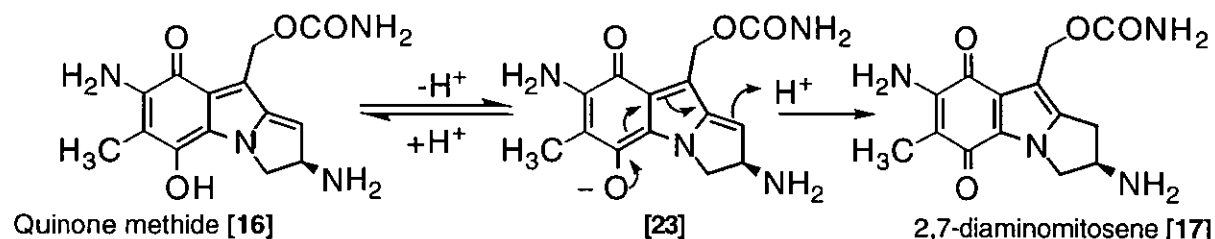
Tomasz *et al.* have proposed a mechanism for monofunctional and bifunctional alkylation of DNA by MMC, **Scheme 8**.³⁷ The monofunctional adduct [18] may react by either of two pathways: i) electron transfer (analogous to the mechanism proposed by Peterson and Fisher for autocatalytic activation of MMC³⁸) with unreacted MMC or oxygen, thereby losing its activated state and giving the monoadduct [22] as the end product, or ii) elimination the C(10) carbamoyl functionality to form the iminium species [20], which is receptive to a second nucleophilic attack giving bifunctionally alkylated end product [19]. However, in DNA only a fraction of the guanines are at cross-linking distance from another guanine, *i.e.*, those in a G*C, CG* or G*G sequence; only that fraction can give the cross-linked adduct [19] upon reaction with the iminium ion [20]. Any iminium ion [20] bound to other guanines will react with H₂O at the activated C(10) position, therefore forming the mono-alkylated product [21].



Scheme 8

Ross *et al.* studied the reductive activation of MMC in a cell free system, in order to confirm the role of individual reducing enzymes in the process.³⁹ Glutathione conjugates and also binding to DNA with interstrand cross-linking were observed during DTD-mediated activation. The major metabolite formed was 2,7-diaminomitosene [17]. However, it was discovered that increasing amounts of DNA cross-linking were observed during DTD-mediated reductive activation of MMC, as the pH was lowered from 7.8 to 5.8. Therefore, at high pH, activation of MMC resulted in the non cross-linking 2,7-diaminomitosene [17], whereas at low pH MMC was activated to form a cross-linking species. These results were in agreement with an earlier report that MMC DNA cross-linking processes were promoted at lower pH. Schiltz and Kohn have speculated that with increasing pH a significant amount of the conjugate base [23] of the quinone methide [16] is formed, **Scheme 9**. Formation of [23] should promote C(1)

electrophilic processes at the expense of C(1) nucleophilic reactions.³⁰ Although the 2,7-diaminomitosenone [17] formed can be activated to form a C(10) mono DNA adduct it is unable to form a cross-linking species. The formation of 2,7-diaminomitosenone [17] leads to a net increase in electrons, thereby increasing the likelihood of redox transformations with any mitomycin hydroquinone species present.

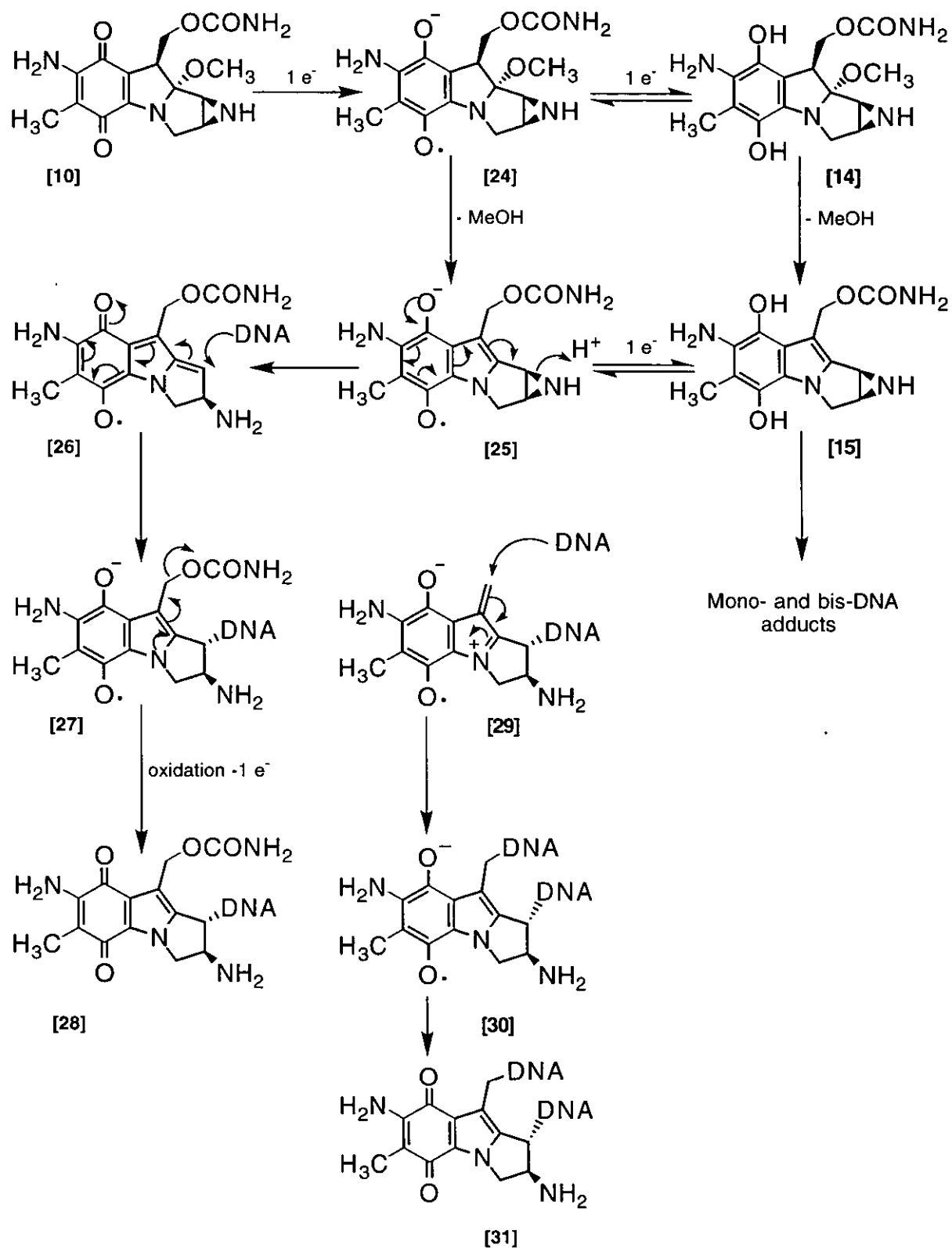


Scheme 9

Evidence has accumulated suggesting that the mitomycin semiquinone radical anion [24] is sufficiently activated to trigger the events that lead to the formation of a bis-alkylating agent.^{28,36,40-42} A proposed mechanism of MMC proceeding *via* one electron reduction to the semiquinone radical anion is shown, **Scheme 10**. The basic mechanisms leading to C(1) and C(10) activation are generally similar to the hydroquinone pathway (**Schemes 4, 7 and 8**). The initial step is single electron reduction of MMC to the semiquinone radical anion [24], which then readily loses methanol. The activated mitosenone semiquinone formed [25] is now rendered electrophilic both at C(1) and C(10) by opening of the aziridine ring which is assisted by the radical anion and by elimination of the carbamate group assisted by the push from the indole nitrogen, respectively. However, the first alkylation of DNA is thought to occur at C(1). Evidence for this comes from the isolation, after oxidation, of the C(1) mono-DNA adduct [28], but not a C(10) mono-DNA adduct. Hence subsequent protonation and aziridine ring opening affords the quinone methide species [26] capable of alkylation at C(1). The resulting C(1) mono-DNA adduct [27] can then be oxidised to the quinone mono-DNA adduct [28], or since it is still a semiquinone radical it can readily lose the carbamoyloxy substituent to afford the iminium compound [29] which can cross-link to give the bis C(1)/C(10) bis-DNA adduct [30]. The products [27] and [30] are probably reoxidised to the quinones [28] and [31] respectively during isolation and / or by electron transfer between various quinones and semiquinone radical anions. The C(1) mono-DNA adduct [27] can be deactivated to the unreactive quinone species [28] in a mechanism similar to the hydroquinone pathway, **Scheme 8**, by a redox reaction between oxygen or a quinone species.

However, the semiquinone radical anion [24] can also be reduced to leucomitomycin [14], either by further enzymatic reduction or by a redox reaction with another semiquinone radical anion. The hydroquinone [14] can then follow the reactive pathway previously mentioned for the mitomycin hydroquinone species, **Schemes 4, 7 and 8**, to form C(1) mono- or bis

C(1)/C(10) bis-DNA adducts. The mitosene semiquinone [25] may also be further reduced to leucoaziridomitosene [15], by a similar mechanism.



Scheme 10: A proposed mechanism for the mode of action of MMC

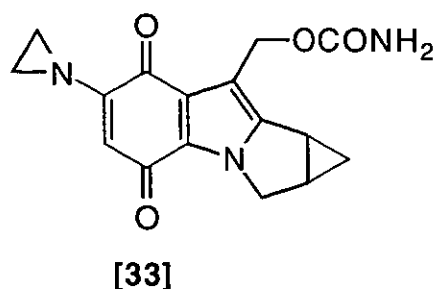
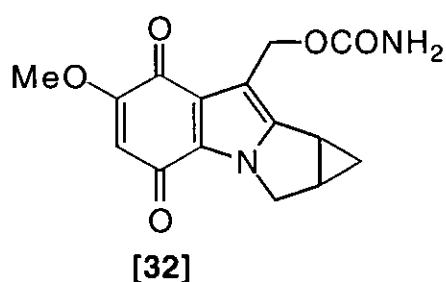
Various reductases have been implicated in the *in vivo* activation of MMC, including NAD(P)H: quinone oxidoreductase (DT-diaphorase (DTD)), NADPH: cytochrome P450 reductase (P450R), NADH: cytochrome *b*₅ (B5R), xanthine oxidase (XO) and xanthine dehydrogenase (XDH).⁴³⁻⁴⁷ MMC activation by P450R, B5R and XO is a one-electron reduction while activation by DTD and XHD is a two-electron reduction.

In toxicity experiments with cell lines high with DTD activity, it has been shown that toxicity of MMC is essentially unchanged under hypoxic versus aerobic conditions.⁴⁸ Conversely, with cell lines that are deficient in DTD, toxicity is markedly enhanced under hypoxic conditions. This suggests that DTD is the major activating enzyme in cells with high DTD expression under both aerobic and hypoxic conditions, but a one electron reduction is the major activating enzyme in DTD deficient cells. Although there still remains controversy as to the exact nature of the initial activation of MMC, what is for certain is that reduction of the quinone provides the driving force for the subsequent formation of electrophilic quinone methide species, and hence cytotoxic products.

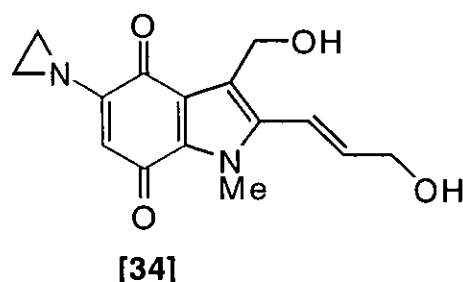
Despite the therapeutic value of MMC, adverse toxicity prohibits its prolonged use. This has led to an intense effort to develop semisynthetic variants with improved efficacy and decreased toxicity. The high level of functionality within MMC exacerbates chemical synthesis, to date only two complete synthesis of MMC have been accomplished. The problems associated with the need to accommodate highly interactive functionality in a rather complex matrix have been nicely summarised by Danishefsky "the synthesis of a mitomycin is the chemical equivalent of walking on egg shells".⁴⁹ However, the indolequinone nucleus appears an attractive target for the construction of bioreductive prodrugs. Compounds based upon reactive intermediates derived from the mechanistic pathways of MMC activation as well as MMC analogues will be briefly discussed.

1.2.5. Synthetic analogues based on the indolequinone moiety of the mitomycin natural products

The so called cyclopropamitosenes [32] are a series of compounds which contain the overall shape of the mitomycins, but the electrophilicity at the C(1) position is greatly reduced, by the substitution of the aziridine ring for the cyclopropane.^{50,51,52} It has been suggested that reduction of the quinone moiety provides a species in which the indole nitrogen lone pair of electrons are "unleashed". This is thought to cause the subsequent elimination of the carbamoyloxy group to provide a iminium species which is receptive to nucleophilic attack by DNA. The aziridino C(7) substituted cyclopropamitosene [33] shows under anaerobic *in vitro* studies in V79 cells 100 times more potency than MMC.

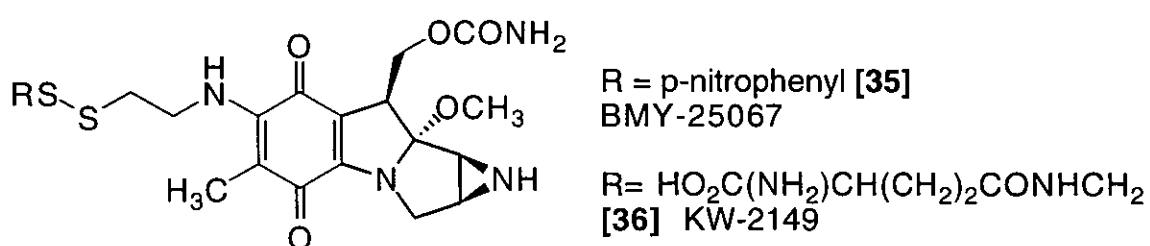


EO9 [34] is the leading compound in a novel class of indolequinones.⁵³ EO9 is considerably more active than MMC under both aerobic and anaerobic conditions and it does not cause the unwanted side effect myelosuppression. Reduction of the quinone is thought to activate the hydroxyl leaving groups and the aziridine, which gives a total of three reactive centres per molecule. Workman *et al.* have shown that EO9 is metabolised by DTD and so initial reduction of EO9 can be considered to result in the formation of the corresponding hydroquinone.⁵⁴



1.2.6. Analogues based on the mitomycin natural products

BMY-25067 [35]⁵⁵ and KW-2419 [36]⁵⁶ are analogues of MMC which bear a substituted disulfide substituent attached to the C(7) position. They have been found to be superior to MMC as anticancer agents and both are presently under clinical trials.



Each was designed on the basis of the hypothesis that their disulfide group may mediate nonenzymatic reduction of the quinone, initiated by thiols such as glutathione (GSH). Tomasz *et al.* have shown that cleavage of the disulfide by GSH resulted in a sulfoxy anion species which can undergo an intramolecular conjugate addition onto the quinone moiety.⁵⁷ The intermediate species can then undergo an internal redox reaction to yield a semiquinone radical anion, a species reminiscent to the semiquinone radical anion [25], Scheme 10. A reactive

pathway similar to the semiquinone radical anion [25] is believed to result in the cytotoxicity of these compounds. However, Kohn and Wang have proposed a novel ionic, nonreductive activation pathway for these mitomycins.⁵⁸

1.3. DNA topoisomerase enzymes

Both the extraordinary length of DNA in individual chromosomes and its double helical nature present a challenge in terms of accessibility, organisation and segregation of the genome in a dense intracellular environment, for example during transcription a copying protein, RNA polymerase remains somewhat stationary while the DNA screws through it, **Figure 2**.⁵⁹ If you have ever been fishing, and the current twists your bait around the end of your line, you will know that there would be an unholy mess, likewise it would cause big trouble if Nature did not find some way to relieve the twisting stress of DNA during its passage through a polymerase.

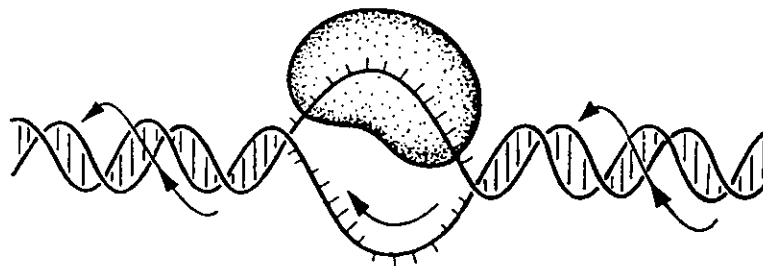


Figure 2: During DNA transcription the DNA screws through the RNA polymerase

Regulation of DNA topology is, thus, of critical importance to normal cellular function, and one of the principle means by which the cell accomplishes this is the DNA topoisomerase enzymes.⁶⁰ Topoisomerase enzymes cut either one or both of the two sugar-phosphate chains to allow some kind of motion in the DNA to relieve the torsional stress; they then re-connect the broken parts and thus leave an intact but relaxed DNA. Repeated application of this process to a tangled-up piece of DNA will eventually unravel it. By opening transient gates in the DNA backbone, DNA topoisomerases assist in solving helical winding and tangling problems of DNA during its replication, transcription, recombination and repair.

DNA topoisomerases can be classified into three evolutionary independent families or types: type I-5', type I-3', and type II.⁶¹ All known DNA topoisomerases share two characteristics. The first is their ability to cleave and reseal the phosphodiester backbone of DNA in two successive transesterification reactions. During the transient DNA cleavage stage, a covalent DNA-protein intermediate is formed between a tyrosine hydroxyl group of the topoisomerase and a DNA phosphate break site. Because the bond energy is conserved in the protein-DNA intermediate, no energy cofactor is required for this DNA breakage and rejoining activity. The

second characteristic is that once a topoisomerase-cleaved DNA intermediate is formed, the enzyme allows the severed DNA ends to come apart, opening a gate for the passage of single- or double-stranded DNA segment.

Type I-5' topoisomerases bind, cleave and open transient gates in single stranded DNA segments, in order to allow the passage of another single strand- or double-stranded DNA segment. In the DNA cleavage stage, the protein-DNA covalent intermediate is formed between a tyrosyl residue and the 5'-phosphate at the DNA break site. Type I-3' topoisomerases bind preferentially to double-stranded DNA, and cleave one of the DNA strands of the duplex by forming a protein-DNA covalent intermediate between a tyrosyl residue and the 3'-phosphate at the break site. During the DNA cleavage stage, the unbroken strand can pass through this enzyme-operated nick and release the twisting stress of a DNA double helix

Unlike type I topoisomerases, type II topoisomerase (topo II) is ATP dependent and functions as a paired molecule. In eukaryotes, the two halves of the enzyme are formed by two identical polypeptide chains, ranging from 160 to 180 kDa each. Harrison *et al.* have recently reported the X-ray crystal structure of a large fragment of yeast DNA topo II, which reveals a heart-shaped dimeric protein with a large central hole.⁶² A molecular model of the enzyme has been posulated, whereby topo II acts as an ATP-modulated clamp with two sets of jaws at opposite ends, connected by multiple joints. An enzyme with bound DNA can admit a second DNA duplex through one set of jaws, transport it through the cleaved first duplex, and expel it through the set of jaws, like a boat passing through a lock.

The two-gate mechanism of type II DNA topoisomerases is shown, **Figure 3**. In (a) the enzyme is in the form of an open clamp, and is bound to a DNA duplex, which will become the DNA gate (the G-segment). A second DNA duplex (the T-segment) can go in and out of the G-segment-bound enzyme so long as the clamp remains in the open conformation. In (b) binding of ATP closes the protein gate composed of the amino-terminal domains of each half of the homodimer. If closure results in the capture of the second DNA duplex, it will pass through the entire interface between the two halves of the topoisomerase, cross the transiently broken G-segment of DNA and exit through a second protein gate located at the opposite site of the entrance gate. In (c) once the T-segment is outside, this second gate closes and the rejoined G-segment remains trapped inside the protein clamp. The enzyme returns to the open-clamp form, bound to a G-segment, after ATP hydrolysis and release of the products. The black dots in (b) indicate the DNA 5'-phosphodiester linkages between the G-segment of DNA and the topoisomerase, and the (N) denotes the amino-terminal domains of the enzyme. If the G- and T-segments reside in the same DNA molecule, the topoisomerase may either eliminate DNA supercoils or interconvert knotted forms in such a DNA molecule. If the G- and T-segments

belong to different DNA molecules, the topoisomerase will catalyse their catenation or decatenation.

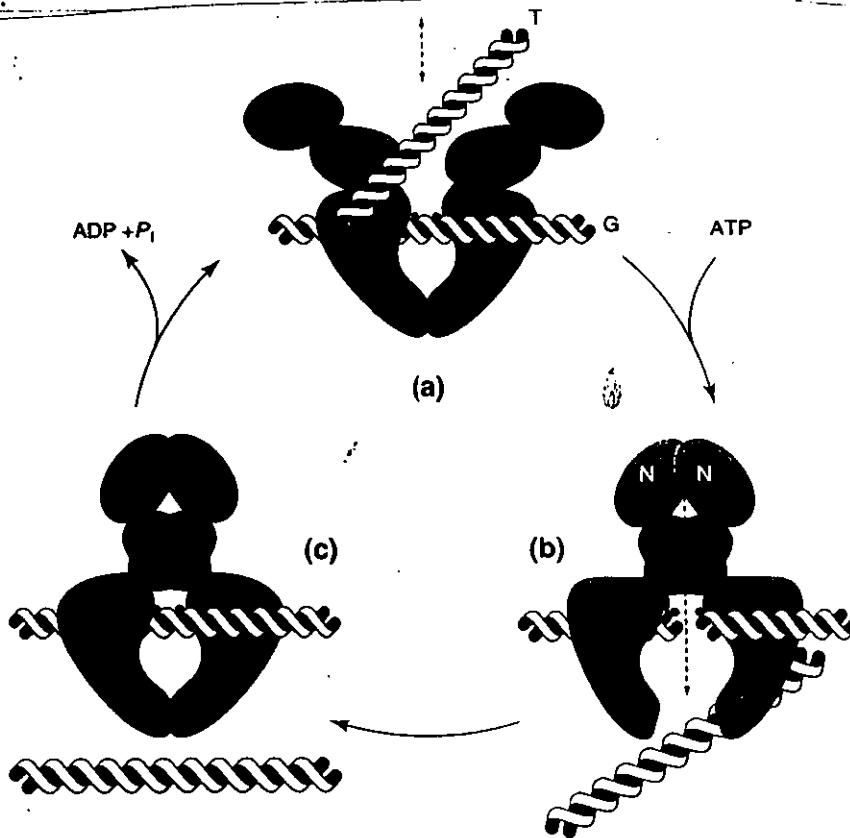


Figure 3: *The two gate mechanism of topo II*

The addition of a strong detergent such as sodium dodecyl sulfate (SDS) to a topo II reaction results in the cleavage of a small proportion of the DNA molecule and the covalent linking of a topoisomerase polypeptide to the 5'-phosphoryl end of each broken DNA strand.⁶³ Both single- and double-strand DNA breaks are produced. The cleavage reaction is rapid and can be reversed by the addition of 0.5 M NaCl prior to detergent addition. DNA remains superhelical after salt reversal, suggesting that no free ends are generated in this partial reaction.⁶³ A simple two state model has been proposed for this partial reaction, **Figure 4**. A topoisomerase II-DNA cleavable complex (C), the presumed key covalent intermediate in the strand-passing reaction, is proposed to be in equilibrium with at least one other topo II-DNA complex, the non-cleavable complex (B).⁶⁴ Exposure of the cleavable complex to a protein denaturant, such as a strong detergent, results in DNA cleavage and the covalent linking of a topo II subunit to the 5'-phosphoryl end of the broken strand. Interaction of a second DNA segment with the cleavable complex presumably triggers the strand passing reaction (D).

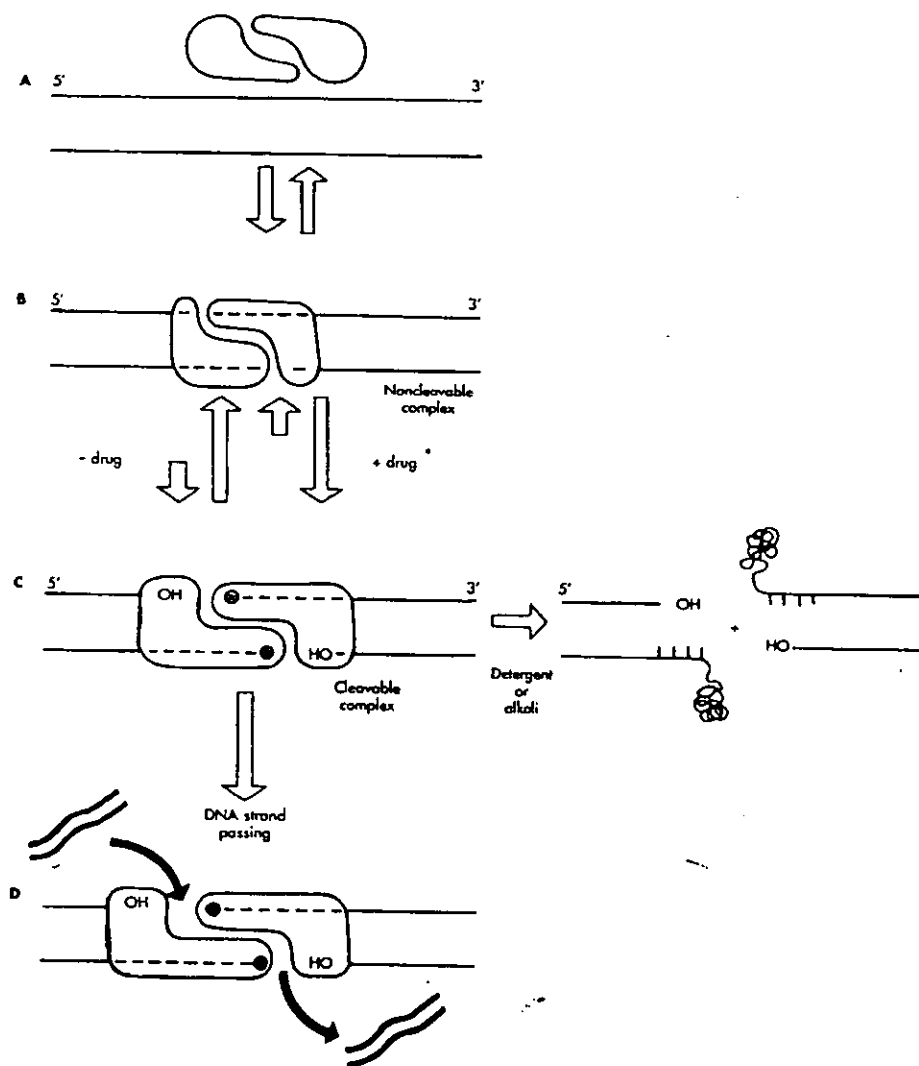


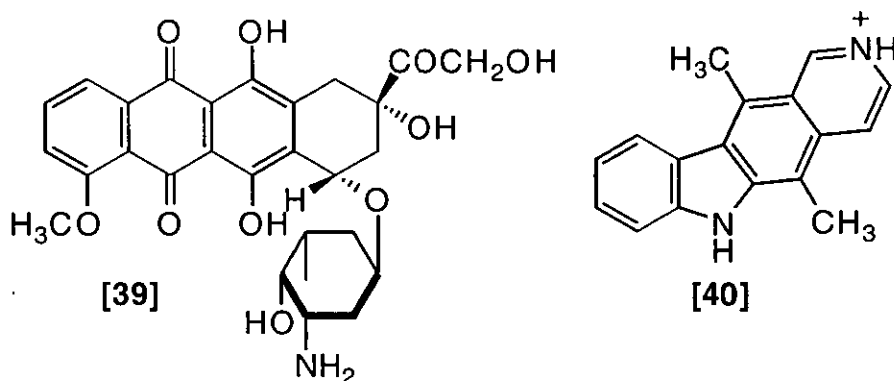
Figure 4: A schematic representation of the mechanism of topoisomerase II

1.3.1. DNA topoisomerase inhibitors as anticancer agents

Studies have shown that both proliferating "normal" and tumour cells contain high levels of topoisomerase II, however, topoisomerase II is regulated very differently in tumour cells.⁶⁵ In tumour cells high topoisomerase II activity is important for cell proliferation and in turn tumour cells have been shown to be sensitive to topoisomerase II poisons. Oestrogen stimulation of human breast cancer cells enhances the induction of DNA cleavage by such topoisomerase II poisons, and the increased DNA cleavage correlates with enhanced cytotoxicity. Since proliferating tumour cells exhibit higher levels of both topoisomerase II and hormone receptors than do "normal" cells, rational new strategies for the selectivity and efficacy of a systemic therapy in hormone-dependent malignancies, such as breast, prostate and testicular cancer, have been suggested.⁶⁶

complex by forming a non-productive drug-enzyme-DNA ternary complex. Studies with other intercalative antitumour drugs, such as adriamycin [39] and ellipticine [40], produced similar results.⁷³

The results imply that it is formation of the cleavable complex and not the subsequent loss of topo II activity which is responsible for lethality. The putative role of topo II in mediating antitumour activity is a somewhat novel one in cancer pharmacology, namely that the drug action does not result from blocking a normal enzyme function but rather by subverting it in such a way as to render the enzyme a lethal instrument. In a sense, the enzyme becomes a required co-factor for drug action.

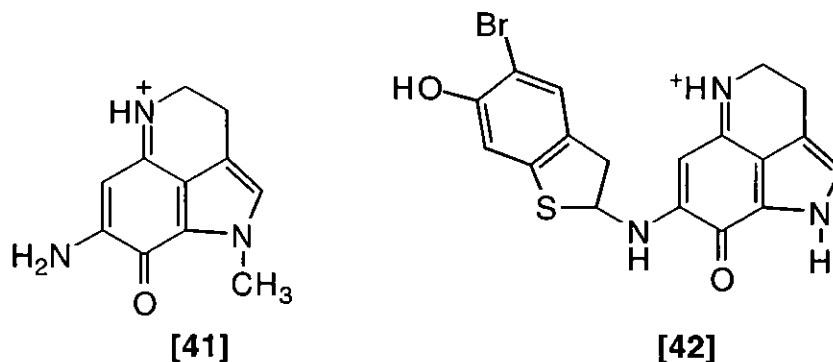


There is little information in mammalian cells regarding the mechanism by which the cleavable complex results in cell death. Inhibition of cell division, DNA synthesis and DNA degradation have all been shown to be associated with topo II poisons.⁷³ It has been speculated that increased sensitivity to oncogenes, expressed in malignant cells, could contribute to the specificity of these antitumour agents.

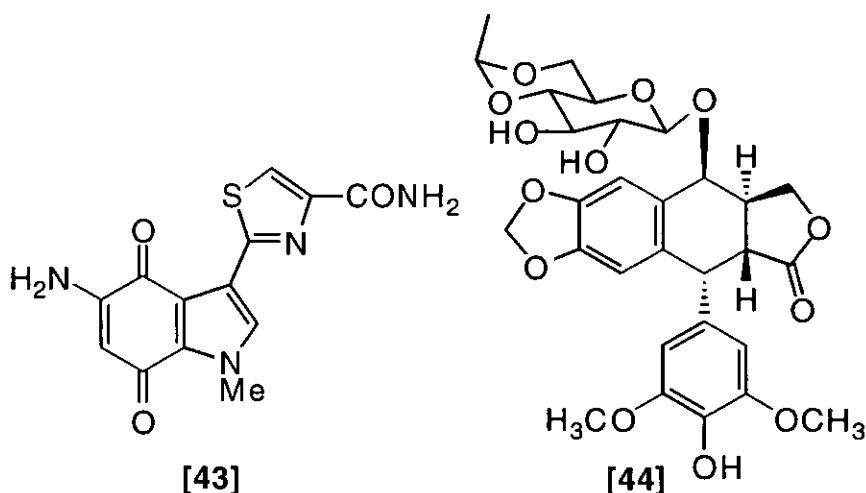
1.3.3. DNA topoisomerase inhibitors

Due to the increasing importance of DNA topoisomerase the search for compounds which have a high inhibitory effect has been initiated. This search has followed screening of new natural products that have been elucidated as well as synthetic analogues derived from known topo II inhibitors.

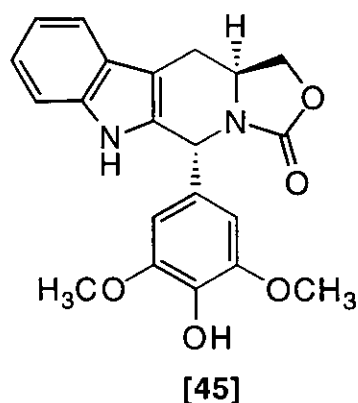
Of the naturally occurring compounds the indolequinones, makaluvamine A [41] and makaluvamine F [42] have been shown to be potent inhibitors of topo II. The malaluvamines were discovered from the Fijian marine sponge *Zyzzya sp.*⁷⁴



Also recently described is the natural product BE 10988 [43], which was isolated from culture broths by Japanese workers, which has been claimed to be a potent inhibitor of topo II.⁷⁵ Of the synthetic topo II inhibitors etoposide [44] (VP 16, 'Vespesid') is the most promising to date, this agent is a semisynthetic analogue of the natural compound podophylotoxin. Etoposide seems to be of most clinical value in non-small-cell lung cancer and certain types of testicular cancer resistant to other therapies.⁷³



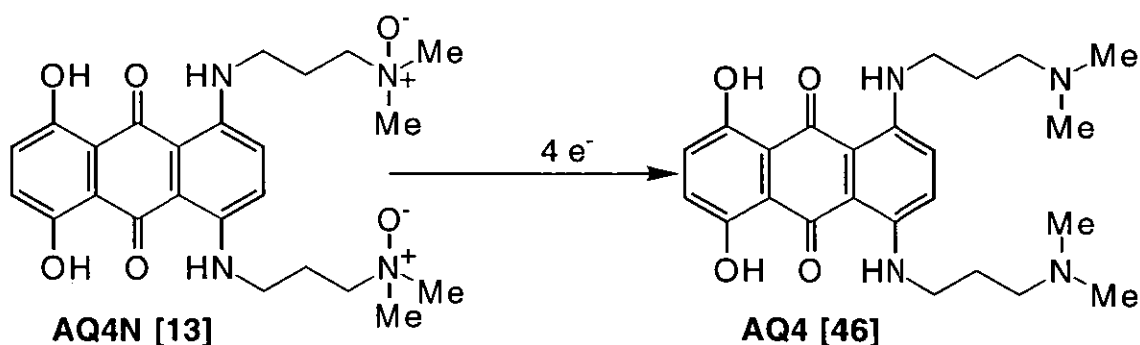
Macdonald *et al.* have hypothesised in the ternary drug-DNA-enzyme 'cleavable complex' the principle interaction of the drug is with DNA and that drug-DNA complexation distorts the DNA duplex in a fashion similar to an intermediate in the enzymes catalytic cycle.⁷⁶ The model postulates three domains: the first, an aromatic domain proposed to be involved in DNA intercalation or 'intercalation like' DNA association; second, a substituent appended to the planar domain, which has hydrogen bonding functionality and is proposed to interact with the DNA minor groove; and thirdly, a domain of considerable structural diversity, which extends above the intercalation region and is also proposed to lie in the DNA minor groove. Macdonald *et al.* have used this model to synthesise hybrid molecules which are composed of subunits of existing topo II active agents. Azatoxin [45] is a unique topo II-directed agent which represents a hybrid of the ellipticine [40] and etoposide [44] nuclei.⁷⁷



The molecular model for the interaction of drugs with topo II and DNA has been refined by Capraico, who has suggested that topo II inhibitors intercalate between the base pairs in the enzyme-DNA complex and that this intercalation interferes with the enzyme's activity and prevents the resealing of DNA.⁷⁸

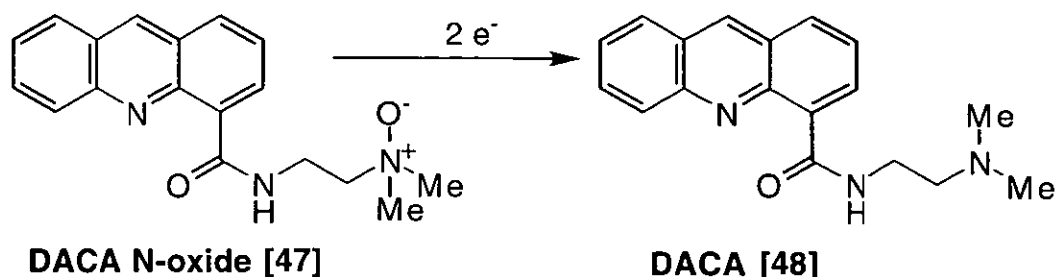
1.3.4. Bioreductive prodrugs of topo II inhibitors

As mentioned previously the need for selective anticancer agents is a crucial factor in the synthesis of novel compounds. Patterson *et al.* have applied the idea of a bioreductive prodrug to a topo II inhibitor, **Scheme 11**.²¹ The alkylaminoanthraquinone **[13]** (AQ4N) is a synthetic DNA affinic agent broadly based on the anthracycline antibiotics. AQ4N **[13]** is metabolised to AQ4 **[46]** which is a potent topo II inhibitor in a 4 electron process, possibly by cytochrome P450.



Scheme 11

Denny *et al.* have also applied the postulate of a bioreductive prodrug to a topo II inhibitor, and again the reduction of an aliphatic *N*-oxide provided the bioreductive focus, **Scheme 12**. DACA **[48]** is a potent cytotoxin, a DNA intercalator, and an inhibitor of topo II. It shows strong activity against some murine solid tumours, and is currently in phase I clinical trials as an anticancer agent. DACA *N*-oxide **[47]** was designed as a non-toxic prodrug which can be reduced to the corresponding amine **[48]** under hypoxic conditions.⁷⁹



Scheme 12

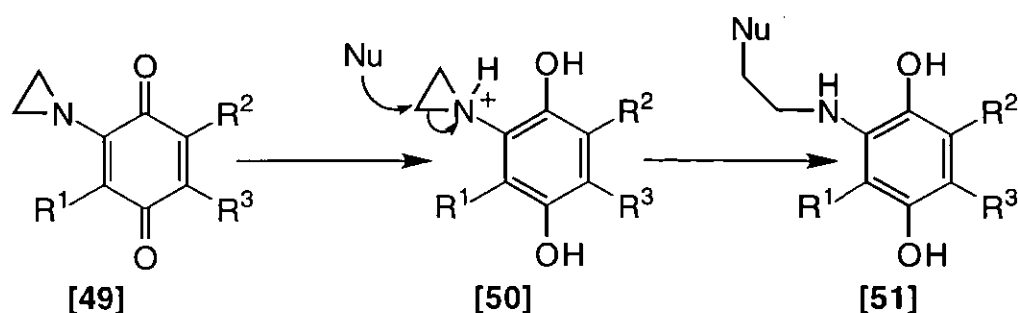
1.4. Research aims

The indolequinone moiety has been shown to be incorporated in a wide range of bioreductive prodrugs, of which MMC [10] is a leading example. Biological reduction of the quinone provides the initial impetus for the activation into a cytotoxic species. The unique thiazolyindolequinone structure of the natural compound BE 10988 [43] suggested that analogues could be synthesised which are specifically activated under a hypoxic environment into cytotoxic agents. The nature of the cytotoxic effect being a consequence of either:

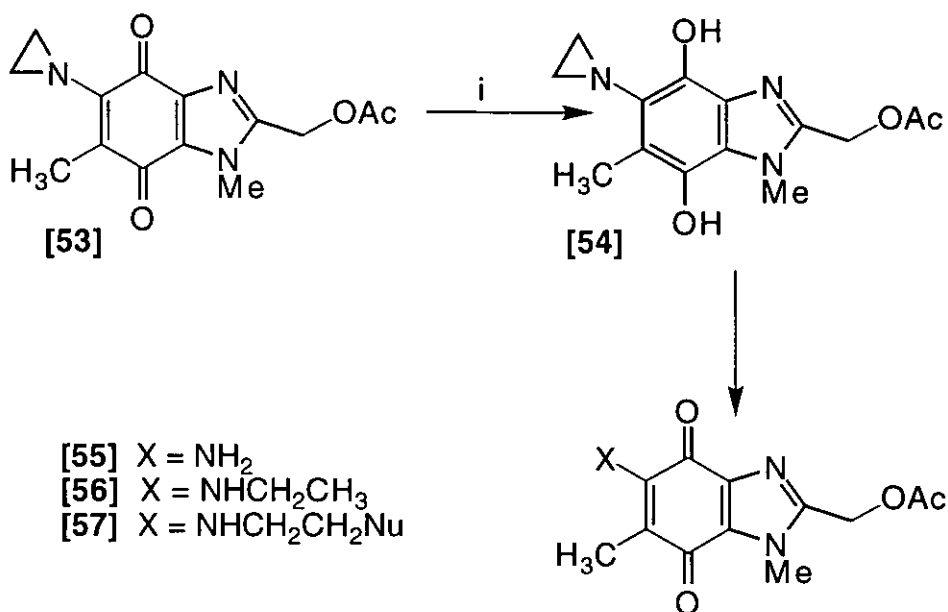
- i) DNA alkylation, as a result of activation into a highly electrophilic species;
- ii) inhibition of the topoisomerase II, based on the reported potent topo II inhibition of BE 10988 [43].

1.4.1 Aziridinyl quinones as bioreductive alkylating agents

Functionality was required on the indolequinone nucleus of BE 10988 [43] which would be activated following reduction of the quinone into a species capable of alkylating DNA. The attachment of a quinone moiety on the nitrogen of an aziridine ring discourages nucleophilic ring opening, as in structure [49], **Scheme 13**. However, reduction of the quinone facilitates protonation to form a aziridinium ion intermediate [50] and hence nucleophilic ring opening to form the ring opened amino derivative [51].⁸⁰

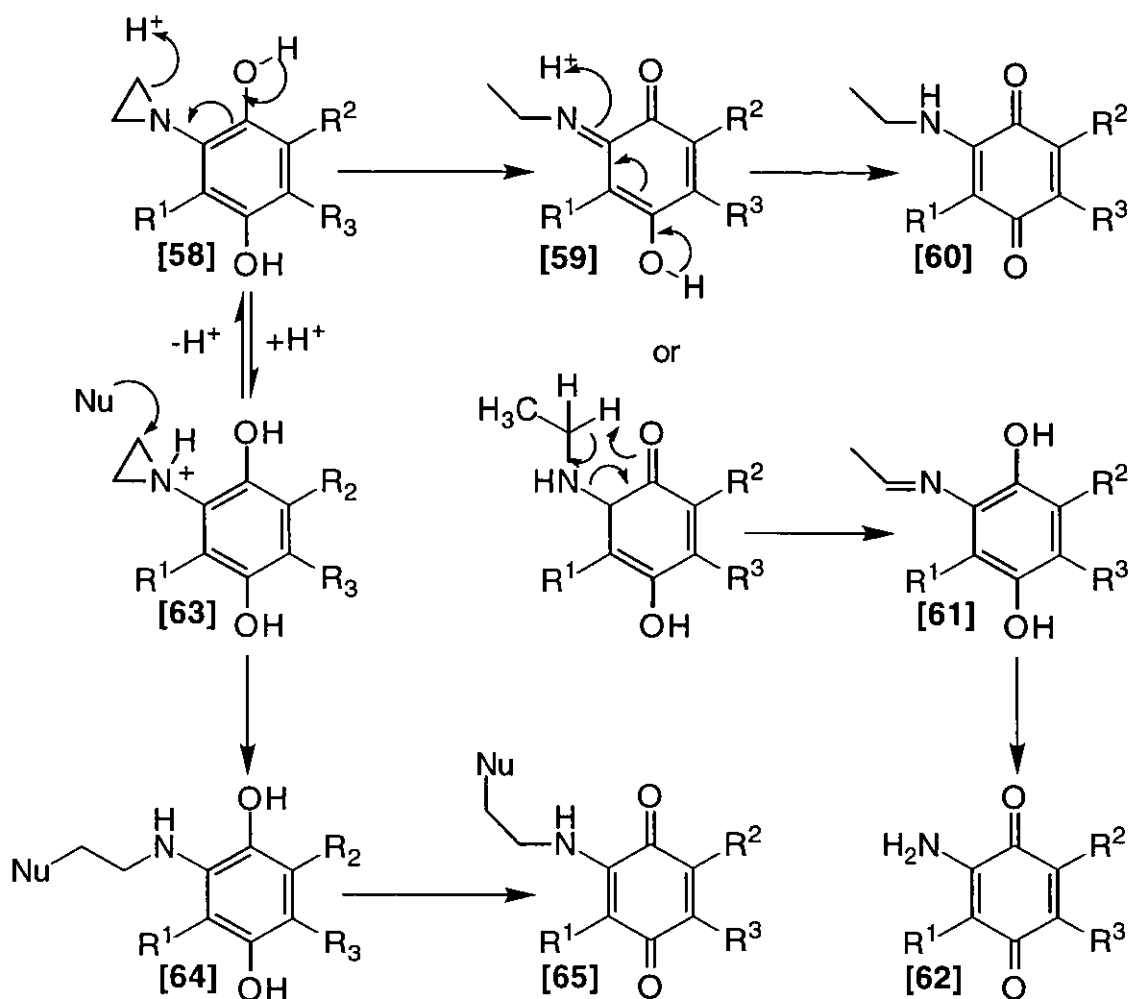


Scheme 13



Scheme 14: Reagents and conditions : i) H₂, Pd on carbon, pH 7.4 tris buffer

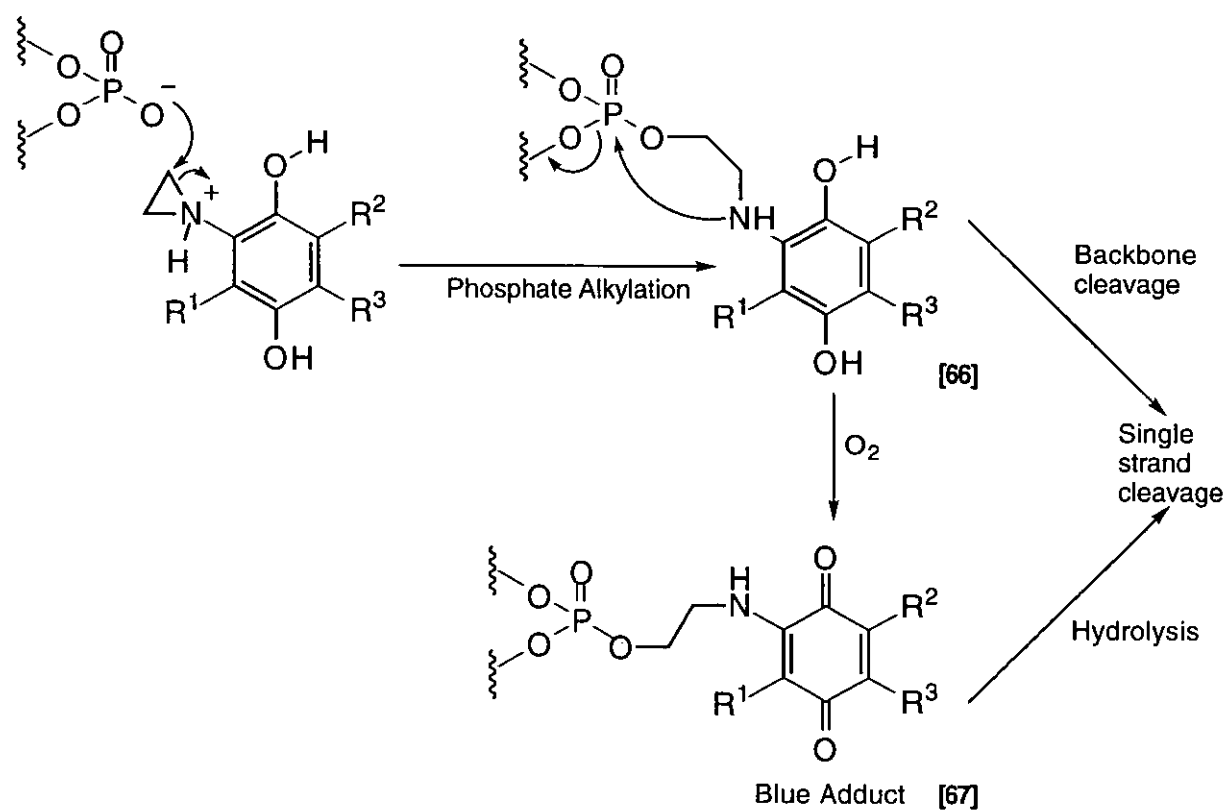
Bourah and Skibo have suggested a possible mechanism for the hydrolysis products formed from the aziridino moiety following initial reduction of the quinone to a hydroquinone, **Scheme 15**.⁸⁵ The aziridiny hydroquinone [58] can either undergo proton assisted ring opening to afford the iminoquinone species [59]. This can then either undergo a further protonation to give the amino quinone [60], or can perform a [3,3] sigmatropic rearrangement to create the intermediate imino species [61]. Hydrolysis of the imino hydroquinone yields the amino quinone [62]. However, protonation of the aziridine forms the aziridinium ion species [63] which is labile to nucleophilic ring opening, and so nucleophilic attack produces the substituted amino hydroquinone [64], which, after oxidation provides the quinone [65].



Scheme 15

The mechanism of BI alkylation involves oxygen alkylation of the DNA phosphate backbone, **Scheme 16**.^{86,87} Trapping of the phosphate oxygen anion by reduced quinone [63] affords a phosphotriester [66]. Aerobic workup of the alkylation reaction then provides a blue DNA adduct [67]. Hydrolysis of this adduct in base results in cleavage of the DNA at phosphotriester linkages. In the absence of oxygen, the hydroquinone form of the adduct [66] can facilitate backbone cleavage by internal nucleophilic attack to result in single-strand cleavage. The single-strand cleavage sites were found to be exclusive to guanine and adenine bases.

The G and A specificity was explained by complexation of the reduced BI [54] in the major groove by hydrogen bonding to G and A bases, resulting in placement of the aziridinyl group proximal to the phosphate backbone. Molecular models with the similar hydroquinone BI analogue [68] showed that major-groove binding as a result of Hoogsteen-type hydrogen bonds could result in phosphate backbone alkylation, **Figure 5**. The PBI hydroquinone [68] contained additional hydrogen bonding functionality in the form of the carbamate group, the aziridine, the benzimidazole nitrogen and the hydroquinone.



Scheme 16

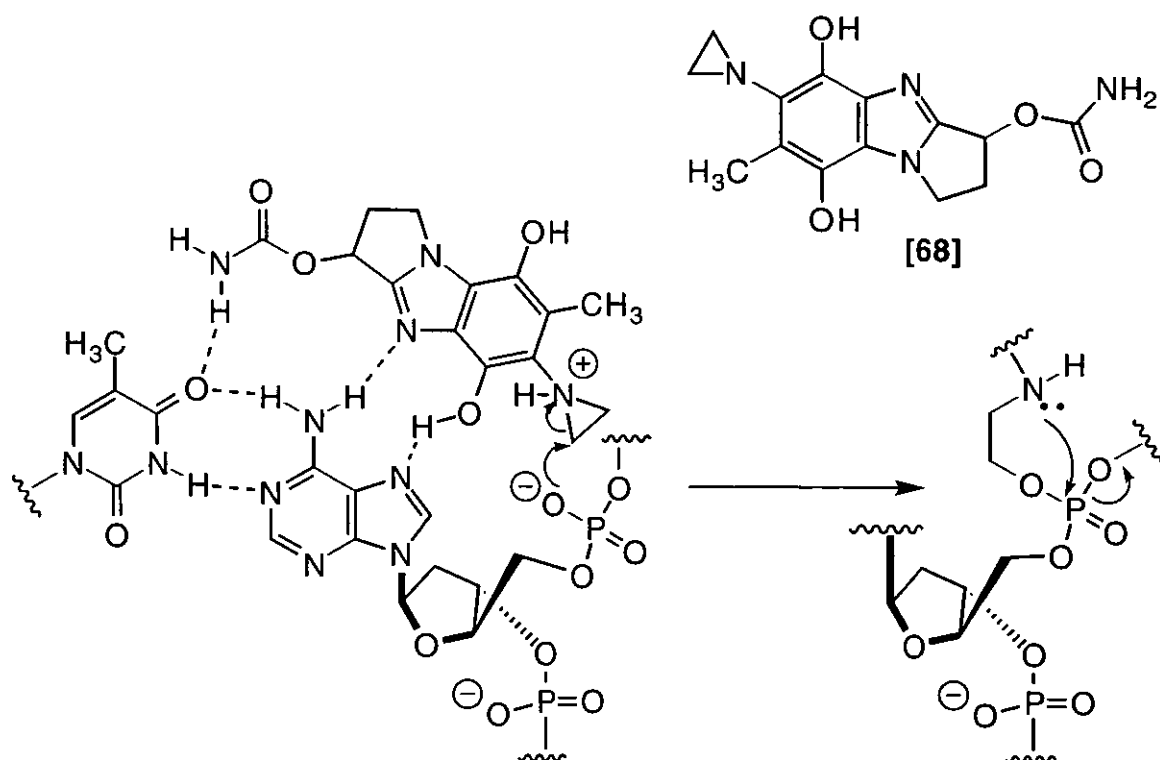
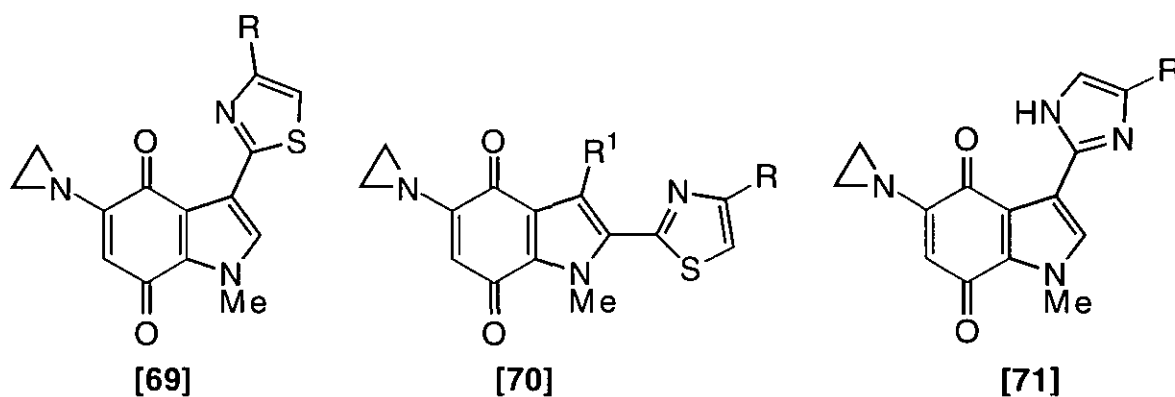


Figure 5: Hoogsten-type hydrogen bonding of the reduced PBI allows phosphate backbone alkylation of DNA

The aziridinyl quinone EO9 [34] has a similar cytotoxicity profile to the benzimidazole-based DNA cleaving agents. In addition, both EO9 and the BI have the unique property of not suppressing bone marrow. Since EO9 possesses some structural similarities to the BI agents, both classes of antitumour agents have been postulated to have a similar cytotoxicity mechanism.

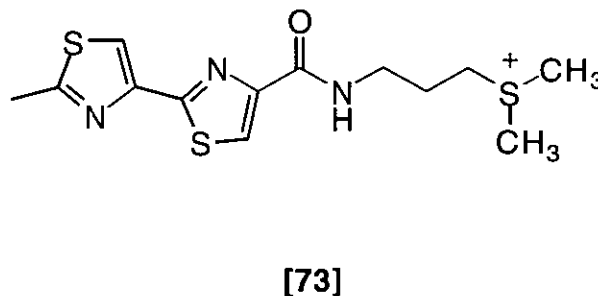
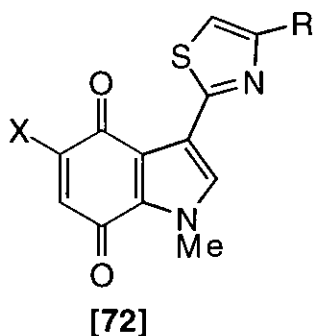
Aziridino quinones have been shown to be reductively activated to result in a species capable of alkylating the phosphate backbone of DNA. Nucleophile-assisted breakdown of the resulting phosphotriester DNA results in cleavage of DNA. The addition of a aziridine moiety at the C(5) position on the thiazolyindolequinone BE 10988 [43] suggested that the synthesis of a bio-reductive alkylating agent could be accomplished. The aziridino thiazolyindolequinone compounds [69] and [70] were therefore selected as synthetic targets. The thiazolyindolequinone [69] being the C(5) aziridinyl analogue of BE 10988. The C(5) aziridinyl thiazolyindolequinone [70] was selected for two reasons: firstly, to study the biological effect produced by changing the position of the thiazole ring; and secondly, the indolequinone C(3) activated to form a iminium ion which can alkylate DNA, *e.g.* MMC [10] contains the carbamate $\text{CH}_2\text{OC(O)NH}_2$ functionality at the indole C(3) position. Therefore the addition of similar functionality at the C(3) position on [70] was hypothesised to result in the formation of an electrophilic species following quinone reduction, *e.g.* $\text{R}^1 = \text{CH}_2\text{OH}$ or $\text{CH}_2\text{OCONH}_2$.



The mechanism of BI cytotoxicity has been shown to involve DNA cleavage as a result of binding to the major-groove followed by phosphate backbone alkylation. The major-groove binding was thought to be a result of Hoogsteen-type hydrogen bonds, between the drug and DNA, **Figure 5**. The pK_a of the thiazole nitrogen is 2.53 and would therefore be expected to show poor hydrogen bonding acceptor properties. The C(5) aziridinyl imidazolyl-indolequinone compound [71] was postulated as a compound more likely to result in hydrogen bond formation with DNA. The pK_a of imidazole is 7.0 whereas for benzimidazole the pK_a is 5.3. The increased basicity of theazole nitrogen in imidazole over thiazole will greatly enhance hydrogen bond acceptor properties.

1.4.2. Investigation into the topo II inhibitory affect of the thiazolyindolequinone natural compound BE 10988 [43]

The thiazolyindolequinone natural compound BE 10988 [43] was reported to act as a inhibitor of topo II. A major requirement for topo II inhibitors is that they possess DNA intercalation or 'intercalation like' DNA association properties. Lerman has proposed a model for DNA intercalators, in that they are flat heteroaromatic species which insert between two adjacent layers of base pairs and are held there by Van der Waals forces.⁸⁸ A consequence of DNA alkylation is that the DNA helix is partially lengthened and thus somewhat unwound, in comparison to the original structure. Upon intercalation the average separation between two stacked base pairs increases from 3-4 Å to approximately 7-8 Å. If there is a sidechain present in the molecule, it can interact with the phosphates of the DNA backbone. The thiazolyindolequinone natural compound BE 10988 [43] contains the aromatic thiazole moiety. Although a single thiazole ring would not be expected to result in DNA intercalation, bithiazoles are known to result in 'intercalation like' DNA association. The bithiazole [73] is a side chain of the chemotherapeutic agent bleomycin A₂.⁸⁹ Stubbe *et al.* have established unambiguously that the mode of binding of Co bleomycin A₂ green involves partial intercalation of the bithiazole side chain and the binding is with the DNA minor groove.⁹⁰ (see Chapter 3 for a more detailed dissusion of bleomycin and related products). Elaboration of the thiazole moiety on the thiazolyindolequinone [72] to the bithiazole [73] would be expected to significantly increase DNA intercalation.



The substituent X attached to the quinone ring in [72] will influence the biological activity in several ways:

- i) the reduction potential of the indolequinone moiety is dependent upon substituents attached to the quinone. The ease of reduction is associated with the electron donating power of the quinone substituent at C(5). Increased electron donation into the quinone moiety consequently increases the electropotential of the quinone, therefore augmenting the energy required for reduction;
- ii) modification of the C(5) substituent may change the partition coefficient of the drug, and hence affect the cell penetration of the chemotherapeutic agent;

- iii) The C(5) can alter the stability, reactivity, and toxicity of the activated drug;
- iv) changes in the steric size and composition of the C(5) substituent may influence the ability of the drug candidate to bind (*i.e.*, van der Waals, hydrogen bond) to DNA.

Therefore, a series of amino and alkoxy derivatives with varying electron donating abilities were selected for incorporation on to the C(5) position.

1.5. Conclusions

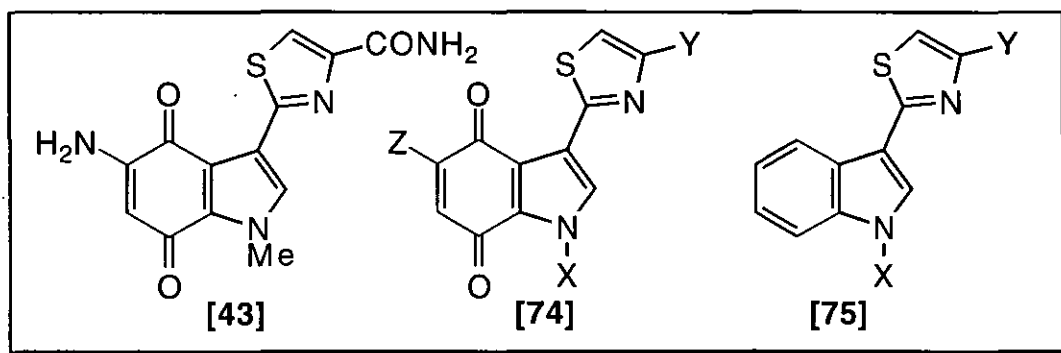
- Although hypoxia can be regarded as being deleterious towards conventional cancer therapy, bioreductive prodrug chemotherapy can be utilised as a means of forming hypoxia selective cytotoxic species.
- Bioreductive prodrugs can be considered to comprise of three domains: trigger and effector units joined by a linker mechanism.
- DNA alkylating agents have been shown to be applicable towards bioreductive prodrug chemotherapy, with particular reference to indolequinones.
- DNA topoisomerase II inhibitors have also been shown to be applicable to a bioreductive activation process.
- Aziridino quinone moieties have been shown to act as bioreductive prodrugs. Hence bioreductive aziridino thiazolylindolequinone analogues based on the natural compound BE 10988 [43] have been hypothesised.
- The incorporation of functionality known to result in 'intercalation like' DNA association onto the thiazolylindolequinone nucleus was hypothesised to result in an increase in topo II activity.

Chapter 2

The synthesis of thiazolylindoles.

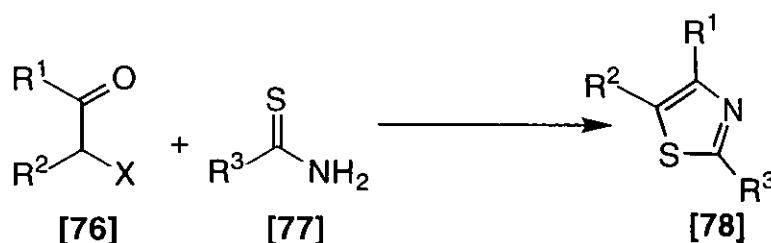
2.1. Introduction

Before embarking upon the synthesis of complicated indolequinone analogues based on BE 10988 [43], with the generic structure [74], it was decided to carry out the synthesis of 'simple' thiazolyindoles loosely based on the structure [75], in order to gain an insight into the chemistry of these species. The synthesis of 'simple' thiazolyindole analogues will be discussed within this chapter and the synthesis of the more structurally challenging indolequinones [74] in chapter 3.

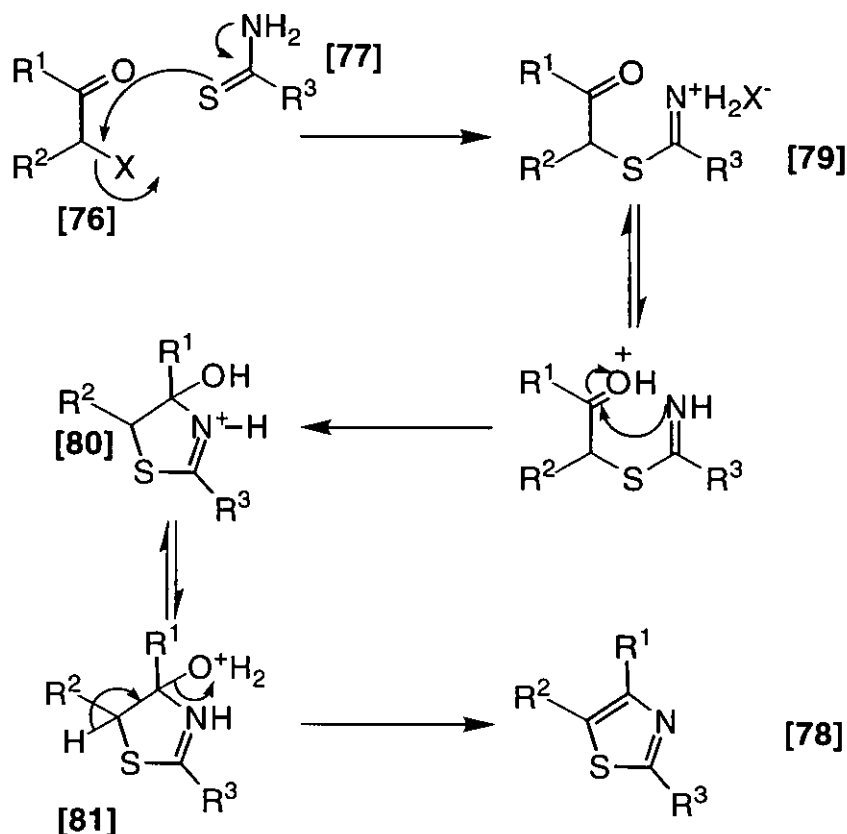


2.2. The synthesis of thiazoles by the Hantzsch reaction

The overall strategy for the construction of the thiazole ring was by a Hantzsch thiazole reaction.⁹¹ The Hantzsch synthesis is the cyclisation of an α -halo or α -hydroxycarbonyl compound [76] ($X = \text{Cl}, \text{Br}, \text{I}$ or OH) with a thioamide [77], shown below.⁹²

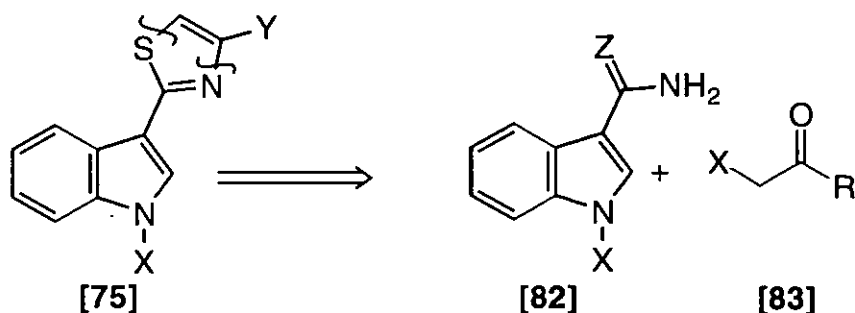


The regiochemistry of the Hantzsch reaction can be explained upon close inspection of the reaction mechanism, **Scheme 17**. Nucleophilic substitution by sulfur, on the halogen of the α -halo ketone, occurs first to give the open chain thioketone [79] which then undergoes proton transfer followed by *5-Exo-Trig* cyclisation to form the intermediate 4-hydroxy- Δ -thiazoline [80]. Acid catalysed dehydration in protic solvents of the protonated intermediate [81] provides the aromatic thiazole species [78]. Thiazole is an electron rich aromatic compound which can be considered to apply to the Hückel ($4n + 2\pi$) electron rule and so the resonance stabilisation energy of the aromatic system provides the driving force for the reaction.



Scheme 17: *The mechanism of the Hantzsch reaction*

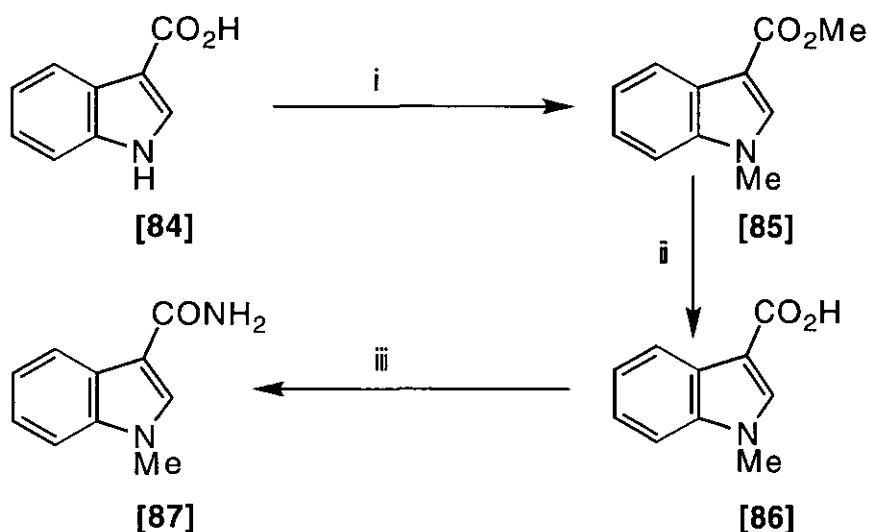
Retrosynthetic analysis of the generic thiazolyindole [75], **Scheme 18**, applying the Hantzsch reaction for the construction of the thiazole moiety yields the indole thioamide [82] (Z= S) and α -haloketone [83] reaction precursors. The indole thioamide [75] can be derived by functional group interconversion from a corresponding amide [82] (Z= O). Unfortunately indole substituted amides were not commercially available, therefore, an independent synthesis of these reaction precursors was required.



Scheme 18: *Retrosynthesis of the generic thiazolyindole [75]*

2.3. The synthesis of various thiazoles containing a indole moiety at the thiazole C(2) position, by the Hantzsch reaction

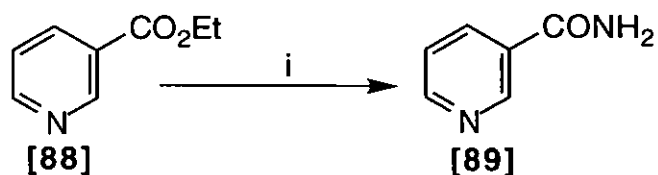
The conventional synthesis of primary amides involves the reaction of an acyl halide with ammonia.⁹³ Commercially available indole-3-carboxylic acid [84] was both *N*- and *O*-methylated by reaction with iodomethane in DMF under a nitrogen atmosphere employing potassium hydride to form [85] in 95% yield, **Scheme 19**. IR spectroscopy of the indole derivative [85] showed the characteristic ester carbonyl C=O absorption at 1687 cm⁻¹, the low value can be explained by electron delocalisation from the indole nitrogen with the carbonyl. Hydrolysis of the methyl ester functionality afforded the indole carboxylic acid compound [86] in 81% yield. Activation of the carboxylic acid functionality to an acyl chloride derivative was accomplished by reaction of the indole carboxylic acid [86] with 20 equivalents of thionyl chloride in refluxing dichloromethane. *in situ* Monitoring of the reaction by IR spectroscopy showed the appearance of the acyl chloride C=O absorption at 1766 cm⁻¹ and the loss of the carboxylic acid C=O absorption at 1710 cm⁻¹ after 2 h. The excess thionyl chloride and dichloromethane were carefully removed by evaporation [CAUTION thionyl chloride is a lachrymator], and the crude indole acyl chloride was reacted with liquid ammonia at -78°C to form the desired indole amide [87] in 60% yield.



Scheme 19: Reagents and conditions i) KH, DMF, MeI, N₂, 95%, ii) KOH, THF, H₂O, reflux, 16 h, 81%, iii) SOCl₂, CH₂Cl₂ then NH₃, CH₂Cl₂, -78°C, 60%

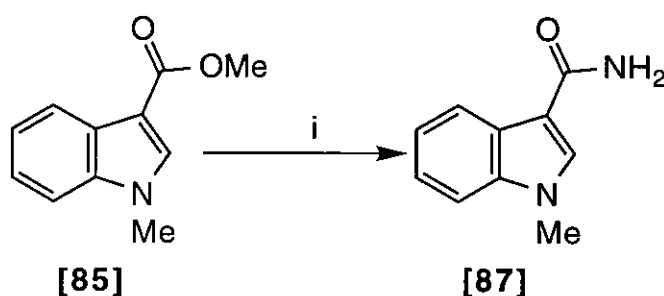
However, upon scale-up, the amination reaction proved capricious and so it was decided to synthesise the key indole amide [87] via a more efficient process. Closer inspection of the literature revealed a variety of methods for the direct conversion of carboxylic esters into primary carboxamides. The most commonly used protocol appeared to be that pioneered by Weinreb *et al.*,^{94, 95} in which an ester is reacted with trimethylaluminum and ammonium

chloride in benzene to form the corresponding primary amide in moderate to good yields, *e.g.* the conversion of ethyl nicotinate [88] to nicotinamide [89] in 32% yield, **Scheme 20**.



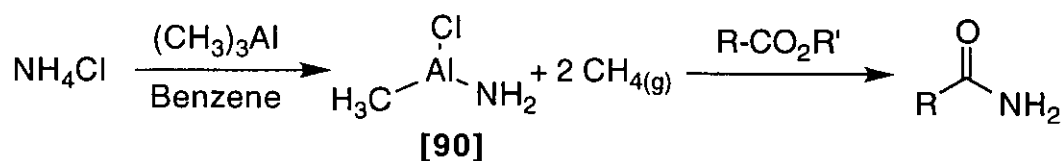
Scheme 20: Reagents and conditions i) Me_3Al , NH_4Cl , benzene, 60°C , 16h, 32%

Reaction of the indole ester derivative [85] under the Wienreb protocol formed the desired 1-methylindole-3-carboxamide [87] in 74% yield, **Scheme 21**.



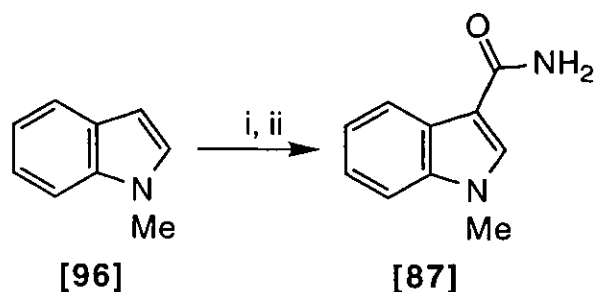
Scheme 21: Reagents and conditions i) Me_3Al , NH_4Cl , toluene, 60°C , 74%

Weinreb has suggested the amination reaction proceeds *via* a methyl chloroaluminum species [90], formed from initial reaction of trimethylaluminum and ammonium chloride, **Equation 1**, although isolation and characterisation has not been reported.⁹⁵ The intermediate aluminum species [90] is then thought to react with the ester to form the amide.



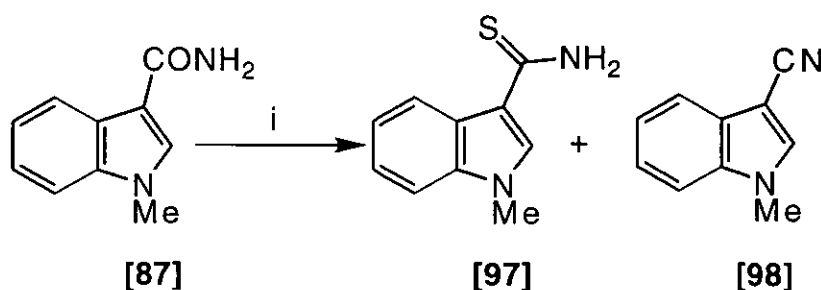
Equation 1: Weinreb's initial postulation as to the reaction intermediate

However, Sidler *et al.* have recently studied the mechanistic implications of the Weinreb amination procedure, with particular reference to the reaction between *N,N*-dimethylamine, trimethylaluminum and methyl benzoate.⁹⁶ Contrary to the original postulation by Weinreb, they failed to find any of the so called trivalent aluminum species [90], however, IR, ^1H NMR and ^{27}Al NMR spectroscopy supported the formation of the tetrahedral complex [91], **Equation 2**.

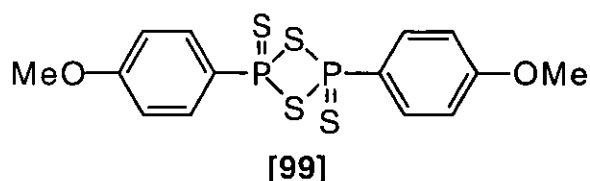


Scheme 24: Reagents and conditions i) CSI, MeCN, 0°C to 25°C, ii) KOH(aq), acetone, 60%

The conversion of 1-methylindole-3-carboxamide **[87]** to the corresponding indole thioamide derivative **[97]**, was achieved by reaction with Lawesson's reagent (LR) **[99]**, **Scheme 25**.⁹⁹ LR was originally chosen as the thionating reagent for the conversion of amides to thioamides because of its reported superior yields and low tendency towards the formation of decomposition products.¹⁰⁰ However, performing the reaction with 0.5 equivalents of LR in refluxing toluene occasionally resulted in the formation of considerable amounts of the unwanted decomposition product **[98]**. In some cases the sole product was the decomposition product **[98]**. Performing the thionation reaction in refluxing benzene, rather than toluene, resulted in a 60% yield of the desired indole thioamide and a significantly decreased yield of the decomposition product, *i.e.*, < 10%. The yield of the decomposition product was found to be dependent upon the reaction time and the purity of the LR. The use of freshly recrystallised LR dramatically reduced the amount of decomposition product.



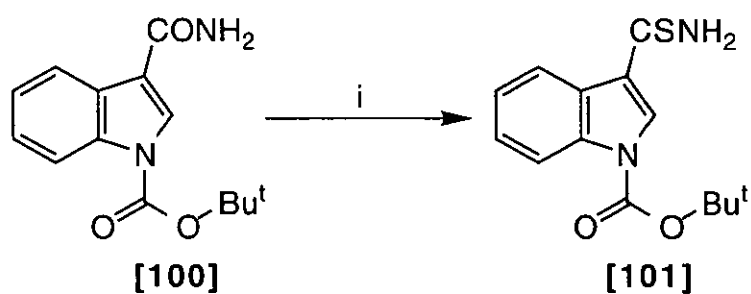
Scheme 25: Reagents and conditions i) Lawesson's reagent **[99]**, either refluxing benzene or toluene, 60%



Superficial studies into the formation of the decomposition product **[98]** suggested that the thioamide **[97]** was not stable under the reaction conditions. The nitrile **[98]** was found to be formed only after the formation of the thioamide, observed by *in situ* IR and TLC analysis.

The thioamide moiety in [97] can be considered to be electron rich, due to conjugation with the lone pair of electrons on the indole nitrogen. This is enhanced by the positive inductive effect of the methyl group attached to the indole nitrogen. Ayer *et al.* have shown that reaction of the indole amide [95] with LR in refluxing benzene leads to complete formation of the decomposition product.¹⁰¹

However, reaction of the *N*-*tert*-butyloxycarbonyl (Boc) protected indole [100] with 0.5 equivalents of LR in refluxing benzene was found to form the thioamide [101] in 94% yield, without a trace of the decomposition product, **Scheme 26**. In the case of [101] the lone pair of electrons on the indole nitrogen are in conjugation with the Boc protecting group, and so the electron density on the thioamide moiety is significantly reduced. These initial observations suggest that the electronic environment of the newly formed thioamide moiety significantly contributes towards the formation of the nitrile decomposition product.



Scheme 26: Reagents and conditions i) LR, toluene, reflux, 94%

Due to the undesirable decomposition of the indole thioamide, synthetic efforts were concentrated on the conversion of indolyl amides to thioamides, by a reactant other than LR. Reaction of the indole amide [97] with phosphorus pentasulfide in refluxing benzene over 2 h, unfortunately resulted in the formation of significant amounts of decomposition product. Woollins *et al.* have reported the synthesis of novel aryl thiophosphine sulfide reagents, which react in a similar fashion to LR.^{102, 103} The *tert*-butyl aryl thiophosphine sulfide [102], naphthyl thiophosphine sulfide [103], and ferrocenyl thiophosphine sulfide [104] thionating reagents were examined as possible alternatives for LR. The reactions were performed in refluxing benzene using 0.5 equivalents of the thionating reagent, **Table 1**. The *tert*-butyl aryl thiophosphine sulfide [102] was found to be the most efficient, forming the desired indole thioamide [97] in 58% yield, however, only a trace of the decomposition product was observed. The naphthyl thiophosphine sulfide [103] and the ferrocenyl thiophosphine sulfide [104] reagents were found to be less efficient than LR, forming the desired indole thioamide in 31 and 26% yield respectively, again the amount of decomposition product formed was significantly reduced, even though the reactions were left for 24 h.

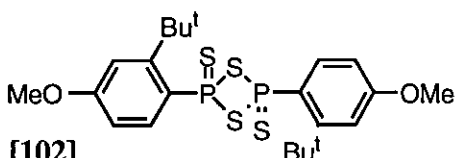
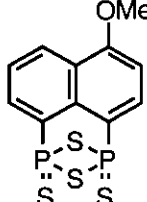
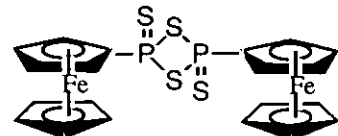
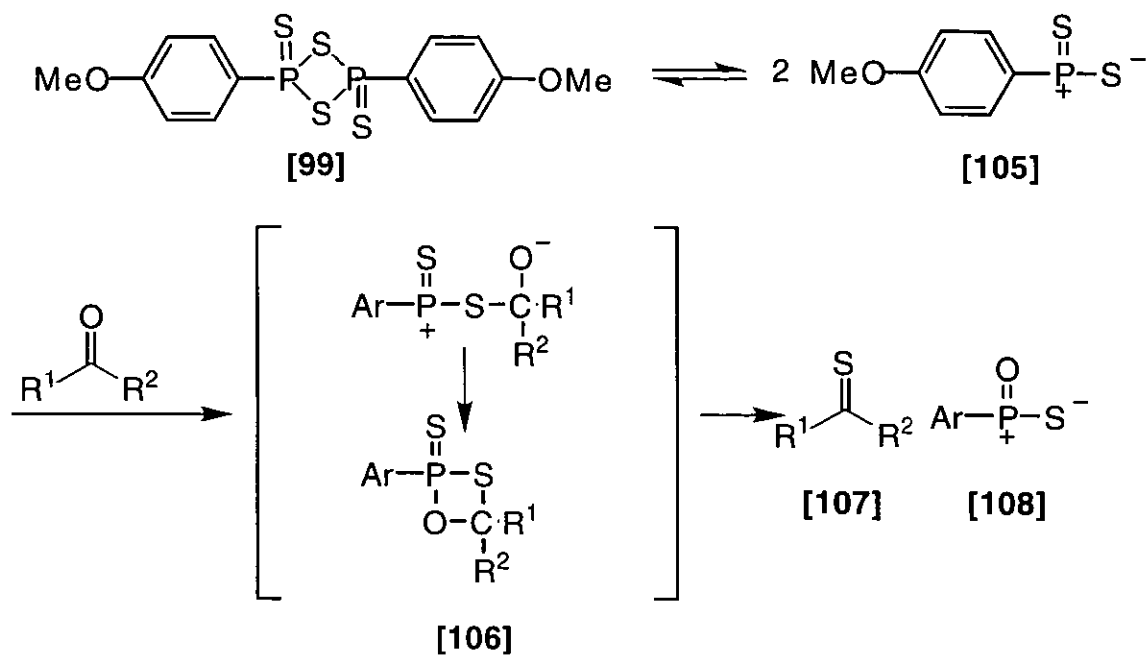
[87]	[97]	[98]		
Thionating reagent	Time (h)	Yield [97] %	Yield [98] %	
 [102]	3	58	Trace	
 [103]	24	31	<5	
 [104]	24	26	<5	

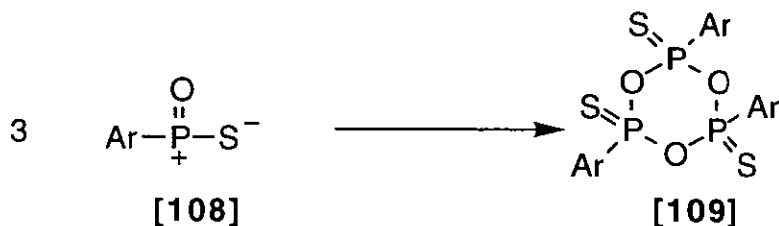
Table 1: The reaction of indole-3-carboxamide [87] with various thionating reagents. All reactions were carried out in refluxing benzene with 0.5 equivalents of thionating reagent

The mechanism of LR is thought to proceed via a betaine type intermediate [106], Scheme 27.¹⁰⁰ Lawesson has suggested the formation of the dipolar species [105] at low concentration in solution, based on ³¹P NMR spectroscopy, although the species has never been fully isolated or characterised. Further evidence for the dipolar species [105] comes from experimental evidence, in that the reaction can proceed efficiently using only 0.5 equivalents of LR. The dipolar species [105] is thought to attack the carbonyl functionality to form the intermediate betaine species which collapses to give the thionated product [107] and the dipolar species [108].



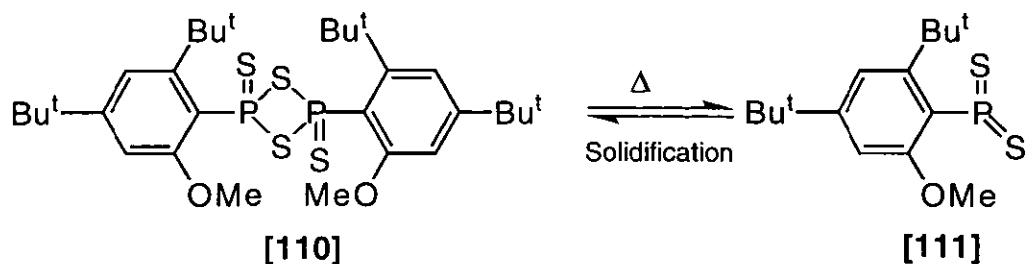
Scheme 27: Proposed mechanism for the reaction of LR with a carbonyl compound

The attendant formation of the trimer [109], isolated as a white crystalline powder of low solubility also lends credibility to the proposed betaine mechanism, **Scheme 28**.¹⁰⁰



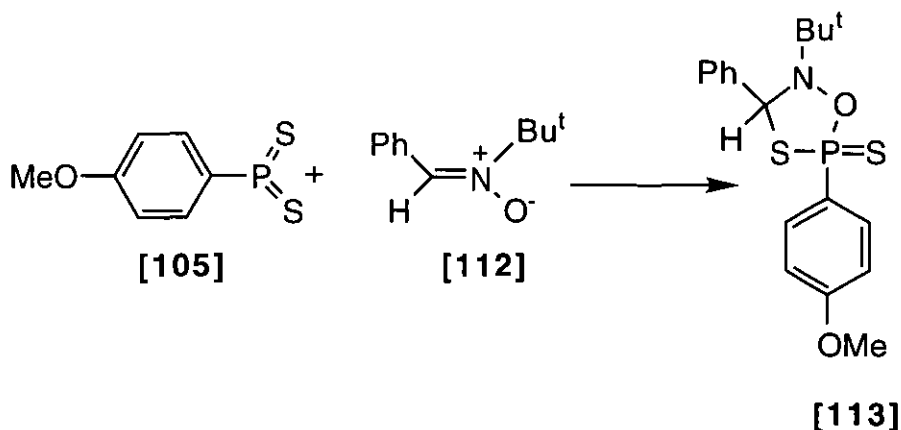
Scheme 28

In order to closely examine the mechanism of LR, Yoshifuji *et al.* have synthesised the sterically challenged thionating reagent 2,4-di-*tert*-butyl-6-methoxyphenyldithioxophosphorane [110] (abbreviated to MOX : methoxy-m-xylene), which, under an inert atmosphere in non-polar solvents, exists almost exclusively as the dithiophosphorane [111], **Scheme 29**. The dithiophosphorane [111] was found to readily thionate benzophenone in refluxing benzene, thus lending credibility to the proposed dipolar species [105].¹⁰⁴



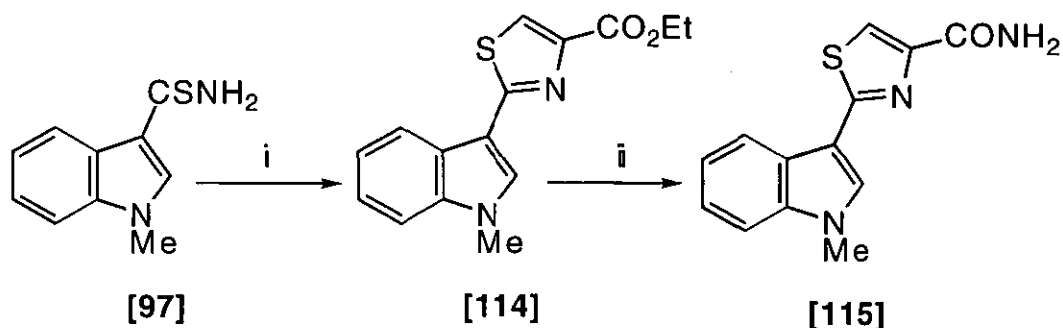
Scheme 29

Further evidence for the proposed proposed species [105] has been provided by Bertrand *et al.* who employed the dithioxophosphorane [105] as a dipolarophile in [2 + 3] cycloaddition reactions. Reaction of [105] with the nitron dipole [112] formed the oxathioxaphospholinine [113] product in 79% yield, Scheme 30.¹⁰⁵



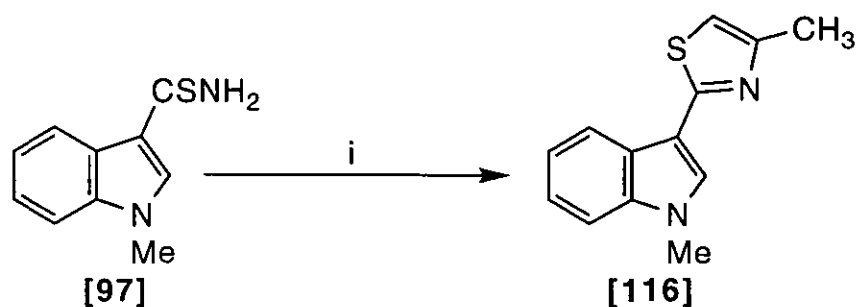
Scheme 30

With the key indole thioamide derivative [97] in hand, synthetic efforts were concentrated on its application in Hantzsch reactions to form indolythiazoles. Reaction of the indole thioamide [97] with commercially available ethyl bromopyruvate in refluxing ethanol formed the thiazolyindole with an ethyl ester functionality positioned at the C(4) thiazole position [114] in 64% yield, Scheme 31. Elaboration of the thiazole ethyl ester into a primary amide was achieved by reaction with a 0.88 solution of ammonium hydroxide and ammonium chloride in a Young's sealed tube at 100°C for 24 h to form [115] in 73% yield.¹⁰⁶



Scheme 31: Reagents and conditions i) ethyl bromopyruvate, ethanol, reflux, 64%, ii) NH₄OH, NH₄Cl, 100°C, sealed tube, 24h, 73%

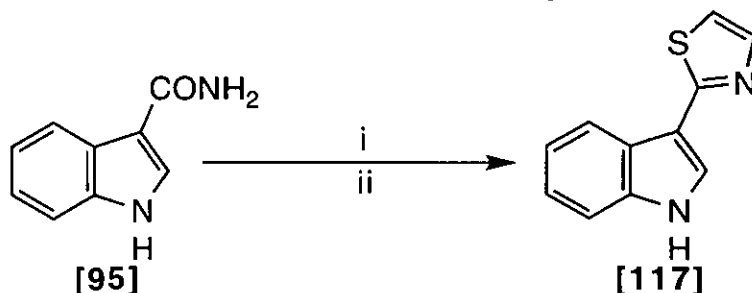
Reaction between the indole thioamide [97] and commercially available chloroacetone in refluxing ethanol formed the indolethiazole [116] containing a methyl substituent at the thiazole C(4) position in 43% yield, Scheme 32.



Scheme 32: Reagents and conditions i) chloroacetone, ethanol, reflux, 3 h, 43%

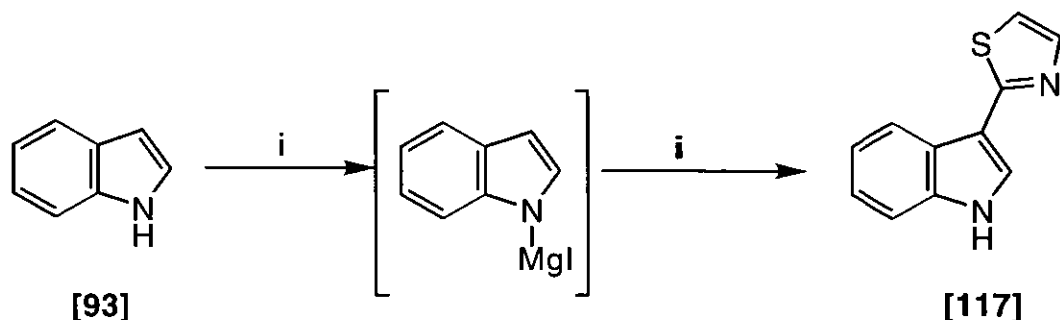
2.4. Nucleophilic displacement reactions by an indolyl anion on 2-bromothiazole

Ayer *et al.* have utilised the Hantzsch reaction as a key step in the synthesis of the natural product camalexin [117], **Scheme 33**.¹⁰¹ Indole-3-carboxamide [95] was reacted with phosphorus pentasulfide in benzene for 3 h to form the corresponding indole-3-thiocarboxamide *in situ* which was then reacted with chloroacetaldehyde diethyl acetal in refluxing ethanol for 15 h, to form camalexin [117] in 35% yield.



Scheme 33: Reagents and conditions i) P₄S₁₀, benzene 3 h, ii) ClCH₂CH(OC₂H₅)₂, ethanol, reflux, 15 h, 35%

Unfortunately the authors found that the above reaction was only sufficient for the preparation of small quantities of camalexin. However, the authors found that a nucleophilic substitution reaction on 2-bromothiazole by an indolyl anion, formed by reaction of indole [93] with methylmagnesium iodide, resulted in the formation of camalexin [117] in a very reasonable 68% yield, **Scheme 34**.



Scheme 34: Reagents and conditions i) Et₂O, methylmagnesium iodide, ii) benzene, 2-bromothiazole, reflux, 67 h, 66%

The indolyl anion can be considered to act as an ambident nucleophile, with the negative charge residing mainly on the nitrogen and the β -carbon, **Figure 6**.¹⁰⁷

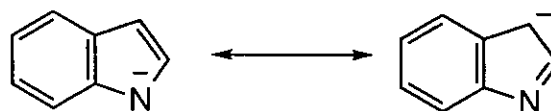
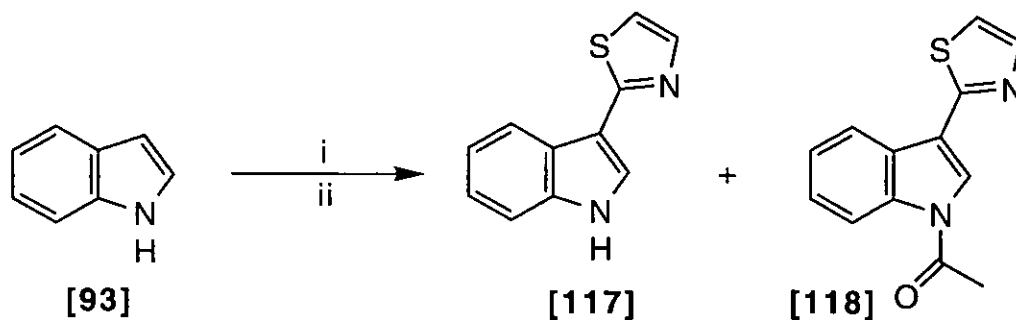


Figure 6

The ratio of nitrogen to β -carbon substitution upon reaction with electrophiles is dependent upon: the associated metal of the base used; the polarity of the solvent; and the nature of the electrophile. Generally, more ionic sodio- and potassio- derivatives tend to react at nitrogen whereas magnesio-derivatives have a greater tendency to react at the β -position.¹⁰⁸ However, reaction of indolyl Grignards in polar solvents, such as HMPA, leads to more attack at nitrogen, whereas non-polar solvents favours attack at the β -carbon. Complementarily, more reactive electrophiles show a greater tendency to react at nitrogen than less electrophilic species.

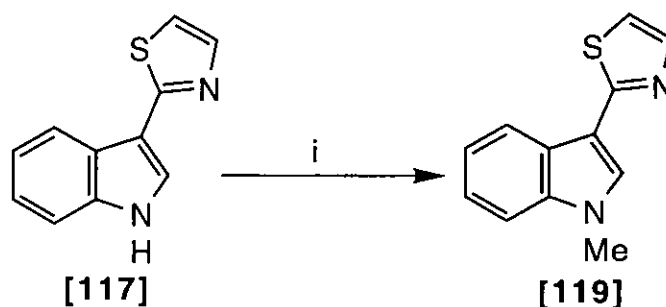
According to electronic theory and MO calculations, the carbon atom of a neutral thiazole ring that is most sensitive to nucleophilic attack is the C(2). The reaction of nucleophiles is not observed unless strong nucleophiles are employed. However, thiazoles with a leaving group at the C(2) position react readily with nucleophiles to give nucleophilic substitution products.¹⁰² The reaction is facilitated by the thiazole nitrogen which can stabilise the reaction intermediate. 2-Chlorothiazole is known to be 100 times more reactive than 2-chloropyridine towards nucleophilic displacement reactions.

The methodology utilised by Ayer *et al.* in the synthesis of camalexin appeared to be ideal for the synthesis of thiazolylindoles, in which the thiazole was substituted by a hydrogen at the thiazole C(4) position.¹⁰¹ However, in our hands, only a 30% yield of camalexin [**117**] was achieved, as well as a 10% yield of the acylated camalexin [**118**] side product, formed by quenching the indolyl Grignard with ethyl acetate, **Scheme 35**. In order to achieve a higher preponderance of attack at the β -carbon on indole, the polar diethyl ether solvent used in the initial formation of the indolyl Grignard was removed and replaced with the non-polar solvent benzene, prior to the addition of 2-bromothiazole.



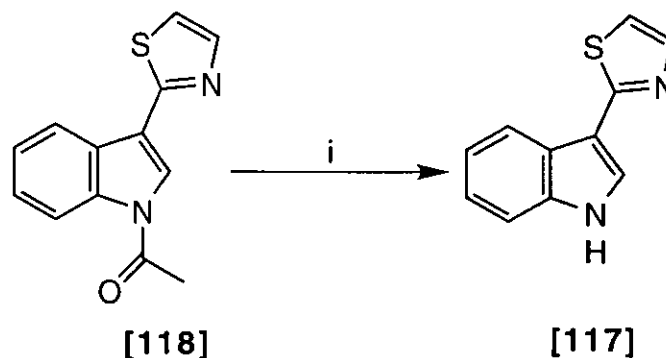
Scheme 35: Reagents and conditions i) methyl magnesium iodide, Et₂O, ii) benzene, 2-bromothiazole, reflux, 67 h, 30% and 10%

N-alkylation of camalexin [117] was accomplished by employment of the Heaney-Ley *N*-methylation protocol by reaction with iodomethane in DMSO with potassium hydroxide as the base to form the *N*-methyl derivative [119] in an excellent 80% yield, **Scheme 36**.¹⁰⁹



Scheme 36: Reagents and conditions i) KOH, DMSO, MeI, 80%

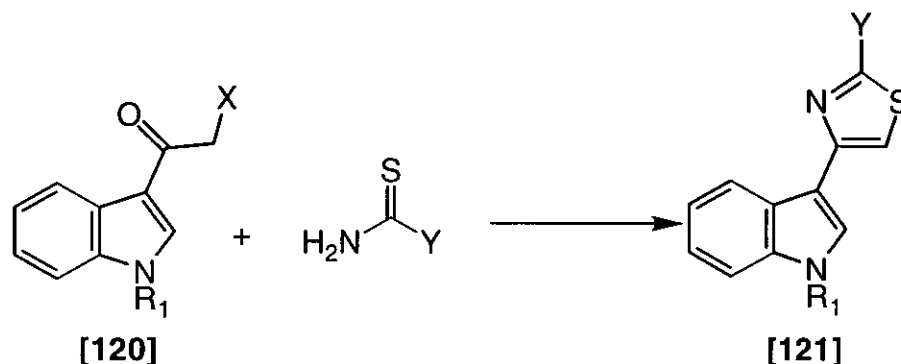
The *N*-acylated camalexin [118] side product was readily converted by basic hydrolysis to form camalexin [117] in 83% yield, **Scheme 37**.



Scheme 37: Reagents and conditions i) NaOH, H₂O, THF, reflux, 83%

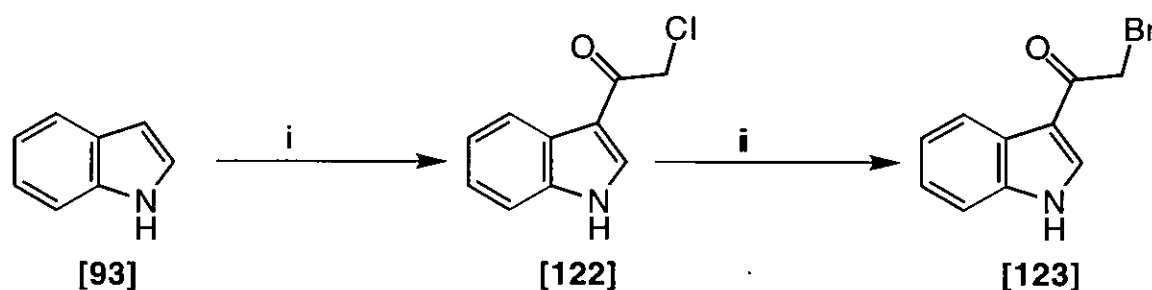
2.5. The synthesis of various thiazoles containing an indole moiety at the thiazole C(4) position, by the Hantzsch reaction

The Hantzsch reactions discussed so far constructed the thiazole ring by reaction between an indole substituted thioamide and a α -halo ketone. However, if the α -halo ketone moiety was attached to the indole derivative, as in [120] (X= Cl or Br), then the resultant thiazole formed would contain the indole substituent attached to the thiazole C(4) position [121], Scheme 38.



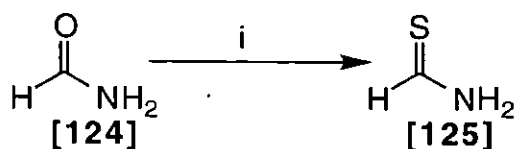
Scheme 38: Proposed synthetic route towards C-4 indole substituted thiazoles

The synthesis of the indole α -halo ketone precursor 3-(2-bromoacetyl)indole [123] was achieved by prior formation of the α -chloro ketone indole derivative [122]¹¹⁰ which was then converted to the α -bromo ketone indole [123] by a Finkelstein reaction in 62% , Scheme 39.¹¹¹



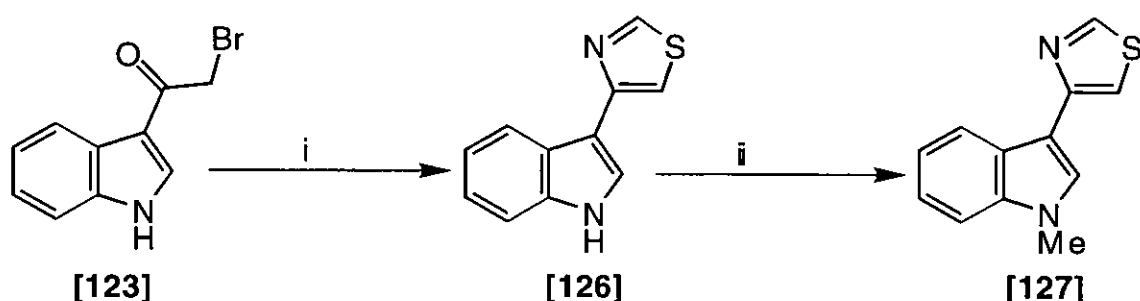
Scheme 39: Reagents and conditions i) ClCH₂COCl, pyridine, toluene, 60°C, 80%, ii) NaBr, acetone, reflux, 62%

The synthesis of an thiazolyindole analogue with no functionality at the thiazole C(2) position, *i.e.* for [121] Y=H, required a Hantzsch reaction between the α -halo ketone indole derivative [123] and thioformamide. Unfortunately thioformamide is not commercially available, however, the synthesis is fairly trivial, Scheme 40.¹¹² Reaction between formamide [124] and phosphorus pentasulfide in refluxing diethyl ether afforded thioformamide [125] in a rather poor 24% yield.



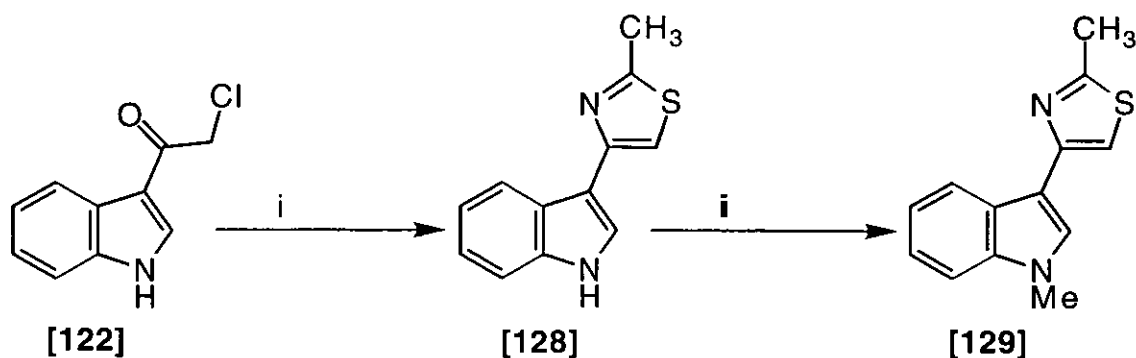
Scheme 40: Reagents and conditions i) P_4S_{10} , diethylether, reflux, 14 h, 24%

Reaction of the α -bromoketone indole derivative [123] with thioformamide [125] in refluxing ethanol gave a modest 30% yield of the desired thiazolyindole [126], **Scheme 41**. The low yield may be explained due to the poor thermal stability of the thioformamide. The thiazolyindole [126] was elaborated to the *N*-methyl derivative [127] by the Heaney-Ley *N*-methylation protocol in 80% yield.



Scheme 41: Reagents and conditions i) thioformamide, ethanol, reflux, 30%, ii) KOH, DMSO, MeI, 80%

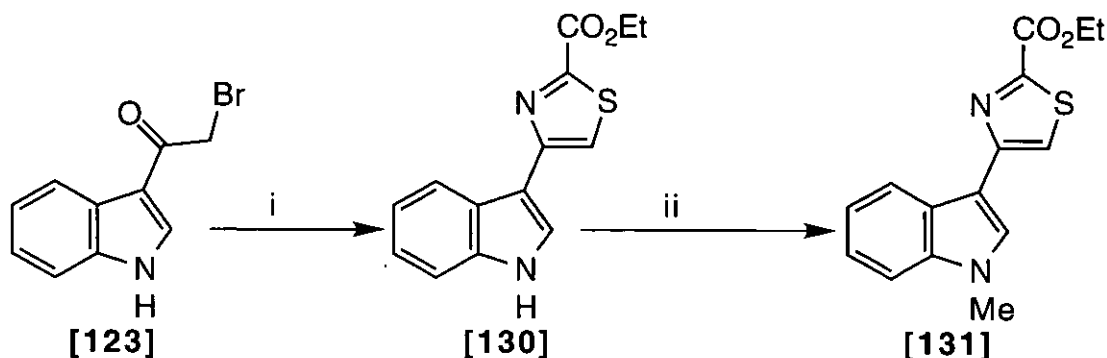
Reaction between the α -chloroketone indole derivative [122] and thioacetamide in refluxing ethanol formed the thiazolyindole [128] in which a methyl group was attached to the thiazole C(2) position in a reasonable 43% yield, **Scheme 42**. *N*-Alkylation of the indole derivative [128] by the Heaney-Ley *N*-methylation protocol formed the *N*-methyl thiazolyindole derivative [129] in 75% yield.



Scheme 42: Reagents and conditions i) thioacetamide, ethanol, reflux, 43%, ii) KOH, DMSO, MeI, 75%

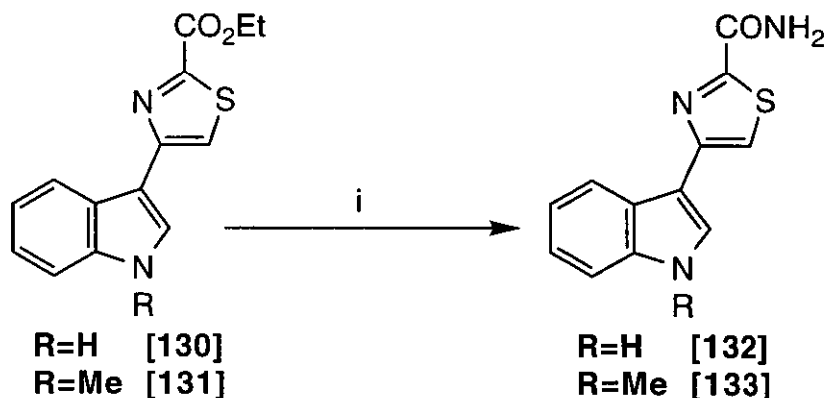
A Hantzsch reaction between the α -bromoketone indole derivative [123] and commercially available ethyl thiooxamate in refluxing acetonitrile,¹¹³ formed the thiazolyindole [130] in 74% yield. The use of ethanol as the solvent failed to form any of the desired product. In this case the thiazole contained an ethyl ester functionality at the thiazole C(2) position, **Scheme**

43. Alkylation of the indole nitrogen to the corresponding *N*-methyl derivative [131] was carried out by reaction with iodomethane in DMF at room temperature under a nitrogen atmosphere with sodium hydride as the base in 90% yield.



Scheme 43: Reagents and conditions i) ethyl thiooxamate, acetonitrile, reflux, 74%, ii) NaH, DMF, MeI, 90%

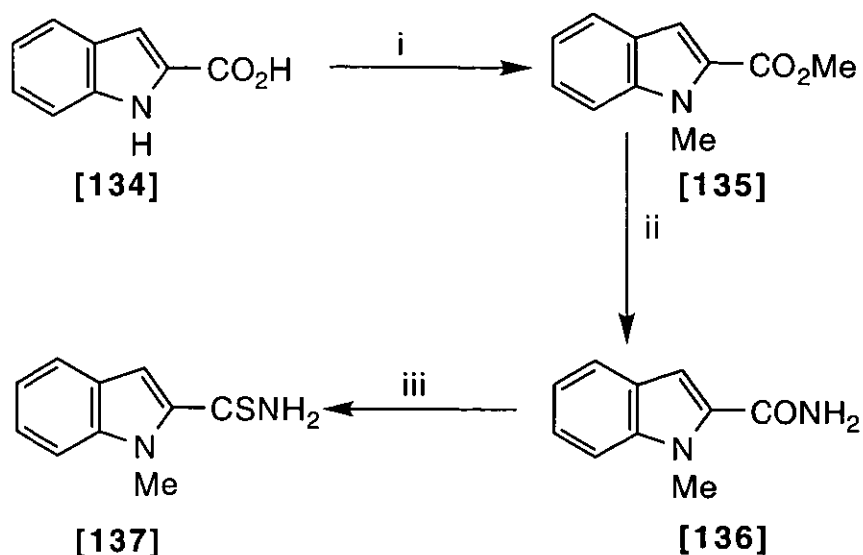
The ethyl ester functionalities on [130] and [131] were smoothly converted to the corresponding primary amide groups under the standard conditions in 47 and 58% yield respectively, **Scheme 44**.



Scheme 44: Reagents and conditions i) NH_4OH , NH_4Cl , 100°C , sealed tube, 58%

2.6. The synthesis of thiazolylindoles, in which the thiazole is attached to the indole C(2) position

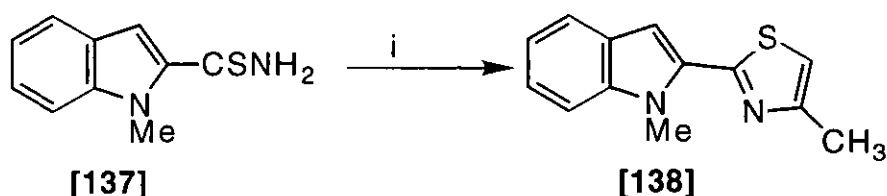
It was anticipated that the Hantzsch reaction could be extended towards the synthesis of thiazolylindoles in which the thiazole moiety was attached to the indole C(2) position.



Scheme 45: Reagents and conditions i) DMF, NaH, MeI, 90%, ii) Me₃Al, NH₄Cl, benzene, 60°C, 16h, 58%, iii) LR, benzene, reflux, 70%

The cheap and commercially available indole-2-carboxylic acid [134] was elaborated by standard chemistry over three steps to the indole thioamide [137], **Scheme 45**. Commercially available indole-2-carboxylic acid was both *N*- and *O*-methylated to form [135] in 90% yield. IR spectroscopy showed the ester C=O absorption at 1704 cm⁻¹ (note the absorbance is higher than the C-3 substituted ester [85] because of decreased electron delocalisation on the carbonyl). The resulting methyl ester derivative [135] was converted to the amide [136] by application of the Weinreb amination protocol in a respectable 58% yield. Reaction of the amide [136] with LR in refluxing benzene formed the indole-2-thioamide [137] in 70% yield. Again there were problems associated with the decomposition of the thioamide to the corresponding nitrile, however, not as pronounced as in the C(3) indole thioamide [97], and generally less than 5% of the decomposition product was formed.

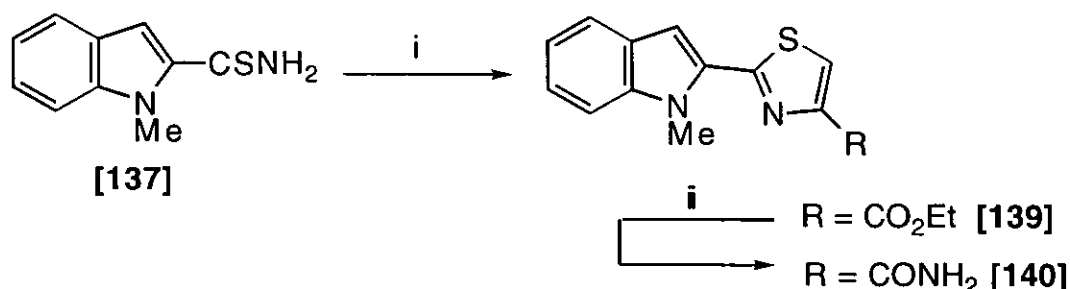
The thiazolyindole, which contained a methyl group at the thiazole C(4) position, was synthesised by reaction of [137] with chloroacetone and sodium bromide in refluxing acetonitrile in 71% yield, **Scheme 46**.



Scheme 46: Reagents and conditions i) chloroacetone, NaBr, acetonitrile, reflux, 71%

A Hantzsch reaction between the indole thioamide derivative [137] and ethyl bromopyruvate in refluxing ethanol constructed the thiazole C(4) ethyl ester substituted derivative [139] in

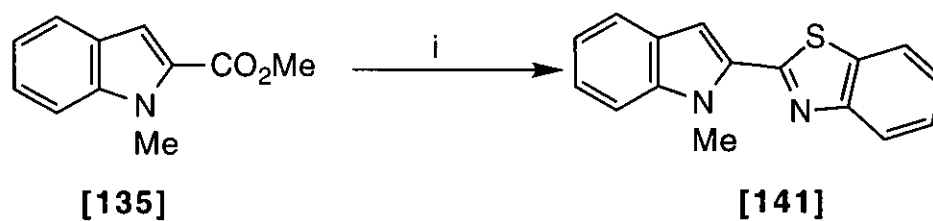
48% yield, **Scheme 47**. Elaboration of the ethyl ester functionality to the primary amide was achieved by reaction with liquid ammonia at room temperature in a Young's sealed tube over a 4 day period to form [140] in 80% yield.



Scheme 47: Reagents and conditions i) ethyl bromopyruvate, ethanol, reflux, 48%, ii) NH₃(l), CH₂Cl₂, sealed tube, rt, 4 days, 80%

2.7. Synthesis of the C(2) substituted indolyl benzothiazole [141]

Hudkins has recently reported the synthesis of aryl substituted benzothiazoles by the reaction of an aryl ester with aminothiophenol and trimethylaluminum.¹¹⁴ It was anticipated that this methodology could be applied towards the synthesis of the novel indolyl benzothiazole [141], **Scheme 48**. Reaction of methyl 1-methylindole-2-carboxylate [135] with trimethylaluminum and 2-aminothiophenol formed the C(2) substituted indolyl benzothiazole [141] in 57 % yield. It was hypothesised that the benzothiazole moiety would facilitate increased interaction of the indole derivative with DNA, this in turn would hopefully lead to enhanced antitumour properties.



Scheme 48: Reagents and conditions i) Me₃Al, 2-aminothiophenol, toluene, 60°C, 57%

2.8. Conclusions

- The Hantzsch thiazole methodology has been shown to be applicable to the synthesis of various thiazolylindole compounds.
- The ester functionality on the thiazole ring can be elaborated into a primary amide.

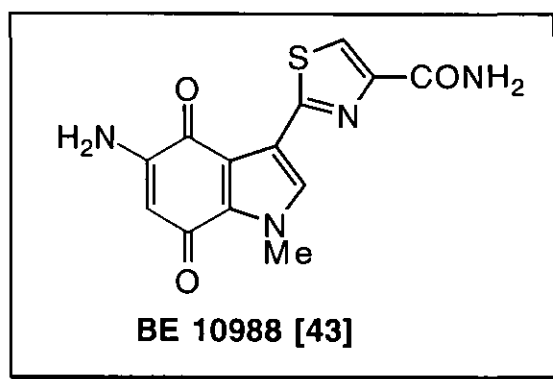
- Nucleophilic substitution of 2-bromothiazole by an indolyl anion has been shown to be applicable towards the synthesis of thiazolylindoles.
- The synthesis of indolyl benzothiazoles has been achieved.

Chapter 3

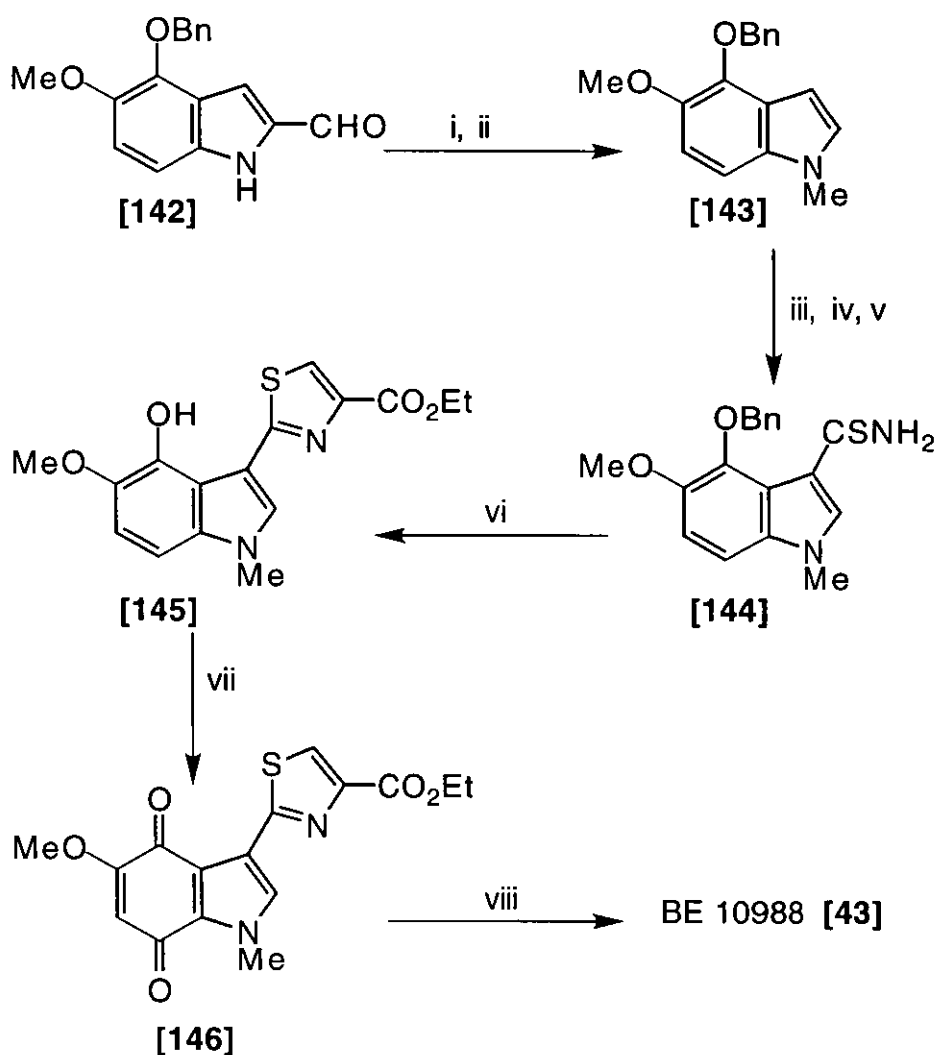
The synthesis of thiazolyloindolequinones.

3.1. The synthesis of BE 10988 [43]

To date there have been two reported total syntheses of BE 10988 [43]: the first, by Moody and Swann, in 1993;¹¹⁵ and the second, by Shizuri *et al.*, also in 1993.¹¹⁶ The thiazole ring in both syntheses was constructed by a Hantzsch reaction.



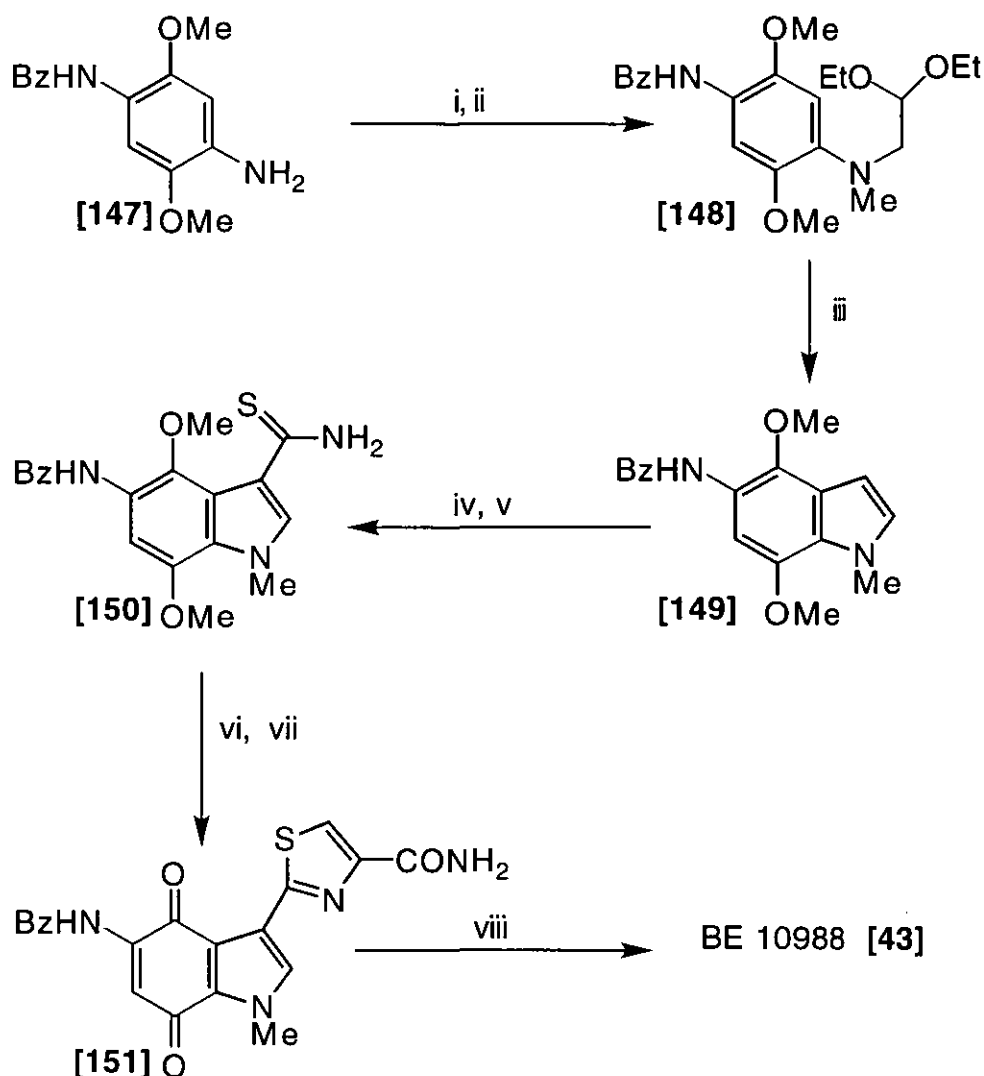
The origin of the Moody and Swann synthesis was the readily available indole carboxaldehyde [142]. Decarbonylation of [142] by the modified Wilkinson's catalyst ((Ph₃P)₂Rh(CO)Cl) and subsequent *N*-methylation formed the *N*-methyl indole [143], **Scheme 49**. Thioamide functionality at the indole C(3) position was introduced by reaction with CSI in diethyl ether followed by decomposition of the intermediate *N*-chlorosulfonylamide with tri-*n*-butyltin hydride in the presence of the radical initiator AIBN in refluxing benzene to form a C(3) substituted amide derivative. Thionation of the amide by reaction with Lawesson's reagent [99] in refluxing benzene formed the corresponding indole thioamide derivative [144]. A Hantzsch reaction between the thioamide [144] and ethyl bromopyruvate formed the indolyl thiazole [145]. The reaction also resulted in cleavage of the the *O*-benzyl protecting group, presumably by hydrobromic acid. Oxidation with Fremy's salt ((KSO₃)₂NO[•]) in a 0.17M sodium phosphate buffered acetone solution formed the indolequinone [146]. Finally ammonolysis of the quinone by reaction with liquid ammonia at rt over 4 days in a Young's sealed tube formed BE 10988 [43], as a bright red solid.



Scheme 49: Reagents and conditions i) $(\text{Ph}_3\text{P})_2\text{Rh}(\text{CO})\text{Cl}$, $\text{Ph}_2\text{P}(\text{CH}_2)_3\text{PPh}_2$, mesitylene, reflux, 82%, ii) KH , DMF , MeI , 91%, iii) CSI , Et_2O , 97%, iv) Bu_3SnH , AIBN , benzene, reflux, 88%, v) LR [99], benzene, reflux, 94%, vi) ethyl bromopyruvate, ethanol, reflux, vii) Fremy's salt, acetone, NaH_2PO_4 , 65%, viii) $\text{NH}_3(\text{l})$, rt, sealed tube, 4 days, 72%

The second reported synthesis, **Scheme 50**, started from commercially available fast blue RR [147]. Monomethylation by the use of an addition-reduction protocol followed by alkylation with bromoacetaldehyde diethylacetal formed [148], which was subjected to a modified Norlander synthesis to form the indole [149]. The C(3) thioamide functionality in [150] was introduced by a Friedel-Crafts thioacylation reaction with ethoxycarbonyl isothiocyanate, followed by hydrolytic cleavage of the intermediate *N*-ethoxycarbonyl thioamide. A Hantzsch reaction with bromopyruvamide afforded the primary amide substituted thiazole and oxidation of the *para*-dimethoxy aryl moiety with ceric ammonium nitrate (CAN) formed the quinone

[151]. Finally the benzamide protecting group on the C(5) amino substituent was removed by an addition-elimination reaction in ammonia saturated methanol to form BE 10988 [43].

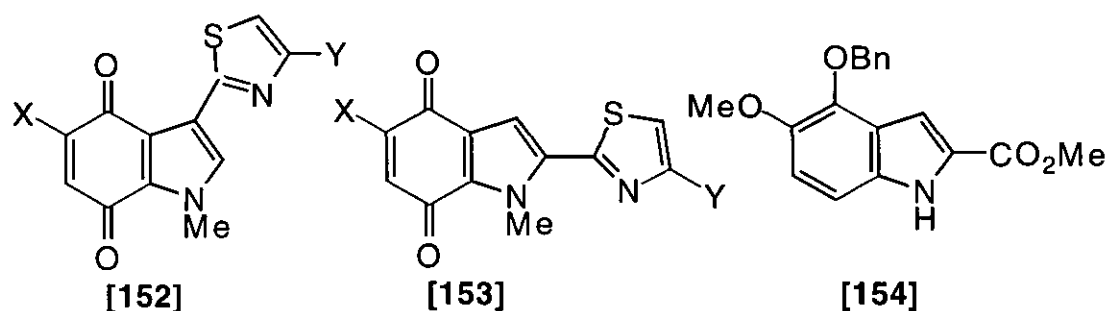


Scheme 50: Reagents and conditions i) $(\text{CH}_2\text{O})_3$, NaOMe, NaBH_4 , 72%, ii) $(\text{EtO})_2\text{CHCH}_2\text{Br}$, K_2CO_3 , $\text{MeOCH}_2\text{CH}_2\text{OH}$, 26%, iii) ZnCl_2 , DMF, 42%, iv) EtCO_2NCS , toluene, 54%, v) KOH , ethanol, 71%, vi) bromopyruvamide, ethanol, 82%, vii) CAN , 78%, viii) NH_4OH , methanol, 96%

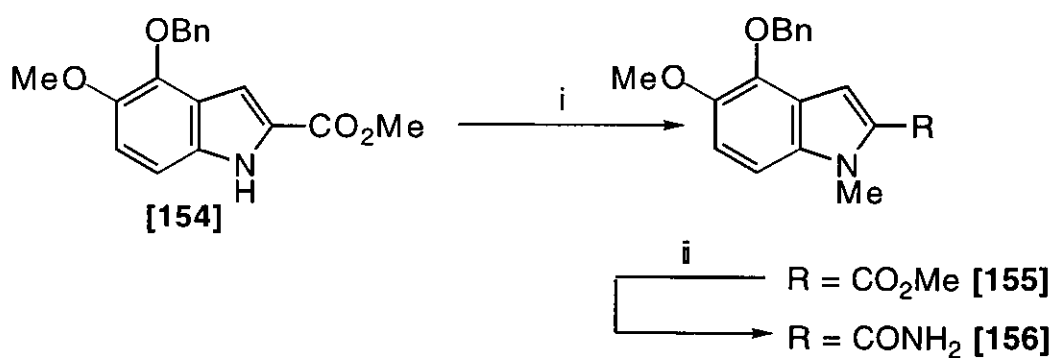
3.2. The synthesis of indolequinone derivatives with a thiazole moiety at the indole C(2) position

It was reasoned that the synthetic methodologies employed by Moody and Swann, **Scheme 49**, as well as those utilised for the construction of the 'simple' indolyl thiazoles, **Chapter 2**, could be combined to provide the generic thiazolyindolequinones [152] and [153]. The readily available functionalised indole [154] appeared to be an attractive precursor for the

synthesis of the C(2) thiazole analogue [153] since indole carboxylic esters have previously been shown to be readily converted to thioamides (for a detailed account towards the synthesis of [154], see Chapter 4).



Initial *N*-methylation of the indole [154] was achieved by a variety of *N*-alkylation conditions, Table 2, Scheme 51. All of the methods attempted formed the desired *N*-methyl indole [155] in yields ranging from 61 to 81%.



Scheme 51: Reagents and conditions i) Table 2, ii) Table 3

Conditions i	Yield [155] %
NaH, DMF, MeI	61
KH, DMF, MeI	77
KOH, DMSO, MeI	81
K ₂ CO ₃ , acetone, MeI, reflux	75

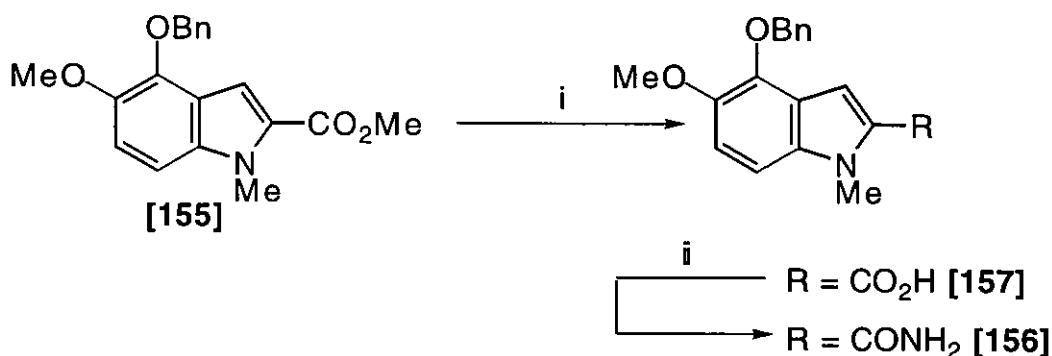
Table 2

The construction of a thiazole ring at the C(2) position by the Hantzsch reaction required elaboration of the methyl ester functionality to a thioamide moiety. The Weinreb amination protocol has been shown to be suitable for the synthesis of simple indolyl amides, however, reaction of the methyl ester derivatised indole [155] under the Weinreb amination conditions resulted in a poor 38% yield of the amide [156], Table 3. The poor yield possibly could be explained by complexation of the proposed tetravalent aluminum intermediate with the oxygen substituents on the indole ring. The method was very messy and TLC indicated several side products. The most efficient method for the synthesis of [156] was found to be application of the Davey amination protocol, by reaction with a 0.88 solution of ammonia and ammonium chloride in a Young's sealed tube at 100°C for 2 days, forming the desired amide [156] in a reasonable 78% yield. Both reaction with liquid ammonia in a sealed tube at rt. and methanolic ammonia solution failed to form any significant amounts of amide product.

Conditions ii	Yield [156] %
Me ₃ Al, NH ₄ Cl, toluene, 60°C	38
NH ₃ (l), sealed tube, rt	trace
NH ₄ OH, NH ₄ Cl, sealed tube, 100°C	78
NH ₄ OH, MeOH, reflux	-

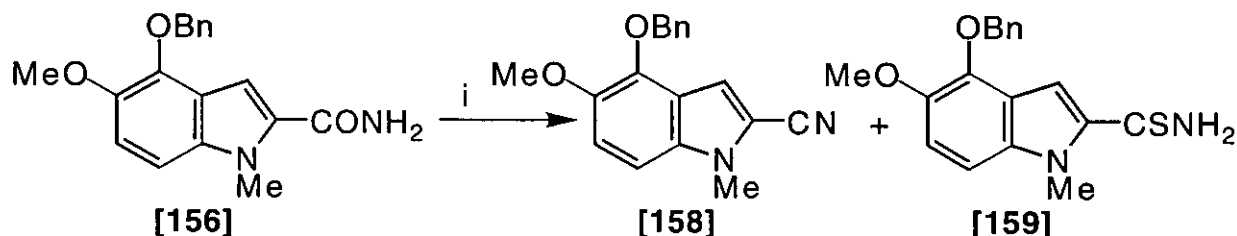
Table 3

A major problem associated with the Davey amination reaction was that it proved to be experimentally difficult during scale up, *i.e.*, the problem associated with a large reaction vessel under pressure at 100°C for up to two days. Indole acyl chlorides have previously been shown to be applicable towards the synthesis of primary amides, Scheme 19. Hydrolysis of the indole methyl ester [155] to the indole acid [157] provided a species capable for carboxylate activation, Scheme 52. However, activation of the carboxylic acid functionality to the corresponding acyl halide proved far from trivial. Conventional methods for the formation of acyl halides, *e.g.* reaction with thionyl chloride or oxalyl chloride, resulted in the formation of a complex mixture of products. It was found that reaction of the indole acid [157] with acetyl chloride and phosphorus pentachloride followed by subsequent reaction with liquid ammonia at -78°C, formed then primary amide [156] in a poor 32% yield.¹¹⁷



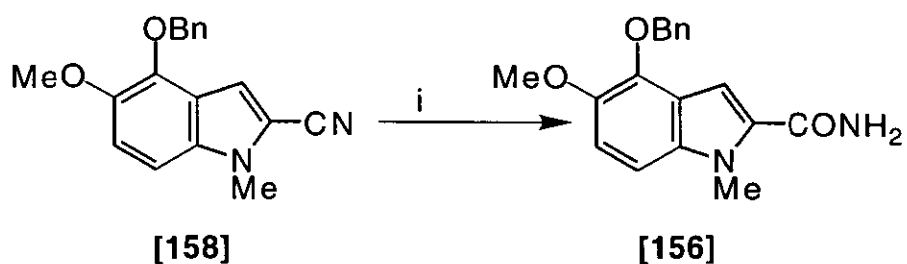
Scheme 52: Reagents and conditions i) NaOH, THF, H₂O, 76%, ii) CH₃COCl, PCl₅, NH₃, CH₂Cl₂, 32%

Reaction of the primary amide [156] with 0.5 equivalents of LR [99] in refluxing benzene proved unreliable and, at best, gave a 64% yield of the desired thioamide [159] as well as varying amounts of the nitrile decomposition product [158], **Scheme 53**. The yield of the decomposition product was dependent upon reaction time and the purity of the LR. Generally after 1 h the yield of the nitrile [158] side product rapidly increased and, if the LR used was not recrystallised prior to use the sole product formed was the nitrile [158]. The thioamide functionality in [159] is electron rich because of electron delocalisation from the C(5) methoxy group. As has been shown previously, in chapter 2, electron rich indole thioamides are prone to decomposition under the reaction with LR.



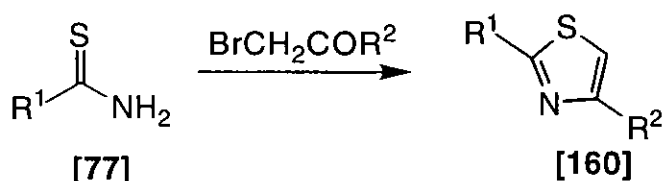
Scheme 53: Reagents and conditions i) LR [99], benzene, reflux, 64% [159]

Due to the undesired formation of the nitrile [158] decomposition product, a method was required to recycle the nitrile back to a useful intermediate. The hydrolysis of nitriles to amides is a well known synthetic reaction, however, problems can be associated with excess hydrolysis of the newly formed amide to the corresponding carboxylic acid. The hydrolysis of the indole nitrile [158] by reaction with potassium hydroxide in *tert*-butanol formed the desired amide [156] in 86% yield, **Scheme 54**.¹¹⁸



Scheme 54: Reagents and conditions i) KOH, (CH₃)₃COH, reflux, 86%

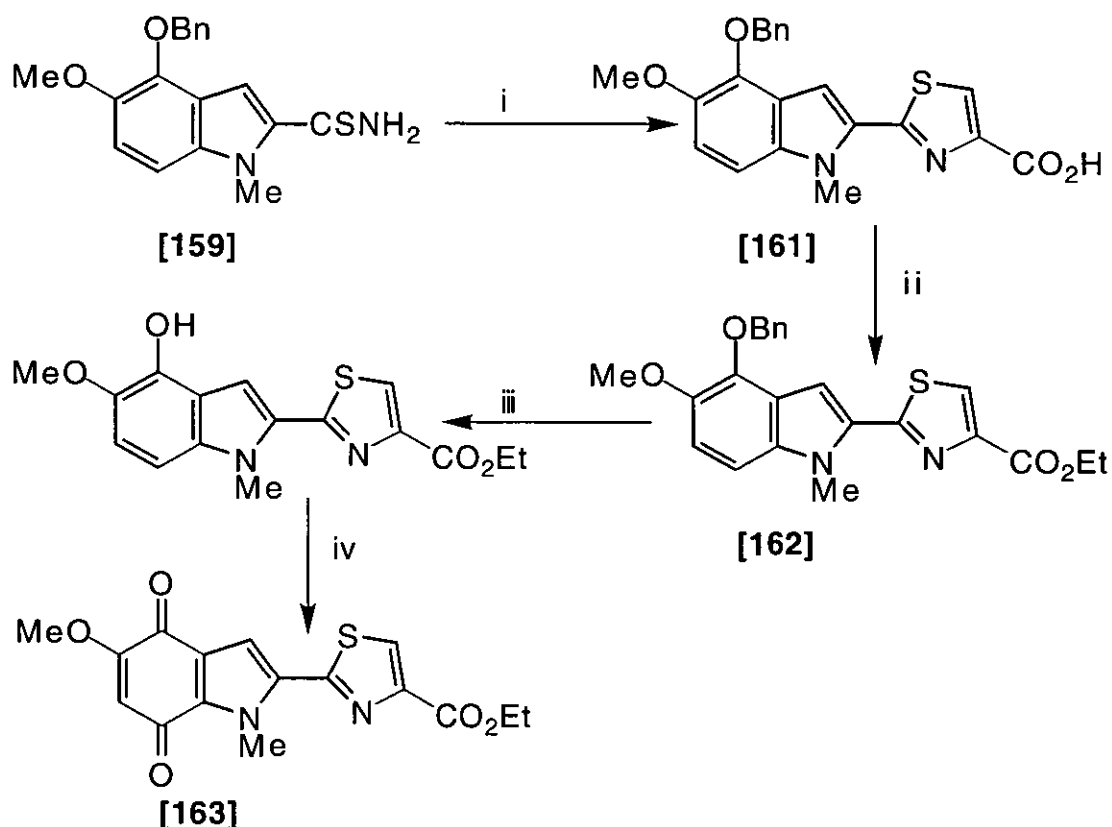
The α -bromoketone used in the Hantzsch reaction dictates the functionality at the thiazole C(4) position [160]. By altering the α -bromoketone various C(4) substituted thiazoles can be prepared, *i.e.* the use of bromopyruvic acid ($R^2 = \text{CO}_2\text{H}$) will form a carboxylic acid substituted thiazole whereas reaction with bromoacetone ($R^2 = \text{CH}_3$) will form the corresponding methyl substituted thiazole.



A Hantzsch reaction between the indole thioamide [159] and bromopyruvic acid formed the carboxylic acid functionalised thiazole [161] in 61% yield and the corresponding ethyl ester functionalised thiazole [162] in 19%, **Scheme 55**. Attempts to directly form of the thiazole ester [162] by reaction with ethyl bromopyruvate resulted in the formation of a complex mixture. Standard acid catalysed esterification of the thiazole carboxylic acid derivative [161] formed the ethyl ester thiazole [162] in 85% yield.

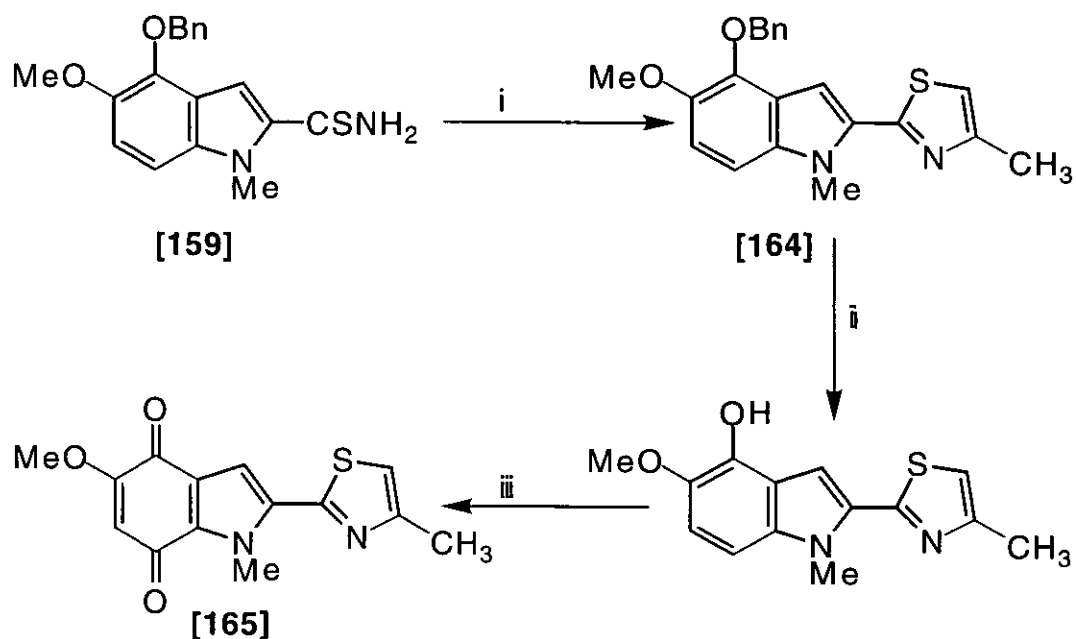
The *O*-benzyl protecting group was originally chosen because of its reported facile removal under hydrogenolysis conditions.¹¹⁹ However, under conventional hydrogenolysis reactions the *O*-benzyl group on the indole derivative [162] proved rather stubborn to removal. Reaction of the benzyl protected indole [162] under a hydrogen atmosphere, at atmospheric pressure, with 10 mol% of a palladium on activated carbon catalyst in either ethanol or ethyl acetate as the solvent (for up to 4 days) gave poor yields of the desired debenzylated product. Performing the hydrogenolysis reactions under three atmospheres of pressure failed to increase the yield of the desired product. However, reaction of the indole [162] under a atmospheric hydrogen atmosphere in ethanol with 10 mol% of palladium hydroxide (Pearlman's catalyst), for 48 h, cleaved the *O*-benzyl protecting group quantitatively. The resultant debenzylated product was found to be somewhat unstable, rapid colourisation occurred only a few hours

after isolation, and so complete characterisation proved fruitless. Therefore immediately after the removal of the *O*-benzyl protecting group the phenol derivative was oxidised to the indolequinone [163] by reaction with a 0.17 M sodium phosphate buffered aqueous acetone solution of the stable free radical species Fremy's salt (potassium nitrodisulfonate ((KSO₃)₂NO)) in 64% yield.



Scheme 55: Reagents and conditions i) bromopyruvic acid, ethanol, reflux, 61% [161] and 19% [162], ii) concentrated. H₂SO₄, ethanol, reflux, 85%, iii) H₂, ethanol, Pd(OH)₂, iv) Fremy's salt ((KSO₃)₂NO), acetone, NaH₂PO₄(aq), 64%

The synthesis of the corresponding methyl substituted thiazole [165] is shown, **Scheme 56**. A Hantzsch reaction between the thioamide [159] and bromoacetone in refluxing ethanol formed the C(4) methyl substituted thiazole derivative [164] in 69% yield. The *O*-benzyl protecting group was cleaved under conditions analogous for the ester thiazole [162]. Again the debenzylated product proved to be somewhat unstable and so the oxidation, carried out under the standard conditions with Fremy's salt, was performed immediately after isolation of the phenol, to form the quinone [165] as orange solid in 59% yield.



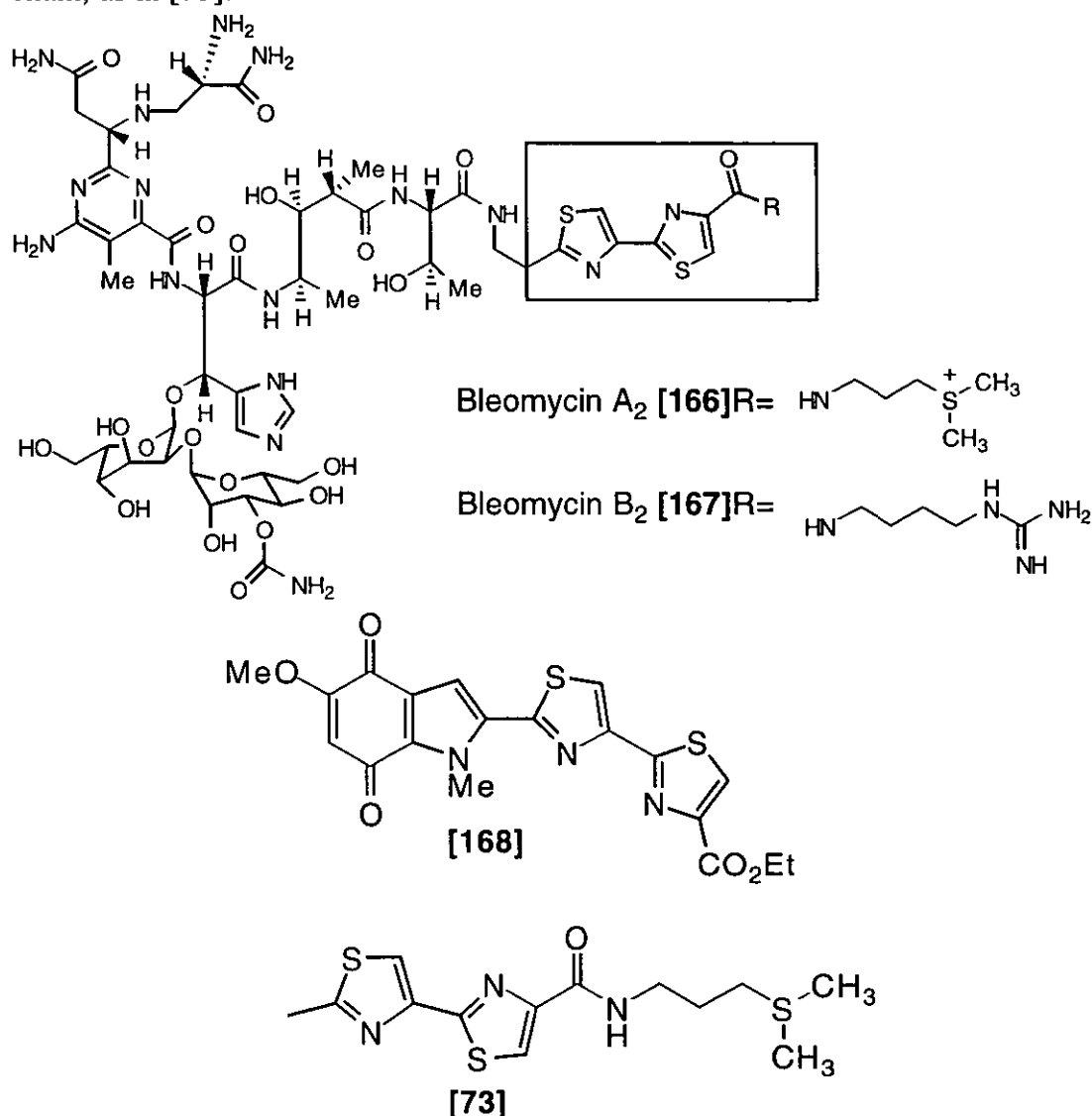
Scheme 56: Reagents and conditions i) bromoacetone, ethanol, reflux, 69%, ii) H₂, ethanol, Pd(OH)₂, iii) Fremy's salt, NaH₂PO₄(aq), acetone, 59%

3.3. Bithiazoles as DNA intercalating agents

The bleomycins (BLM) are a series of compounds which are currently used in the clinic as anticancer agents, mainly for the treatment of head, neck and testicular cancers.⁸⁹ The bleomycins require two cofactors: a metal (particularly iron and cobalt); and oxygen (O₂) in order to induce double-strand cleavage sites in DNA. Bleomycin A₂ [166] and B₂ [167], contain a bithiazole side chain, shown in the box, and it is this side chain that is thought to bind with the DNA helix and so bring the active centre of the BLM into close proximity with DNA. Povirik *et al.* initially reported the unwinding and lengthening of DNA upon binding of BLM, this provided the first experimental evidence for the intercalation of BLM with DNA.¹²⁰ Stubbe *et al.* have established unambiguously that the mode of binding of Co BLM A₂ green involves partial intercalation of the bithiazole side chain.⁹⁰ 2D NMR studies showed that BLM binds *via* partial intercalation of the bithiazole tail, observed by an upfield shift in the ¹H NMR of the bithiazole protons.

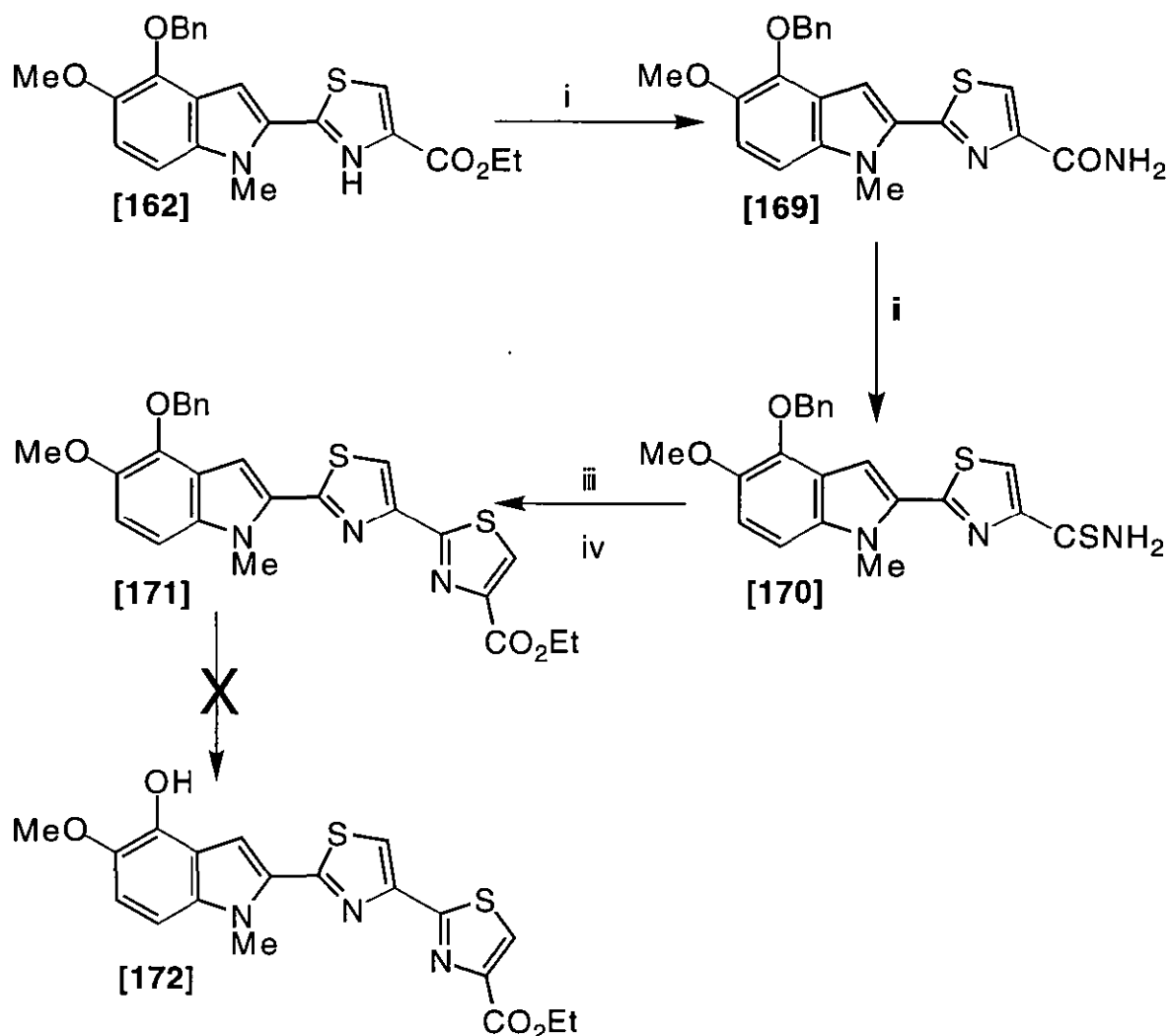
It was hypothesised that the bithiazole moiety could be incorporated into the indolequinone compounds. This would provide increased binding of the indolequinone agents to DNA, and hopefully increase DNA topoisomerase II inhibitory activity. Synthetic efforts were initially concentrated towards the synthesis of the indolequinone bithiazole [168]. With the aim of

elaborating the ethyl ester functionality at the thiazole C(4) position to the sulfide containing side chain, as in [73].



It was anticipated that two successive Hantzsch reactions could be employed for the construction of the bithiazole moiety. The ethyl ester functionality at the thiazole C(4) position on the indole derivative [162] was initially elaborated into a thioamide, which was then subjected to a Hantzsch reaction, **Scheme 57**. Reaction of the indole derivative [162] under the Davey amination conditions formed the thiazole substituted amide [169] in 76% yield. The primary amide functionality was poorly converted to the corresponding thioamide [170] by reaction with 0.5 equivalents of LR in refluxing benzene, however, reaction with 0.5 equivalents of LR under an inert atmosphere in THF at rt formed the desired thioamide in 55% yield. The bithiazole [171] was constructed in two steps in 60% yield from the thioamide [170]: first, by reaction of the thioamide with bromopyruvic acid in refluxing ethanol followed

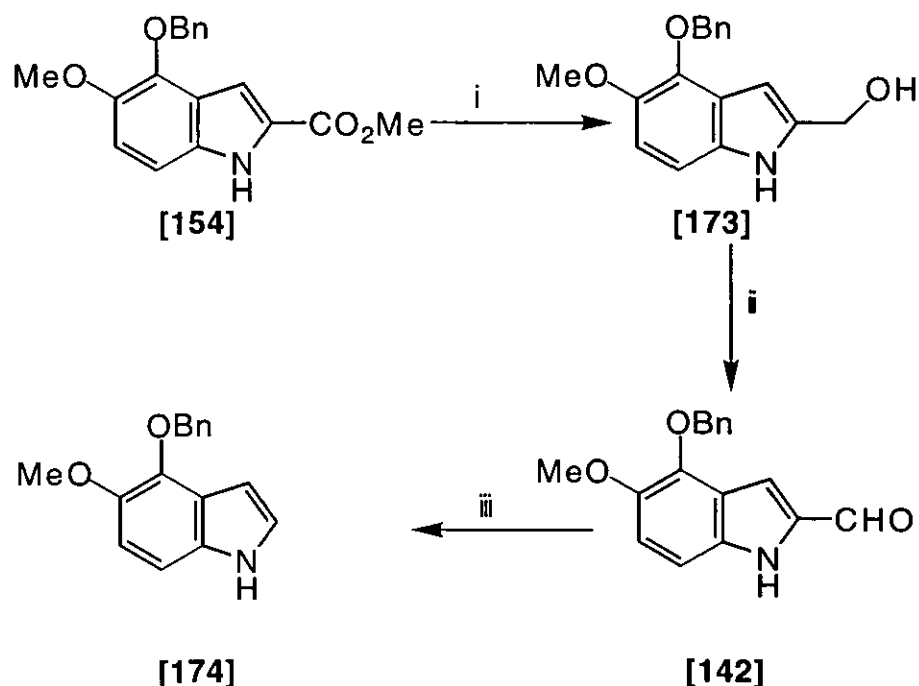
by acid catalysed esterification. However, deprotection of the *O*-benzylated indole derivative [171] under a hydrogen atmosphere, either at atmospheric pressure or three atmospheres, in ethanol with 10 mol% Pearlman's catalyst failed to form cleaved product [172]. Holmes *et al.* recently suggested the use of the Lewis acid complex boron trichloride dimethylsulfide as a selective reagent for the cleavage of *O*-benzyl ethers.¹²¹ Unfortunately reaction of the bithiazole [171] with boron trichloride dimethylsulfide in dichloromethane at 0°C resulted in complete decomposition of starting material and therefore, work on this compound was curtailed.



Scheme 57: Reagents and conditions i) NH₄OH, NH₄Cl, 100°C, sealed tube, 2 days, 76%, ii) LR [99], THF, rt, 55%, iii) bromopyruvic acid, ethanol, reflux, iv) ethanol, H₂SO₄, reflux, 60%

3.4 . Studies towards the synthesis of [175]

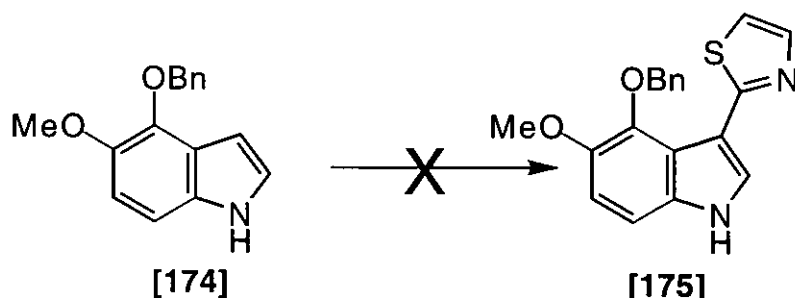
It was envisaged that the nucleophilic substitution reaction previously discussed in **Scheme 34** could be applied towards the construction of the more complicated thiazolyindole [175]. In this case the indolyl anion would be derived from the highly substituted indole [154]. Reduction of the methyl ester side chain on the indole [154] using lithium aluminium hydride in refluxing THF formed the alcohol [173] in 93% yield, **Scheme 58**. Oxidation to the aldehyde [142] was achieved by reaction with manganese(IV)oxide in refluxing dichloromethane in 50% yield. Manganese(IV)oxide is well known as a oxidising agent for benzylic alcohols. In the case of the indole alcohol [173] the low yield can probably be explained by the electron rich nature of the indole. Attempts to directly form the aldehyde [142] from the indole ester [154] by reduction with DIBAL-H resulted in complete reduction to the alcohol [173]. The indole carboxaldehyde was decarbonylated by reaction with the modified Wilkinson's catalyst $(\text{Ph}_3\text{P})_2\text{Rh}(\text{CO})\text{Cl}$ in 84% yield.¹²² The ligand bis(diphenylphosphino)propane is thought to exchange *in situ* with the triphenylphosphine ligands on the catalyst to form a more active decarbonylating reagent.



Scheme 58: Reagents and conditions i) LiAlH_4 , THF, reflux, 93%, ii) MnO_2 , CH_2Cl_2 , 50%, iii) $(\text{Ph}_3\text{P})_2\text{Rh}(\text{CO})\text{Cl}$, $\text{Ph}_2\text{P}(\text{CH}_2)_3\text{PPh}_2$, mesitylene, reflux, 84%

Unfortunately attempts to form the C(3) indole thiazole [175] resulted in failure, **Scheme 59**. Reaction of the indole [174] with commercially available methylmagnesium iodide in diethyl

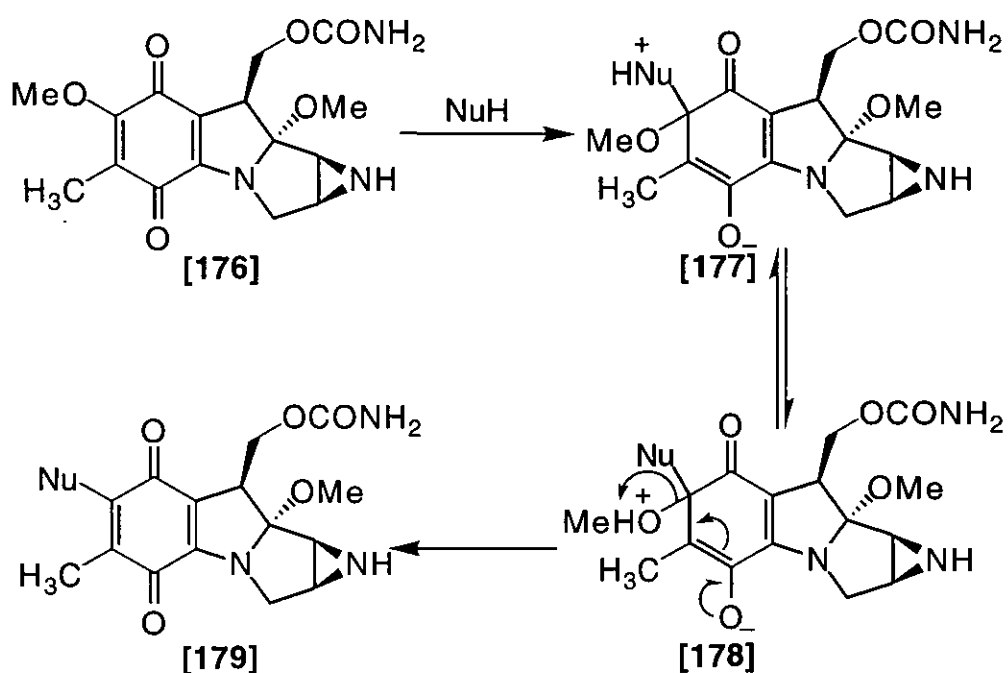
ether to form the indolyl anion and subsequent removal of the solvent followed by reaction with a benzene solution of 2-bromothiazole at reflux for 72 h resulted in a complex mixture. Decreasing the reaction time and performing the coupling reaction at rt failed to form any of the desired product.



Scheme 59: *Reagents and conditions* i) methylmagnesium bromide, diethyl ether, ii) 2-bromothiazole benzene or toluene

3.5 Methoxyquinone exchange reactions on various thiazolylindolequinones

The C(7) methoxy group on mitomycin A [176] has been shown to undergo a variety of nucleophilic substitution reactions, especially with alkoxides¹²³ and amines¹²⁴ including aziridines.¹²⁵ The reaction with any nucleophile can be envisaged as a three step addition-elimination reaction.¹²⁶ Initially a nucleophile adds at the C(7) end of the conjugated carbonyl system (Michael type addition) affording the intermediate [177], **Scheme 60**. A proton is then transferred to the C(7) methoxy group generating the intermediate [178], and methanol is eliminated to afford the desired C(7) substituted mitomycin A derivative [179].



Scheme 60: Reaction of MMA with a nucleophile

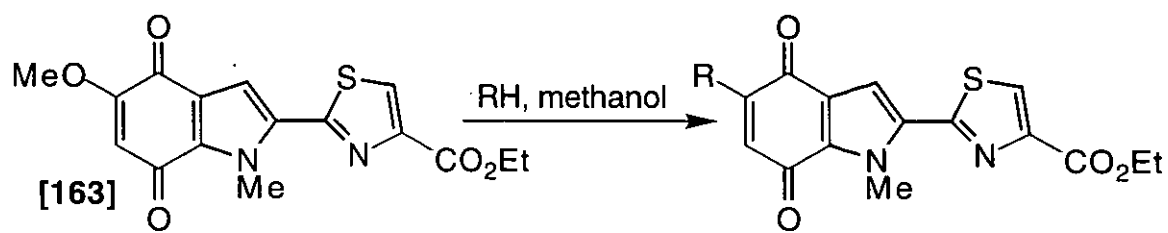
It was envisaged that the thiazoleindolequinone compounds [163] and [165] could be subjected to C(5) nucleophilic exchange reactions with a range of nitrogen nucleophiles to form the corresponding 5-(substituted-amino) indolequinone compounds.

The substituent attached to the C(5) position on the quinone ring will influence the biological activity in several ways:

- the reduction potential of the indolequinone moiety is dependent upon substituents attached to the quinone. The ease of reduction is associated with the electron donating power of the quinone substituent at C(5). Increased electron donation into the quinone moiety consequently increases the electropotential of the quinone, therefore augmenting the energy required for reduction;
- modification of the C(5) substituent may change the partition coefficient of the drug, and hence affect the cell penetration of the chemotherapeutic agent;
- The C(5) substituent can alter the stability, reactivity, and toxicity of the activated drug;
- changes in the steric size and composition of the C(5) substituent may influence the ability of the drug candidate to bind (*i.e.*, van der Waals, hydrogen bond) to DNA.
- the introduction of an aziridinyl substituent at the C(5) position would result in DNA cleavage, with the order of reactivity towards the cleavage of DNA being, unsubstituted > mono-methyl > bi-methyl > ring open.

The ester substituted thiazole [163] was treated with an excess of a variety of primary and secondary amines, ranging from acyclic amines such as cyclopropylamine, to cyclic secondary amines such as aziridine and piperidine, **Table 4**. All the reactions were carried out in methanol at rt and the products were purified by column chromatography on silica gel followed by size exclusion chromatography on Sephadex® LH20 gel. From **Table 4** it can be seen that the desired 5-(substituted-amino) derivatives of the indolequinone [163] were formed when the amines: aziridine, 2-methylaziridine, pyrrolidine, piperidine and cyclopropylamine were used, in yields ranging from 37-81%. Attempts to form the desired *cis*-dimethyl aziridine, tetramethyl aziridine and azetidione derivatives resulted in failure, and in the case of reaction with tetramethyl aziridine, complete decomposition of the SM resulted. As can be seen all the aziridine containing products were red solids and the pyrrolidine, piperidine and cyclopropylamine derivatives were purple solids.

All the amines were commercially available except aziridine, which was prepared according to the literature procedure of Allen *et al.* in which 2-aminoethanesulfonic acid is cyclised to aziridine by reaction with 40% sodium hydroxide.¹²⁷ *Cis*-dimethyl aziridine and tetramethyl aziridine were kindly supplied by the MRC Unit, Didcot. Extreme care was taken at all times when handling the aziridines, as these compounds are extremely toxic, and are potent alkylating agents of DNA. All the reactions were carried out in an efficient fume cupboard and contaminated glassware was washed in dilute acid. Also because of the sensitivity of aziridines towards acidic media, the excess aziridines were removed by evaporation, carried out under strict safety precautions. In the case of pyrrolidine, piperidine and cyclopropylamine reactions the excess amine was removed by an acidic workup.

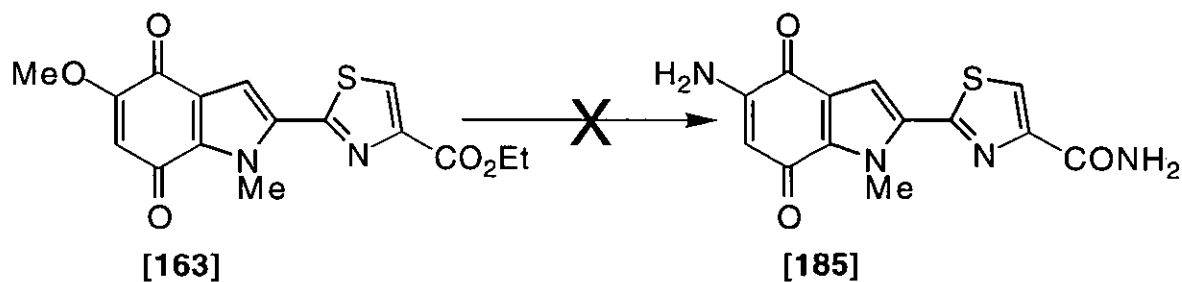


R	Time (h)	Yield %	Colour	Product No.
	24	42	Red	[180]
	24	37	Red	[181]
	24	-	-	-
	24	-	-	-
	24	-	-	-
	12	81	Purple	[182]
	12	60	Purple	[183]
	12	80	Purple	[184]

Table 4

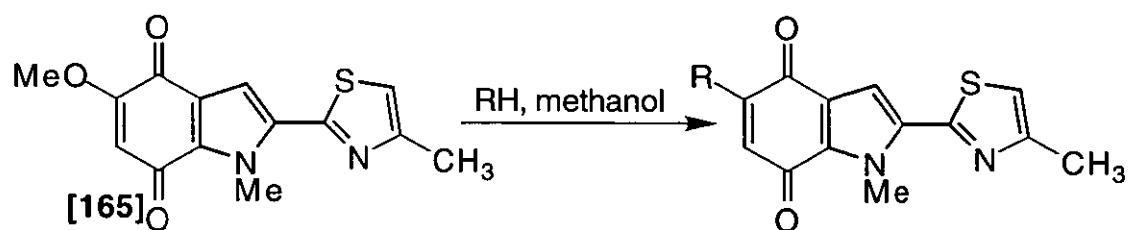
It has previously been shown, **Scheme 49**, that the C(5)-methoxy substituent on the BE 10988 precursor [146] can be exchanged by an amino group. It was anticipated that this methodology could be extended towards the synthesis of the BE 10988 analogue [185], in which the thiazole is attached to the C(2) position of the indolequinone, **Scheme 61**. Unfortunately, reaction of the quinone [163] with liquid ammonia at room temperature in a Young's sealed tube resulted in the formation of a complex mixture of products. Alternative procedures, *e.g.* reaction of the indolequinone with a 0.88 solution of ammonium hydroxide; reaction with a 0.88 solution of ammonium hydroxide and ammonium chloride at 100°C in a

sealed tube; and reaction with ammonium chloride in refluxing methanol, all failed to form any of the desired indolequinone [185].



Scheme 61

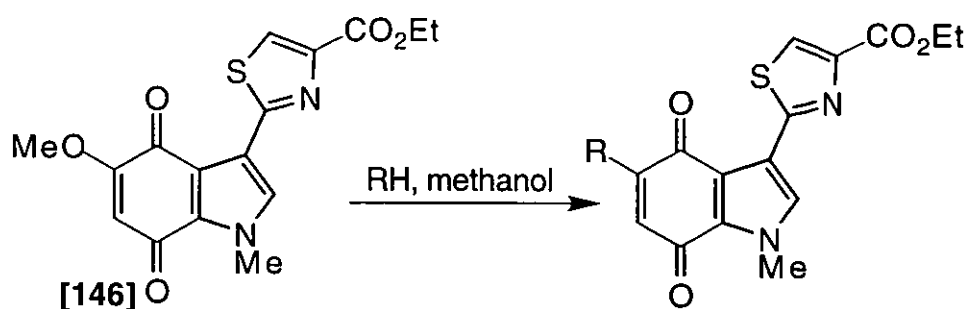
It was also possible to exchange the C(5)-methoxy group of the indolequinone derivative [165] with a variety of primary and secondary amine nucleophiles, in yields ranging from 27 to 85%, **Table 5**. Reaction of [165] with tetramethyl aziridine resulted in complete decomposition of the SM. However, contrary to the ester substituted thiazole derivative [163], reaction of the indolequinone [165] with *cis*-dimethylaziridine and azetidine formed the desired 5-(substituted-*cis*-dimethylaziridino) derivative [188] and 5-(substituted-azetidino) product [189] in 27% and 43% yields respectively. The ring strain in azetidine is less than that in the three membered aziridine series, and as a result azetidines show few of the exceptional properties associated with aziridines, however, ring cleavage reactions occur with greater ease than in the larger cyclic amines.¹²⁸ It was envisaged that the azetidine substituent would be activated in a manner similar to the aziridine series, although because of the decrease in ring strain the formation of drug-DNA alkylation sites would not be as pronounced as for the aziridino derivatives. The colour of the indolequinone derivatives was dependent upon the substituent at the C(5) position *i.e.*, the methoxy derivative [165] was orange, the aziridino derivatives were all red and the amino derivatives were purple.



R	Time (h)	Yield %	Colour	Product No.
	24	85	red	[186]
	48	40	red	[187]
	48	27	red	[188]
	24	-	-	-
	12	43	purple	[189]
	12	63	purple	[190]
	12	73	purple	[191]
	12	67	purple	[192]
	12	57	purple	[193]

Table 5

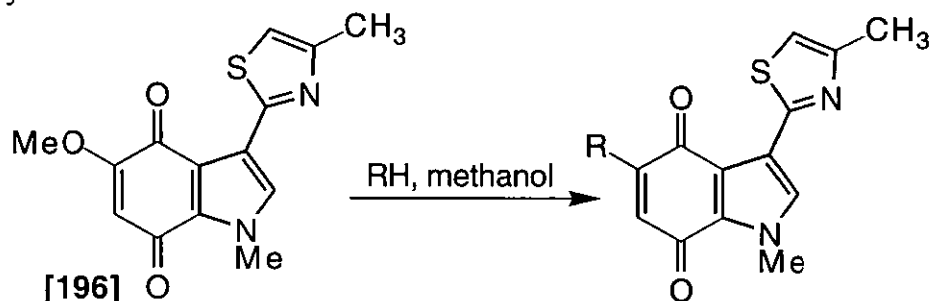
The thiazolyndolequinones [146], [196] and [199] have previously been synthesised within these laboratories.¹²⁹ It was envisaged that C(5)-methoxy quinone exchange reactions could be utilised for the formation of various C(5)-aziridino and 2-methylaziridino analogues.



R	Time (h)	Yield (%)	Product colour	Product No.
	24	95	red	[194]
	48	84	red	[195]

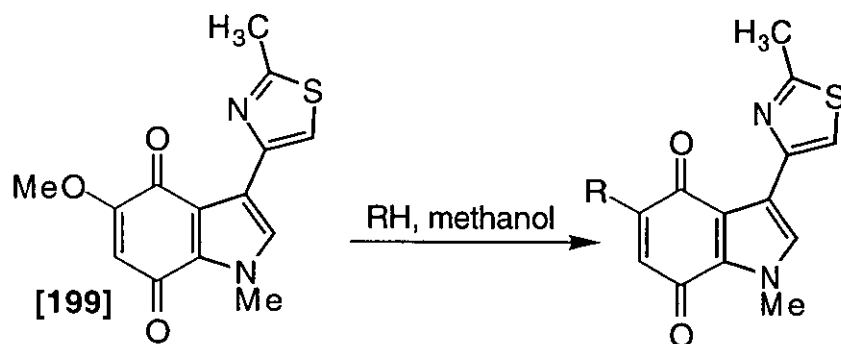
Table 6

The thiazolyloindolequinones [146], [196] and [199] were reacted with an excess of aziridine in methanol at rt over 24 h to form the desired 5-(substituted-aziridine) derivatives [194], [197] and [200] in 95, 92 and 87% yields respectively, **Tables 6, 7** and **8**. Likewise reaction with an excess of 2-methylaziridine in methanol at rt over a 48 h period formed the 5-(substituted-methylaziridine) derivatives [195], [198] and [201] in 84, 72 and 78% yields respectively.



R	Time (h)	Yield (%)	Product colour	Product No.
	24	92	red	[197]
	48	72	red	[198]

Table 7



R	Time (h)	Yield (%)	Product colour	Product No.
	24	87	red	[200]
	48	78	red	[201]

Table 8

3.6. Physical properties of C(5) substituted thiazoylindolequinone compounds

One of the striking properties of the C(5) substituted thiazoylindolequinone compounds is their colour, for example, orange for the 5-methoxy derivatives, purple for the 5-(substituted-amino) analogues and red for the C(5) aziridino derivatives. These physical properties can be rationalised as follows. Both the oxygen of the 5-methoxy indolequinones ($X = \text{OMe}$) and the nitrogen of the 5-(substituted-amino) derivatives ($X = \text{NR}^1\text{R}^2$) can rehybridise from sp^3 [202] to sp^2 [203], planar geometry, **Figure 7**. Since the lone pair of electrons on nitrogen is not so tightly bound, this leads to more extensive delocalisation of the chromophore, and a shift to a longer wavelength (red shift).

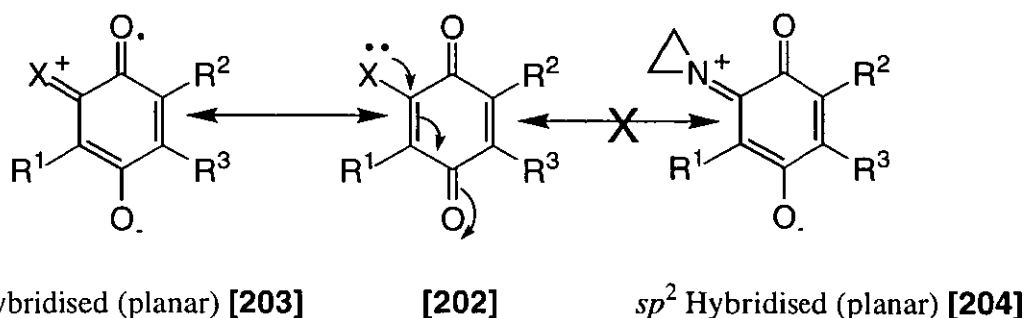


Figure 7

However, in the case of the aziridine derivative it is not favourable for the aziridine nitrogen atom to rehybridise from sp^3 to sp^2 , planar geometry, as this would increase the strain in the three-membered ring [204]. Hence, in the aziridino thiazoylindolequinone analogues, there is no extensive delocalisation of the chromophore, and a shift to a shorter wavelength compared with other amino derivatives is observed. Thus, these compounds appear red in colour.

A consequence of extensive delocalisation also results in an increase in quinone electropotential. This is reflected in the σ_p values (aziridine is -0.25,¹³⁰ methoxy = -0.28¹³¹), whereas other amine substituents are much more electron releasing (e.g., pyrrolidiny = -0.77¹³⁰), and as a result amino quinones are more difficult to reduce.

The various substituents attached to the C(5) position on the thiazoylindolequinones radically effect the NMR spectrum of the quinone moiety, **Figure 8**. The electron distribution within the enone portion is displaced by a series of inductive effects, which operate within the σ -framework (and fall off with distance) and conjugative effects, which operate in the π -system (and alternate along the conjugated chain).

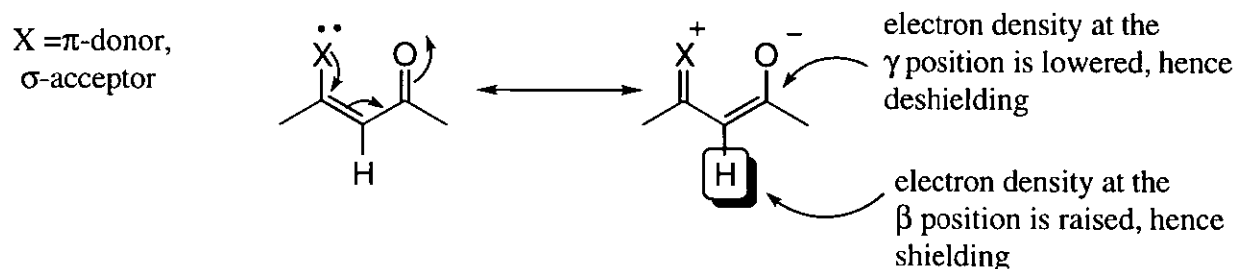


Figure 8

Thus, were X= a methoxy or amino (not aziridino) substituent the π -donor, σ -acceptor effect is enhanced, such that the nuclei at the β -position are shielded, causing an upfield shift in the spectrum and the carbonyl carbon at the γ -position is deshielded, causing a downfield shift. As previously mentioned, the lone pair of electrons on the nitrogen of aziridino substituents act less efficiently than other amines with conjugated substituents, and so the π -donor property is lowered, such that the shielding and deshielding effects at the β - and γ -positions are reduced.

The δ_H and δ_C values for the β -hydrogen and γ -carbonyl carbon resonances for a series of C(5) substituted thiazoylindolequinones are shown, **Table 9**. Consequently the more π -donating cyclopropylamino substituted derivative [193] shows an upfield shift in the 1H NMR spectrum at the H6 β -position and a downfield shift in the ^{13}C NMR of the C7 γ -

carbonyl position in comparison with the poor π -donating aziridino substituted derivative [186].


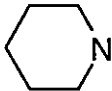
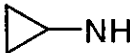
R	δ H ppm	δ C ppm	colour	No.
	5.88	179.43	red	[186]
MeO	5.75	179.21	orange	[165]
	5.59	180.29	purple	[191]
	5.56	180.31	purple	[193]

Table 9

3.7. Conclusions

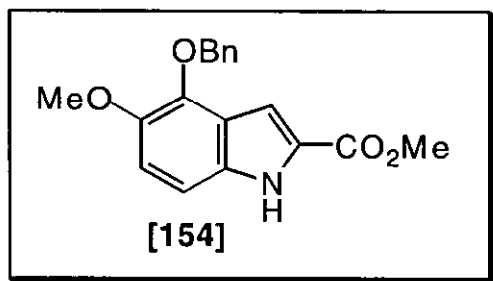
- The Hantzsch thiazole reaction can be applied towards the synthesis of the structurally challenging indolequinones [163] and [165].
- The Hantzsch thiazole reaction can be applied towards the synthesis of a indole substituted bithiazole
- Application of a indolyl anion nucleophilic substitution reaction on 2-bromothiazole, failed in the case of the highly substituted indole [174].
- C(5) methoxy quinone exchange reactions were found to proceed readily with a wide range of nitrogen nucleophiles on various thiazolyindolequinone compounds.

Chapter 4

**The synthesis of highly
substituted indoles.**

4.1. Introduction

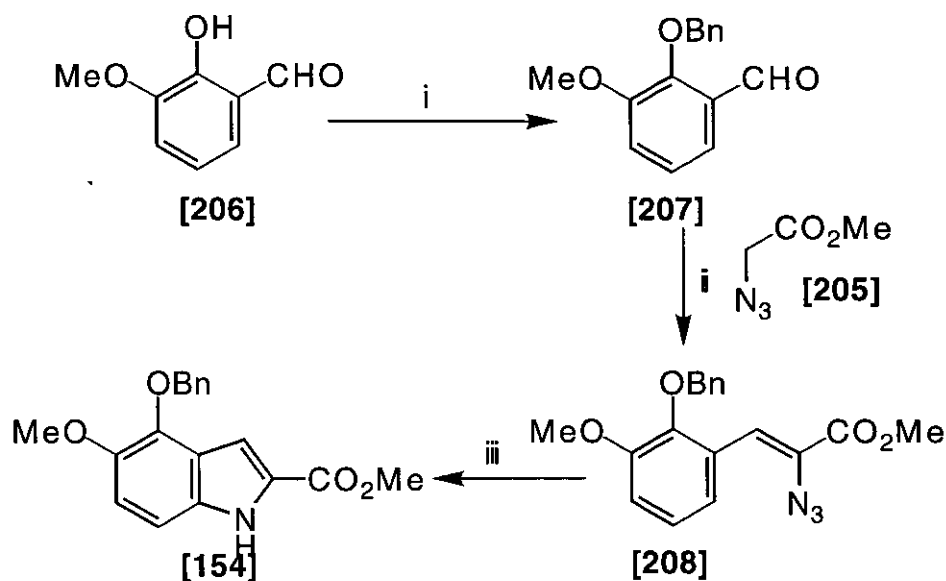
The indole methyl ester [154] is not only a key intermediate in the synthesis of BE 10988 [43] and its analogues^{115,129}, Chapter 3, but also the useful anticancer compound EO9 [34],¹⁴⁰ and the cyclopropamitosenes [32, 33].^{50-52, 132}



The methyl ester functionality at the C(2) position on the indole [154] can be readily transformed into useful functionality, *e.g.* either to a primary amide [156], Scheme 51; an aldehyde [142], Scheme 58; or a hydrogen [143], Scheme 58.

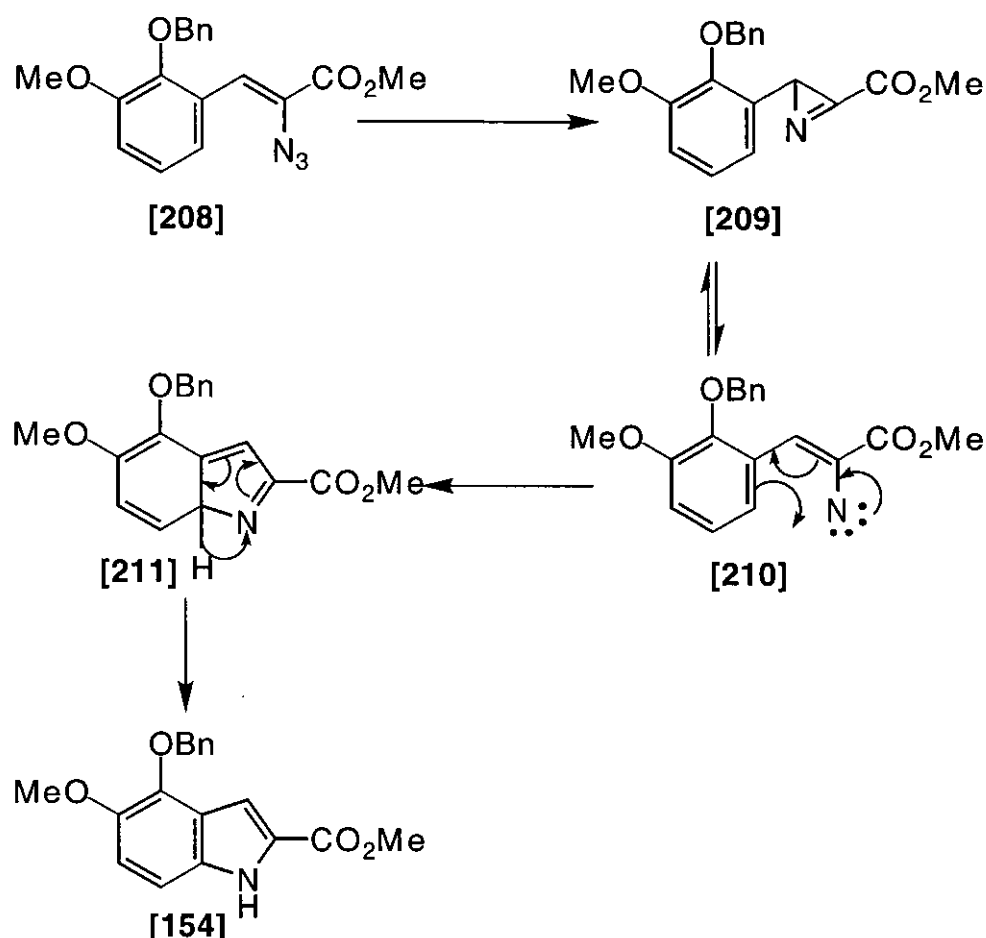
4.2. The synthesis of methyl 4-benzyloxy-5-methoxyindole-2-carboxylate [154] via a vinyl nitrene cyclisation

The Moody-Rees vinyl azide cyclisation was employed for the synthesis of the indole [154] as this allowed the introduction of carboxylate functionality at the indole C(2) position.¹³³ The synthesis of the indole [154] started from the cheap and readily available *ortho*-vanillin [206] which was *O*-benzylated by reaction with benzyl chloride in refluxing ethanol using potassium hydroxide as the base, in 74% yield, Scheme 62. The benzyl protecting group was originally chosen due to its facile removal under hydrogenolysis and its relative stability to both acidic and basic media.¹¹⁹ The *O*-benzylated aldehyde [207] was then condensed with methyl azidoacetate [205] using sodium methoxide as the base in methanol, to form the vinyl azide [208] in 82% yield. The resulting vinyl azide was thermolysed in refluxing xylene to afford the desired indole [154] in 66% yield.



Scheme 62: Reagents and conditions i) BnCl, KOH, ethanol, reflux, 74%, ii) methylazidoacetate [205], methanol, MeONa, -15°C, 82%, iii) xylene, reflux, 66%

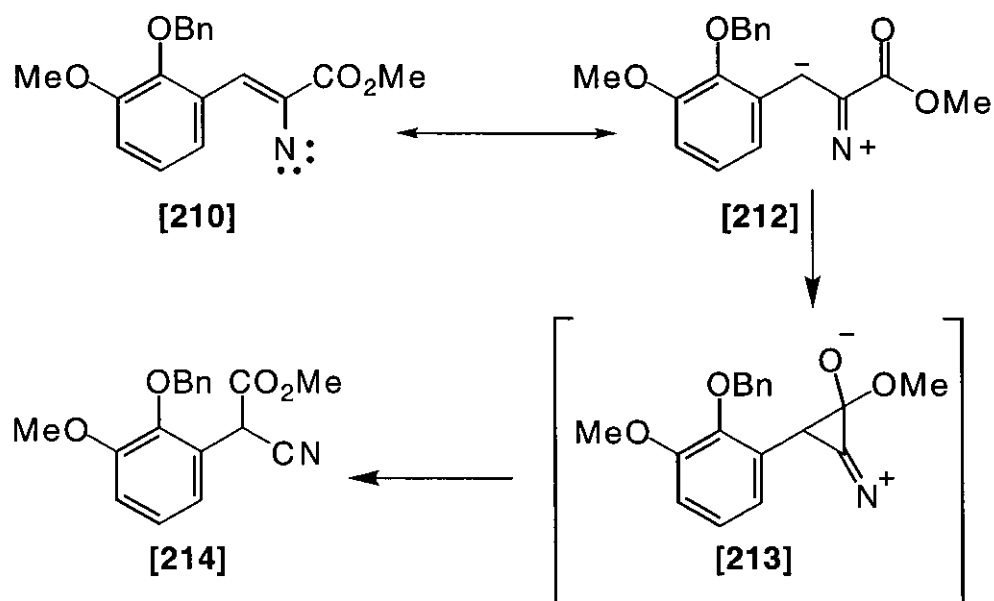
The geometry of the vinyl azide [208] is of no significance in the final outcome of the thermolysis reaction. The mechanism of the indole forming reaction, **Scheme 63**, is considered to proceed via the 2*H*-azirine [209] intermediate. Hemetsberger *et al.* have shown in the thermolysis of a series of azidocinnamates, the 2*H*-azirine intermediate accumulates at 80°C (shown by IR and ¹H NMR spectroscopy) and is converted upon further heating at 110°C to the corresponding indole.¹³⁴ The 2*H*-azirine is in equilibrium with the vinyl nitrene [210], and molecular orbital (MO) studies support the assumption that the C-N bond breaking requires less energy than C-C bond breaking, with no energy barrier for the conversion of nitrene back to the 2*H*-azirine.¹³⁵ The vinyl nitrene [210] then undergoes a 6π electrocyclisation to form [211] which, after a [1,5] H shift, forms the indole [154].



Scheme 63: Mechanism for the formation of indoles via a vinyl nitrene cyclisation

However, there are three major problems associated with the synthesis of the indole [154] via the thermolysis of the vinyl azide [208]: firstly, the occurrence of unwanted side products; secondly, the need for high dilution during the thermolysis reaction; and thirdly, the use of dangerous reactants, namely the use of large amounts of sodium azide required for the synthesis of methyl azidoacetate [205].

Analysis of the reaction mixture has shown the formation of the benzylic nitrile [214], formed as a by product from the vinyl nitrene intermediate [210], **Scheme 64**. In some cases up to 15% of the unwanted nitrile [214] has been observed.¹³² Resonance of the intermediate vinyl nitrene [210] provided the charged compound [212], which can be stabilised by the adjacent carbonyl group to form the intermediate [213], which can collapse to form the nitrile side product [214].



Scheme 64: Mechanism for the formation of the benzylic nitrile side product

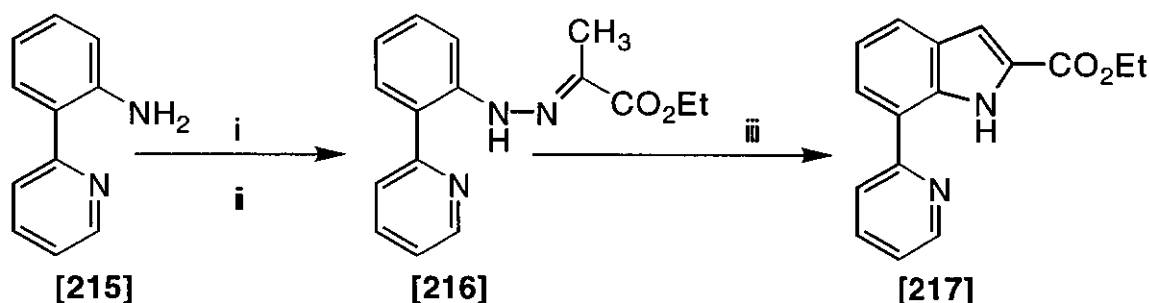
Perhaps the biggest problem associated with the thermolysis of the vinyl azide **[210]** has been the need for high dilution, it was found that the yield of the indole **[154]** decreased as the concentration of the vinyl azide increased. Optimised conditions for the reaction were found, in which the vinyl azide was ≥ 0.023 M. However this proved to be experimentally difficult, in that large quantities of xylene were required in order to produce 10-15g of the indole **[154]**.

Because of the importance of the indole **[154]** an alternative synthesis was required. The main criteria for the surrogate synthesis was to construct the indole **[154]** on a large scale without the need for chromatography and the use of dangerous reagents.

4.3. The synthesis of indole-2-carboxylates

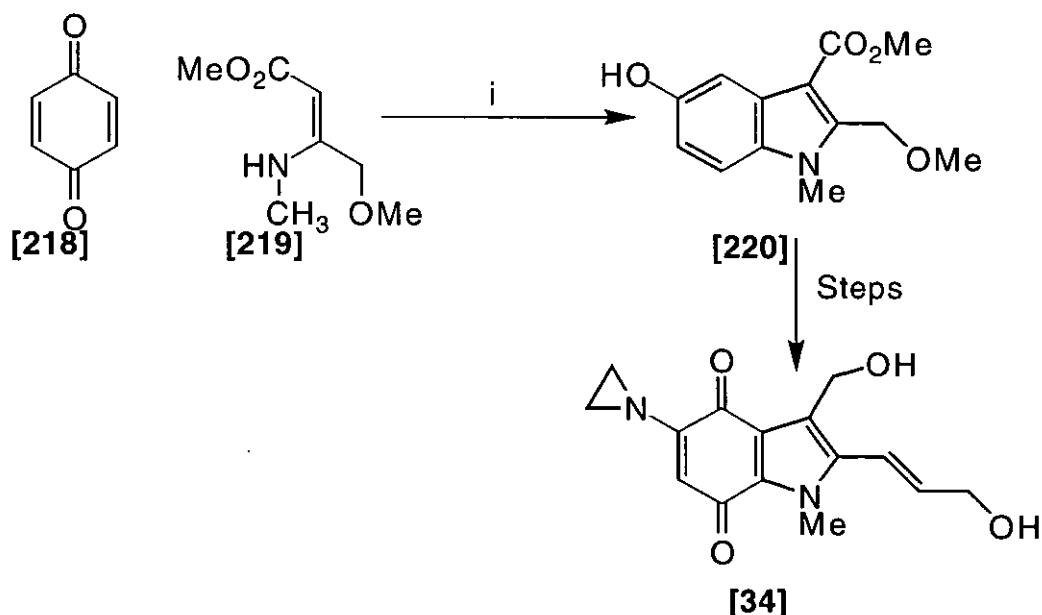
The synthesis of an indole moiety substituted at the C-2 position with a formyl or carboxylate functionality is far from trivial. The natural site for electrophilic substitution reaction on indole is at the C(3) position. Whereas Vilsmeier formylation proceeds with ease at the activated C(3) position,¹³⁶ Vilsmeier formylation at C(2), via a blocked C(3) indole does not.¹³⁷ It has been shown that certain protected indoles (namely *N*-methyl, *N*-benzenesulfonyl, and *N*-2-(trimethylsilyl)methoxymethyl)¹³⁸ can be selectively lithiated at the C(2) position and subsequently reacted with a range of electrophiles, however, the formation of the corresponding C(2) formyl derivatives have proved elusive.¹³⁸ The scarcity of methodology for the introduction of carboxylate functionality at the C(2) position on indole meant that a synthesis incorporating this functionality was required.

Black *et al.* have shown that the Japp-Klingemann reaction can be used as a entry to ester substituted hydrazones [216], **Scheme 65**.¹³⁹ The hydrazone was formed by conversion of the aniline [215] to the diazonium salt by reaction with sodium nitrite and hydrochloric acid and a Japp-Klingemann reaction with ethyl pyruvate formed the hydrazone [216], which was then subjected to a Fischer indole reaction in polyphosphoric acid (PPA) to form the corresponding C(2) ester substituted indole [217].



Scheme 65: Reagents and conditions i) NaNO_2 , HCl , ii) ethylpyruvate, iii) PPA

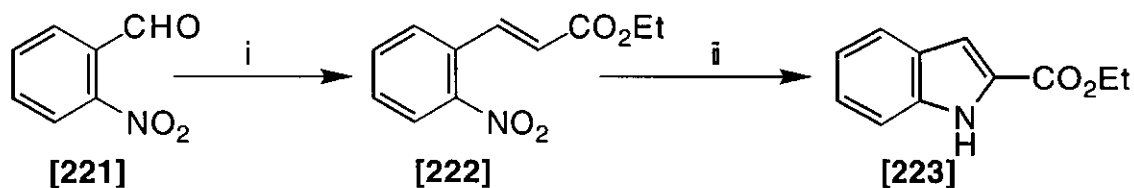
Kasai *et al.* have recently shown that a Nenitzescu reaction on quinone [218] in acetic acid at 50°C with the highly substituted amine [219], prepared from commercially available 4-methoxyacetoacetate and methylamine, formed the indole [220] in reasonable yield, **Scheme 66**.¹⁴⁰ Oxidation of the C(2) side chain with DDQ resulted in the formation of the formyl functionality. The indole derivative was subsequently converted into the anticancer agent EO9 [34].



Scheme 66: Reagents and conditions i) ethyl acetate, acetic acid, 50°C

Mali *et al.* have shown that a Cadogan-Sundberg reaction can be applied towards the synthesis of indole-2-carboxylates, **Scheme 67**.¹⁴¹ The cinnamate derivative [222] was formed by a

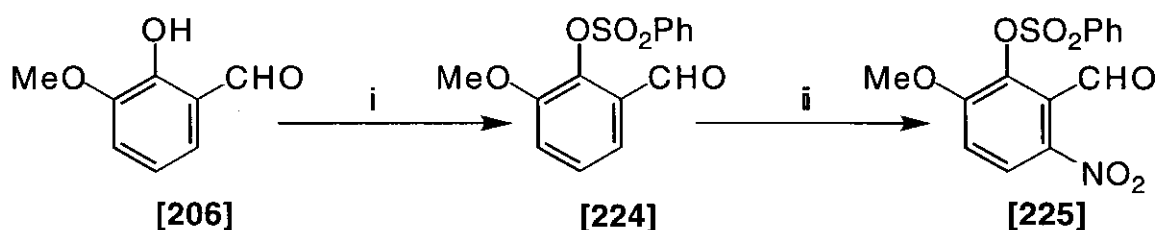
Wittig reaction between *ortho*-nitrobenzaldehyde [221] and carbomethoxymethylene triphenylphosphorane in refluxing benzene and was subsequently reacted with three equivalents of refluxing triethyl phosphite to form the indole-2-carboxylate [223] in reasonable yield.



Scheme 67: Reagents and conditions i) $(\text{C}_6\text{H}_5)_3\text{P}=\text{CHCO}_2\text{Et}$, benzene, reflux, ii) $(\text{EtO})_3\text{P}$, 170°C

4.4. Synthesis of methyl 4-benzyloxy-5-methoxyindole-2-carboxylate [154], via a Cadogan-Sundberg reaction

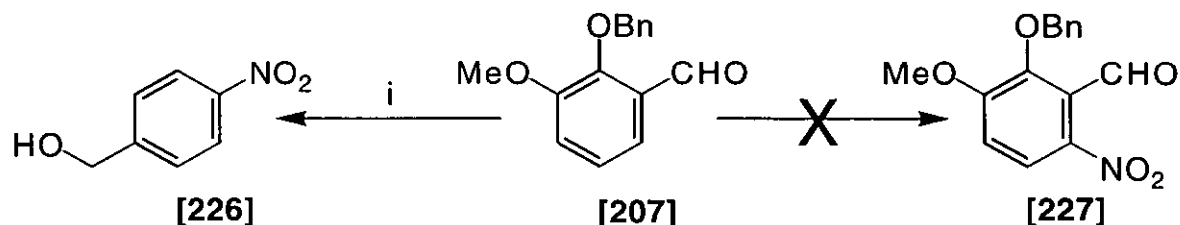
Out of the methods discussed the Cadogan-Sundberg methodology appeared to be most applicable towards the synthesis of the important indole [154]. Reid and Schiller have reported that nitration of benzenesulfonyl protected *ortho*-vanillin derivative [224] with fuming nitric acid results in nitration at the C-6 position, **Scheme 68**.¹⁴² The functionality on [225] suggested that elaboration to the indole [154] could be achieved, via the Cadogan-Sundberg methodology. *ortho*-Vanillin was shaken with a 15% solution of sodium hydroxide and benzenesulfonyl chloride to form the benzenesulfonyl protected *ortho*-vanillin [224] in 89% yield. The reaction could be performed on a large scale (>20g) and the product [224] was recrystallised to analytical purity. Reaction of the benzenesulfonyl *ortho*-vanillin [224] with fuming nitric acid, maintaining the temperature between $5\text{-}15^\circ\text{C}$, formed the desired nitro compound [225] in 61% yield, again the product was recrystallised from methanol. ^1H NMR spectroscopy of the nitro compound [225] revealed the H4 and H5 resonances as doublets at δ_{H} 7.09 and 7.56 ppm respectively, with J values of 6.7 Hz.



Scheme 68: Reagents and conditions i) benzenesulfonyl chloride, 15%, shaken not stirred, NaOH, 89%, ii) fuming HNO_3 , 61%

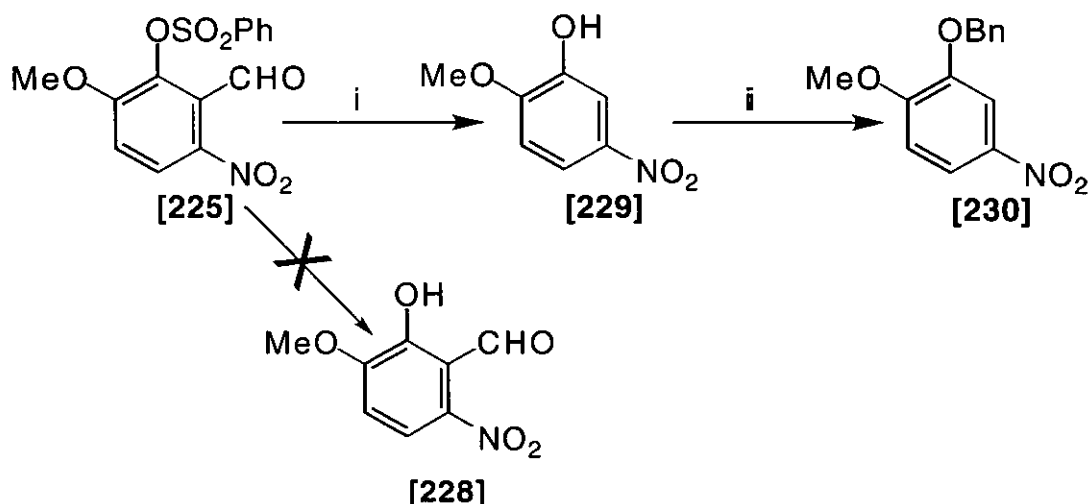
It was expected that the *O*-benzyl derivative [207] would be nitrated in a similar fashion to form [224]. However, attempted nitration of the *O*-benzyl aldehyde [207] with fuming nitric acid, maintaining the temperature between $5\text{-}15^\circ\text{C}$, failed to form any of the desired nitrated

compound [227], **Scheme 69**. The only product that was separated and characterised from the reaction mixture was the 4-nitrobenzyl alcohol [226] in 60% yield, presumably formed by nitration of the *O*-benzyl protecting group and cleavage under the extreme acidic conditions. Attempts to nitrate the *O*-benzyl aldehyde under more facile conditions, *e.g.* by reaction with trifluoromethanesulfonic acid and nitric acid in dichloromethane as the solvent at -60°C resulted in a complex mixture of products.¹⁴³



Scheme 69: Reagents and conditions i) fuming HNO₃, 5-15°C, 60%

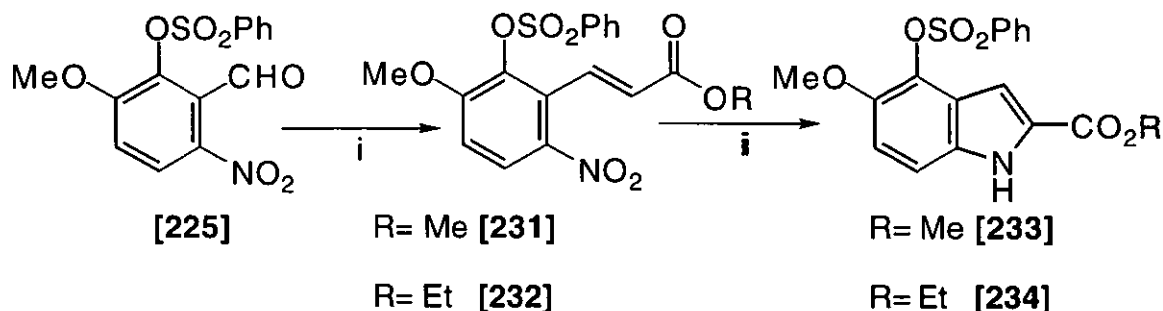
As an alternative synthesis towards the *O*-benzylated nitro compound [227] it was envisaged that the *O*-benzenesulfonyl protecting group on [225] could be cleaved and subsequently replaced with the *O*-benzyl functionality. However, basic hydrolysis of [225] with a 15% solution of potassium hydroxide in refluxing ethanol resulted in the formation of the decarbonylated phenol [229] in 90% yield, rather than the expected phenol [228], a mechanism for which has so far proved elusive, **Scheme 70**. *O*-Benzylation of the phenol [229] under standard conditions formed the protected phenol [230] in 96% yield.



Scheme 70: Reagents and conditions i) 15% KOH, MeOH, 90%, ii) KOH, BnBr, THF, 96%

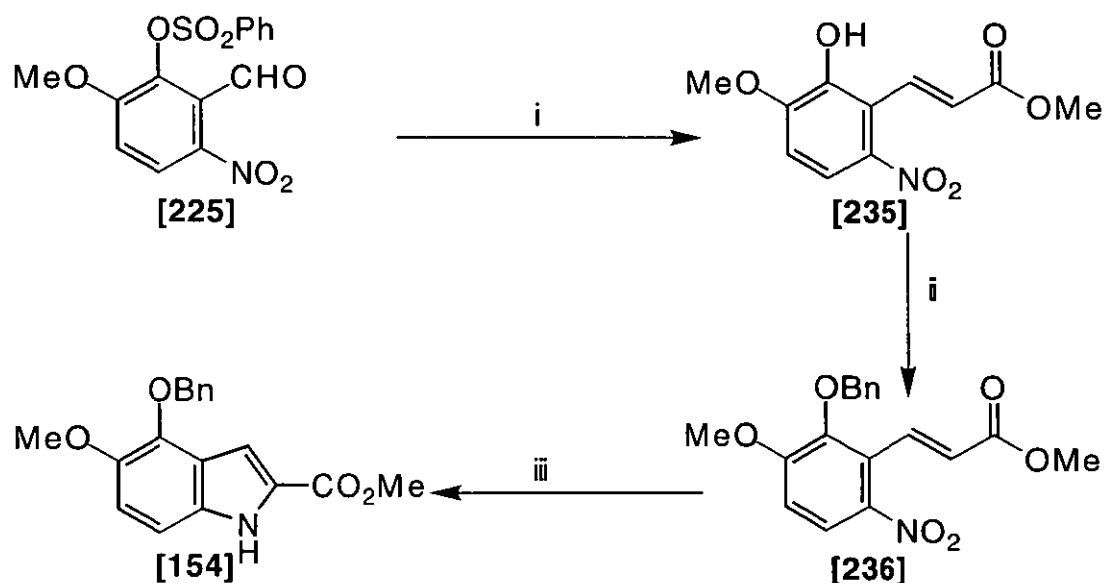
Elaboration of the nitro compound [225] by a Wadsworth-Emmons olefination, by reaction with methyl diethyl phosphonoacetate or triethyl phosphonoacetate in THF at rt with sodium hydride as the base formed the methyl and ethyl cinnamate derivatives [231] and [232] in 85 and 51% yield respectively, **Scheme 71**. The geometry of the olefin formed was predominately *trans*, identified by a coupling constant of 16 Hz between the olefinic protons in

^1H NMR analysis. A Cadogan-Sundberg reaction of the methyl cinnamate derivative [231] with three equivalents of triethyl phosphite at 170°C formed the C(2) methyl ester indole [233] in a respectable 66% yield. The corresponding C(2) ethyl ester functionalised indole [234] was formed in 79% yield under the same conditions.



Scheme 71: Reagents and conditions i) methyl diethylphosphonoacetate, NaH, THF, rt, 85% [231] or triethyl phosphonoacetate, NaH, THF, 51%, [232] iii) (EtO)₃P, 170°C , 66% [233] and 79% [234]

However, upon scale-up, using three equivalents of sodium hydride and methyl diethyl phosphonoacetate, it was rather fortuitously found that the resulting cinnamate derivative was the deprotected phenol [235] formed in 58% yield, **Scheme 72**. ^1H NMR revealed the phenolic OH resonance as a singlet at δ_{H} 6.38 ppm, which disappeared after a D₂O shake. The phenol [235] was subsequently protected as the *O*-benzylcinnamate derivative [236] in 68% yield by reaction with benzyl bromide in refluxing THF with potassium carbonate as the base. A Cadogan-Sundberg reaction of the cinnamate derivative [236] with three equivalents of triethyl phosphite at 170°C formed the desired indole [154] in 54% yield.



Scheme 72: Reagents and conditions i) methyl diethylphosphonoacetate, NaH, THF, rt, 58%, ii) BnBr, K₂CO₃, THF, reflux, iii) (EtO)₃P, 170°C , 54%

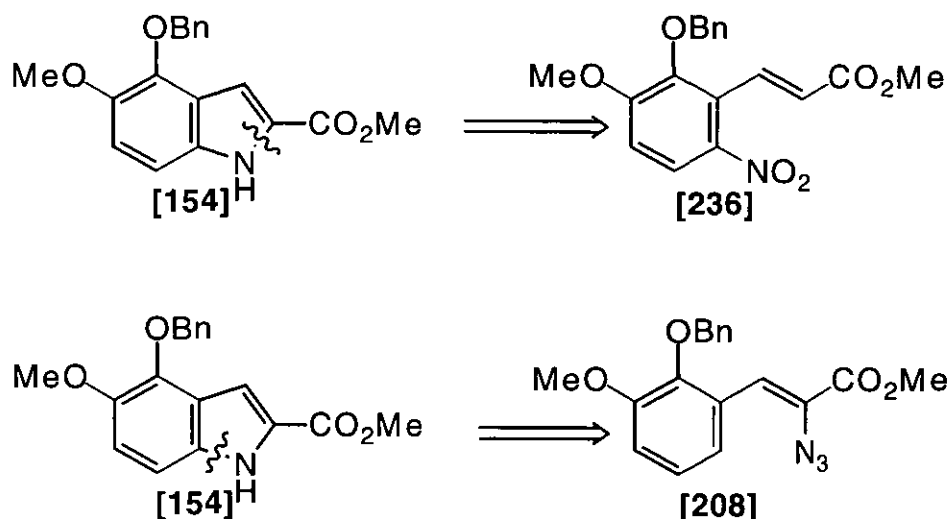


Figure 9: Retrosynthesis of the indole [154] via the Cadogan-Sundberg reaction and vinyl nitrene cyclisation reactions

It was proposed that an indole-2-carboxylate derivative could be synthesised by an intramolecular Wadsworth-Emmons olefination to form the indole C(2) / C(3) bond, **Figure 10**, in this case between the aldehyde and the nitrogen substituted phosphonate [239]. Couture *et al.* have recently applied an intramolecular Wittig reaction towards the synthesis of C(3) substituted indoles.¹⁴⁴

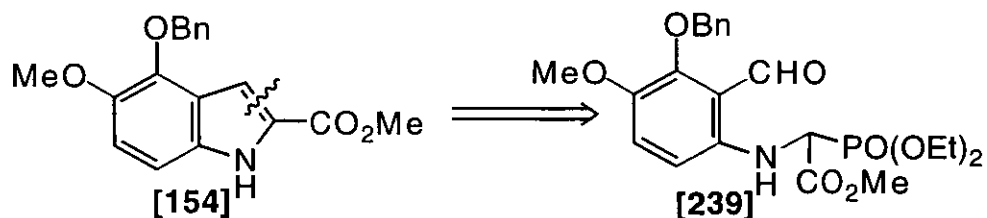
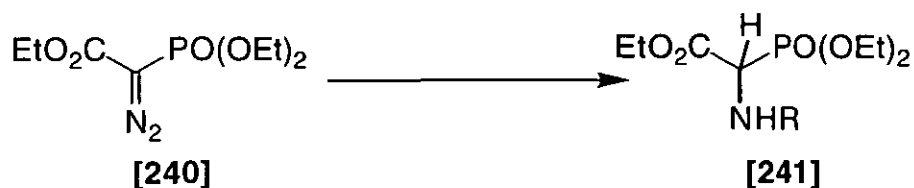


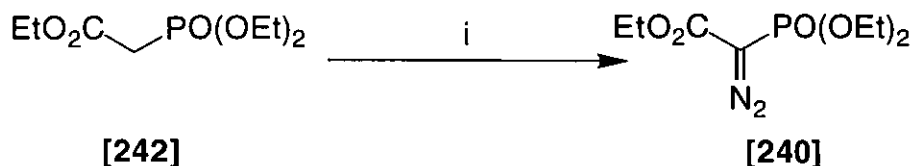
Figure 10: Retrosynthesis of an indole 2-carboxamide via an intramolecular Wadsworth-Emmons reaction

It has been shown within our research group at Loughborough, that anilines can be converted to *N*-phosphonoacetates [241], **Scheme 75**, by rhodium(II) acetate catalysed decomposition of triethyl diazophosphonoacetate [240] and anilines.¹⁴⁵



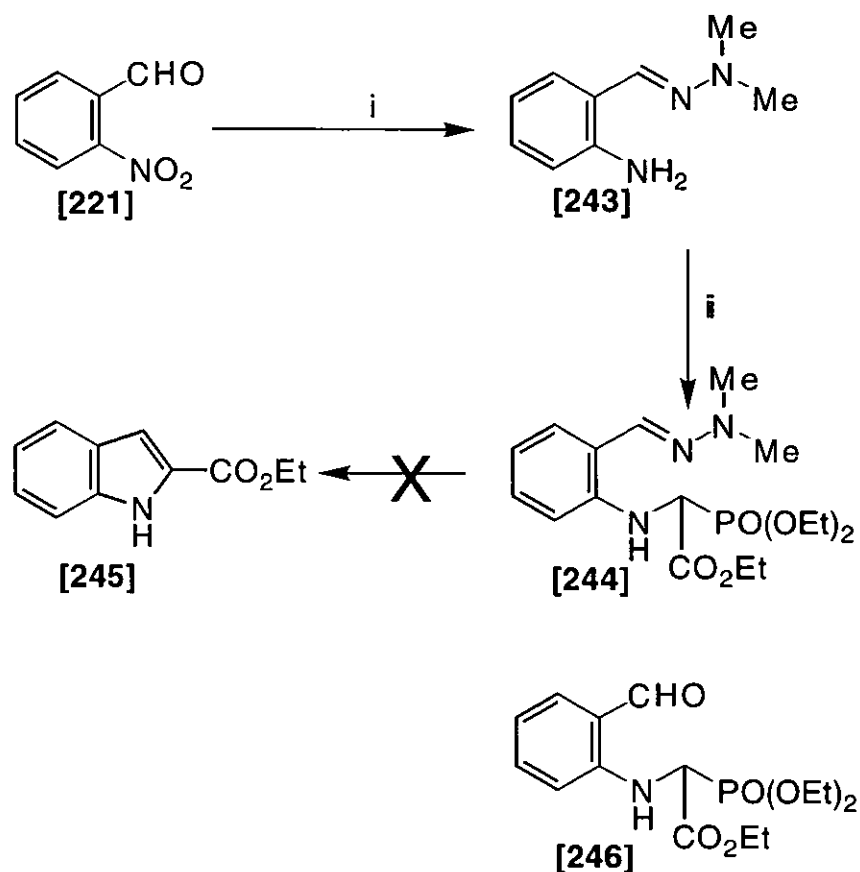
Scheme 75: Reagents and conditions i) $\text{Rh}_2(\text{OAc})_4$, toluene, RNH_2 , reflux

Triethyl diazophosphonoacetate [240] was prepared by the method described by Lee and Yuk,¹⁴⁶ in which *para*-toluenesulfonyl azide was added to a solution of triethyl phosphonoacetate [242] and cesium carbonate in THF to form the diazocompound in 74% yield, **Scheme 76**.



Scheme 76: Reagents and conditions i) CsCO₃, THF, *para*-toluene-sulfonylazide, 74%

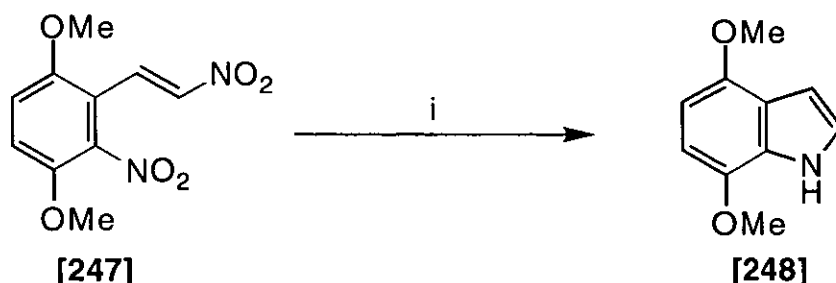
It was envisaged that the triethyldiazophosphonoacetate [240] could be used as the source of the phosphonoacetate functionality in [239]. A model reaction was originally carried out on *ortho*-nitrobenzaldehyde [221] to assess the feasibility of this methodology. Boothroyd and Kerr have shown that aryl nitro compounds can be reduced in excellent yield by reaction with *N,N*-dimethylhydrazine and ferric chloride in refluxing methanol, this reaction has the advantage that carbonyl functionality is tolerated due to *in situ* protection as the hydrazone.¹⁴⁷ *ortho*-Nitrobenzaldehyde [221] was reduced to the aniline derivative [243] in 50% yield by reaction with *N,N*-dimethylhydrazone and ferric chloride in refluxing methanol, **Scheme 77**. Rhodium(II) acetate catalysed decomposition of diazophosphonate [240] in the presence of the aniline [243] resulted in the formation of the *N*-phosphonoacetate [244] in 47% yield. It was anticipated that an intramolecular Wadsworth-Emmons olefination onto the hydrazone would result in the formation of the indole. However, reaction of the *N*-phosphonoacetate [244] with a variety of bases, *e.g.* sodium hydride, potassium hydride or LDA in either THF or DMF failed to form any of the desired indole [245]. Due to time constraints it was not possible to attempt to deprotect the hydrazone [244] to form the corresponding aldehyde [246] and then attempt the intramolecular Wadsworth-Emmons olefination.



Scheme 77: Reagents and conditions i) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, methanol, *N,N*-dimethylhydrazine, 50%, ii) [243], $\text{Rh}_2(\text{OAc})_4$, toluene, reflux, 47%

4.6. The synthesis of indoles by reductive cyclisation of *ortho*, β -dinitrosyrenes

Showalter and Pohlman have reported the synthesis of 4,7-dimethoxyindole [248] by a reductive cyclisation from the *ortho*, β -dinitrostyrene compound [247], by reduction with iron powder in refluxing acetic acid, **Scheme 78**.¹⁴⁸

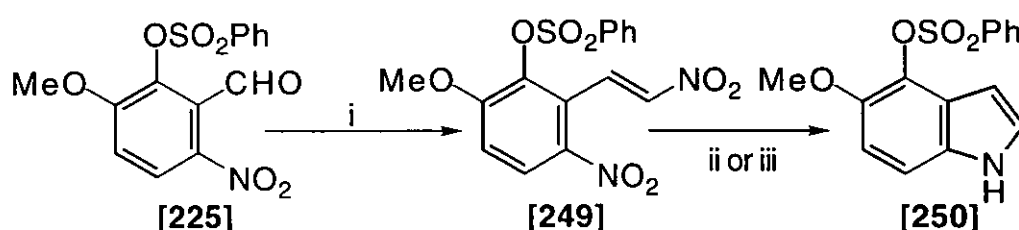


Scheme 78: Reagents and conditions i) $\text{CH}_3\text{CO}_2\text{H}$, Fe, reflux

It was contemplated that the reductive cyclisation methodology could be applied towards the synthesis of the indole [167], which has been used in the synthesis of BE 10988, **Scheme**

49, and mitomycin analogues. A Knoevenagel reaction between the aldehyde [225] and nitromethane in refluxing acetic acid with ammonium acetate formed the *ortho*, β -nitrostyrene [249] in a mediocre 47% yield, **Scheme 79**. Various alternative conditions for the formation of *ortho*, β -nitrostyrenes resulted in either poor yields of [249] or in the formation of a complex mixture of products.

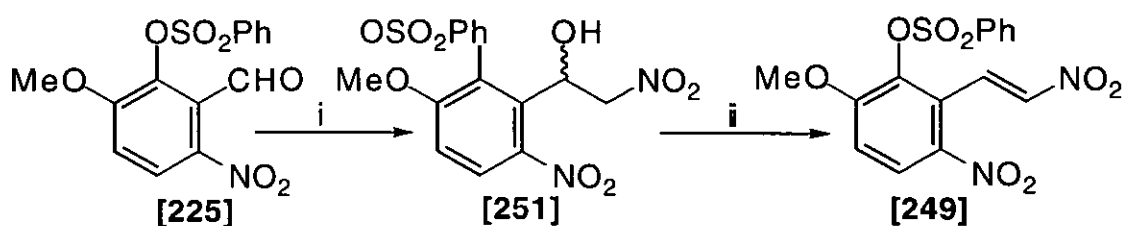
Reduction of the *ortho*, β -nitrostyrene [249] with iron powder in refluxing ethanol and acetic acid gave a poor 20% yield of the desired indole [250]. Burchardt and Sinhababu have reported, similar poor yields for the reductive cyclisation of alkoxy-*ortho*, β -nitrostyrenes to alkoxyindoles. However, they showed that the yields were greatly increased by the addition of silica gel and performing the reaction in a nonpolar solvent.¹⁴⁹ They reasoned that the poor yield in the iron / acetic acid reductive cyclisation was due to intermolecular reactions involving basic (neutral or negatively charged) intermediates and the starting material, to form dimeric and or intractable polymeric by-products. As silica gel binds polar materials strongly it was hypothesised that it would be possible to maintain the relatively nonpolar but reactive starting material in the solvent phase and the polar intermediates on the silica gel surface, thereby minimising interactions between the polar and the non polar species and also between the polar intermediates themselves. Harley-Mason has observed up to 14% of a highly insoluble dimeric indole, whilst using iron in acetic for the reductive cyclisation of various *ortho*, β -nitrostyrenes to indoles.¹⁵⁰ Reductive cyclisation of the *ortho*, β -nitrostyrene [249] with iron, acetic acid and silica gel in refluxing toluene resulted in the formation of the indole [250] in a much improved 85% yield.



Scheme 79: Reagents and conditions i) CH₃NO₂, toluene, CH₃CO₂H, CH₃CO₂NH₄, reflux, 47%, ii) CH₃CO₂H, Fe, EtOH, 20% or iii) CH₃CO₂H, toluene, Fe, SiO₂, 85%

The poor formation of the *ortho*, β -nitrostyrene [249], by various Knoevenagel reactions, prompted the search for an alternative procedure. The Henry reaction is a versatile procedure for the formation of α -hydroxynitro alkanes.¹⁵¹ Dehydration of the α -hydroxynitro alkane will provide the required nitroalkene functionality. Kamba and Yasuda have shown that potassium fluoride can be used to promote the Henry reaction towards unreactive aldehydes.¹⁵² The use of 5 mol% potassium fluoride in isopropanol at rt were found to be the optimum conditions. Reaction of the aldehyde [225] under the Kamba and Yasuda conditions at rt for 24 h resulted in the formation of the nitroalcohol [251] in 71% yield, **Scheme 80**.

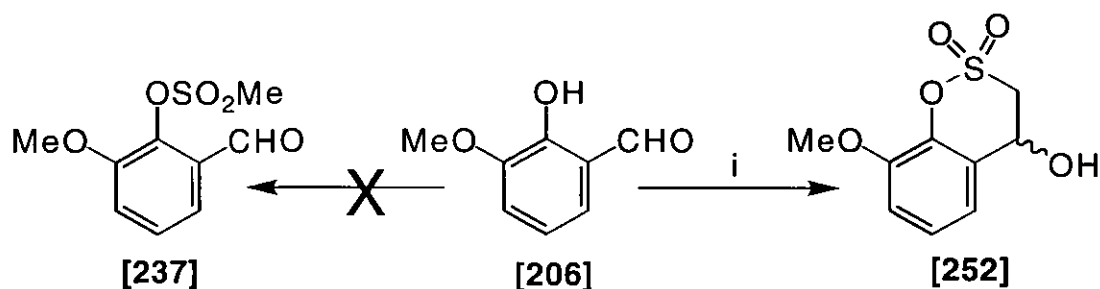
Dehydration of the nitroalcohol [251] employing the Melton and McMurry protocol,¹⁵³ by reaction with methanesulfonyl chloride and triethylamine in dichloromethane at rt formed the *ortho*, β -nitrostyrene [249] in 59% yield.



Scheme 80: Reagents and conditions i) KH, (CH₃)₂CH₂OH, CH₃NO₂, 71% ii) CH₃SO₂Cl, CH₂Cl₂, (CH₃)₃N, 59%

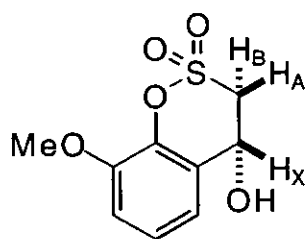
4.7. The serendipitous synthesis of a cyclic sulfonate

Attempted synthesis of the *O*-methanesulfonyl protected *ortho*-vanillin [237] by employment of the standard conditions for the synthesis of the benzenesulfonyl *ortho*-vanillin [224], *i.e.* shaking with a 15% solution of sodium hydroxide and methanesulfonyl chloride, resulted in the formation of the cyclic sulfonate [252] in 34% yield, **Scheme 81**, rather than the desired *O*-methanesulfonyl protected compound [237].

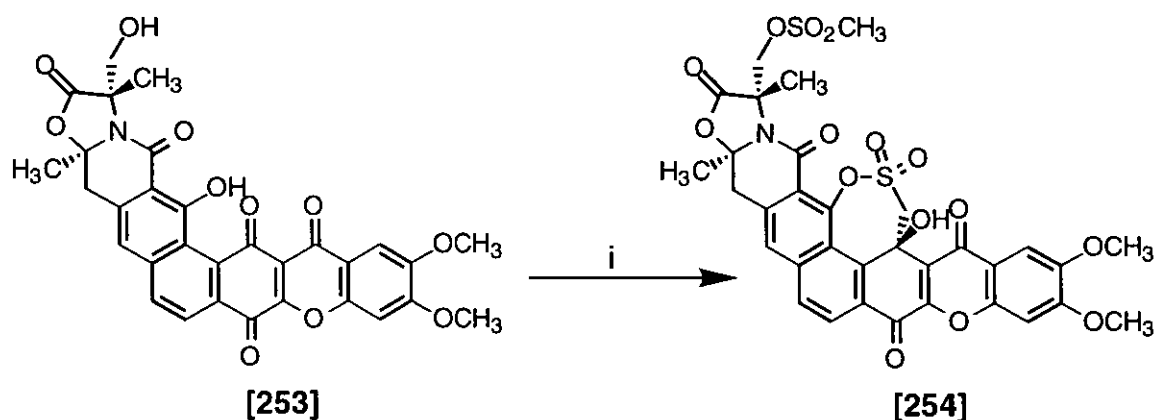


Scheme 81: Reagents and conditions i) 15% NaOH, methanesulfonylchloride, shaken not stirred, 34%

The cyclic sulfonate [252] was initially identified by ¹H NMR spectroscopy. The protons H_a, H_b and H_x formed a classic ABX system with the following coupling constants: *J*_{ab} 8.6, *J*_{AX} 5.0 Hz; *J*_{BA} 8.5, *J*_{BX} 1.2 Hz and *J*_{XA} 5.0, *J*_{XB} 1.2 Hz.

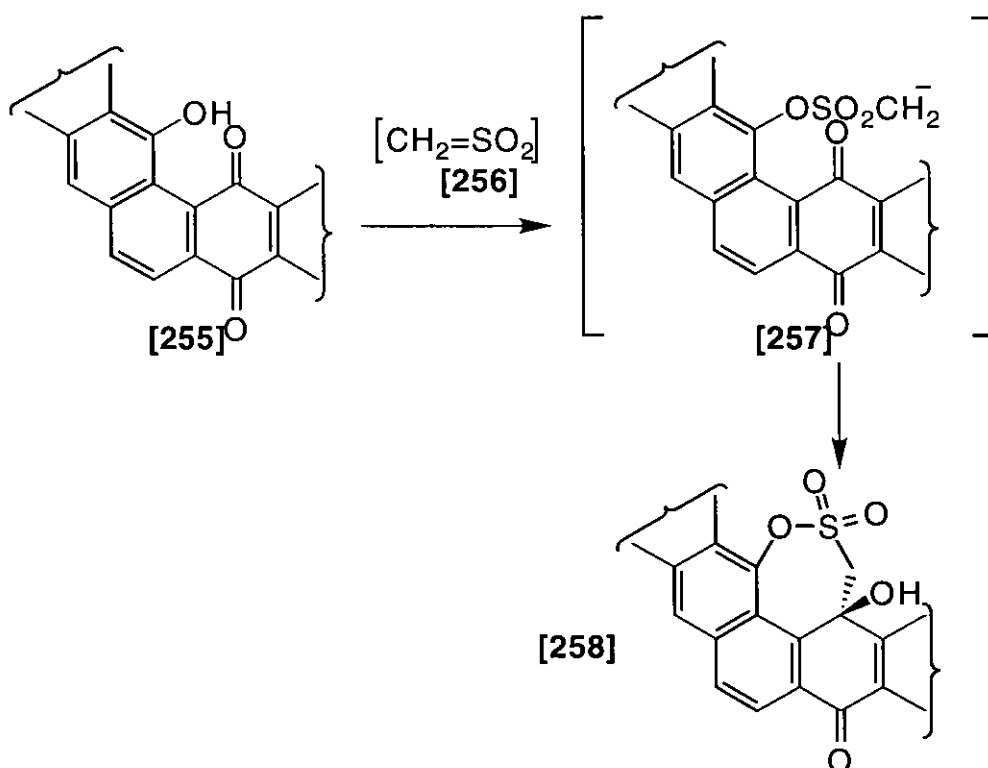


A similar reaction has been reported by Reich *et al.* during attempted *O*-methanesulfonyl protection of the hydroxyl groups on Citreamicin η [253]. Reaction with methanesulfonyl chloride and triethylamine resulted in the formation of the 7-membered cyclic sulfonate [254], **Scheme 82**.¹⁵⁴



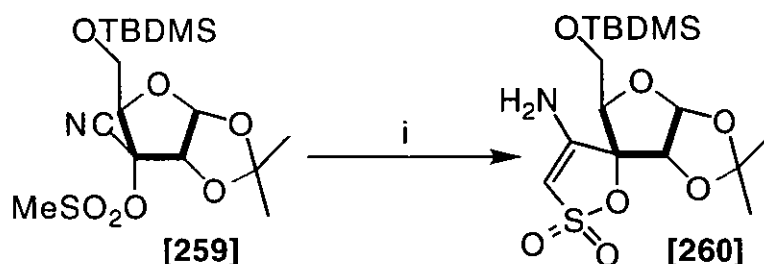
Scheme 82: Reagents and conditions i) $\text{CH}_3\text{SO}_2\text{Cl}$, $(\text{CH}_3)_3\text{N}$

They proposed that reaction of methanesulfonyl chloride and triethylamine initially formed sulfene [256]. Nucleophilic attack on sulfene by the phenoxo anion [255] formed the intermediate carbanion [257], **Scheme 83**. The nature of the polycyclic ring system allowed the positioning of the carbanionic species within bonding distance of the quinone carbonyl carbon and facilitated the formation of the seven membered cyclic sulfonate [258].



Scheme 83: Proposed mechanism for the formation of the cyclic sulfonate [258]

A related two-step sequence in carbohydrate chemistry has also been described by de las Heras *et al.* who found that mesylation of furanose cyanohydrins with methanesulfonyl chloride and pyridine, yielded α -(mesyloxy) nitriles [259].¹⁵⁵ Treatment with the base, DBU in acetonitrile, produced a spiro 4-amino-1,2-oxathiole 2,2-dioxide [260] formed via nucleophilic attack of a carbanionic $\text{OSO}_2\text{CH}_2^-$ species on the nitrile, **Scheme 84**.



Scheme 84: Reagents and conditions i) DBU, acetonitrile

4.8 Conclusions

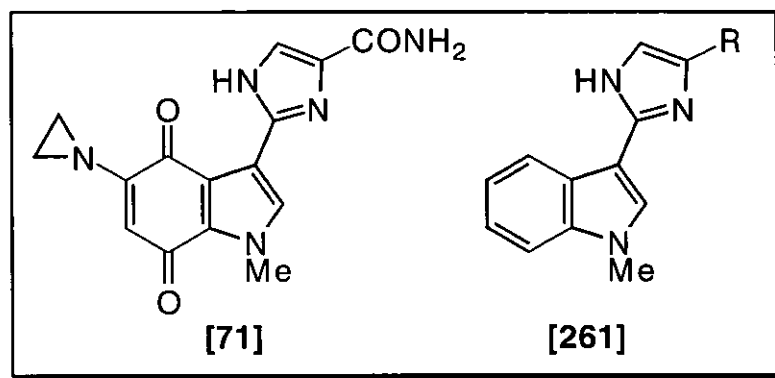
- The Moody-Rees vinyl azide cyclisation can be applied towards the indole [154], however, problems associated with scale up prompted the requirement for a more efficient synthesis.
- The Cadogan-Sundberg indole synthesis can be applied towards the synthesis of methyl 4-benzyloxy-5-methoxyindole-2-carboxylate [154].
- Reductive cyclisation of *ortho*, β -dinitrosyrenes can be applied towards the synthesis of highly substituted indoles.
- The serendipitous synthesis of cyclic sulfonates has been acknowledged.

Chapter 5

**Studies towards the synthesis
of the nortopsentins.**

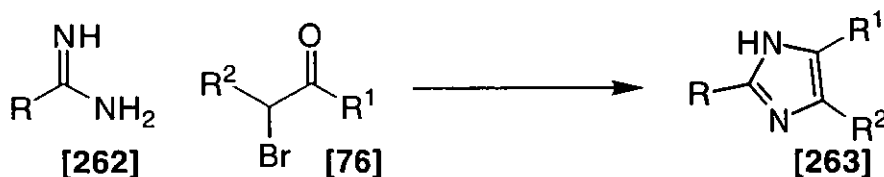
5.1. Introduction

The indolequinone [71] has been suggested as a possible synthetic target, **Chapter 1**. As in the indolequinone thiazole series of compounds, model studies were required in order to gain an insight into the synthetic methodology required towards the synthesis of the novel imidazolyindole structure. Therefore before embarking upon the synthesis of [71] it was decided to synthesise a series of simple imidazolyindoles, based on the generic structure [261].



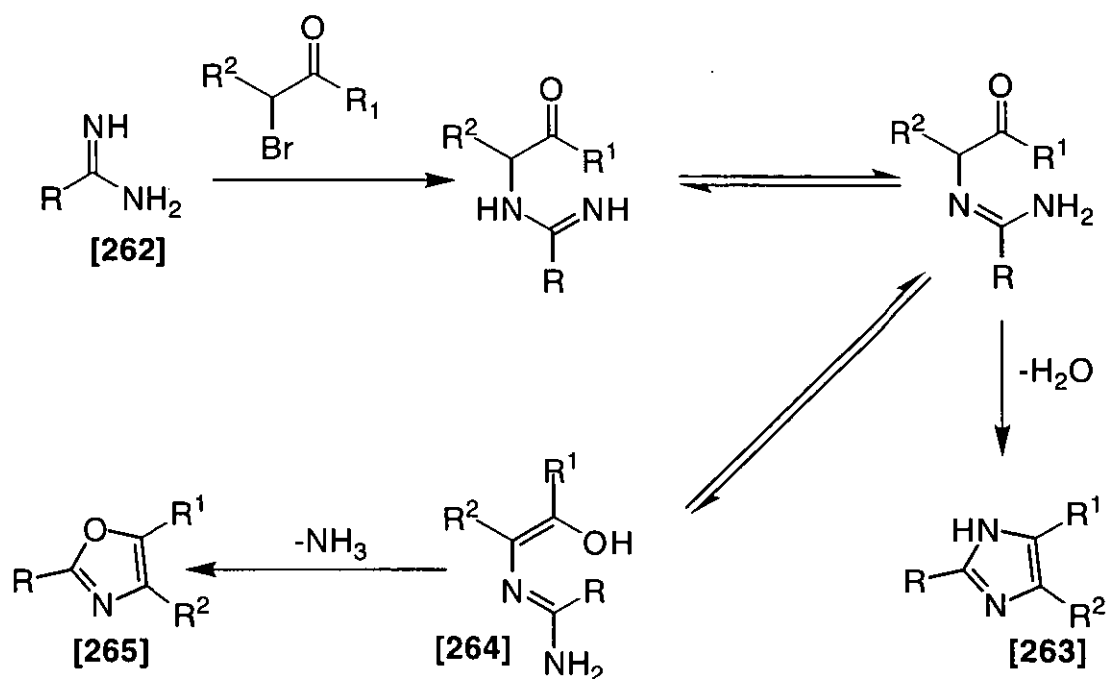
5.2. The synthesis of 2-4(5)-disubstituted imidazoles

The reaction between a suitable α -haloketone [76] and an amidine [262] has been shown by Brederek *et al.* to result in the formation of 2-4(5)-disubstituted imidazoles [263], **Scheme 85**.¹⁵⁶



Scheme 85: The synthesis of imidazole via the Brederek amidine cyclisation

The mechanism for the Brederek imidazole reaction is shown below, **Scheme 86**. In the case where the amidine [262] is formamidine (*i.e.* R=H) the nature of the α -halocarbonyl compound [76] has a major effect on the outcome of the reaction.¹⁵⁷ α -Haloketones in which the substituents R¹ and R² are aliphatic give almost exclusive formation of the imidazole [263], however, if the α -haloketone bears aromatic substituents then the major product from the reaction is the oxazole [265]. It is thought that the aromatic substituents favour the formation of the enol [264]. Fortunately amidines more complex than formamidine give imidazoles exclusively, this is thought to be a consequence of steric hindrance to the reaction of the enolic oxygen with the amidine carbon atom in the enol intermediate.



Scheme 86: The proposed mechanism for the Brederek amidine cyclisation

It must be noted that NH imidazoles bearing a substituent at either the C(4) [266] or the C(5) [267] position exist as a pair of tautomers, **Figure 11**, this is because of rapid proton exchange between the *sp*² NH and the *sp* C=N nitrogen atoms.¹⁵⁶

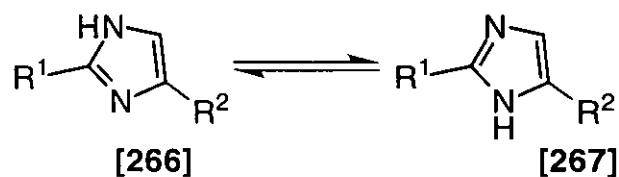


Figure 11

In neutral or organic solvents this proton exchange can be explained as an intermolecular process involving two or more imidazole molecules, **Figure 12**. In protic solvents, such as water, the solvent itself is involved. Thus this protropy makes the C(4) and C(5) positions of NH imidazoles magnetically and chemically equivalent.

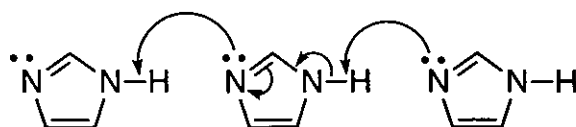
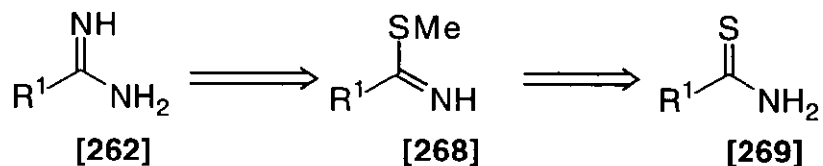


Fig 12

It was contemplated that the Brederek amidine cyclisation could be applied towards the synthesis of the required model imidazolyindole [261] and hence extended towards the synthesis of the more structurally challenging indolequinone imidazole [71].

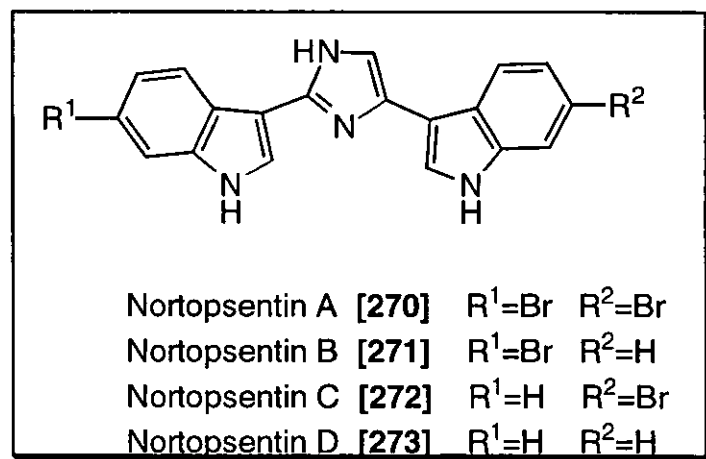
A general route for the synthesis of amidines involves functional group interconversion of a thioimidate [268], which can be formed by alkylation of a corresponding thioamide [269], **Scheme 87**.¹⁵⁸ This methodology appeared attractive because of our previous familiarity in the synthesis of indolyl thioamides (see **Chapter 2** and **Chapter 3**).



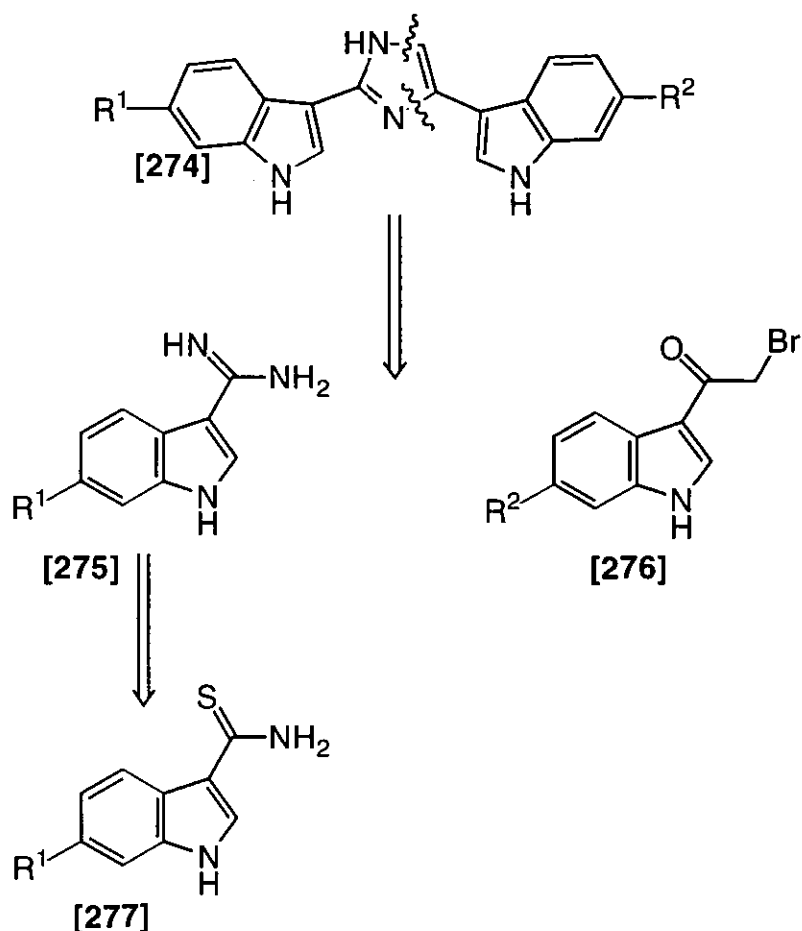
Scheme 87

5.3. The Nortopsentins

The nortopsentins A [270], B [271] and C [272] have recently been isolated from the deep sea sponge *Spongosorites ruetzler* by Sun *et al.*, and were found to possess cytotoxic and antifungal properties.^{159,160} Nortopsentin D [273] was formed by catalytic hydrogenation of either nortopsentin A, B and C at atmospheric pressure and rt. The imidazole bis[indole] skeleton of the natural compounds suggested that the model studies towards the desired imidazolyindole [261] could be extended to the synthesis of these complex multiheterocyclic natural products.

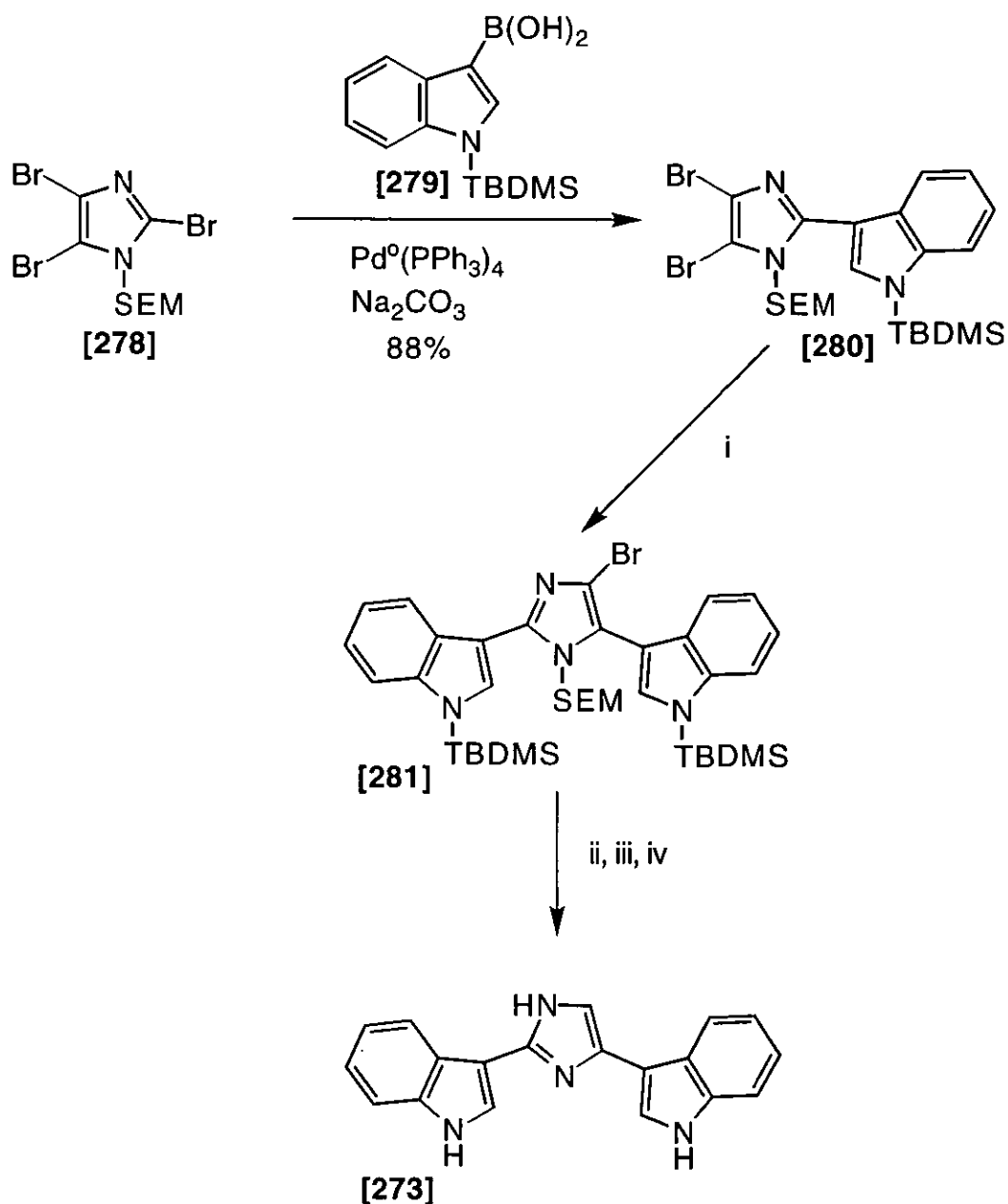


Retrosynthetic analysis of the general nortopsentin skeleton [274], employing the Brederick reaction as the strategem for imidazole formation, revealed the indolyl amidine fragment [275] (R¹= H or Br), which in turn can be derived from the corresponding indolyl thioamide [277] (again R¹= H or Br) and the indolyl α -bromoketone [276] (R²= H or Br), **Scheme 88**. The synthesis of indole containing compounds with similar functionality has been previously been described in **Chapter 2**. It was predicted that the bromine atom at the C(6) position on the naturally occurring nortopsentins would not dramatically alter the chemistry of the indoles with regards to the formation of the intermediates [275] (R¹ = Br) and [276] (R² = Br).



Scheme 88: Retrosynthesis of the nortopsentins

Ohta *et al.* have recently reported the total synthesis of nortopsentin D [273] by the use of successive palladium mediated Suzuki cross-coupling reactions, **Scheme 89**.¹⁶¹ The (trimethylsilyl)ethoxymethyl (SEM) protected tribromoimidazole [278] was found to undergo a palladium catalysed cross-coupling reaction with the *tert*-butyldimethylsilyl protected indole-3-boronic acid [279] to form the indole-3-imidazole [280]. Repetition of the cross-coupling reaction resulted in the formation of the imidazole bis[indole] [281]. Subsequent removal of the imidazole bromine and the indole and imidazole protecting groups formed nortopsentin D [273].

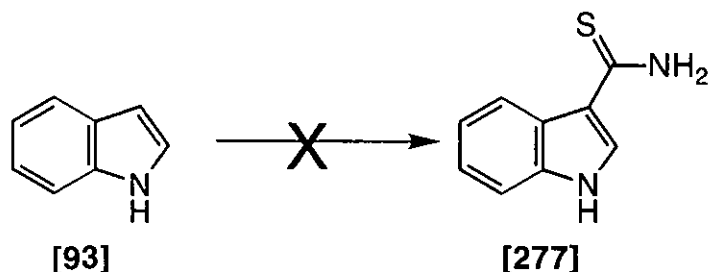


Scheme 89: Reagents and conditions i) [279], Pd(PPh₃)₄, Na₂CO₃, 70%, ii) nBuLi, iii) H₂O, iv) [CH₃(CH₂)₃]₄NF, 67% (from [281])

5.4. Synthetic routes to indole-3-amidines

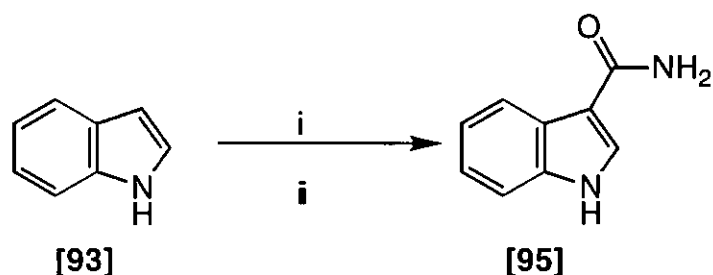
Papadopoulos has shown that a Friedel-Crafts thioacylation reaction can be applied towards the synthesis of aromatic thioamides, by reaction of ethoxycarbonyl isothiocyanate with a nucleophilic aromatic compound and anhydrous aluminium chloride.¹⁶² The product is a *N*-ethoxycarbonyl thioamide which is readily hydrolysed to a thioamide. Indole is known to undergo electrophilic substitution at the C(3) position and so this appeared an attractive synthetic route towards indole thioamides. This methodology was utilised by Shizuri *et al.* to form the indole C(3) thioamide in the synthesis of BE 10988, **Scheme 50**.¹¹⁶

However, reaction of indole [93] with ethoxycarbonyl isothiocyanate in refluxing toluene and anhydrous aluminium chloride, followed by subsequent basic hydrolysis failed to form any of the desired thioamide [277], **Scheme 90**. Altering the Lewis acid, reaction solvent and reaction conditions failed to form any product by this method.



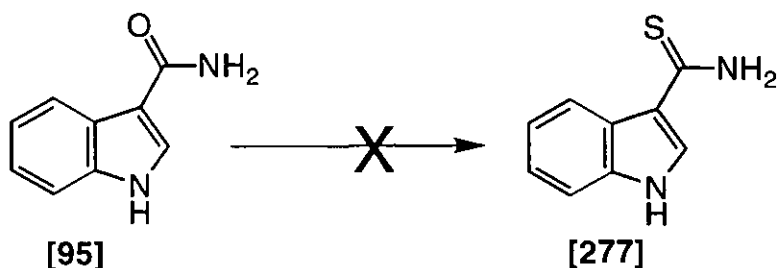
Scheme 90

Owing to the failure to form [277] by a direct route, synthetic efforts were concentrated on the synthesis *via* a thionation reaction of the corresponding indole-3-carboxamide [95]. The indole amide [95] was synthesised according to the method of Mehta, in 61% yield, **Scheme 91**.⁹⁷



Scheme 91: Reagents and conditions i) CSI, CH₃CN, 0°C, ii) KOH, acetone, H₂O, 61%

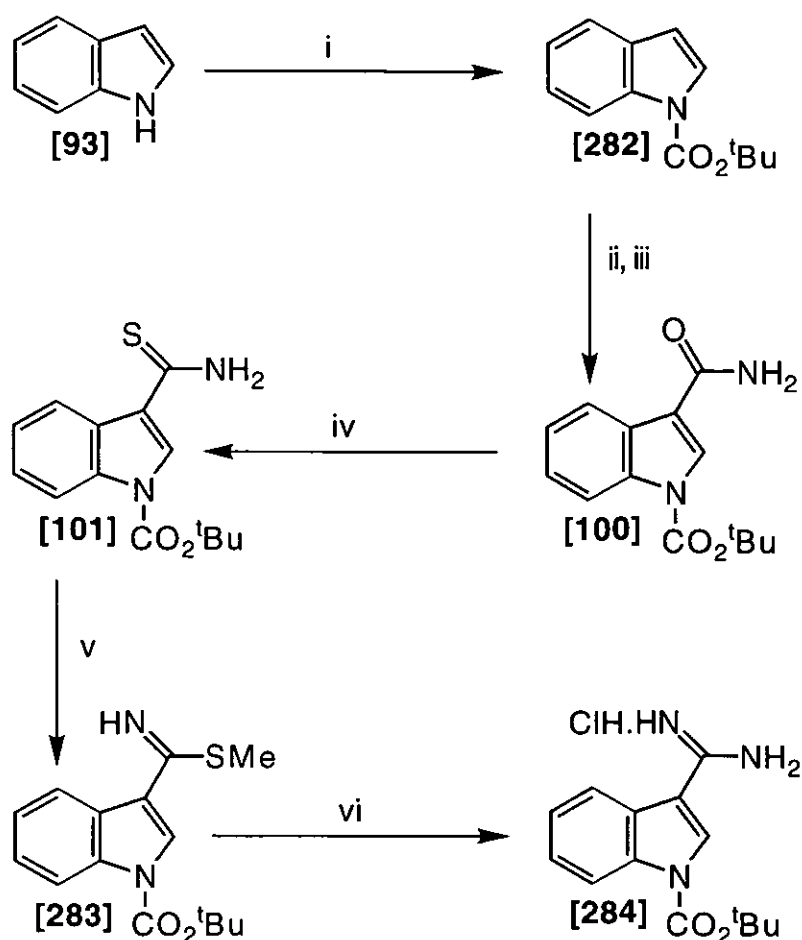
However, reaction of the amide [95] with 0.5 equivalents of LR in either refluxing toluene or benzene failed to form any of the desired thioamide [277], **Scheme 92**, and contrary to the findings of Ayer *et al.* reaction of the indole [95] with phosphorus pentasulfide in refluxing benzene resulted in a complex mixture of products.¹⁰¹



Scheme 92

It has previously been shown, **Scheme 26**, that *N*-Boc-protected indole-3-amide [100] was readily converted by reaction with LR to the corresponding thioamide [101] in 94% yield without forming any decomposition products. The *tert*-butyloxycarbonyl (Boc) protecting group has found widespread use in indole chemistry and can be cleaved either under mild acidic conditions or by reaction with sodium methoxide.¹⁶³

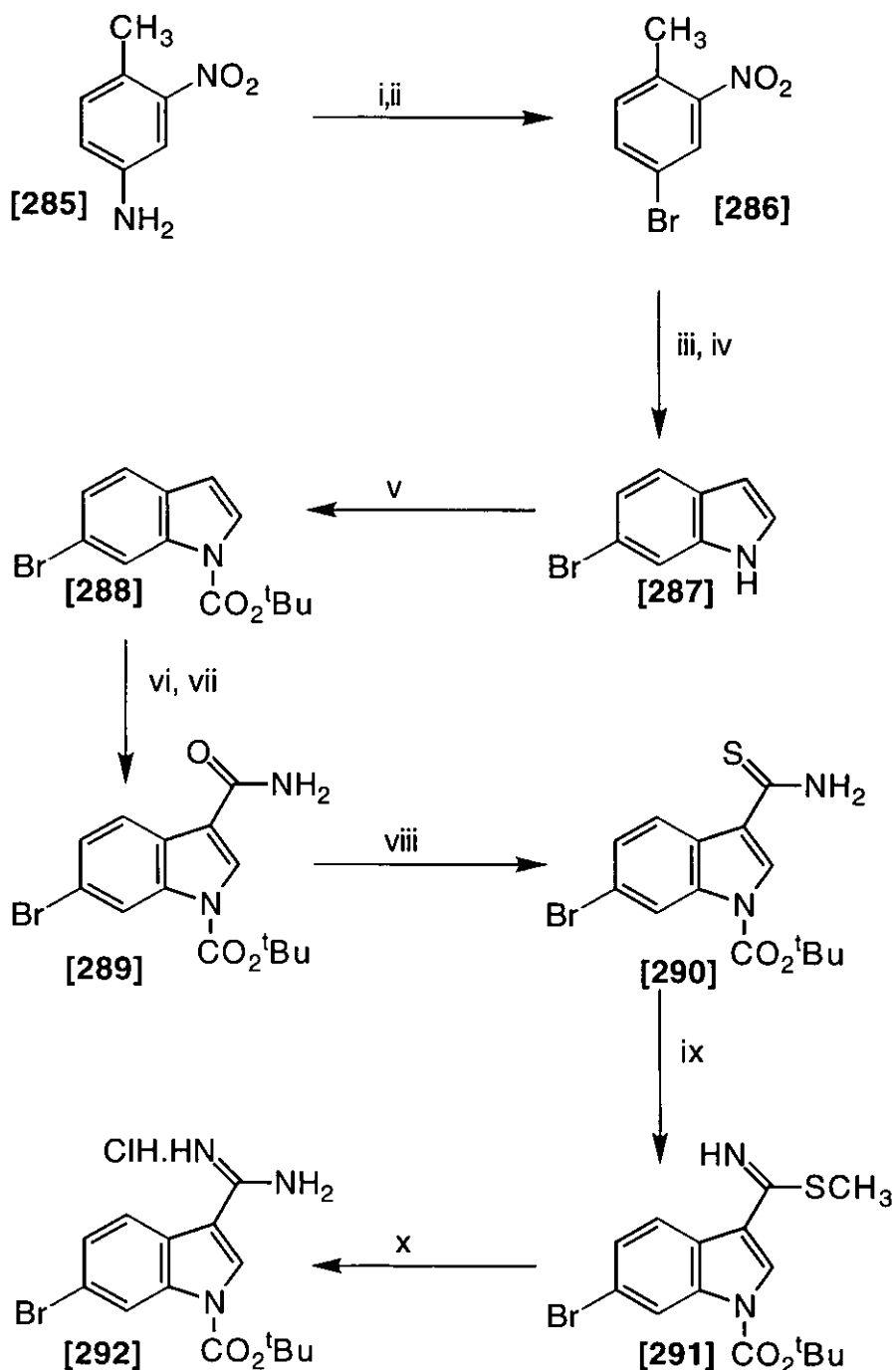
The Boc protecting group was introduced by reaction of indole [93] with di-*tert*-butyl dicarbonate and a catalytic amount of dimethylaminopyridine (DMAP), used as a hypernucleophilic acylation catalyst, in acetonitrile at rt to form [282] in 96% yield, **Scheme 93**. The *N*-Boc protecting group stabilises indole as a result of conjugation with the lone pair of electrons on the indole nitrogen, thus reducing the nucleophilicity at the indole C(3) position. Upon initial inspection, the use of such a protecting group would be expected to significantly reduce the yield of electrophilic substitution product. However, reaction of the protected indole [282] with CSI and subsequent basic hydrolysis formed the indole-3-carboxamide [100] in 64% yield. Thionation of the indole-3-carboxamide [100] to the thioamide [101] was achieved by reaction with 0.5 equivalents of LR in refluxing benzene in excellent 94% yield. Alkylation of the thioamide [101] by reaction with iodomethane in dichloromethane at rt for 24h formed the required thioimidate [283] in a respectable 74% yield. The labile methylthiol functionality in thioimidates activates them towards nucleophilic attack at the imine carbon. Ammoniolysis by reaction with ammonium chloride in refluxing methanol resulted in the formation of the amidine [284], as the hydrochloride salt, in 62% yield. Methanethiol is liberated and so extreme care was taken. A potassium permanganate trap was attached to the reaction vessel, in order to oxidise the anticipated smelly methanethiol to methanesulfonic acid.



Scheme 93: Reagents and conditions i) $\text{O}[\text{CO}_2\text{C}(\text{CH}_3)_3]_2$, DMAP, CH_3CN , 96%, ii) CSI, CH_3CN , 0°C , iii) KOH, acetone, H_2O , 94%, iv) LR [99], benzene, reflux, 94%, v) CH_3I , CH_2Cl_2 , 74%, vi) NH_4Cl , CH_3OH , reflux, 62%

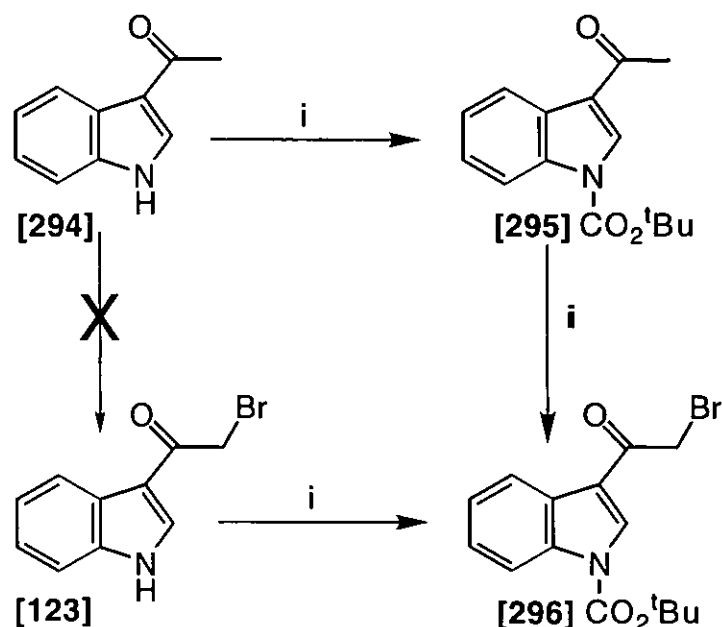
The indolyl amidine reaction precursor for the synthesis of nortopsentin A [270] and B [271] required a bromine atom at the indole C(6) position. Unfortunately 6-bromoindole is not commercially available and so an independent synthesis was required. Out of the many reported methodologies for the synthesis of 6-bromoindole, employment of a modified Leimgruber-Batcho reaction appeared the most promising.¹⁶⁴ 4-Amino-2-nitrotoluene [285] was converted to 4-bromo-2-nitrotoluene [286] via a Sandmeyer reaction, **Scheme 94**.¹⁶⁵ The intermediate diazo compound was formed by reaction of the aniline [285] with 48% hydrobromic acid in refluxing water followed by addition of aqueous sodium nitrite, maintaining the temperature between $0\text{--}5^\circ\text{C}$, and the resultant diazo intermediate was then reacted with cuprous bromide (copper(I)bromide). Steam distillation of the crude reaction mixture afforded the desired product [286] in 88% yield. The arylbromo derivative [286] was subjected to a modified Leimgruber-Batcho indole synthesis by initial reaction with pyrrolidine and DMF-dimethylacetal in refluxing DMF to form a intermediate β -dialkylamino-2-nitrostyrene, this was then reacted under reductive conditions with 80% acetic acid and zinc

dust to form 6-bromoindole [287] in 54% yield. The desired indole amidine derivative [292] was formed under analogous conditions for the synthesis of [284].



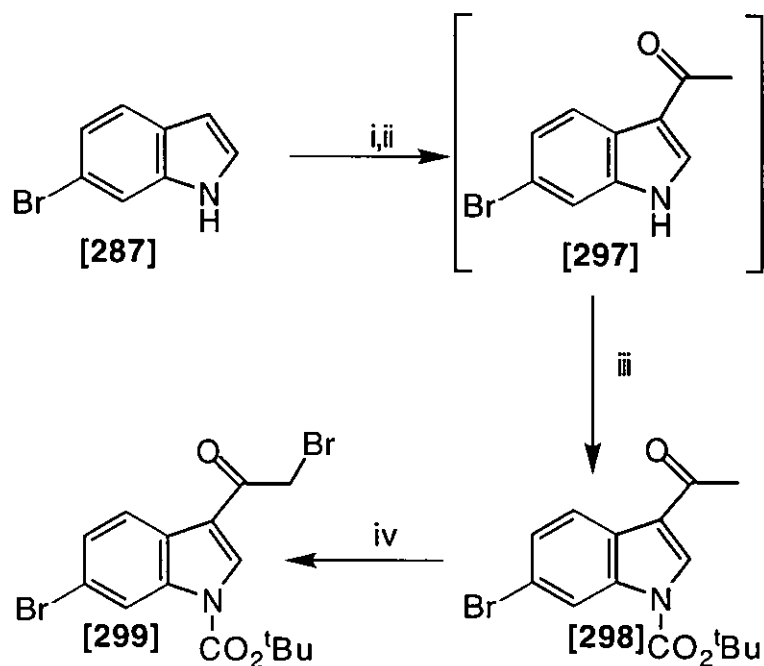
Scheme 94: Reagents and conditions i) 48% HBr, H₂O, NaNO₂, ii) CuBr, iii) pyrrolidine, (CH₃)₂NCH(OC₂H₅)₂, DMF, reflux, 88%, iv) Zn, CH₃CO₂H, H₂O, 80°C, 54%, v) O[CO₂C(CH₃)₃]₂, DMAP, CH₃CN, 77%, vi) CSI, CH₃CN, 0°C, vii) KOH, acetone, H₂O, 64%, viii) LR [99], benzene, reflux, 49%, ix) CH₃I, CH₂Cl₂, 91% crude, x) NH₄Cl, CH₃OH, reflux, 45%

a catalytic amount of DMAP in acetonitrile, independently formed the desired bromo compound [296] in 92% yield.



Scheme 96: Reagents and conditions i) $\text{O}[\text{CO}_2\text{C}(\text{CH}_3)_3]_2$, DMAP, 82% [295] and 92% [296] ii) CuBr_2 , CH_3Cl , ethyl acetate, reflux, 45%

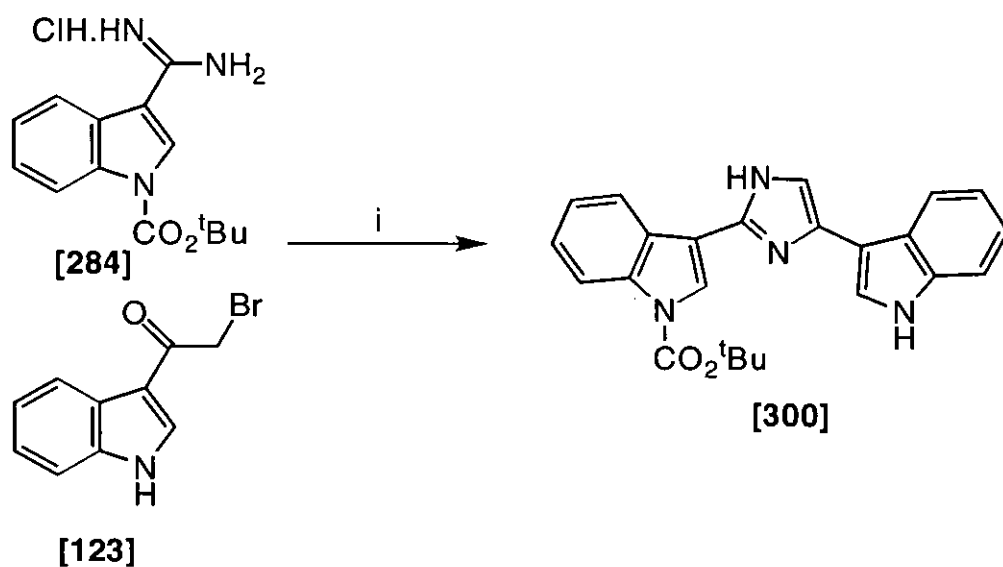
The corresponding 6-bromo indole derivative [299] was formed *via* an initial Vilsmeier acetylation on 6-bromoindole [287], by reaction with *N,N*-diethylacetamide and phosphorus oxychloride in chloroform to form, after basic work-up, the acetyl indole derivative [297] in a poor 8% yield, **Scheme 97**.¹⁶⁶ The acetyl derivative [297] appeared to be unstable and so standard *N*-Boc protection was immediately carried out to provide the *N*-Boc protected derivative [298] in 40% yield. Bromination of the acetyl derivative [298] by reaction with copper(II)bromide in a 1:1 mixture of refluxing chloroform and ethyl acetate over 4 h formed the desired bromo compound [299] in a poor 45% yield.



Scheme 97: Reagents and conditions i) $\text{CH}_3\text{CON}(\text{CH}_3)_2$, POCl_3 , CH_3Cl , reflux, ii) 40% KOH , iii) $\text{O}[\text{CO}_2\text{C}(\text{CH}_3)_3]_2$, DMAP , 40%, iv) CuBr_2 , CH_3Cl , ethyl acetate, reflux, 45%

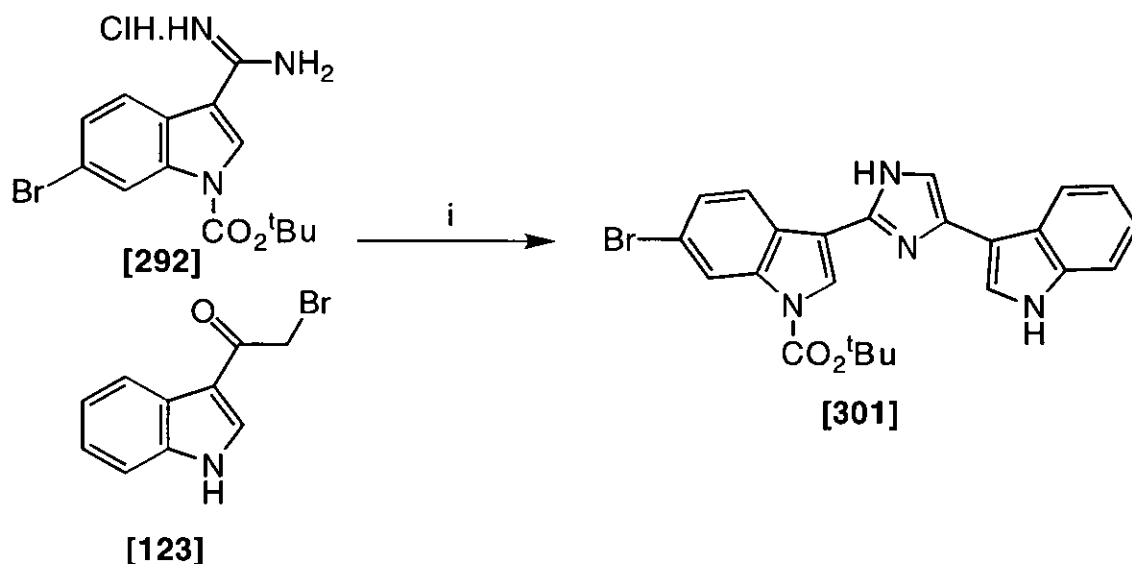
5.6. Synthesis of BOC protected nortopsentins

A Brederik imidazole reaction between the indolyl amidine [284] and the α -bromoketone [123] with potassium carbonate in refluxing acetonitrile resulted in the formation of the *N*-Boc imidazole bis[indole] derivative [300] in a reasonable 41% yield, **Scheme 98**. The imidazole bis[indole] [300] is *N*-Boc protected nortopsentins D [273].



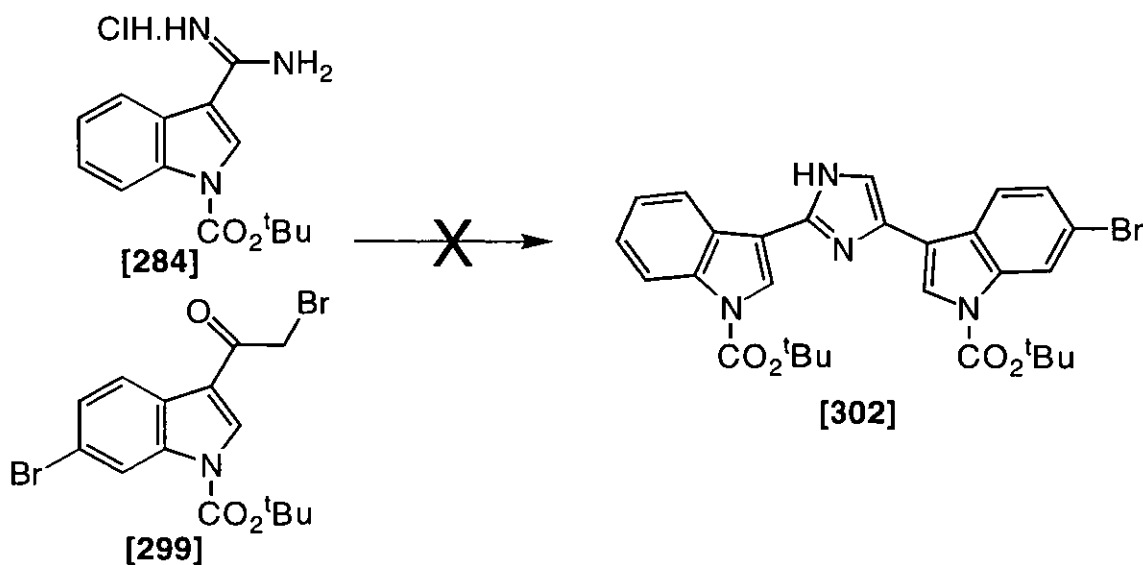
Scheme 98: Reagents and conditions i) K_2CO_3 , CH_3CN , reflux, 41%

Reaction of the 6-bromoindolyl amidine derivative [292] in a Brederik imidazole reaction with the α -bromoketone [123] and potassium carbonate in refluxing acetonitrile produced in a modest 34% yield *N*-Boc protected imidazole bis[indole] [302], **Scheme 99**. The imidazole bis[indole] [301] is *N*-Boc protected nortopsentin B [271].

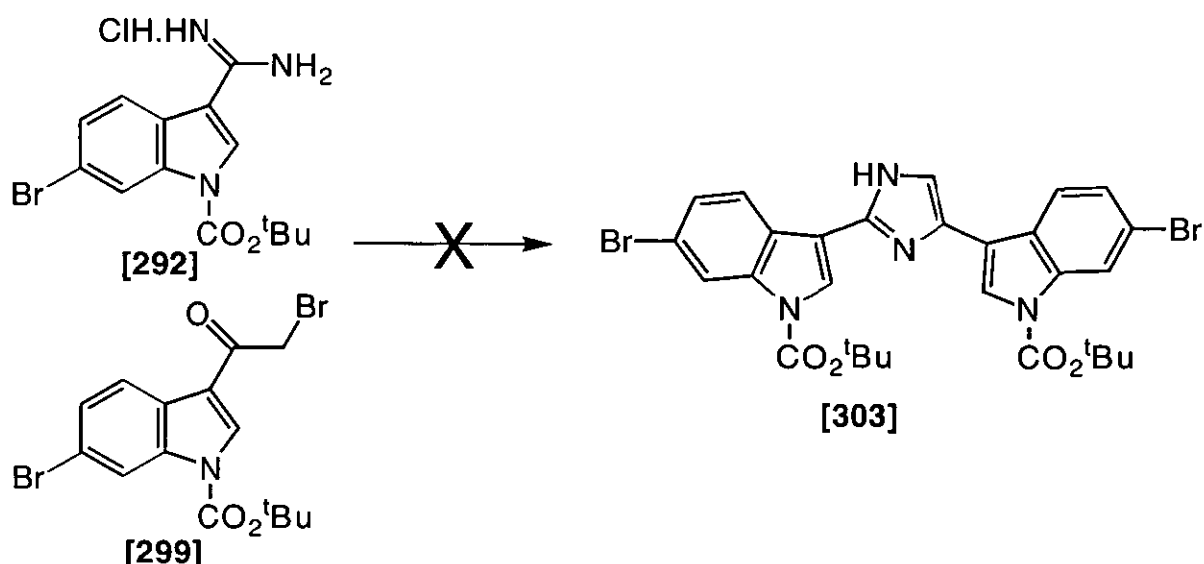


Scheme 99: Reagents and conditions i) K_2CO_3 , CH_3CN , reflux, 34%

However, attempts to form the *N*-Boc nortopsentin C derivative [302], **Scheme 100**, by a Brederik imidazole reaction between the indolyl amidine [284] and the 6-bromoindole α -bromoketone [299], and to form the *N*-Boc protected nortopsentin A derivative [303], **Scheme 101**, by reaction of the 6-bromoindolyl amidine derivative [292] and the α -bromoketone indole derivative [299] both failed to form any identifiable products. TLC indicated the formation of three products and efforts to identify the desired nortopsentin products [302] and [303] proved fruitless.

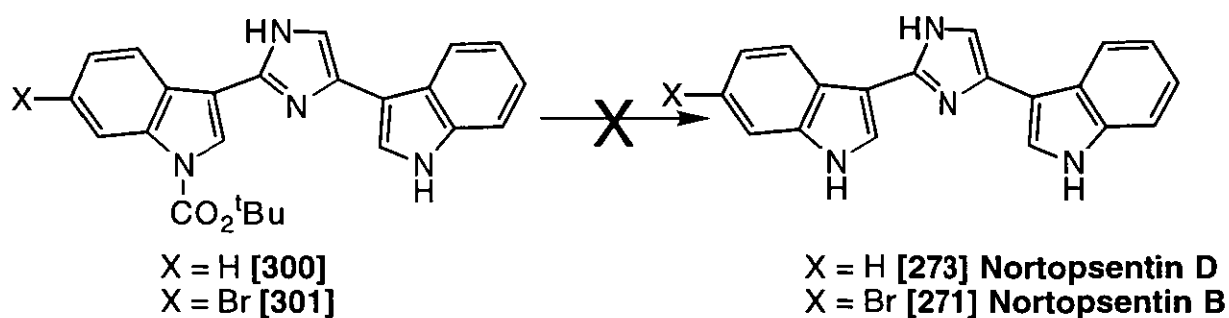


Scheme 100



Scheme 101

With the *N*-Boc protected nortopsentin B and D derivatives [300] and [301] in hand, synthetic efforts were concentrated on cleavage of the *N*-Boc protecting groups to reveal the natural compounds, **Scheme 102**. However, attempted deprotection, of both [300] and [301], either by reaction with trifluoroacetic acid or by reaction with sodium methoxide in THF failed to form any of the coveted natural products [273] or [271].¹⁶³ Under both deprotection methodologies a bright blue solution was formed. ¹H NMR analysis of the crude reaction mixture provided no evidence for the formation of the natural products.



Scheme 102: Attempted Boc deprotection of the imidazole bis[indole] compounds [311] and [312]

Because of the problems associated with the Brederick imidazole reaction towards the synthesis of the nortopsentins, the synthesis of the imidazolylindole [261] and the required indolequinone imidazole [71] was discontinued.

5.7. Conclusions

- Literature precedence has shown that the cyclisation of an amidine with a α -haloketone may be applied to the synthesis of the the naturally occurring nortopsentins.
- The *N*-Boc protected indole-3-amidines [284] and [292], derived from indole [93] and 6-bromoindole [287] respectively, can be synthesised.
- The 3-(2-bromoacetyl)indoles [123] and [299] can be synthesied, from indole [93] and 6-bromoindole [287] respectively, however, in the case of [299] in great difficulty.
- The Brederek imidazole reaction has been shown to be applicable towards the synthesis of the imidazole bis[indole] compounds [300] and [301]. Unfortunately, deprotection of the indole *N*-Boc protecting groups, to provide the nortopsentins D [273] and B [271], failed.

Chapter 6

Biological results.

6.1. Introduction

The synthesis of a series of the so called "simple" thiazolyindoles has been discussed in chapter 2. Employment of the Hantzsch reaction allowed the construction of a variety of functionalised thiazole derivatives. Such functionality included methyl [116], ethyl ester [114] and primary carboxamide [115] moieties. A series of thiazolyindole compounds were also synthesised in which structural diversification was centred on the indole nitrogen, *e.g.* the *N*-H [117], *N*-methyl [119] and *N*-acetyl [118] camalexin derivatives. The position of the thiazole ring, relative to the indole moiety, was also varied. A class of compounds were synthesised in which the thiazole derivative was attached to the indole C(3) position, *e.g.* [116] and [129] in conjunction with a sequence of analogues where the thiazole ring was attached to the indole C(2) position, *e.g.* [138]. The synthesis of the miscellaneous benzothiazolyindole [141] was also discussed. The compound [141] was synthesised on the hypothesis that the benzothiazole moiety would enhance binding of the drug to DNA, possibly by intercalation, thus extending the mechanism of action of these compounds.

The synthesis of a range of thiazolyindolequinone analogues, structurally related to the natural compound BE 10988 [43], has been discussed in chapter 3. Again a set of structurally diverse compounds was synthesised. A major proportion of the synthetic effort was concentrated upon the synthesis of the ethyl ester substituted thiazole [163] and the methyl substituted thiazole [165]. Both [163] and [165] contain a thiazole moiety attached to the indolequinone C(2) position. C(5)-Methoxy quinone exchange reactions with a variety of nitrogen nucleophiles, ranging from aziridine to morpholine, allowed the construction of various C(5) amino substituted thiazolyindolequinones. C(5)-Methoxy quinone exchange reactions, with aziridine and 2-methylaziridine as the nucleophile, were also shown to take place readily on the previously synthesised BE 10988 analogues [146], [196] and [199].

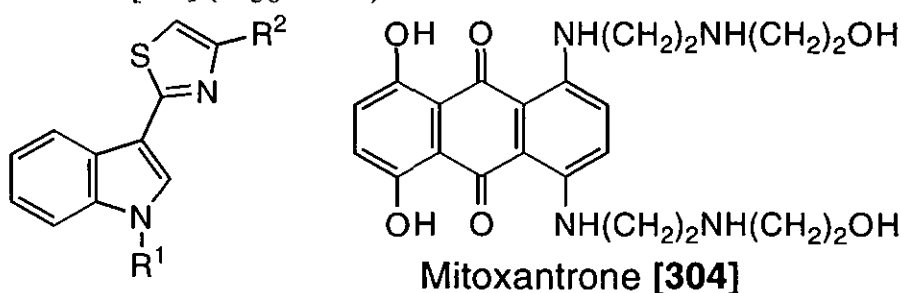
6.2. Inhibition of DNA topoisomerase II

The structurally "simple" thiazolyindole, the miscellaneous benzothiazolyindole [141] and the thiazolyindolequinone analogues were initially assessed for their ability to inhibit the action of the nuclease topoisomerase II. To evaluate this, the compounds were tested for *in vitro* activity against a human breast cancer cell line (SKBr3).¹⁶⁹ The SKBr3 cell line over expresses the topo II α gene and is recognised to be extremely sensitive to known topo II inhibitors, such as m-AMSA [37] and mitoxantrone [304]. It is thought that the topoisomerase II agents stabilise the so called cleavable complex, resulting in DNA double-strand cleavage. *in vitro* Values of IC₅₀ for each compound are given and comparison is made with the toxicity of the known topo II inhibitor mitoxantrone [304] and the thiazolyindolequinone natural product BE 10988 [43]. The IC₅₀ value indicates the

concentration of drug required to inhibit survival by 50% following exposure to SKBr3 cells incubated for 4 days at 37°C.

6.2.1. *in vitro* Activities (IC_{50} / μM) of the "simple" thiazolyindole and the benzothiazolyindole compounds against SKBr3 cell lines

The *in vitro* activities for the thiazolyindole compounds [114] - [117], *i.e.* analogues with a variety of functionality on the indole nitrogen (R^1) and the thiazole (R^2), are shown, **Table 10**. The activities for the natural compound BE 10988 [43] and mitoxantrone [304] are also shown. Surprisingly the camalexin analogues [117], [118] and [119] were more potent than the natural product BE 10988 [43]. The most active compound [117] was 3.2 times more active than [43]. The most active analogue [117] showed approximately 12 fold activity over the least effective agent [115]. The activities for the *N*-methyl analogues ($R^1 = CH_3$) were found to be dependent upon the thiazole functionality (R^2). The order of activity being $R^2 = H$ [117], ($IC_{50} = 2.7$) > methyl [116] ($IC_{50} = 6.3$) > ethyl ester [114] ($IC_{50} = 19.6$) > primary carboxamide [115] ($IC_{50} = 32.0$).

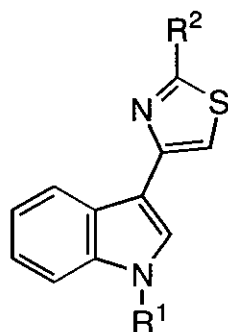


Compound No.	R^1	R^2	$IC_{50}(\mu M)$ / SKBr3
[117]	H	H	2.7
[119]	CH ₃	H	3.8
[118]	Ac	H	3.6
[116]	CH ₃	CH ₃	6.3
[114]	CH ₃	CO ₂ Et	19.6
[115]	CH ₃	CONH ₂	32.0
[43] BE 10988	-	-	8.7
[304] Mitoxantrone	-	-	0.016

Table 10

Mitoxantrone [304] was found to be approximately 550 times more active than the supposedly potent topo II agent [43]. The significant reduction in activity of [43] compared with the recognised topo II inhibitor [304] suggested that inhibition of topo II was not a consequence of the cytotoxicity of [43], for a more detailed account into the spurious topo II inhibitory effect of [43], see section 6.2.3.

The *in vitro* activities of isomeric thiazolyindole derivatives, against the SKBr3 cell line are shown in **Table 11**. Compound [128] was found to be the most active ($IC_{50} = 4.9$) and it displayed an approximate two fold increase in activity over [43]. The compound [129] ($IC_{50} = 9.5$) was approximately equipotent in activity with [43]. The range in activity over the thiazolyindoles was seven fold. Both sequences of compounds in which the substituent attached to the indole nitrogen (R^1) remained constant were shown to be dependent upon the nature of the thiazole functionality R^2 . The order for both the *N*-H and *N*-methyl derivatives was: $CH_3 > H > CONH_2 > CO_2Et$.

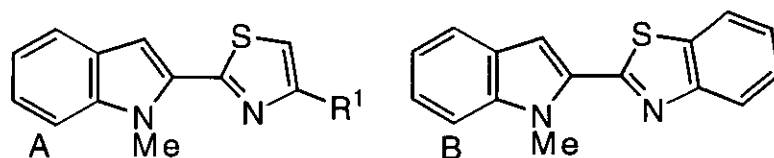


Compound No.	R^1	R^2	$IC_{50}(\mu M) / SKBr3$
[126]	H	H	18.0
[127]	CH_3	H	12.0
[128]	H	CH_3	4.9
[129]	CH_3	CH_3	9.5
[130]	H	CO_2Et	35.5
[131]	CH_3	CO_2Et	35.5
[132]	H	$CONH_2$	32.3
[133]	CH_3	$CONH_2$	30.0

Table 11

The *in vitro* activities for the indole C(2) thiazole analogues [138] - [140], designated structure A, and the benzothiazolyindole [141], structure B, are shown, **Table 12**. All the

thiazolylyndole compounds were found to possess poor activity, the order of activity being, $R^1 = \text{CH}_3$ [138] > CO_2Et [139] > CONH_2 . However, the benzothiazolylyndole [141] displayed a dramatic increase in activity ($\text{IC}_{50} = 2.6$), such that it was the most active "simplified" compound synthesised so far. The compound [141] showed approximately three times more activity than the natural product BE 10988 [43] and was 15 times more potent than the least active compound [140].



Compound No.	Structure	R1	$\text{IC}_{50}(\mu\text{M}) / \text{SKBr3}$
[138]	A	CH_3	20.0
[139]	A	CO_2Et	30.0
[140]	A	CONH_2	40.06
[141]	B	-	2.6

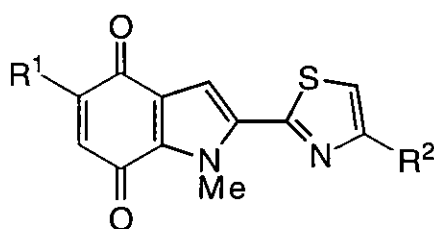
Table 12

From the results obtained so far the following generalisations can be made: indolylthiazole moieties bearing either an ethyl ester or primary carboxamide functionality on the thiazole show much lower activities towards the SKBr3 cell lines than methyl or nonfunctionalised thiazoles. The natural product [43] contains a primary carboxamide group attached to the C(4) position on the thiazole, from these preliminary results it can be postulated that substitution of the primary amide for a methyl or hydrogen would substantially increase the activity. Also attachment of the thiazole at the indole C(2) position reduced the activity against the SKBr3 cell line.

6.2.2. *in vitro* Activities ($\text{IC}_{50} / \mu\text{M}$) of the thiazolylyndolequinone compounds against SKBr3, A 549 and PV9 cell lines

The thiazolylyndolequinone analogues were screened for *in vitro* topo II inhibitory activity against the human breast cancer SKBr3, the human lung carcinoma A549 and PV9 cell lines. The A549 cell line also shows amplification of the topo II α gene and has been shown to be hypersensitive towards recognised topo II inhibitors, such as mitoxantrone [304] and etoposide [44]. Whereas the PV9 cells were selected as a stably resistant population. Following long term etoposide [44] exposure the PV9 cell lines are found to become drug

resistant. This cell line exhibits significant cross-resistance to etoposide [44], adriamycin [39], m-AMSA [37], mitoxantrone [304] and all agents thought to act through topoisomerase. Therefore topoisomerase inhibitors are expected show increased activities in the SKBr3 and A459 cell lines and poor activities in the PV9 cell line. The *in vitro* activities, reported as IC₅₀ values, for the thiazolyindolequinone analogues against these cell lines are shown, **Table 13**. The compounds tested were derived from the indolequinones [163] ($R^2 = \text{CO}_2\text{Et}$) and [165] ($R^2 = \text{CH}_3$), see structure below. The functionality at the indolequinone C(5) position (R^1) ranged from a simple methoxy group to acyclic amines (such as cyclopropyl amine) and three, four, five and six-membered cyclic amines.



The most striking observation in this series of compounds is the high activities of the aziridino derivatives [180] and [186], both of which were significantly more active than BE 10988 in the SKBr3 cell line. In the case of [186] a 40 fold increase in activity was noted. However, for [186] high activities were observed in all the cell lines. The similar activity in the PV9 cell line to the SKBr3 and A549 indicated that the mechanism of cytotoxicity was probably not a result of topoisomerase II initiated cleavage of DNA. The order of reactivity for the aziridino compounds was: unsubstituted > mono-methyl > bi-methyl, which corresponds to the studies of Lafleur *et al.* Both the methoxy derivatives [163] and [165] showed approximately two fold increase in activity over BE 10988. The remaining amino derivatives were all significantly less active than the aziridino compounds. Generally all the derivatives were found to be more active than the simple C(2) indolethiazole compounds (**Table 12**), with the exception of the azetidiny [189] and the cyclopropylamine [193] derivatives. Likewise the methyl thiazole compounds were more active than the ethyl ester thiazole compounds (a similar trend was observed in the simple C(2) indolethiazole compounds, **Table 12**).



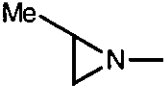
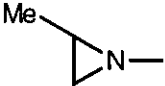
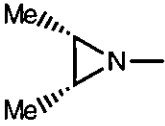
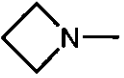
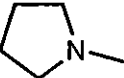
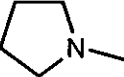
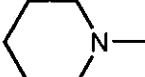
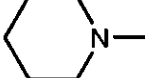
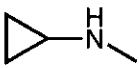
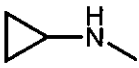
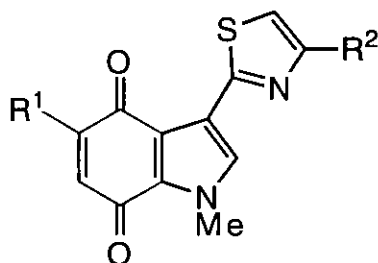
Compound No.	R ¹	R ²	IC ₅₀ (μ M)		
			SKBr3	A549	PV9
[163]	OCH ₃	CO ₂ Et	3.9	-	-
[165]	OCH ₃	CH ₃	3.2	-	-
[180]		CO ₂ Et	0.42	-	-
[186]		CH ₃	0.2	0.7	0.5
[181]		CO ₂ Et	4.2	-	-
[187]		CH ₃	7.0	25.7	27.1
[188]		CH ₃	8.9	19.0	19.7
[189]		CH ₃	30.1	26.1	36.3
[182]		CO ₂ Et	11.3	13.9	24.3
[190]		CH ₃	8.5	14.6	7.5
[183]		CO ₂ Et	21.5	20.1	31.4
[191]		CH ₃	12.4	23.2	21.3
[184]		CO ₂ Et	10.1	22.6	38.0
[193]		CH ₃	22.3	38.1	37.5

Table 13

The *in vitro* activities of the aziridino [194], [197] and the 2-methylaziridino [195], [198] thiazolyndolquinone derivatives against the SKBr3, A459 and PV9 cell lines are shown, **Table 14**. Also included, for comparison, are the previously synthesised C(5) methoxy [146], [196] compounds and the C(5) amino [305] derivative. An initial striking observation is the notable difference in activity between the two methoxy derivatives [146] and [196]. The only structural difference between the compounds is the functionality on the thiazole ring. Switching the ethyl ester moiety in [146] ($R^2 = \text{CO}_2\text{Et}$) to a methyl group [196] ($R^2 = \text{CH}_3$) results in a 20 fold increase in activity. The aziridino substituted compounds [194] and [197] both showed an impressive increase in activity, in all cell lines. The aziridine indolequinone [194] ($\text{IC}_{50} = 0.1$) is 87 times more potent than BE 10988. However, all the aziridine containing compounds were similarly active in all three cell lines. Thus, implying that topoisomerase II mediated DNA cleavage was not the mechanism of cytotoxicity. Again the order of activities were: unsubstituted aziridine > mono-methyl > amino.



Results from the analogous 'simple' thiazolyndole compounds (**Table 10**) suggested that the activities were dependent upon the nature of the functionality attached to the thiazole ring, with hydrogen and methyl groups showing significantly more activity than the ethyl ester and primary carboxamide functionalities. An approximate 10 fold increase in activity was found in the methyl derivatised thiazolyndole [119] over the primary carboxamide derivatised thiazolyndole [119]. Likewise the methyl thiazole derivative [305] showed an approximate 10 fold increase in activity over the natural product BE 10988 [43].

The thiazolyndolequinone compounds derivatised with a thiazole moiety at the indole C(3) position (**Table 14**) were generally more active than the corresponding indole C(2) thiazole derivatised compounds (**Table 13**). A similar trend was found in the simple indolethiazole series. Although a number of compounds were synthesised with major increases in activity over BE 10988, serious questions have to be asked about the true cytotoxic nature of these compounds, including the original statement that BE 10988 was a potent topo II inhibitor. The recognised topo II inhibitor mitoxantrone [304] showed a significant increase in activity over all the compounds synthesised and the few compounds that possessed promising activities in the SKBr3 and A549 cell lines were similarly active in the PV9 cell line.



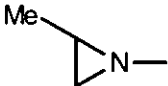
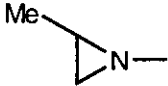
Compound No.	R ¹	R ²	IC ₅₀ (μ M)		
			SKBr3	A549	PV9
[146]	OCH ₃	CO ₂ Et	27.8	-	-
[196]	OCH ₃	CH ₃	1.4	-	-
[194]		CO ₂ Et	0.1	0.41	1.3
[197]		CH ₃	0.12	0.31	0.76
[195]		CO ₂ Et	0.33	1.80	2.0
[198]		CH ₃	0.66	1.1	2.3
[43] BE 10988	NH ₂	CONH ₂	7.8	-	-
[305]	NH ₂	CH ₃	0.73	-	-

Table 14

6. 2. 3 . Is the thiazolyindolequinone BE 10988 [43] a topo II inhibitor ?

The cell lines used to determine the *in vitro* topo II activity of the thiazolyindole and thiazolyindolequinone compounds are sensitive to the formation of drug induced topo II DNA strand breaks. Total inhibition of topo II function has been shown not to result in DNA cleavage, however, total inhibition of topo II would seriously effect cellular metabolism. With the exact nature of the cytotoxic effect of BE 10988 and related analogues appearing rather hazy, a method was required to prove precisely whether topo II inhibition was involved.¹²⁹

The topo II-mediated decatenation of kDNA assay can be used to ascertain precisely whether an agent acts as a topo II inhibitor.¹²⁹ kDNA consists of a series of interlinked rings of DNA. During incubation with nuclear extracts that contain topo II, those links become separated. Electrophoresis of this decatenated DNA shows that it runs approximately one-half the distance of the dye front. An effective inhibitor of topo II activity will prevent the decatenation from occurring, and the electrophoretic pattern will appear the same as for kDNA alone. It was found that 10 μ M mitoxantrone [304] completely inhibited the action of topo II by this assay, in contrast 150 μ M of BE 10988 [43] had no effect on the decatenating

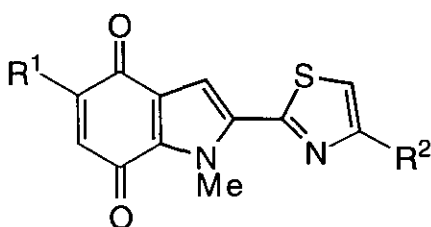
activity of topo II. Thus suggesting that the mechanism of action of BE 10988 does not involve topo II inhibition.

6.3. Bioreductive compounds

Bioreductive drugs are defined as compounds that are selectively toxic to hypoxic tumour cells. These bioreductive drugs, can selectively target hypoxic tumour populations that are resistant to both radiation and chemotherapy. Bioreductive drugs are initially activated to toxic species under hypoxic conditions by enzymatic metabolism. The ease of bioreduction of a given drug will depend upon the ability of the drug to act as a substrate for the intracellular reductase(s) and the expression of these enzyme(s) within the particular cell type. Further more depending upon whether or not the bioreductive drug is reduced in a one or two electron step, the reduction may be reversed by oxygen. In the case of the quinone moiety, one electron reduction results in the formation of a semiquinone radical anion which will be reoxidised back to a quinone in the presence of oxygen.

6.3.1. *in vitro* Activities (IC_{50} / μM) of the thiazolyindolequinone analogues in V79 cell lines under hypoxia and air

Selective *in vitro* toxicity toward hypoxic V79 cells were determined for all compounds using the MTT assay. Cells derived from exponentially growing culture were treated with varying concentrations of drugs for 3 h at 37°C under hypoxic (N_2) or aerobic conditions.¹⁷⁰ Then following removal of the drug the cells were allowed to proliferate for 3 days prior to MTT assay. The activities are expressed as values of IC_{50} which are the concentrations required to kill 50% of the cells under the conditions of the initial treatment. The ratio of $IC_{50}(\text{air})$ vs $IC_{50}(N_2)$ enables quantitative comparison to be made of the O_2 -dependent bioreductive activities of these compounds. The *in vitro* activities, reported as IC_{50} values, for the C(2) thiazolyindolequinone analogues against these cell lines are shown, **Table 15**.



The functionality on the indolequinone C(5) position (R^1) ranged from a simple methoxy group to the three, four and six-membered cyclic amines. All the ethyl ester ($R^2 = CO_2Et$) thiazolyindolequinone analogues were found to be more active under hypoxic (N_2) conditions, with air/ N_2 ratios ranging from 5 to 14, **Figure 12**. These results suggested that the quinone moieties were reduced by a one electron reduction to a semiquinone radical

anion. However, further studies are required to unambiguously confirm the postulate. The most active compound was the aziridino derivative [180], however, the most hypoxic selective compound was the 2-methyl aziridino derivative [181] which was found to be 14 times more active against hypoxic (N_2) cell lines. The methyl ($R^2 = CH_3$) thiazolyindolequinone analogues did not show the same levels of activity. The aziridino [186] and azetidino compounds were 5 and 4 times more active under hypoxia, again suggesting a one electron reduction of the quinone. Whereas the 2-methylaziridinyl [181] and morpholinyl [192] derivatives were found to possess similar activities under both hypoxia and air, thus suggesting a two electron reduction of the quinone to the hydroquinone species.



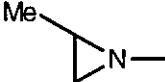
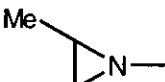
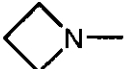
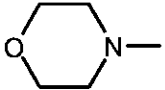
Compound No.	R ¹	R ²	IC ₅₀ (μ M)		
			Air	N ₂	Air/N ₂
[163]	OCH ₃	CO ₂ Et	200	20	10.0
[165]	OCH ₃	CH ₃	54	76	<1
[180]		CO ₂ Et	10.8	2.2	5.0
[186]		CH ₃	17.4	3.4	5.0
[181]		CO ₂ Et	112	7.9	14.0
[187]		CH ₃	30.0	30.0	1.0
[189]		CH ₃	100	25	4.0
[192]		CH ₃	300	200	1.5

Table 15

A rather curious result is found in the case of the methoxy derivatives [163] and [165]. The ethyl ester thiazole derivative [163] is significantly more active under hypoxia, whereas the methyl thiazole derivative [165] was deactivated under hypoxia. The methoxy compound [163] appears to be devoid of functionality capable of alkylating DNA, therefore suggesting a different mechanism is responsible for the cytotoxic effect. It could be speculated that

reduction of the quinone provides hydrogen bonding sites, thus allowing binding of the compound to DNA. Indeed this may be the mechanism of action for all the compounds. Further studies to unambiguously prove DNA alkylation and strand cleavage for the aziridine compounds are required.

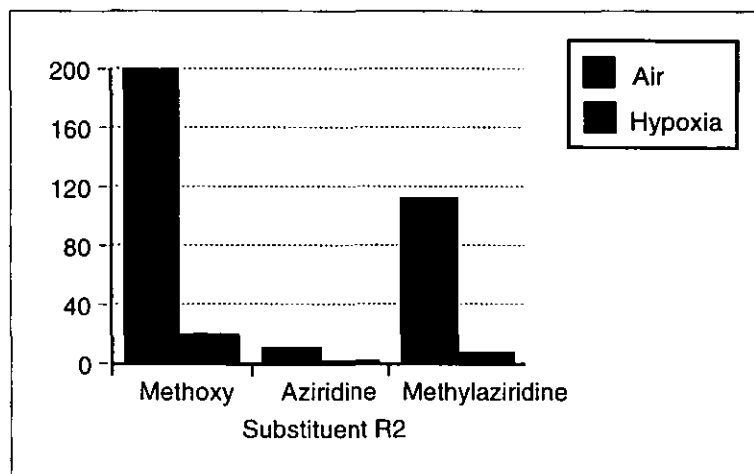
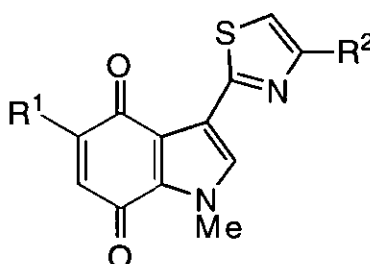


Figure 12: *in vitro* IC₅₀ (µM) values, under air and hypoxia, for the thiazolyndolquinone C(5) methoxy [163], aziridinyl [180] and 2-methylaziridinyl [181] derivatives.

The *in vitro* activities of the aziridino [194], [197] and the 2-methylaziridino [195], [198] thiazolyndolequinone derivatives against the hypoxic (N₂) and air V79 cell lines are shown, **Table 16**. Also included, for comparison, are the previously synthesised C(5) methoxy [146], [196] compounds and the C(5) amino [305] derivative. Activities for the known bioreductive alkylators MMC [10] and EO9 [34] are also provided for comparison. Initial observation reveals the poor selectivity for the hypoxic cell lines, with the most selective compound [197] being twice as active against the hypoxic cell lines. The poor selectivity towards hypoxia in all the compounds suggested that they may be activated by a two electron process. The aziridino and 2-methylaziridino compounds were significantly more active than the methoxy derivatives and the natural product BE 10988. The aziridino derivative [194] was 420 and 340 times more active than the methoxy derivative [146] under air and hypoxia respectively.



Although the aziridinyl thiazolyindolequinone [197] was approximately 7 times more active than the natural compound BE 10988 [43] under hypoxia, the activity must be put into perspective with the bioreductive alkylators MMC [10] and EO9 [34]. As can be seen from Table 16, the the aziridino thiazolyindolequinone [197] was approximately two and 200 fold less active under hypoxic conditions than MMC and EO9 respectively.



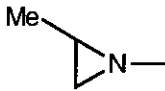
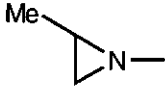
Compound No.	R ¹	R ²	IC ₅₀ (μ M)		
			Air	N ₂	Air/N ₂
[146]	OCH ₃	CO ₂ Et	420	340	1.2
[196]	OCH ₃	CH ₃	73	48	1.5
[194]		CO ₂ Et	1.0	1.0	1.0
[197]		CH ₃	1.5	0.75	2.0
[195]		CO ₂ Et	2.0	2.0	1.0
[198]		CH ₃	3.0	3.0	1.0
[305]	NH ₂	CH ₃	10.3	6.0	1.7
[43] BE 10988	NH ₂	CONH ₂	6.2	5.1	1.2
[10] MMC	-	-	0.8	0.4	2
[34] EO9	-	-	0.19	0.0038	50.3

Table 16

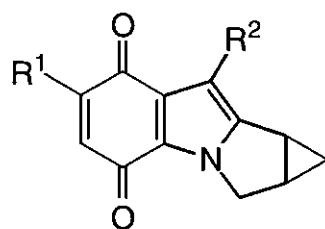
The thiazolyindolequinone derivatives in which the thiazole ring was attached to the indole C(3) (Table 16) were generally more active than the corresponding C(2) thiazolyindolequinone derivatives (Table 15). However, the C(5) methoxy ethyl ester thiazole derivative [146] is significantly less active than the isomeric analogue [163] under hypoxia, approximately 17 times less active, whereas, in air it is only two times less active. Thus suggesting that the conformation of the thiazole moiety in [146] may inhibit DNA binding. The C(2) thiazolyindolequinone derivatives were significantly more selective under hypoxia, thus, suggesting they are reduced predominantly by a one electron process.

The C(3) thiazolyloindolequinone derivatives all showed poor hypoxic cell selectivity, suggesting a two electron reduction. Thus the attachment of a thiazole ring at the C(2) position on the indolequinone appears to activate reduction of the indolequinone moiety *via* a one electron process, thus forming hypoxia selective agents. This may be a result of altering the electropotential of the quinone or a factor of drug conformation, *i.e.*, increasing the compounds to act as a substrate for one electron reductase enzymes.

6.4. Future work, the synthesis of bifunctional thiazolyloindolequinone alkylating agents

Alkylating agents that cause cross linking of DNA are generally more cytotoxic than agents that result in monofunctional DNA alkylation. The thiazolyloindolequinone analogues (**Tables 15, 16**) contain one site for bioreductive alkylation (the aziridino functionality) and so incorporation of functionality onto these molecules which will result in possible bifunctional alkylation would be expected to dramatically increase cytotoxicity.

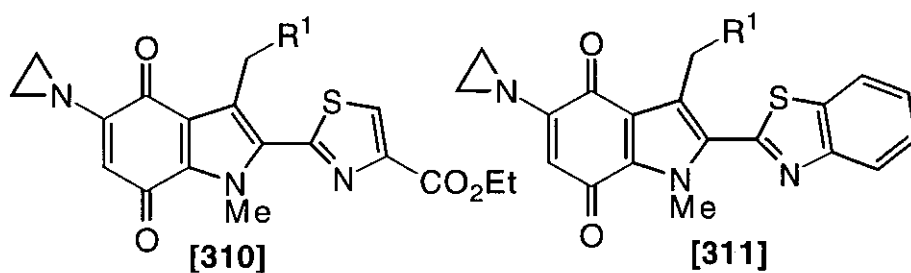
The cyclopropamitosenes have previously been synthesised within these laboratories. As can be seen in **Table 17** the compounds containing two possible sites for alkylation [**33**] and [**309**] are significantly more cytotoxic under hypoxia *in vitro* than the monofunctional agents [**307**] and [**308**] respectively, *i.e.*, incorporation of an aziridine attached to the quinone and a carbamoyl group at the indole C(3) position.



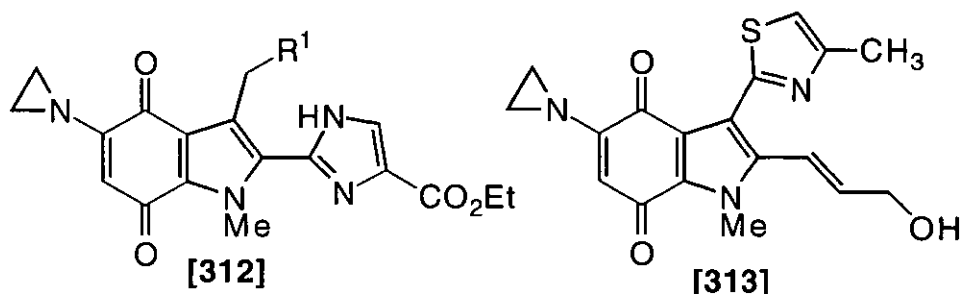
Compound No.	R ¹	R ²	IC ₅₀ (μM)		
			Air	N ₂	Air/N ₂
[306]	OCH ₃	H	200	200	1.0
[32]	OCH ₃	CH ₂ OCONH ₂	4.8	0.14	34
[307]		H	7.0	2.0	3.5
[33]		CH ₂ OCONH ₂	0.003	0.003	1.0
[308]		H	90	35	2.5
[309]		CH ₂ OCONH ₂	1.2	0.055	22

Table 17

The indole C(3) position is "free" in the thiazolyndolequinone analogues, in which the thiazole ring is attached to the indole C(2), therefore the incorporation masked electrophilic functionalities could be envisaged to provide species capable of forming DNA cross-links upon reductive activation. The compounds [310] and [311] (were R¹ = OH or OCONH₂) were envisaged to act as bifunctional alkylating agents. The derivative [311] can be regarded as a hybrid of the benzothiazolyndole [141] and the thiazolyndolequinone analogue [186].



The C(2) imidazole analogue [312] is also a synthetic possibility, such that the imidazole ring may augment drug binding to DNA. The compound [312] can be considered to be a hybrid of the thiazolyindolequinone [197] and EO9 [31]. All of these compounds can be synthesised from the highly functionalised flexible indole [154].



6.5. Conclusions

- A number of the "simple" thiazolyindole compounds were found to have greater *in vitro* activity in the SKBr3 cell lines than the natural compound BE 10988 [43], notably [117] (Table 10) and the benzothiazolyindole [141] (Table 12).
- A number of the C(2) thiazolyindolequinone compounds were found to have greater *in vitro* activity in the SKBr3 and A 549 cell lines than the natural compound BE 10988 [43], notably the C(5) aziridiny derivatives [180] and [186] (Table 13). However, significant activities in the PV9 cell lines indicated that topo II inhibition was not a consequence of cytotoxicity.
- A number of the C(3) thiazolyindolequinone compounds were found to have greater *in vitro* activity in the SKBr3 and A 549 cell lines than the natural compound BE 10988 [43], notably the C(5) aziridiny derivatives [194], [197] and the 2-methylaziridiny derivatives [195], [198] (Table 14). However, significant activities in the PV9 cell lines also indicated that topo II inhibition was not a consequence of cytotoxicity.
- The "simple" thiazolyindole and thiazolyindolequinone compounds were found to have significantly reduced *in vitro* activity in the SKBr3 cell lines, compared to the known topo II inhibitor mitoxantrone [304], thus suggesting inhibition of topo II was not the cytotoxic effect. Independent studies into the topo II-mediated decatenation of kDNA revealed that the natural compound was not a topo II inhibitor.
- A number of the C(2) thiazolyindolequinone compounds were found to have increased *in vitro* activity under hypoxia in the V79 cell lines, notably the C(5) 2-methylaziridiny

derivative [181] which was 14 times more active under hypoxia (Table 15). Thus, suggesting an oxygen inhibited one electron bioreduction of the quinone moiety.

- A number of the C(3) thiazolyldiolequinone compounds were found to have increased *in vitro* activity in the V79 cell lines than the natural compound BE 10988 [43], notably the C(5) aziridinyI derivatives [194], [197] and the C(5) 2-methylaziridinyI derivatives [195], [198] (Table 16). However, the *in vitro* activities were similar under air and hypoxia, thus, suggesting a two electron bioreduction of the quinone moiety.
- The *in vitro* activities in the V79 cell lines of the most active thiazolyldiolequinone compounds were less than the common bioreductive agents MMC [10] and EO9 [31]. However, precedent suggests incorporation of a second alkylation site on the thiazolyldiolequinone compounds would lead to a significant increase in activity. The hypothetical compounds [310], [311], [312] and [313] were suggested as possible synthetic targets.

Chapter 7

Experimental.

7.1. General Information

Solvents and Reagents: Commercially available solvents and reagents were used without further purification, except for those detailed below which were purified as described. 'Light petroleum' refers to the fraction of petroleum ether boiling between 40°C and 60°C and was distilled through a 36 cm Vigreux column before use. Diethyl ether, benzene, mesitylene and xylene were dried were necessary by standing over sodium wire for several days. THF was distilled from sodium benzophenone ketyl under nitrogen, prior to use. Dichloromethane and chloroform were distilled from phosphorus pentoxide, prior to use. Ethyl acetate was distilled from potassium carbonate, prior to use. DMF was dried by stirring over calcium hydride for 15 h, decanted, and distilled under reduced pressure before storing over activated 4Å molecular sieves under nitrogen. Methanol and ethanol were distilled from magnesium turnings and iodine and stored over activated 4Å molecular sieves under nitrogen. Aziridine, 2-methyl aziridine and *cis*-dimethyl aziridine were distilled from potassium hydroxide pellets and stored over sodium hydroxide pellets under nitrogen in the refrigerator. Unless otherwise stated, all reagents were used as supplied.

Chromatographic Procedures: Analytical thin layer chromatography (TLC) was carried out using aluminium backed plates coated with Merck Kieselgel 60 GF254. Flash column chromatography was carried out using Merck Kieselgel 60 H silica. Pressure was applied at the column head with hand bellows. Size exclusion chromatography was carried out using Sephadex LH20® gel, with a 1:1 solution of methanol and dichloromethane as the eluent.

Spectroscopic techniques: Infra red (IR) spectra were recorded in the range 4000-600 cm⁻¹ using a Nicolet FT-205 spectrometer, with internal calibration. Spectra were recorded as either solutions in chloroform, as thin films or as nujol mulls. Thin films and nujol mulls were recorded between sodium chloride plates. ¹H NMR spectra were recorded using Bruker AC-250 (250 MHz), and Bruker DPX-400 (400 MHz) instruments. ¹³C spectra were recorded using Bruker AC-250 (62.5 MHz), and Bruker DPX-400 (100 MHz) instruments. High and low resolution mass spectra were recorded on a Kratos MS80 instrument.

Other data: Melting points were measured on a Reichert-Kofler hot stage apparatus or on an electrothermal digital melting point apparatus. Elemental analyses were carried out on a Perkin Elmer 2400 Elemental Analyser.

7.2. Synthesis or preparation of general reagents

Methyl Azidoacetate [205]

Methyl chloroacetate (50 g, 0.461 mol) was added followed by sodium azide (37.65 g, 0.579 mol, **CAUTION** with a plastic spatula) to a stirred mixture of water (50 ml) and acetone (75 ml). The stirred mixture was heated at reflux for 16 h. The mixture was cooled and evaporated under reduced pressure. The yellow mixture was extracted with diethyl ether (3 x 150 ml), brine (150 ml), dried (MgSO_4) and evaporated under reduced pressure to give the *title compound* (44.2 g, 83%) as a pale yellow liquid. Care was taken at all times when handling this compound, methyl azidoacetate is potentially explosive.

Fremy's salt [$\text{K}_2\text{ON}(\text{SO}_3)_2$]

A solution of sodium nitrite (5.8 g, 0.08 mol) in water (15 ml) was cooled in an ice bath, crushed ice (approx. 35 g) was added with continuous stirring. A solution of sodium metabisulphite (7.3 g, 0.04 mol) in water (15 ml) was added, followed by glacial acetic acid (3.5 ml). The mixture was rendered alkaline by adding ammonia (0.88 solution, 2.5 ml), and was then stirred continuously in the ice bath during the addition of an ice cold solution of potassium permanganate (2.1 g, 0.013 mol) in water (65 ml). The precipitate of manganese dioxide was filtered off through a bed of Celite. To the cooled violet filtrate was added a saturated solution (85 ml) of potassium chloride (33 g / 100 ml of water). The orange precipitate was collected by filtration and washed several times with 5% potassium hydroxide, then twice with ethanol containing approximately 5% v / v 0.88 ammonia and finally with acetone. Air was not drawn through the solid but it was spread on a watch glass and the acetone allowed to evaporate over 10-15 min. The product was dried in a desiccator over ammonium carbonate and calcium oxide.

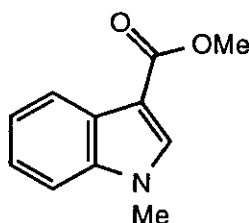
Aziridine

2-aminoethyl hydrogen sulfate (35.28 g, 0.25 mol) was added to a solution of sodium hydroxide (40%, 77 ml). The mixture was gently heated until it just began to boil. Heating was resumed and half the volume was collected by distillation, **CAUTION** aziridine is extremely toxic. Potassium hydroxide pellets (31.2g, 0.55 mol) were added and the distillate was cooled in a refrigerator for 15 h. The mixture was separated and potassium hydroxide pellets (25 g, 0.44 mol) were added. The mixture was left in a refrigerator for a further 3 h. The mixture was separated and distilled twice to give the *title compound* (3.65 g, 34%) as a colourless oil. The product was stored over potassium hydroxide pellets in a refrigerator.

Thioformamide [125]

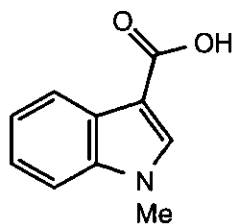
Formamide (1 g, 22.2 mmol), phosphorus pentasulfide (2.63 g, 5.9 mmol) and diethyl ether (20 ml) were heated at reflux for 48 h. The reaction mixture was cooled and evaporated under reduced pressure to give the title compound as a yellow oil.

7.3. Chapter 2 experimental



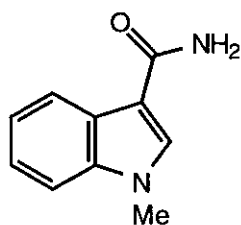
Methyl 1-methylindole-3-carboxylate [85]

Dry DMF (23 ml) was added to potassium hydride (1.17 g, 29.1 mmol), under a nitrogen atmosphere, at 0°C. A solution of indole-3-carboxylic acid [84] (0.94 g, 5.8 mmol) in DMF (13 ml) was added dropwise and the solution stirred at 0°C for 5 min then at rt for 45 min. The solution was cooled to 0°C and methyl iodide (4.15 g, 29.2 mmol) added, the mixture was warmed to rt and stirred for a further 45 min. A saturated solution of ammonium chloride (50 ml) was added and the mixture extracted with diethyl ether (3 x 50 ml). The extracts were combined, washed with water (2 x 50 ml), brine (50 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* [85] (1.090 g, 95%) as an off white solid; m.p. 85-87°C; (Found: C, 69.82; H, 5.80; N, 7.38. C₁₁H₁₁NO₂ requires C, 69.83; H, 5.86; N, 7.40%); ν_{\max} . (nujol)/cm⁻¹ 1687, 1554 and 1232; δ_{H} (250 MHz; CDCl₃) 3.83 (3H, s, NCH₃), 3.91 (3H, s, OCH₃), 7.33 (3H, m), 7.78 (1H, s) and 8.15 (1H, m); δ_{C} (62.5 MHz; CDCl₃) 33.31, 50.90, 107.12, 109.77, 121.58, 122.73, 127.13, 127.24, 135.16, 136.91 and 166.78; m/z 189 (M⁺, 70%) and 158 (100); (Found M⁺, 189.0800. C₁₁H₁₁NO₂ requires M, 189.07897).



1-Methylindole-3-carboxylic acid [86]

Methyl 1-methylindole-3-carboxylate [85] (0.97 g, 5.18 mmol), THF (14 ml) and sodium hydroxide (12 ml, 5% solution) were heated under reflux for 16 h. The reaction mixture was cooled and made acidic by the addition of hydrochloric acid (6 M). The mixture was extracted with dichloromethane (2 x 50 ml). The extracts were combined, washed with brine (50 ml), dried (MgSO₄) and evaporated under reduced pressure to give the *title compound* [86] (0.73 g, 81%) as an off white solid; m.p. 95-97°C; δ_{H} (250 MHz; CDCl₃) 3.86 (3H, s, NCH₃), 7.30 (2H, m), 7.38 (1H, m), 7.88 (1H, s, H₂) and 8.24 (1H, m), carboxylic acid OH unobserved.



1-Methylindole-3-carboxamide [87]

1-Methylindole-3-carboxylic acid [86] (0.2 g, 1.15 mmol), thionyl chloride (2.68 g, 22.4 mmol) and dichloromethane (10 ml) were heated under reflux for 2 h. The reaction mixture was cooled and evaporated under reduced pressure, **CAUTION**. Dichloromethane (10 ml) was added to the crude red oil and the mixture cooled to -78°C . Ammonia gas was bubbled into the mixture (approx. 30 ml). The mixture was left to stir at -78°C for 2 h then allowed to warm to rt over 12 h. The mixture was evaporated under reduced pressure and flash column chromatography (ethyl acetate elution) gave the *title compound* [87] (0.12 g, 60%) as an off white solid; m.p. $178\text{--}179^{\circ}\text{C}$; (Found M^+ , 174.0794. $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}$ requires M , 174.0793); ν_{max} (nujol)/ cm^{-1} 1604, 1465 and 1377; δ_{H} (250 MHz; CDCl_3) 3.83 (3H, s, NCH_3), 5.77 (2H, broad, NH_2), 7.36 (3H, m), 7.7 (1H, s) and 7.96 (1H, m); δ_{C} (62.9 MHz; CDCl_3) 33.12, 109.56, 109.66, 121.1, 121.24, 122.22, 126.47, 132.56, 136.97 and 167.15; m/z 174 (M^+ , 80%), 158 (100), 130 (10), and 77 (10).

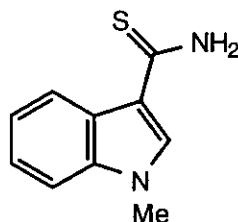
Alternative procedure for 1-methylindole-3-carboxamide [87]

Trimethyl aluminium (2M solution in toluene, 7.95 ml, 15.9 mmol) was added dropwise to a solution of ammonium chloride (0.85 g, 15.9 mmol) and benzene (15.9 ml) at 5°C under a nitrogen atmosphere. The mixture was warmed to rt and stirred for 1 h. A solution of methyl 1-methylindole-3-carboxylate [85] (1.0 g, 5.3 mmol) in benzene (50 ml) was added and the solution stirred at 60°C for 12 h. Hydrochloric acid (2M, 30 ml) was added and the solution extracted with ethyl acetate (3 x 50 ml). The extracts were combined, washed with brine (50 ml), dried (MgSO_4) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* [87] (0.68 g, 74%) as a pale yellow solid, with analytical data identical to that previously prepared.

Alternative procedure for 1-methylindole-3-carboxamide [87]

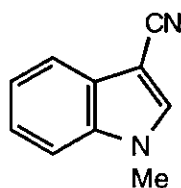
A solution of CSI (4.31 g, 30.5 mmol) in acetonitrile (10 ml) was added dropwise to a solution of 1-methylindole [96] (4 g, 30.5 mmol) in acetonitrile (40 ml) at 0°C . The mixture was stirred at 0°C for 30 min then warmed to rt and stirred for a further 1 h. The precipitate was collected by filtration and dissolved in a mixture of acetone (20 ml) and water (6 ml). The mixture was made basic (10% potassium hydroxide). The mixture was extracted with dichloromethane (3 x 50 ml), the extracts were combined, washed with brine (2 x 50 ml), dried (MgSO_4) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate

elution) gave the *title compound* [87] (3.09 g, 60%) as a colourless solid, with analytical data identical to that previously prepared.



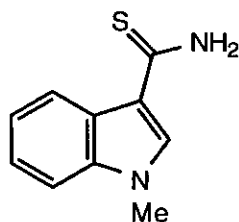
1-Methylindole-3-thiocarboxamide [97]

A solution of 1-methylindole-3-carboxamide [87] (120 mg, 0.68 mmol), Lawesson's reagent [99] (163 mg, 0.4 mmol) and benzene (10 ml) were heated under reflux for 1 h. The crude reaction mixture was evaporated under reduced pressure and flash column chromatography (dichloromethane : diethyl ether elution) gave the *title compound* [97] (120 mg, 60%) as a yellow solid; m.p. 125-126°C; (Found M^+ , 190.2651. $C_{10}H_{10}N_2S$ requires M , 190.2646); ν_{\max} (nujol)/ cm^{-1} 3496, 2968 and 1596; δ_H (250 MHz; $CDCl_3$) 3.85 (3H, s, NCH_3), 7.36 (5H, m), 7.86 (1H, m) and 8.07 (1H, s); δ_C (62.5 MHz; $CDCl_3$) 33.45, 110.58, 116.08, 119.89, 122.49, 122.88, 123.75, 137.14, 137.69 and 194.88; m/z 190 (M^+ , 60%), 156 (100).



and *1-methylindole-3-nitrile* [98]

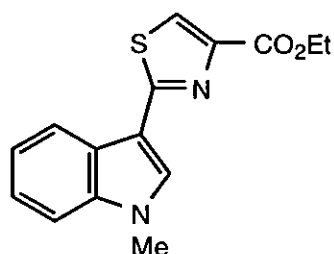
m.p. 61°C; ν_{\max} (CH_3Cl)/ cm^{-1} 2218, 1531, 1380, 1249 and 742; δ_H (250 MHz, $CDCl_3$) 3.81 (3H, NCH_3), 7.24 (3H, m), 7.49 (1H, s, 2H) and 7.72 (1H, m); δ_C (62.5 MHz; $CDCl_3$) 33.56, 85.01, 110.36, 115.98, 119.63, 122.05, 123.77, 127.67, 135.58 and 135.92.



General procedure for the formation of 1-methylindole-3-thiocarboxamide [97]

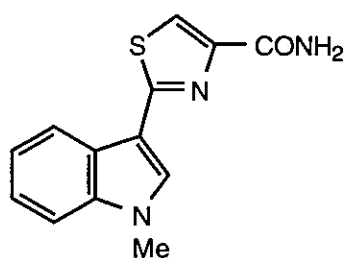
A solution of 1-methylindole-3-carboxamide [87] (30 mg, 0.17 mmol), benzene (5 ml) and thionating agent (0.086 mmol, see **Table 1**) were heated under reflux. The reaction mixture was checked by TLC until there appeared to be no more consumption of 1-methylindole-3-

carboxamide [97]. The reaction mixture was cooled and evaporated under reduced pressure. Flash column chromatography (dichloromethane : diethyl ether elution) gave the *title compound* [97] (for yield see **table 1**), with analytical data identical to that previously prepared.



Ethyl 2-(1-methylindol-3-yl)thiazole-4-carboxylate [114]

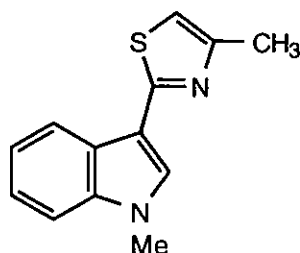
1-Methylindole-3-thiocarboxamide [97] (100 mg, 0.53 mmol), ethyl bromopyruvate (155 mg, 0.79 mmol) and ethanol (25 ml) were heated under reflux for 0.5 h. The reaction mixture was evaporated under reduced pressure to give a yellow solid. Recrystallisation (methanol : water) gave the *title compound* [114] (100 mg, 66% yield) as pale brown crystals; m.p. 117-118°C; (Found: C, 63.07; H, 4.73; N, 9.51. C₁₅H₁₄N₂OS Requires C, 62.94; H, 4.90; N, 9.79%); ν_{\max} (nujol)/cm⁻¹ 3050, 2930 and 1730; δ_{H} (250 MHz; CDCl₃) 1.44 (3H, t, *J* 7.2 Hz, OCH₂CH₃), 3.85 (3H, s, NCH₃), 4.43 (2H, q, *J* 7.12 Hz, OCH₂CH₃), 7.34 (3H, m), 7.90 (1H, s), 8.05 (1H, s) and 8.19 (1H, m); δ_{C} (62.5 MHz; CDCl₃) 14.32, 23.18, 61.26, 109.88, 110.06, 120.390, 121.35, 122.75, 124.06, 125.03, 129.96, 137.19, 146.52, 162.32 and 163.52; *m/z* 286 (M⁺, 100%), 214 (40), 174 (10), 77 (50), 158 (30) and 120 (12); (Found M⁺, 286.0785. C₁₅H₁₄N₂O₂S requires M, 286.0776).



2-(1-Methylindol-3-yl)thiazole-4-carboxamide [115]

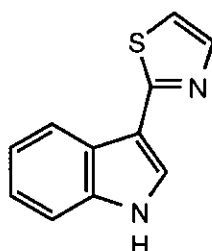
Ethyl 2-(1-methylindol-3-yl)thiazole-4-carboxylate [114] (218 mg, 0.77 mmol), ammonium chloride (10.5 mg, 0.19 mmol) and ammonium hydroxide (15 ml, of a 0.88 solution) were heated at 100°C in a Young's sealed tube for 48 h. The crude mixture was extracted with ethyl acetate (3 x 20 ml), the extracts were combined and washed with brine (20 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* [115] (142 mg, 73%) as a pale brown solid; m.p. 184-185°C; (Found M⁺, 257.0617. C₁₄H₁₂N₂O₂S requires M, 257.0617); ν_{\max} (CHCl₃)/cm⁻¹ 3019, 1680, 1422, 1216 and 929; δ_{H} (250 MHz; CD₃OD) 3.62 (3H, s, NCH₃), 7.12 (3H, m), 7.54 (1H, s),

7.80 (1H, s) and 7.98 (1H, m), amide NH₂ unobserved; δ_C (62.5 MHz; CD₃OD) 32.65, 109.56, 109.67, 120.19, 120.83, 121.08, 122.66, 124.71, 129.58, 137.12, 148.52, 163.44 and 164.22; m/z 257 (M⁺, 100%), 174(10), 157(60), 101(20) and 44(18).



3-(4-Methyl-2-thiazolyl)-1-methylindole [116]

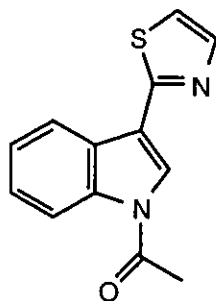
1-Methylindole-3-thiocarboxamide [97] (115 mg, 0.61 mmol), chloroacetone (113 mg, 1.2 mmol) and ethanol (28 ml) were heated under reflux for 3 h. The reaction mixture was evaporated under reduced pressure to give a yellow solid. Flash column chromatography (light petroleum : ethyl acetate elution) gave the *title compound* [116] (60 mg, 43%) as a pale yellow solid; m.p. 74-76°C; (Found M⁺, 228.0718. C₁₃H₁₂N₂S requires M, 228.0721); δ_H (250 MHz; CDCl₃) 2.52 (3H, s, CH₃), 3.34 (3H, s, NCH₃), 6.76 (1H, s), 7.35 (3H, m), 7.56 (1H, s) and 8.19 (1H, m); δ_C (62.5 MHz; CDCl₃) 15.01, 33.69, 106.14, 109.69, 110.66, 119.55, 122.38, 123.41, 124.68, 133.79, 137.67, 146.94 and 163.68; m/z 228 (M⁺, 100%), 156 (90), 114 (20) and 72 (30).



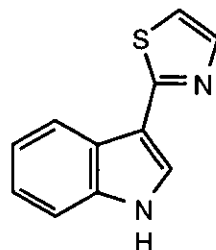
3-(2-Thiazolyl)indole [117]

A solution of methyl iodide (3.37 g, 23.7 mmol) in diethyl ether (10 ml) was added over a 5 min period to magnesium turnings (0.42 g, 17.5 mmol) and a few crystals of iodine, under a nitrogen atmosphere. The mixture was heated under reflux for 1 h. The solvent was removed by increasing the nitrogen flow and benzene (20 ml) added. A solution of indole [93] (1.74 g, 14.8 mmol) in benzene (8 ml) was added and the solution stirred for 10 min. 2-Bromothiazole (1.23 g 7.5 mmol) was added and the mixture was heated at reflux for 65 h, under a nitrogen atmosphere. Ethyl acetate (60 ml) was added and the mixture washed with a saturated solution of ammonium chloride (2 x 20 ml), brine (2 x 20 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (light petroleum : acetone elution) gave the *title compound* [117] (0.896 g, 30% yield) as a pale brown solid; m.p. 134-138°C; (lit., 134-

137°C); (Found M^+ , 200.0489. $C_{11}H_8N_2S$ requires M , 200.0482); ν_{\max} (nujol)/ cm^{-1} 1456, 1377 and 1243; δ_H (250 MHz; $CDCl_3$) 7.30 (3H, m), 7.44 (1H, m), 7.83 (1H, m), , 7.88 (1H, m), 8.24 (1H, m) and 8.57 (1H, broad, *NH*); δ_C (62.5 MHz; $CDCl_3$) 111.69, 112.57, 116.01, 118.52, 120.43, 121.39, 123.12, 124.65, 136.44, 142.37 and 164.43; m/z 200 (M^+ , 100%), 142 (30), 115 (15), 100 (10) and 58 (30).

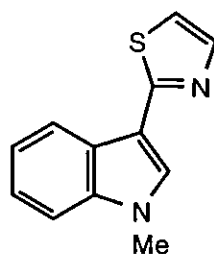


and **1-acetyl-3-(2-thiazolyl)indole [118]** (270 mg, 10%) as colourless crisyalline solid; m.p. 117.5°C; (lit., 116-118°C); (Found M^+ , 242.0511. $C_{13}H_{10}N_2OS$ requires M , 242.0513); ν_{\max} (nujol)/ cm^{-1} 1712, 1450, 1376 and 1248; δ_H (250 MHz; $CDCl_3$) 2.73 (3H, s, $COCH_3$), 7.16 (1H, s), 7.42 (2H, m), , 7.92 (1H, m), 8.06 (1H, m), 8.23 (1H, s) and 8.53 (1H, m); δ_C (62.5 MHz; $CDCl_3$) 24.09 ($COCH_3$), 116.81, 116.97, 117.81, 120.69, 124.38, 124.62, 126.16, 127.24, 127.44, 136.65, 143.04 and 169.02; m/z 242 (M^+ , 30%), 200 (100), 142 (20), 115 (10), 58 (30) and 43 (10).



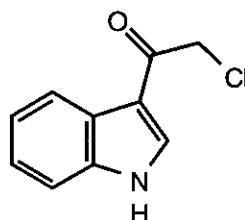
3-(2-Thiazolyl)indole [117]

1-Acetyl-3-(2-thiazolyl)indole [118] (100 mg, 0.41 mmol), THF (2 ml) and sodium hydroxide (1 ml, of a 5% solution) were heated at reflux for 16 h. The reaction mixture was cooled and made acidic by the addition of hydrochloric acid (6M). The mixture was extracted with ethyl acetate (3 x 10 ml), the extracts were combined, washed with brine (20 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (diethyl ether elution) gave the *title compound* [117] (68 mg, 83%), with analytical data identical to that previously reported.



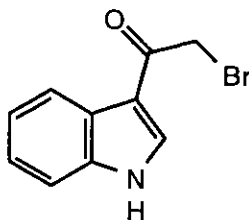
1-Methyl-(2-thiazolyl)indole [119]

3-(2-Thiazolyl)indole [117] (100 mg, 0.49 mmol) was added to a stirred solution of potassium hydroxide (111 mg, 1.98 mmol) and DMSO (1 ml). The solution was stirred at rt for 45 min then methyl iodide (141 mg, 1.0 mmol) was added and the mixture stirred for a further 45 min. Water (5 ml) was added and the mixture extracted with ethyl acetate (3 x 10 ml). The extracts were combined, washed with water (3 x 10 ml), brine (10 ml), dried (MgSO_4) and evaporated under reduced pressure. Recrystallisation (hexane) gave the *title compound* [119] (82 mg, 78% yield) as a brown solid; m.p. 69-70°C; (Found M^+ , 214.0575. $\text{C}_{12}\text{H}_{10}\text{N}_2\text{S}$ requires M , 214.0564); ν_{max} (nujol)/ cm^{-1} 1552, 1468 and 1376 ; δ_{H} (250 MHz; CDCl_3) 3.68 (3H, s, NCH_3), 7.15 (1H, s), 7.28 (3H, m), 7.65 (1H, s), 7.77 (1H, m) and 8.23 (1H, s); δ_{C} (62.5 MHz; CDCl_3) 33.04 (NCH_3), 109.75, 110.37, 115.43, 120.67, 121.10, 122.66, 125.31, 128.96, 137.42, 142.43 and 163.82; m/z 214 (M^+ , 100%), 156 (40), 107 (10) and 58 (10).



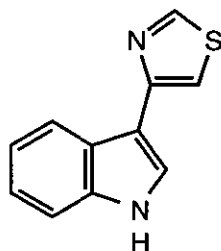
3-Chloroacetylindole [122]

Chloroacetyl chloride (11.29 g, 100 mmol) was added to a stirred solution of pyridine (7.91 g, 100 mmol), indole [93] (11.7 g, 100 mmol) and toluene (250 ml) over 1 h at 60°C. The mixture was stirred for 1 h, then water (300 ml) and methanol (50 ml) were added. The mixture was stirred at 60°C for 1 h, whereupon a brown solid separated. The brown solid was removed by filtration, and recrystallisation (ethanol) gave the *title compound* [122] (13.92 g, 72% yield) as a pale brown solid; m.p. 230-232°C (lit., 230-232); ν_{max} (nujol)/ cm^{-1} 1645, 1459, 1436, 1377 and 751; δ_{H} (250 MHz; CD_3OD) 4.72 (2H, s, COCH_2Cl), 7.25 (2H, m), 7.49 (1H, m) and 8.24 (2H, m), indole NH unobserved; δ_{C} (62.5 MHz; CD_3OD) 45.09 (COCH_2Cl), 111.83, 120.95, 120.97, 121.92, 122.94, 122.97, 133.55, 133.76 and 202.37; m/z 193 (M^+ , 30%), 144 (100), 89 (20) and 148 (20).



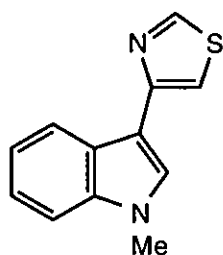
3-Bromoacetylindole [123]

3-Chloroacetylindole [122] (1 g, 5.2 mmol), sodium bromide (5.35 g, 52 mmol) and acetone (30 ml) were heated under reflux for 24 h. The reaction mixture was filtered through a glass sinter and the residue washed with acetone (100 ml), the filtrate was evaporated under reduced pressure and recrystallisation (ethyl acetate) gave the *title compound* [123] (0.78 g, 62%) as a brown solid; m.p. 192-194°C; (Found M^+ , 238.9777. $C_{10}H_8^{80}BrNO$ requires M , 238.9779; Found M^+ , 236.9787. $C_{10}H_8^{78}BrNO$ requires M , 236.9799); δ_H (250 MHz; $CDCl_3$: CD_3OD) 4.21 (2H, s, $COCH_2Br$), 7.08 (2H, m), 7.30 (1H, m, Ar), 7.90 (1H, m) and 8.09 (1H, m), indole NH unobserved; δ_C (62.5 MHz; $CDCl_3$: CD_3OD) 31.39 ($COCH_2Br$), 104.32, 11.83, 114.62, 121.56, 122.47, 123.44, 125.01, 133.92 and 188.91; m/z 239 (M^+ , 20%), 237 (10), 144 (100) and 89 (20).



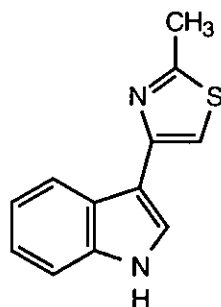
3-(4-Thiazolyl)indole [126]

3-Bromoacetylindole [123] (1.07 g, 4.5 mmol), thioformamide [125] (0.64 g, 9 mmol) and ethanol (30 ml) were heated under reflux for 3 h. The reaction mixture was evaporated under reduced pressure to give a yellow solid. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [126] (0.252 g, 28% yield) as pale yellow solid; m.p. 139-140°C; ν_{max} ($CHCl_3$)/ cm^{-1} 3066, 1522, 1476, 1423, 1225 and 929; δ_H (250 MHz; $CDCl_3$) 7.24 (3H, m), 7.42 (1H, d J 1.9 Hz), 7.80 (1H, d J 2.7 Hz), 8.04 (1H, m), 8.45 (1H, s, NH) and 8.91 (1H, d J 1.9 Hz); δ_C (62.5 MHz; $CDCl_3$) 109.66, 111.75, 111.80, 119.36, 120.18, 122.07, 124.23, 124.40, 137.29, 151.03 and 152.75.



1-Methyl-3-(4-thiazolyl)indole [127]

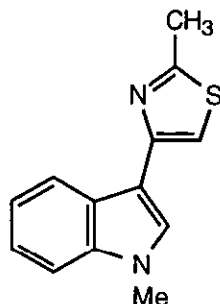
3-(4-Thiazolyl)indole [126] (50 mg, 0.25 mmol) was added to a stirred solution of potassium hydroxide (56 mg, 1 mmol) and DMSO (1 ml). The solution was stirred at rt for 45 min then methyl iodide (141 mg, 1.0 mmol) was added and the mixture stirred for a further 45 min. Water (5 ml) was added and the mixture extracted with ethyl acetate (3 x 10 ml). The extracts were combined, washed with water (3 x 10 ml), brine (10 ml), dried (MgSO_4) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* [127] (44 mg, 83% yield) as a brown solid; m.p. 54-55°C; ν_{max} (Found M^+ , 214.0562. $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}$ requires M, 214.0565); $(\text{CHCl}_3)/\text{cm}^{-1}$ 3020, 1522, 1421, 1228 and 929; δ_{H} (250 MHz; CDCl_3) 3.69 (3H, s, NCH_3), 7.19 (3H, s), 7.26 (1H, d J 1.75 Hz), 7.55 (1H, s), 7.92 (1H, m) and 8.75 (1H, d J 1.62 Hz); δ_{C} (62.5 MHz; CDCl_3) 32.86, 108.94, 109.66, 110.88, 120.07, 120.26, 122.07, 125.48, 128.53, 137.36, 151.65 and 152.03; m/z 214 (M^+ , 40%), 186 (25), 155 (45), 113 (40), 70 (30), 51 (100) and 31 (85).



3-(2-Methyl-4-thiazolyl)indole [128]

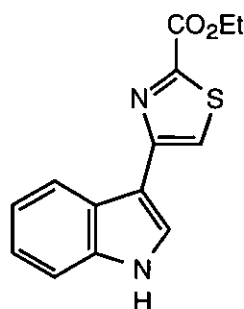
A solution of thioacetamide (1.15 g, 15.2 mmol) and ethanol (60 ml) was added dropwise to a stirring solution of 3-chloroacetylindole [122] (1.5 g, 7.7 mmol) in ethanol (100 ml) at reflux and the solution was stirred under reflux for 3 h. The reaction mixture was cooled and sodium hydroxide (20% solution, 50 ml) was added. The mixture was extracted with ethyl acetate (2 x 200 ml) and the extracts were combined and washed with brine (50 ml), dried (MgSO_4) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [128] (650 mg, 43% yield) as a pale brown solid; m.p. 122-124°C; (Found M^+ , 214.0564. $\text{C}_{12}\text{H}_{10}\text{N}_2\text{S}$ requires M, 214.0559); ν_{max} (CHCl_3)/ cm^{-1} 3448, 1617, 1575, 1482 and 1360; δ_{H} (250 MHz; CDCl_3) 2.28 (3H, s, CH_3), 7.22 (3H, m), 7.38 (1H, m), 7.74 (1H, m), 7.99 (1H, m) and 8.45 (1H, broad, NH); δ_{C} (62.5 MHz;

CDCl₃) 19.06 (CH₃), 109.57, 111.64, 112.22, 119.82, 120.44, 122.35, 123.96, 125.01, 136.58 and 150.29; *m/z* 214 (M⁺, 100%), 173 (70), 98 (50), 91 (40) and 84 (40).



3-(2-Methyl-4-thiazolyl)-1-methylindole [129]

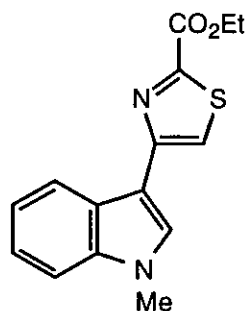
3-(2-Methyl-4-thiazolyl)indole [128] (200 mg, 0.93 mmol) was added to a stirred solution of potassium hydroxide (209 mg, 3.73 mmol) and DMSO (2 ml). The resultant red solution was stirred at rt for 45 min then methyl iodide (246 mg, 1.86 mmol) added and the mixture stirred for a further 45 min. Water (5 ml) was added and the mixture extracted with ethyl acetate (3 x 10 ml). The extracts were combined, washed with water (3 x 10 ml), brine (10 ml), dried (MgSO₄) and evaporated under reduced pressure. Recrystallisation (hexane) gave the *title compound* [129] (160 mg, 75% yield) as a yellow solid; m.p. 120-121°C; (Found: C, 68.48; H, 5.24; N, 12.18. C₁₃H₁₂N₂S requires C, 68.39; H, 5.3; N, 12.27%); δ_H (250 MHz; CDCl₃) 2.77 (3H, s, CH₃), 3.82 (3H, s, NCH₃), 7.19 (1H, s), 7.31 (3H, m), 7.96 (1H, s) and 8.0 (1H, m); *m/z* 228 (M⁺, 100%), 187 (50), 172 (30), 98 (25) and 84 (25); (Found M⁺, 228.0707. C₁₃H₁₂N₂S requires M, 228.0721).



Ethyl 4-(indol-3-yl)thiazole-2-carboxylate [130]

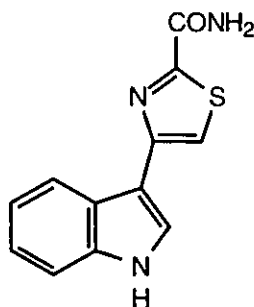
3-Bromoacetylindole [123] (500 mg, 2.1 mmol), ethyl thiooxamate (290 mg, 2.15 mmol) and acetonitrile (30 ml) were heated under reflux for 3 h, further ethyl thiooxamate (60 mg, 0.45 mmol) was added and the mixture heated under reflux for a further 2 h. The reaction mixture was evaporated under reduced pressure to give a yellow solid. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [130] (417 mg, 74% yield) as pale yellow solid; m.p. 170-171°C; (Found M⁺, 272.0619. C₁₄H₁₂N₂O₂S requires M, 272.0619); ν_{max} (nujol)/cm⁻¹ 3683, 3019, 1719, 1522, 1422, 1221 and 929; δ_H (250 MHz;

CDCl₃) 1.44 (3H, t, *J* 7.5 Hz, OCH₂CH₃), 4.42 (2H, q, *J* 7.1 Hz, OCH₂CH₃), 7.23 (2H, m), 7.47 (1H, s), 7.64 (1H, s), 7.86 (1H, d *J* 2.71 Hz), 8.09 (1H, m) and 8.98 (1H, s, NH); δ_C (62.5 MHz; CDCl₃) 16.65, 66.02, 115.23, 119.58, 123.12, 123.76, 125.31, 125.58, 127.21, 128.66, 128.71, 140.91, 160.09 and 164.82; *m/z* 272 (M⁺, 90%), 141 (100) and 29 (20).



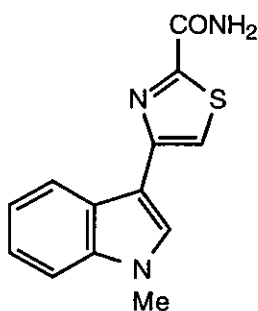
Ethyl 4-(1-methylindol-3-yl)thiazole-2-carboxylate [131]

Dry DMF (1.5 ml) was added to potassium hydride (44 mg, 1.83 mmol), under a nitrogen atmosphere, at 0°C. A solution of ethyl 4-(indol-3-yl)thiazole-2-carboxylate [130] (100 mg, 0.37 mmol) in DMF (2 ml) was added dropwise and the solution stirred at 0°C for 5 min, then at rt for 45 min. The solution was cooled to 0°C and methyl iodide (261 mg, 1.84 mmol) added, the mixture was warmed to rt and stirred for a further 45 min. A saturated solution of ammonium chloride (10 ml) was added and the mixture extracted with diethyl ether (3 x 20 ml), the extracts were combined, washed with water (2 x 20 ml), brine (20 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (diethyl ether elution) gave the *title compound* [131] (95 mg, 90%) as an off white solid; m.p. 131-132°C; (Found M⁺, 286.0777. C₁₅H₁₄N₂O₂S requires M, 285.0776); ν_{max.} (nujol)/cm⁻¹ 1687, 1554 and 1232; δ_H (250 MHz; CDCl₃) 1.44 (3H, t, *J* 7.4 Hz, OCH₂CH₃), 4.01 (3H, s, NCH₃), 4.42 (2H, q, *J* 7.1 Hz, OCH₂CH₃), 7.25 (3H, m), 7.56 (1H, s), 7.73 (1H, s) and 7.98 (1H, m); δ_C (62.9 MHz; CDCl₃) 14.24, 32.87, 62.40, 109.73, 111.76, 115.42, 119.64, 120.44, 122.12, 124.99, 129.37, 137.30, 153.39, 157.08 and 160.21; *m/z* 286 (M⁺, 100%), 172 (18), 154 (20) and 29 (20).



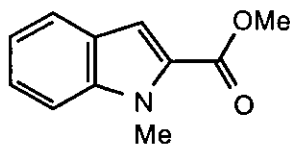
2-(Indol-3-yl)thiazole-4-carboxamide [132]

Ethyl 4-(indol-3-yl)thiazole-2-carboxylate [130] (100 mg, 0.4 mmol), ammonium chloride (5 mg, 0.1 mmol) and ammonium hydroxide (5 ml, of a 0.88 solution) were heated at 100°C in a Young's sealed tube for 48 h. The crude mixture was extracted with ethyl acetate (3 x 10 ml), the extracts were combined and washed with brine (15 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* [132] (43 mg, 47%) as a pale brown solid; m.p. 215-216°C; ν_{\max} (nujol)/cm⁻¹ 3683, 3019, 1719, 1522, 1422, 1221 and 929; δ_{H} (250 MHz; CD₃OD : CDCl₃) 7.13 (2H, m), 7.33 (1H, m), 7.36 (1H, s), 7.64 (1H, s) and 7.90 (1H, m), indole NH and amide NH₂ unobserved; δ_{C} (62.5 MHz; CD₃OD : CDCl₃) 114.81, 115.34, 119.02, 123.35, 123.79, 125.64, 125.81, 128.40, 128.72, 140.92, 157.30 and 166.12; *m/z* 243 (M⁺ 100%), 225 (30), 173 (50), 141 (40) and 44 (30).



4-(1-Methylindol-3-yl)thiazole-2-carboxamide [133]

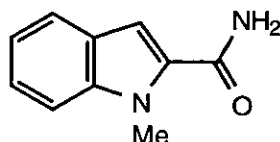
Ethyl 4-(1-methylindol-3-yl)thiazole-2-carboxylate [132] (233 mg, 0.82 mmol), ammonium chloride (20 mg, 0.37 mmol) and ammonium hydroxide (10 ml, of a 0.88 solution) were heated at 100°C in a Young's sealed tube for 48 h. The crude mixture was extracted with ethyl acetate (3 x 10 ml), the extracts were combined and washed with brine (15 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* [133] (120 mg, 58%) as a pale brown solid; m.p. 186-187°C; (Found M⁺, 257.0617. C₁₃H₁₁N₃OS requires M, 257.0623); ν_{\max} (nujol)/cm⁻¹ 1687, 1554 and 1232; δ_{H} (250 MHz; CDCl₃); *m/z* 257 (M⁺, 100%), 187 (20), 172 (15), 155 (15), 128 (10) and 69 (15).



Methyl-1-methylindole-2-carboxylate [135]

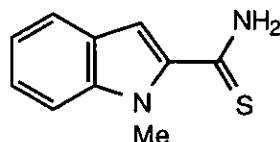
Dry DMF (70 ml) was added to potassium hydride (5.542 g, 138 mmol), under a nitrogen atmosphere, at 0°C. A solution of indole-2-carboxylic acid [134] (4.44 g, 27.5 mmol) in DMF (70 ml) was added dropwise. The solution was stirred at 0°C for 5 min then stirred at rt

for 45 min. The solution was cooled to 0°C and methyl iodide (19.45 g, 138 mmol) added. The mixture was warmed to rt and stirred for a further 45 min. A saturated solution of ammonium chloride (150 ml) was added and the mixture extracted with diethyl ether (3 x 150 ml), the extracts were combined, washed with water (2 x 150 ml), brine (150 ml), dried (MgSO₄) and evaporated under reduced pressure. Recrystallisation (hexane) gave the *title compound* [135] (4.64 g, 90%) as a colourless solid; m.p. 95-97°C; (Found: C, 70.3; H, 6.06; N, 7.46. C₁₁H₁₁NO₂ requires C, 69.84; H, 5.82; N, 7.41%); ν_{\max} (nujol)/cm⁻¹ 1704, 1516, 1467, 1253 and 752 ; δ_{H} (250 MHz; CDCl₃) 3.90 (3H, s, OCH₃), 4.07 (3H, s, NCH₃), 7.12 (1H, m), 7.31 (1H, s), 7.34 (2H, m) and 7.66 (1H, dt, *J* 7.9, 1.0 Hz); δ_{C} (62.5 MHz; CDCl₃) 31.51, 51.45, 110.16, 110.17, 120.5, 122.55, 124.95, 125.84, 127.61, 139.92 and 163.32; *m/z* 189 (M⁺, 100%), 158 (40), 89 (50), 57 (48) and 43 (42); (Found M⁺, 189.0785. C₁₁H₁₁NO₂ requires M, 189.0789).



***1-Methylindole-2-carboxamide* [136]**

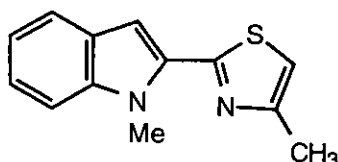
Trimethyl aluminium (2M solution in toluene, 19.87 ml, 39.75 mmol) was added dropwise to a solution of ammonium chloride (2.125 g, 39.75 mmol) and benzene (39.75 ml) at 5°C, under a nitrogen atmosphere. The mixture was warmed to rt and stirred for 1 h. A solution of methyl-1-methylindole-2-carboxylate [135] (2.5 g, 13.25 mmol) in benzene (125 ml) was added and the solution stirred at 60°C for 12 h. Hydrochloric acid (2M, 200 ml) was added and the solution extracted with ethyl acetate (3 x 150 ml). The extracts were combined, washed with brine (200 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* [136] (1.32 g, 58% yield) as a colourless solid; m.p. 167-169°C; (Found: C, 68.88; H, 5.71; N, 16.13. C₁₀H₁₀N₂O requires C, 68.97; H, 5.75; N, 16.09%); ν_{\max} (nujol)/cm⁻¹ 1652, 1616, 1464 and 1332; δ_{H} (250 MHz; CDCl₃) 4.07 (3H, s, NCH₃), 5.91 (2H, broad, NH₂), 6.93 (1H, s), 7.16 (1H, m), 7.34 (2H, m) and 7.66 (1H, m.); *m/z* 174 (M⁺, 100%), 158 (40), 130 (40) and 89 (50); (Found M⁺, 174.0776. C₁₀H₁₀N₂O requires M, 174.0793).



***1-Methylindole-2-thiocarboxamide* [137]**

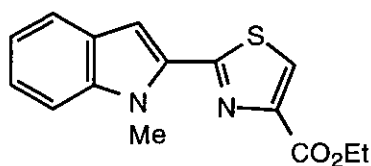
A solution of 1-methylindole-2-carboxamide [136] (300 mg, 1.74 mmol) and Lawesson's reagent [99] (351 mg, 0.87 mmol) in benzene (30 ml) were heated under reflux for 1 h. The

crude reaction mixture was evaporated under reduced pressure and flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [137] (252 mg, 77% yield) as a yellow solid; m.p. 139-140°C; (Found M^+ , 190.0545. $C_{13}H_{12}N_2S$ requires M , 190.0565); ν_{max} (nujol)/ cm^{-1} 1615, 1464, 1377 and 1351; δ_H (250 MHz; $CDCl_3$) 4.09 (3H, s, NCH_3), 6.71 (1H, s), 7.21 (3H, m), 7.34 (2H, s) and 7.64 (1H, m); δ_C (62.5 MHz; $CDCl_3$) 32.23, 103.49, 110.44, 120.97, 122.22, 124.76, 125.57, 138.03, 140.52 and 193.24; m/z 190 (M^+ , 90%), 157 (100) and 130 (50).



2-(4-Methyl-2-thiazolyl)-1-methylindole [138]

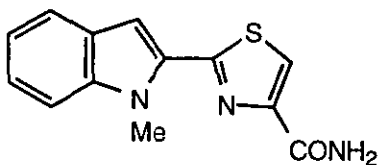
1-Methylindole-2-thiocarboxamide [137] (79 mg, 0.4 mmol), chloroacetone (75 mg, 0.81 mmol), sodium bromide (414 mg, 4.03 mmol) and acetonitrile (5 ml) were heated under reflux for 2 h. The reaction mixture was cooled, filtered and washed with dichloromethane (20 ml). The filtrate was washed with brine (2 x 20 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow solid. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [138] (68 mg, 71% yield) as pale yellow solid; m.p. 83-84°C; (Found M^+ , 228.0723. $C_{13}H_{12}N_2S$ requires M , 228.0721); ν_{max} ($CHCl_3$)/ cm^{-1} 2957, 1522, 1224 and 884, δ_H (250 MHz; $CDCl_3$) 2.56 (3H, s, CH_3), 4.19 (3H, s, NCH_3), 6.87 (1H, s), 7.14 (1H, s), 7.20 (1H, m), 7.35 (2H, m) and 7.65 (1H, dd, J 7.8, 1.4 Hz); δ_C (62.5 MHz; $CDCl_3$) 17.32 (CH_3), 31.75 (NCH_3), 104.54, 109.85, 113.04, 120.22, 121.07, 123.16, 127.27, 132.76, 139.81, 153.58 and 159.96; m/z 228 (M^+ , 100%), 213 (30), 130 (25), 98 (40) and 44 (15).



Ethyl 2-(1-methylindol-2-yl)thiazole-4-carboxylate [139]

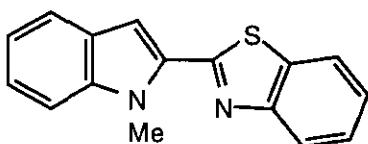
1-Methylindole-2-thiocarboxamide [137] (100 mg, 0.53 mmol), ethylbromopyruvate (155 mg, 0.79 mmol) and ethanol (25 ml) were heated under reflux for 0.5 h. The reaction mixture was evaporated under reduced pressure to give a yellow solid. Flash column chromatography (light petroleum : ethyl acetate elution) gave the *title compound* [139] (74 mg, 48% yield) as pale yellow solid; m.p. 122-123°C; (Found M^+ , 286.0771. $C_{15}H_{14}N_2O_2S$ requires M , 286.0776); ν_{max} ($CHCl_3$)/ cm^{-1} 1733, 1420, 1098 and 668, δ_H (250 MHz; $CDCl_3$) 1.47 (3H, t, J 7.15 Hz, CH_2CH_3), 4.44 (3H, s, NCH_3), 4.44 (2H, q, J 7.2 Hz, OCH_2CH_3), 7.24 (2H,

m.), 7.37 (2H, m.), 7.61 (1H, m) and 8.29 (1H, s); δ_C (62.5 MHz; $CDCl_3$) 14.31 (OCH_2CH_3), 32.86 (NCH_3), 61.45 (OCH_2CH_3), 110.17, 120.19, 121.07, 124.97, 126.74, 127.42, 127.53, 138.72, 143.21, 157.98, 161.67 and 187.07; m/z 286 (M^+ , 100%), 214 (50), 157 (30) and 77 (15).



2-(1-Methylindol-2-yl)thiazole-4-carboxylate [140]

Ethyl 2-(1-methylindol-2-yl)thiazole-4-carboxylate [137] (15 mg, 0.057 mmol) was dissolved in dichloromethane (1 ml) and placed in a Young's tube. The mixture was cooled to $-78^\circ C$ and ammonia gas was bubbled into the mixture (approx. 30 ml). The Young's tube was sealed and allowed to warm to rt. The mixture was stirred at rt for 4 days. The reaction mixture was cooled to $-78^\circ C$ and the Young's tube opened, **CAUTION**. The mixture was left to warm to rt over a 4 h period. Ethyl acetate (20 ml) was added and the mixture was washed with brine (2 x 20 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give a pale brown solid. Flash column chromatography (ethyl acetate elution) gave the *title compound* [140] (11 mg, 80%) as a pale brown solid; m.p. $190^\circ C$; (Found M^+ , 257.0617. $C_{13}H_{11}N_3OS$ requires M , 257.0617) ν_{max} ($CHCl_3$)/ cm^{-1} 1686, 1420, 1216 and 853; δ_H (250 MHz; CD_3OD) 4.04 (3H, s, NCH_3), 7.10 (3H, m), 7.21 (1H, m), 7.43 (1H, m) and 8.03 (1H, m); amide NH_2 unobserved; m/z 257 (M^+ , 100%), and 213 (40), 120 (20) and 77 (10).

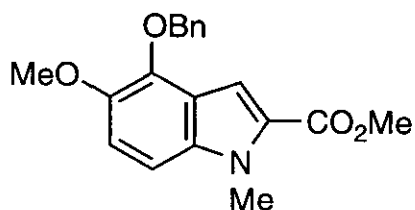


2-(2-Benzothiazolyl)-1-methylindole [141]

Trimethyl aluminium (2M solution in toluene, 6.76 ml, 13.53 mmol) was added dropwise to toluene (25 ml) at $0^\circ C$, under a nitrogen atmosphere. 2-Aminothiophenol (504 mg, 4.02 mmol) was added dropwise maintaining the temperature at $0^\circ C$. The mixture was stirred at $0^\circ C$ for 0.5 h then warmed to rt. A solution of methyl-1-methylindole-2-carboxylate [135] (500 mg, 2.67 mmol) in toluene (10 ml) was added and the solution stirred at $60^\circ C$ for 14 h. The mixture was cooled to $0^\circ C$ and water (5 ml) followed by methanol (50 ml) were added. The mixture was filtered and washed with methanol (100 ml) the filtrate was evaporated under reduced pressure to give a brown solid. Recrystallisation (diethyl ether) gave the *title compound* [141] (401 mg, 57%) as a pale brown solid; m.p. $135-136^\circ C$; (Found M^+ , 264.0721. $C_{16}H_{12}N_2S$ requires M , 264.0721) ν_{max} ($CHCl_3$)/ cm^{-1} 1549, 1424, 1345, 1312,

975 and 929; δ_{H} (250 MHz; CDCl_3) 4.3 (3H, s, NCH_3), 7.19 (2H, m), 7.4 (3H, m), 7.54 (1H, m), 7.67 (1H, dd, J 7.9, 1.0 Hz), 7.9 (1H, dd, J 7.8, 1.3 Hz) and 8.07 (1H, dd, J 8.7, 1.4 Hz); δ_{C} (100 MHz; CDCl_3) 32.65, 107.96, 110.51, 120.97, 121.68, 121.96, 123.60, 124.51, 125.66, 126.65, 127.69, 132.62, 134.90, 140.22, 154.69 and 161.00; m/z 264 (M^+ , 90%), 263 (100), 155 (20), 51 (20) and 28 (35).

7.4. Chapter 3 experimental



Methyl 4-benzyloxy-5-methoxy-1-methylindole-2-carboxylate [155]

Dry DMF (10 ml) was added to potassium hydride (644 mg, 16.05 mmol), under a nitrogen atmosphere, at 0°C. A solution of methyl-4-benzyloxy-5-methoxy-2-carboxylate [154] (1.0 g, 3.21 mmol) in DMF (10 ml) was added dropwise. The solution was stirred at 0°C for 5 min then stirred at rt for 45 min. The solution was cooled to 0°C and methyl iodide (2.27 g, 16.05 mmol) was added, the mixture was warmed to rt and stirred for a further 45 min. A saturated solution of ammonium chloride (30 ml) was added and the mixture extracted with diethyl ether (4 x 30 ml), the extracts were combined, washed with water (2 x 30 ml), brine (30 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [155] (0.807 g, 77% yield) as an off white solid; m.p. 51-53°C; (Found: C, 70.44; H, 5.72; N, 4.17. C₁₉H₁₉NO₄ requires C, 70.14; H, 5.89; N, 4.3%); ν_{\max} (nujol)/cm⁻¹ 1717, 1377, 1237 and 743; δ_{H} (250 MHz; CDCl₃) 3.85 (3H, s, NCH₃), 3.86 (3H, s, OCH₃), 3.99 (3H, s, CO₂CH₃), 5.25 (2H, s, OCH₂Ar), 7.02 (1H, d, *J* 9.02 Hz), 7.12 (1H, d, *J* 8.93 Hz), 7.35 (5H, m) and 7.52 (1H, d, *J* 8.19); δ_{C} (62.5 MHz; CDCl₃) 31.77 (NCH₃), 51.56 (CO₂CH₃), 58.57 (OCH₃), 75.57 (OCH₂Ar), 105.39, 107.24, 107.38, 115.96, 121.38, 127.85, 128.01, 128.15, 128.35, 137.07, 138.05, 145.11 and 162.48; *m/z* 325 (M⁺, 20%), 234 (45), 91 (100) and 65 (15); (Found M⁺, 325.1325. C₁₉H₁₉NO₄ requires M, 325.1314).

Alternative procedure for the preparation of

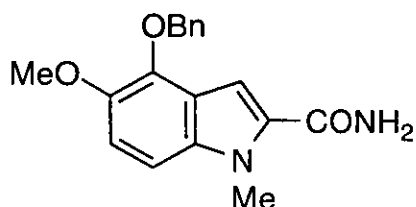
methyl 4-benzyloxy-5-methoxy-1-methylindole-2-carboxylate [155]

Methyl-4-benzyloxy-5-methoxy-2-carboxylate [154] (1.54 g, 4.94 mmol), potassium carbonate (4.1 g, 29.6 mmol), acetone (40 ml) and methyl iodide (0.769 g, 5.42 mmol) were heated under reflux for 24 h. The reaction mixture was cooled and diethyl ether (100 ml) Added. The mixture was washed with water (2 x 100 ml), brine (2 x 100 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [155] (1.21 g, 75%) as a colourless oil, with analytical data identical to that previously prepared.

Alternative procedure for the preparation of

methyl 4-benzyloxy-5-methoxy-1-methylindole-2-carboxylate [155]

DMSO (20 ml) was added to freshly crushed potassium hydroxide pellets (1.85 g, 33.12 mmol) at 0°C, the mixture was stirred for 10 min then methyl-4-benzyloxy-5-methoxy-2-carboxylate [154] (2.57 g, 8.28 mmol) was added. The mixture was stirred at 0°C for 10 min then at rt for 45 min. The solution was cooled to 0°C and methyl iodide (9.4 g, 66.24 mmol) was added, the mixture was allowed to warm to rt and stirred for a further 45 min. Saturated ammonium chloride (100 ml) was added and the mixture extracted with diethyl ether (3 x 50 ml). The extracts were combined and washed with water (5 x 100 ml), brine (2 x 100 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [155] (2.24 g, 84%) as a pale yellow oil, with analytical data identical to that previously prepared.



4-Benzyloxy-5-methoxy-1-methylindole-2-carboxamide [156]

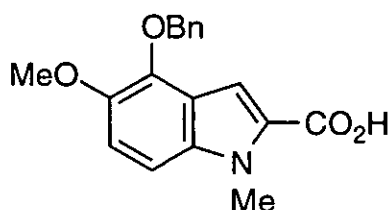
Ammonium hydroxide (15 ml, of a 0.88 solution) was added to methyl-4-benzyloxy-5-methoxy-1-methyl-2-carboxylate [155] (410 mg, 1.32 mmol) and ammonium chloride (18 mg, 0.336 mmol), in a Young's pressure tube. The tube was sealed and stirred at 100°C for 48 h. The crude reaction mixture was evaporated under reduced pressure to give a brown oil. Dichloromethane (100 ml) was added and the mixture was washed with brine (2 x 40 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* [156] (264 mg, 68%) as a pale brown solid; m.p. 148-150°C; (Found: C, 69.29; H, 5.79; N, 8.89. C₁₈H₁₈N₂O₃S requires C, 69.65; H, 5.85; N, 9.03%); ν_{\max} (nujol)/cm⁻¹ 1678, 1462, 1377 and 1229 ; δ_{H} (250 MHz; CDCl₃) 3.88 (3H, s, NCH₃), 4.01 (3H, s, OCH₃), 5.23 (2H, s, OCH₂Ar), 5.72 (2H, broad, NH₂), 6.83 (1H, s), 7.05 (1H, d, *J* 8.99 Hz), 7.09 (1H, d, *J* 8.92 Hz), 7.33 (4H, m) and 7.50 (1H, m); δ_{C} (62.5 MHz; CDCl₃) 31.72 (NCH₃), 58.32 (OCH₃), 75.11 (OCH₂Ar), 102.30, 105.42, 114.97, 121.44, 127.28, 127.84, 128.09, 128.31, 130.66, 136.49, 138.02, 145.13 and 164.19; *m/z* 310 (M⁺, 70%), 267 (30), 148 (55), 91 (100) and 65 (30); (Found M⁺, 310.1317. C₁₈H₁₈N₂O₃S requires M, 310.1317).

Alternative procedure for the preparation of

4-benzyloxy-5-methoxy-1-methylindole-2-carboxamide [156]

Ammonium chloride (0.519 g, 9.7 mmol) was added to a solution of trimethyl aluminium chloride (2M solution in hexanes, 4.84 ml, 9.7 mmol) and benzene (9.7 ml) at 0°C under a nitrogen atmosphere. A solution of methyl-4-benzyloxy-5-methoxy-1-methyl-2-carboxylate

[155] (1 g, 3.22 mmol) in benzene (30 ml) was added dropwise and the mixture stirred at 0°C for 10 min. The mixture was then stirred at 60°C for 14h. Hydrochloric acid (2M, 50 ml) was cautiously added and the mixture extracted with ethyl acetate (3 x 40 ml). The extracts were combined and washed with brine (2 x 40 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [156] (366 mg, 38%) as a pale yellow solid, with analytical data identical to that previously prepared.



4-Benzyloxy-5-methoxyindole-2-carboxylic acid [157]

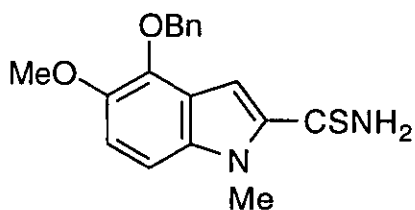
Methyl-4-benzyloxy-5-methoxy-2-carboxylate [155] (200 mg, 0.65 mmol), THF (4 ml) and sodium hydroxide (5% solution, 1.5 ml) were heated at reflux for 16h. The mixture was cooled, made acidic, by the addition of dilute hydrochloric acid, then extracted with dichloromethane (3 x 10 ml). The extracts were combined, washed with brine (20 ml), dried (MgSO₄) and evaporated under reduced pressure to give a yellow solid. Flash column chromatography (ethyl acetate elution) gave the *title compound* [157] (164 mg, 86%) as a pale yellow solid; m.p. 177-179°C; (Found M⁺, 311.1150. C₁₈H₁₇NO₄ requires M, 311.1157); ν_{\max} (CHCl₃)/cm⁻¹ 1681, 1520, 1349, 1216 and 752; δ_{H} (250 MHz; CDCl₃) 3.90 (3H, s, OCH₃), 4.03 (3H, s, NCH₃), 5.29 (2H, s, OCH₂Ph), 7.03 (1H, d, *J* 8.5 Hz), 7.14 (1H, d, *J* 8.5 Hz), 3.34 (3H, m) and 7.49 (3H, m), carboxylic acid OH unobserved; δ_{C} (62.5 MHz; CDCl₃ : CD₃OD) 31.62, 58.34, 75.07, 105.51, 107.65, 112.96, 115.59, 121.32, 127.65, 128.02, 128.23, 128.79, 137.07, 137.63, 141.34 and 164.0; *m/z* 311 (M⁺, 35%), 267 (15), 220 (100), 206 (25), 91 (75) and 63 (20).

Alternative procedure for the preparation of

4-benzyloxy-5-methoxy-1-methylindole-2-carboxamide [156]

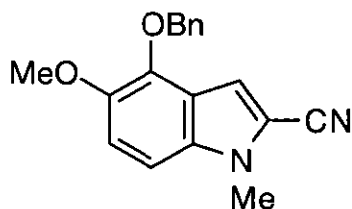
A solution of 4-benzyloxy-5-methoxy-1-methylindole-2-carboxylic acid [157] (55 mg, 0.18 mmol) and acetyl chloride (326 mg, 4.16 mg) was cooled to 0°C. Powdered phosphorus pentachloride (40 mg, 0.19 mmol) was added and the mixture stirred at rt for 4 h. The resulting yellow solution was evaporated under reduced pressure. Dichloromethane (5 ml) was added and the solution was cooled to -78°C, ammonia gas was bubbled into the reaction mixture (approx. 30 ml). The reaction mixture was stirred at -78°C for 2 h, then warmed to rt over a 12 h period. Dichloromethane (20 ml) was added and the mixture washed with brine (2 x 10 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column

chromatography (ethyl acetate elution) gave the *title compound* [156] (17 mg, 31%) as a pale brown solid, with analytical data identical to that previously prepared.



4-Benzyloxy-5-methoxy-1-methylindole-2-thiocarboxamide [159]

A solution of 4-benzyloxy-5-methoxy-1-methylindole-3-amide [156] (462 mg, 1.49 mmol), Lawesson's reagent [99] (356 mg, 0.88 mmol) and toluene (25 ml) were heated under reflux for 1 h. The crude reaction mixture was evaporated under reduced pressure and flash column chromatography (dichloromethane : diethyl ether elution) gave the *title compound* [159] (318 mg, 65% yield) as a yellow solid; m.p. 176-177°C; (Found: C, 66.50; H, 5.44; N, 8.44. $C_{18}H_{18}N_2O_2S$ requires C, 66.24; H, 5.56; N, 8.59%); ν_{max} (nujol)/ cm^{-1} 1642, 1596, 1523 and 1463; δ_H (250 MHz; $CDCl_3$) 3.89 (3H, s, NCH_3), 4.03 (3H, s, OCH_3), 5.21 (2H, s, OCH_2Ar), 6.77 (1H, s), 7.03 (3H, m), 7.12 (3H, m) and 7.49 (3H, m); δ_C (62.5 MHz; $CDCl_3$) 32.34 (NCH_3), 58.21 (OCH_3), 75.16 (OCH_2Ar), 100.42, 105.63, 115.13, 122.31, 127.85, 128.13, 128.31, 128.75, 138.07, 138.13, 142.01, 146 and 192; m/z 326 (M^+ , 40%), 235 (75), 201 (30), 148 (20), 91 (100) and 65 (30); (Found M^+ , 326.1096. $C_{18}H_{18}N_2O_2S$ requires M, 326.1089).

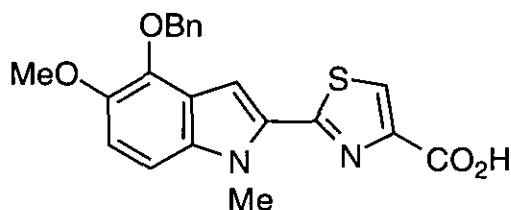


and **4-benzyloxy-5-methoxy-1-methylindole-2-nitrile [158]**; (Found M^+ , 292.1216. $C_{18}H_{16}N_2O_2$ requires M, 292.1212); $\nu_{max}(CHCl_3)/cm^{-1}$ 2224, 1601, 1519, 1215 and 752; δ_H ($CDCl_3$; 250 MHz) 3.84 (3H, s, OCH_3), 3.91 (3H, s, NCH_3), 5.24 (2H, s, OCH_2Ph), 6.98 (1H, m), 7.10 (2H, m), 7.20 (1H, s), 7.36 (2H, m) and 7.48 (2H, m); δ_C ($CDCl_3$; 62.5 MHz) 31.65, 75.13, 85.41, 105.16, 109.80, 113.50, 116.38, 121.96, 123.43, 128.01, 128.08, 128.24, 128.53, 134.98, 137.56 and 145.63; m/z 292 (M^+ , 20%), 201 (40) and 91 (100).

Alternative procedure for the preparation of

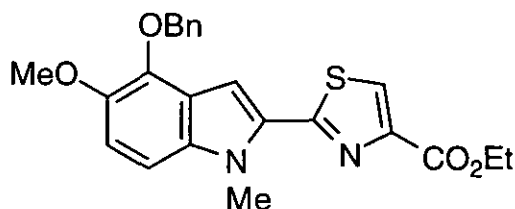
4-benzyloxy-5-methoxy-1-methylindole-2-carboxamide [156]

4-benzyloxy-5-methoxy-1-methylindole-2-nitrile **[158]** (137 mg, 0.49 mmol), potassium hydroxide (101 mg, 0.18 mmol) and *tert*-butanol (5 ml) were heated under reflux for 30 min. Brine (10 ml) was added and the mixture extracted with dichloromethane (3 x 30 ml). The extracts were combined washed with brine (2 x 10 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* **[156]** (126 mg, 86%) as a pale brown solid, with analytical data identical to that previously prepared.



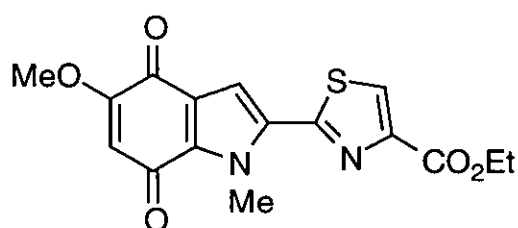
2-(4-Benzyloxy-5-methoxy-1-methylindol-2-yl)thiazole-4-carboxylic acid [161]

4-Benzyloxy-5-methoxy-1-methylindole-3-thioamide **[159]** (674 mg, 2.06 mmol), bromopyruvic acid (725 mg, 4.34 mmol) and ethanol (90 ml) were heated under reflux for 0.5 h. The crude reaction mixture was evaporated under reduced pressure, to give an oil, water (30 ml) was added and the mixture extracted with ethyl acetate (3 x 50 ml), washed with brine (30 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (dichloromethane : ethyl acetate elution) gave the *title compound* **[161]** (509 mg, 62% yield) as a pale yellow solid; m.p. 143-145°C; (Found M⁺, 394.0853. C₂₁H₁₈N₂O₄S requires M, 394.0988); δ_H (250 MHz; CDCl₃) 3.92 (3H, s, NCH₃), 4.11 (3H, s, OCH₃), 5.26 (2H, s, OCH₂Ar), 6.99 (1H, s), 7.07 (2H, m), 7.35 (3H, m), 7.50 (2H, m) and 8.24 (1H, s), carboxylic acid OH unobserved; δ_C (62.5 MHz; CDCl₃ : CD₃OD) 31.86 (NCH₃), 58.16 (OCH₃), 75.12 (OCH₂Ar), 102.8, 105.35, 113.9, 122.9, 127.23, 127.82, 128.08, 128.2, 132.12, 136.7, 137.3, 140.8, 145.2, 148.3, 162.3 and 164.3; *m/z* 394 (M⁺, 5%), 350 (20), 320 (25), 304 (30), 260 (25), 217 (20), 155 (25), 113 (20), 91 (100), 51 (45) and 44 (100); as well as the ester **[162]** (175 mg, 19%).

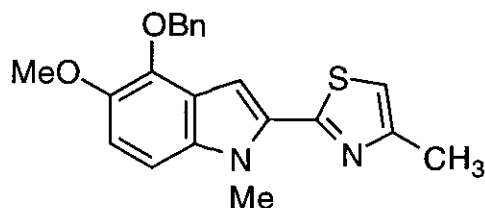


Ethyl 2-(4-benzyloxy-5-methoxy-1-methylindol-2-yl)thiazole-4-carboxylate [162]

2-(4-Benzyloxy-5-methoxy-1-methylindol-2-yl)thiazole-4-carboxylic acid [161] (471 mg, 1.19 mmol), ethanol (20 ml) and sulphuric acid (0.5 ml, of a concentrated solution) were heated under reflux for 14 h. Brine (20 ml) was added and the mixture extracted with dichloromethane (3 x 40 ml). The extracts were combined and washed with brine (30 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (dichloromethane : ethyl acetate elution) gave the *title compound* [162] (428 mg, 85%) as a pale yellow solid; m.p. 113-114°C; (Found M⁺, 422.1257. C₂₃H₂₂N₂O₄S requires M, 422.1300; ν_{\max} (CHCl₃/cm⁻¹) 1722, 1553, 1421, 1321, 1215 and 757; δ_{H} (250 MHz; CDCl₃) 1.43 (3H, t, *J* 7.2 Hz, OCH₂CH₃), 3.97 (3H, s, NCH₃), 4.15 (3H, s, OCH₃), 4.42 (2H, q, *J* 7.15, OCH₂CH₃), 5.25 (2H, s, OCH₂Ar), 6.96 (1H, s), 7.07 (2H, s), 7.35 (4H, m), 7.50 (1H, m) and 8.11 (1H, s); δ_{C} (62.5 MHz; CDCl₃) 14.29 (OCH₂CH₃), 31.86 (NCH₃), 58.27 (OCH₃), 61.39 (OCH₂CH₃), 75.06 (OCH₂Ar), 102.91, 105.24, 114.04, 122.49, 126.63, 127.83, 128.09, 128.28, 128.74, 131.62, 136.53, 138.03, 145.32, 147.71, 161.15 and 161.24. *m/z* 422 (M⁺, 5%), 332 (70), 317 (45), 111 (30), 97 (40), 83 (50), 69 (70), 55 (100), 39 (70) and 27 (40).

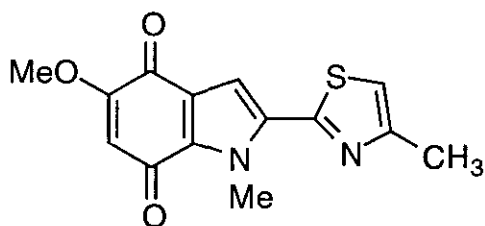


***Ethyl 2-(5-methoxy-1-methyl-4,7-dioxoindol-2-yl)thiazole-4-carboxylate* [163]**
 Ethyl 2-(4-benzyloxy-5-methoxy-1-methylindol-2-yl)thiazole-4-carboxylate [162] (0.6 g, 1.42 mmol), ethanol (10 ml) and palladium hydroxide (Pearlman's catalyst) (0.2 g, 0.14 mmol) were stirred under a hydrogen atmosphere at atmospheric pressure for 48 h. The reaction mixture was filtered, washed with dichloromethane (100 ml), the filtrate and washings were combined and evaporated under reduced pressure. Acetone (20 ml) and sodium dihydrogen phosphate (0.17 M, 20 ml) were added and the mixture stirred at rt for 10 min. Fremy's salt (1.9 g, 2.5 mmol) and water (20 ml) were added and the mixture stirred at rt for 16 h. The mixture was extracted with dichloromethane (3 x 30 ml). The extracts were combined, washed with brine (30 ml), dried (Na₂SO₄) and evaporated under reduced pressure. Flash column chromatography (dichloromethane : ethyl acetate elution) gave the *title compound* [163] (0.317 g, 64%) as an orange solid; (Found M⁺, 346.0645. C₁₆H₁₄N₂O₅S. requires M, 346.0623); δ_{H} (250 MHz; CDCl₃) 1.42 (3H, t, *J* 7.2 Hz, OCH₂CH₃), 3.8 (3H, s, OCH₃), 4.39 (3H, 3H, s, NCH₃), 4.42 (2H, q, *J* 7.2 Hz, OCH₂CH₃), 7.78 (1H, s, *H*₆), 7.96 (1H, s) and 8.2 (1H, s); δ_{C} (62.5 MHz; CDCl₃) 14.24, 35.19, 56.57, 61.59, 108.06, 110.83, 116.12, 121.04, 124.32, 127.40, 127.92, 129.21, 148.17, 160.07, 178.93 and 179.62; *m/z* 364 (M⁺, 10%), 97 (40), 81 (50), 69 (85), 55 (60), 41 (100) and 27 (50).



2-(4-Benzyloxy-5-methoxy-1-methylindol-2-yl)-4-methylthiazole [164]

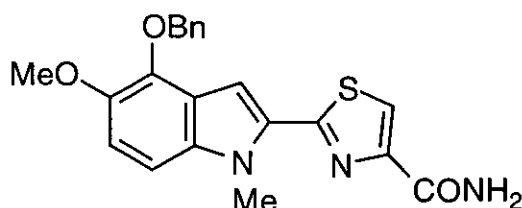
4-Benzyloxy-5-methoxy-1-methylindole-3-thioamide [159] (173 mg, 0.53 mmol), bromoacetone (145 mg, 1.06 mmol) and ethanol (20 ml) were heated under reflux for 1 h. The reaction mixture was evaporated under reduced pressure and flash column chromatography (dichloromethane : ethyl acetate elution) gave the title compound [164] as a pale yellow solid (133 mg, 69%); m.p. 115-117°C; (Found M^+ , 364.126. $C_{21}H_{20}N_2O_2S$ requires M , 363.1245); ν_{\max} ($CHCl_3/cm^{-1}$) 1503, 1415, 1341, 1215, 1003 and 771; δ_H (250 MHz; $CDCl_3$) 2.32 (3H, s, CH_3), 3.92 (3H, s, NCH_3), 4.01 (3H, s, OCH_3), 5.25 (2H, s, OCH_2Ar), 6.85 (1H, s), 6.94 (1H, s), 7.04 (2H, s), 7.33 (3H, m) and 7.55 (2H, m); δ_C (62.5 MHz; $CDCl_3$) 17.26 (CH_3), 31.94 (NCH_3), 58.33 (OCH_3), 51.96, 75.02 (OCH_2Ar), 101.72, 102.25, 105.07, 113.09, 113.36, 113.75, 117.09, 122.52, 127.7, 128.07, 128.28, 132.85, 136.41, 145.26 and 13.46; m/z 311 (M^+ , 40%), 364 (20), 273 (100), 230 (20) and 91 (40).



2-(5-Methoxy-1-methyl-4,7-dioxoindol-2-yl)-4-methylthiazole [165]

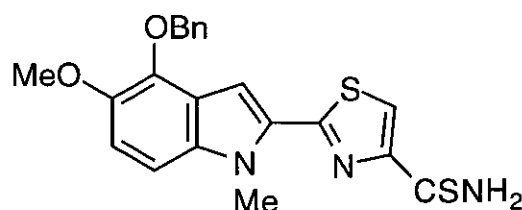
2-(4-Benzyloxy-5-methoxy-1-methylindol-2-yl)-4-methylthiazole [164] (0.58 g, 1.60 mmol), ethanol (10 ml) and palladium hydroxide (Pearlman's catalyst) (0.223 g, 0.16 mmol) were stirred under a hydrogen atmosphere at atmospheric pressure for 48 h. The reaction mixture was filtered, washed with dichloromethane (100 ml), the filtrate and washings were combined and evaporated under reduced pressure. Acetone (20 ml) and sodium dihydrogen phosphate (0.17 M, 20 ml) was added and the mixture stirred at rt for 10 min. Fremy's salt (2.14 g, 8.0 mmol) and water (20 ml) were added and the mixture stirred at rt for 16 h. The mixture was extracted with dichloromethane (3 x 30 ml). The extracts were combined, washed with brine (30 ml), dried (Na_2SO_4) and evaporated under reduced pressure. Flash column chromatography (dichloromethane : ethyl acetate elution) gave the *title compound* [165] (0.272 g, 59%) as an orange solid; (Found M^+ , 288.0573. $C_{14}H_{12}N_2O_3S$ requires M , 288.0567); ν_{\max} ($CHCl_3/cm^{-1}$) 1741, 1692, 1569, 1481, 1208 and 741; δ_H (250 MHz; $CDCl_3$) 2.04 (3H, s, CH_3), 3.85 (3H, s, OCH_3), 4.36 (3H, NCH_3), 5.75 (1H, s, H_6), 6.93 (1H, s) and

7.02 (1H, s); δ_C (62.5 MHz; $CDCl_3$) 17.16, 35.03, 56.50, 107.92, 109.91, 113.40, 114.14, 125.01, 131.24, 154.08, 156.91, 160.04, 178.82 and 179.21.



2-(4-Benzyloxy-5-methoxy-1-methyl-indol-2-yl)thiazole-4-carboxamide [169]

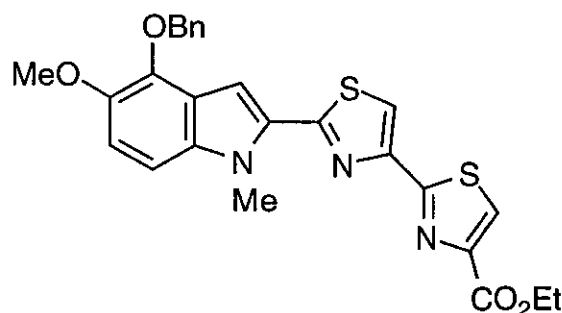
Ethyl 2-(4-benzyloxy-5-methoxy-1-methylindol-2-yl)thiazole-4-carboxylate.[162] (385 mg, 0.91 mmol), ammonium hydroxide (5 ml) and ammonium chloride (12.4 mg, 0.23 mmol) were placed in a Young's sealed tube and heated at 100°C for 48 h. The reaction mixture was cooled and dichloromethane (30 ml) was added. The mixture was washed with water (2 x 10 ml), brine (10 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (dichloromethane : ethyl acetate elution) gave the *title compound* [169] (272 mg, 79%) as a pale yellow solid; m.p. 193-194°C; (Found M^+ , 393.1144. $C_{21}H_{19}N_3O_3S$ requires M , 393.1147); ν_{max} ($CHCl_3$)/ cm^{-1} 1639, 1500, 1459, 1417, 1215 and 757; δ_C (62.5 MHz; $CDCl_3$: CD_3OD) 35.85, 62.03, 79.09, 106.84, 109.22, 117.92, 125.31, 127.91, 131.82, 132.07, 132.18, 138.78, 140.38, 141.66, 144.51, 149.21, 153.52, 163.41 and 165.29; m/z 393 (M^+ , 5%), 305 (100), 105 (30), 91 (90), 77 (35), 65 (25) and 44 (90).



2-(4-Benzyloxy-5-methoxy-1-methyl-indol-2-yl)thiazole-4-thiocarboxamide [170]

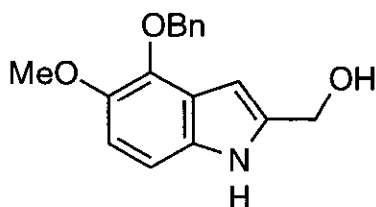
2-(4-Benzyloxy-5-methoxy-1-methyl-indol-2-yl)thiazole-4-carboxamide [169] (265 mg, 0.67 mmol), Lawesson's reagent [99] (136 mg, 0.34 mmol) and THF (8 ml) were heated under reflux for 0.5 h under a nitrogen atmosphere. The reaction mixture was cooled and evaporated under reduced pressure. Flash column chromatography (dichloromethane : methanol elution) gave the *title compound* [170] (150 mg, 55%) as a pale yellow solid; m.p. 194-195°C; (Found M^+ , 409.0864. $C_{21}H_{19}N_3O_2S_2$ requires M , 409.0919); δ_H (250 MHz; $CDCl_3$) 3.73 (3H, s, OCH_3), 3.89 (3H, s, NCH_3), 5.15 (2H, OCH_2Ph), 6.76 (1H, s), 6.86 (2H, m), 7.13 (3H, m), 7.30 (2H, m) and 8.24 (1H, s), thioamide NH_2 unobserved; δ_C (62.5 MHz; $CDCl_3$) 32.47, 58.64, 66.23, 103.69, 105.69, 109.21, 114.65, 118.93, 122.91, 127.43, 128.35,

128.57, 128.71, 131.79, 132.03, 136.96, 138.54, 145.79 and 161.21; m/z 409 (M^+ , 5%), 318 (15), 284 (60), 241 (20), 109 (10), 91 (60), 77 (20), 60 (20) and 44 (100).



Ethyl 2-(2-(4-benzyloxy-5-methoxy-1-methylindol-2-yl)thiazol-4-yl)thiazole-4-carboxylate [171]

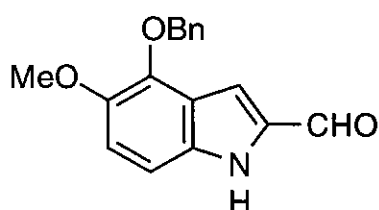
2-(4-Benzyloxy-5-methoxy-1-methyl-indol-2-yl)thiazole-4-thiocarboxamide [170] (150 mg, 0.37 mmol), bromopyruvic acid (92 mg, 0.55 mmol) and ethanol (10 ml) was heated under reflux for 1 h. The reaction mixture was cooled and dichloromethane (50 ml) was added. The mixture was washed with water (2 x 20 ml), brine (20 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give a brown oil. Ethanol (20 ml) and a few drops of concentrated sulphuric acid were added and the mixture was heated at reflux for 16 h. The reaction mixture was cooled and diethyl ether (20 ml) was added. The mixture was washed with brine (2 x 10 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give a brown oil. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [171] (110 mg, 60%) as a pale yellow solid; m.p. 132-134°C; (Found M^+ , 505.1126. $C_{26}H_{23}N_3O_4S_2$ requires M , 505.1130); ν_{max} ($CHCl_3$)/ cm^{-1} 1726, 1529, 1421, 1215 and 736; δ_H (250 MHz; $CDCl_3$) 1.45 (3H, t, J 7.2 Hz, OCH_2CH_3), 3.84 (3H, s, OCH_3), 4.10 (3H, s, NCH_3), 4.33 (2H, q, J 7.2 Hz, OCH_2CH_3), 5.26 (2H, s, OCH_2Ar), 7.0 (1H, s), 7.08 (1H, s), 7.36 (4H, m), 7.51 (2H, m), 8.12 (1H, s) and 8.20 (1H, s); δ_C (62.5 MHz; $CDCl_3$) 14.3, 32.14, 58.18, 61.53, 75.09, 100.91, 102.83, 104.79, 105.15, 114.08, 116.52, 122.54, 127.71, 127.87, 128.11, 131.43, 136.56, 138.02, 140.85, 145.34, 147.96, 148.61, 161.23 and 163.29; m/z 505 (M^+ , 5%), 292 (30), 201 (40) and 91 (100).



4-Benzyloxy-5-methoxyindole-2-methanol [173]

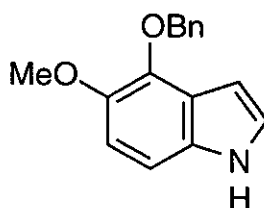
A solution of methyl-4-benzyloxy-5-methoxyindole-2-carboxylate [154] (4.92 g, 16.62 mmol) in dry THF (150 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (1.225 g, 32.1 mmol) in dry THF (60 ml), such that the mixture achieved gentle

reflux. After 30 min, water (1.2 ml), sodium hydroxide (15% solution, 1.2 ml) and water (2 ml), were added to the mixture, and the resultant precipitate removed by filtration (through a bed of Celite). The filtrate was dried (MgSO_4), then evaporated under reduced pressure to give the *title compound* [173] (4.134 g, 93%) as a colourless crystalline solid; m.p. 82-85°C; (Found M^+ , 283.1214. $\text{C}_{17}\text{H}_{17}\text{NO}_3$ requires M , 283.1208); ν_{max} (nujol)/ cm^{-1} 1467 and 1090; δ_{H} (250 MHz; CDCl_3) 3.89 (3H, s, OCH_3), 4.69 (3H, s, CH_2OH), 5.19 (2H, s, OCH_2Ar), 6.37 (1H, s), 6.91 (1H, d, J 8.71 Hz), 6.93 (1H, d, J 8.73 Hz), 7.33 (3H, m), 7.48 (1H, m) and 8.28 (1H, broad, NH), hydroxyl OH unobserved; δ_{C} (62.5 MHz; CDCl_3) 58.25 (CH_2OH), 58.41 (OCH_3), 74.98 (OCH_2Ar), 97.54, 106.33, 111.77, 123.15, 127.76, 128.02, 128.28, 128.74, 133.60, 138.14, 138.36 and 144.95; m/z 283 (M^+ , 20%), 192 (100), 174 (30), 146 (20), 91 (55).



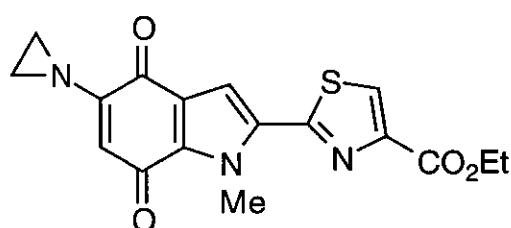
4-Benzyloxy-5-methoxyindole-2-carboxaldehyde [142]

Manganese dioxide (11.89 g, 136.7 mmol) was added to a stirred solution of 4-benzyloxy-5-methoxyindole-2-methanol [173] (3.98 g, 14.06 mmol) in dichloromethane (450 ml). The suspension was refluxed for 12 h, then the mixture was filtered, and the residue washed with dichloromethane (3 x 250 ml). The combined filtrate and washings were evaporated to give an oil, which was purified by flash column chromatography (dichloromethane elution) to give the *title compound* [142] (1.98 g, 50%) as a yellow crystalline solid; m.p. 143-144°C; (Found M^+ , 281.1023. $\text{C}_{17}\text{H}_{15}\text{NO}_3$ requires M , 281.1050); ν_{max} (nujol)/ cm^{-1} 1665, 1453 and 1377; δ_{H} (250 MHz; CDCl_3) 3.92 (3H, s, OCH_3), 5.28 (2H, s, OCH_2Ar), 7.15 (2H, m), 7.22 (3H, m), 7.34 (2H, m), 7.47 (1H, m), 8.13 (1H, s, NH) and 9.75 (1H, s, CHO); δ_{C} (62.5 MHz; CDCl_3) 58.41 (OCH_3), 75.14 (OCH_2Ar), 107.48, 112.33, 118.41, 123.39, 127.93, 128.04, 128.33, 128.74, 135.06, 136.17, 137.76, 145.20 and 183.24 (CHO); m/z 281 (M^+ , 40%), 253 (30), 190 (50), 91 (100) and 65 (15).



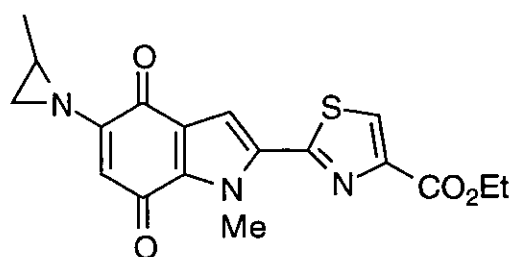
4-Benzyloxy-5-methoxyindole [174]

Bis(triphenylphosphine)carbonylrodium chloride (100.3 mg, 0.146 mmol) was suspended in dry degassed mesitylene (6 ml) and warmed to 80°C. After 10 min 1,3-bis(diphenylphosphino)propane (117 mg, 0.28 mmol) was added. After a further 10 min 4-benzyloxy-5-methoxyindole-2-carboxaldehyde [**142**] (500 mg, 1.79 mmol) was added. The resultant yellow solution was plunged into a Wood's metal bath at 190°C and refluxed for 2 h. The mixture was evaporated under reduced pressure and flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [**174**] (379 mg, 84%) as a pale yellow solid; m.p. 81-83°C; (Found M⁺, 253.1102. C₁₆H₁₅NO₂ requires M, 253.1102); δ_H (250 MHz; CDCl₃) 3.89 (3H, s, OCH₃), 5.24 (2H, s, OCH₂Ar), 6.55 (1H, s), 6.95 (1H, d, *J* 8.68 Hz), 7.04 (2H, m), 7.33 (3H, m), 7.52 (2H, m) and 8.08 (1H, broad, NH); *m/z* 253 (M⁺, 70%), 163 (40), 148 (20), 134 (40), 119 (30), 91 (100) and 65 (20).



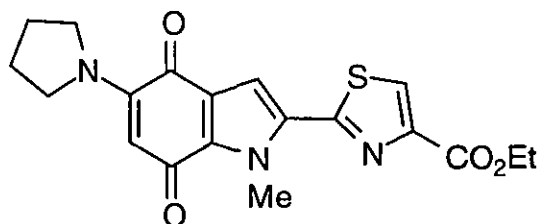
***Ethyl 2-(5-(aziridin-1-yl)-1-methyl-4,7-dioxoindol-2-yl)thiazole-4-carboxylate* [**180**]**

Ethyl 2-(5-methoxy-1-methyl-4,7-dioxoindol-2-yl)thiazole-4-carboxylate [**163**] (25.6 mg, 0.074 mmol), aziridine (0.5 ml) and methanol (3 ml) were stirred at rt for 16 h. The reaction mixture was evaporated under reduced pressure, **CAUTION**. Flash column chromatography (dichloromethane : ethyl acetate elution) followed by size exclusion chromatography (sephadex LH 20 gel filtration dichloromethane : methanol elution) gave the *title compound* [**180**] (11.2 mg, 42%) as a red solid; (Found M⁺, 357.0817. C₁₇H₁₅N₃O₄S requires M, 357.0783); ν_{max} (CHCl₃)/cm⁻¹ 1717, 1674, 1641, 1398, 1355, 1260 and 756; δ_H (250 MHz; CDCl₃) 1.42 (3H, t, *J* 7.2 Hz, OCH₂CH₃), 2.24 (4H, s, N(CH₂)₂), 4.4 (3H, s, NCH₃), 4.45 (2H, q, *J* 7.2 Hz, OCH₂CH₃), 5.9 (1H, s, H₆), 7.06 (1H, s) and 8.17 (1H, s); δ_C (100 MHz; CDCl₃) 14.34, 29.19, 35.19, 61.68, 110.83, 118.20, 125.31, 127.36, 128.41, 131.93, 141.80, 148.19, 157.39, 162.54 179.01 and 179.74; *m/z* 357 (M⁺, 10%), 267 910), 217 (10), 148 (100), 77 (60), 51 (40) and 31 (30).



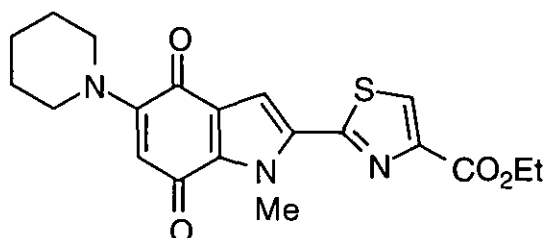
Ethyl 2-(1-methyl-5-(2-methylaziridin-1-yl)-4,7-dioxoindol-2-yl)thiazole-4-carboxylate [181]

As a red solid; (Found M^+ , 371.0905. $C_{18}H_{17}N_3O_4S$ requires M , 371.0939); ν_{max} ($CHCl_3$)/ cm^{-1} 1715, 1668, 1620, 1591, 1397, 1242 and 747; δ_H (250 MHz; $CDCl_3$) 1.35 (3H, t, J 7.3 Hz, OCH_2CH_3), 1.53 (3H, d, J 6.7 Hz, $CHCH_3$), 3.12 (2H, m, NCH_2), 3.98 (1H, m, NCH), 4.35 (3H, s, NCH_3), 4.41 (2H, q, J 7.3 Hz, OCH_2CH_3), 6.01 (1H, s, H_6), 6.97 (1H, s) and 8.09 (1H, s); m/z 371 (M^+ , 5%), 317 (15), 267 (25), 217 (20), 155 (50), 51 (100) and 31 (80).



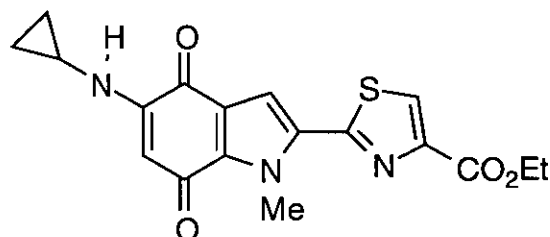
Ethyl 2-(1-methyl-5-(pyrrolidin-1-yl)-4,7-dioxoindol-2-yl)thiazole-4-carboxylate [182]

Ethyl 2-(5-methoxy-1-methyl-4,7-dioxoindol-2-yl)thiazole-4-carboxylate [163] (15 mg, 0.043 mmol), pyrrolidine (0.5 ml) and methanol (3 ml) were stirred at rt for 16 h. Water (10 ml) was added and the mixture extracted with dichloromethane (3 x 10 ml). The extracts were combined, washed with hydrochloric acid (2M, 3 x 10 ml), brine (10 ml), dried (Na_2SO_4) and evaporated under reduced pressure. Flash column chromatography (dichloromethane : ethyl acetate elution) followed by size exclusion chromatography (sephadex LH 20 gel filtration dichloromethane : methanol elution) gave the *title compound* [182] (13 mg, 81%) as a purple solid; (Found M^+ , 385.1103. $C_{19}H_{19}N_3O_4S$ requires M , 385.1096); ν_{max} ($CHCl_3$)/ cm^{-1} 1725, 1667, 1613, 1545, 1385, 1272, 1205 and 752; δ_H (250 MHz; $CDCl_3$) 1.42 (3H, t, J 7.2 Hz, OCH_2CH_3), 1.65 (4H, m, $(CH_2)_2$), 3.45 (4H, m, $N(CH_2)_2$), 4.39 (3H, s, NCH_3), 4.47 (2H, q, J 7.2 Hz, OCH_2CH_3), 5.61 (1H, s, H_6), 6.98 (1H, s) and 8.14 (1H, s); m/z 385 (M^+ , 25%), 267 (10), 217 (10), 155 (25), 113 (20), 70 (20), 51 (50) and 28 (100).



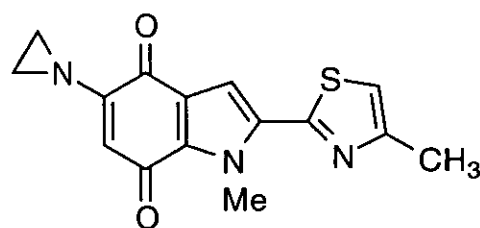
Ethyl 2-(1-methyl-5-(piperidin-1-yl)-4,7-dioxoindol-2-yl)thiazole-4-carboxylate [183]

As a purple solid; (Found M^+ , 399.1262. $C_{20}H_{21}N_3O_4S$ requires M , 399.1253); ν_{max} ($CHCl_3$)/ cm^{-1} 1724, 1668, 1628, 1548, 1391, 1244 and 757; δ_H (250 MHz; $CDCl_3$) 1.41 (3H, t, J 7.2 Hz, OCH_2CH_3), 1.61 (2H, m, CH_2), 1.65 (4H, m, $(CH_2)_2$), 2.95 (4H, m, $N(CH_2)_2$), 4.39 (3H, s, NCH_3), 4.44 (2H, q, J 7.2, OCH_2CH_3), 5.61 (1H, s, H_6), 6.99 (1H, s) and 8.1 (1H, s); δ_C (100 MHz; $CDCl_3$) 14.67, 24.65, 29.93, 35.32, 51.29, 61.71, 109.91, 111.40, 125.19, 127.30, 128.43, 131.55, 148.49, 154.28, 159.60, 161.44, 178.79 and 179.21; m/z 399 (M^+ , 100%), 176 (10), 155 (15), 103 (20), 84 (40), 51 (35), 41 (40) and 28 (50).



Ethyl 2-(5-cyclopropylamino-1-methyl-4,7-dioxoindol-2-yl)thiazole-4-carboxylate [184]

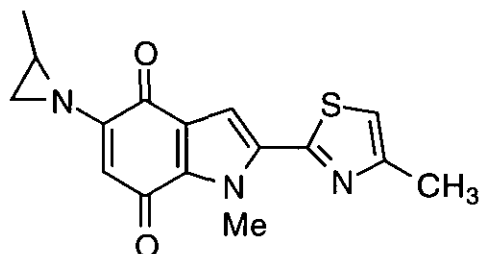
As a purple solid; (Found M^+ , 371.0938. $C_{18}H_{17}N_3O_4S$ requires M , 371.0937); ν_{max} ($CHCl_3$)/ cm^{-1} 1725, 1674, 1620, 1593, 1504, 1398, 1257 and 780; δ_H (400 MHz; $CDCl_3$) 0.81 (4H, m, $(CH_2)_2$), 1.36 (3H, t, J 7.2 Hz, OCH_2CH_3), 2.39 (1H, m, CH), 4.36 (3H, s, NCH_3), 4.39 (2H, q, J 7.2 Hz, OCH_2CH_3), 5.60 (1H, s, H_6), 5.87 (1H, br, NH), 6.94 (1H, s) and 8.09 (1H, s); δ_C (100 MHz; $CDCl_3$) 7.45, 14.68, 24.63, 35.57, 61.96, 100.90, 110.93, 122.66, 127.35, 127.61, 131.42, 148.52, 149.60, 159.45, 161.44, 178.34 and 179.42; m/z 371 (M^+ , 70%), 217 (20), 186 (20), 155 (30), 113 (25), 80 (40), 51 (100) and 31 (70).



2-(5-(Aziridin-1-yl)-1-methyl-4,7-dioxoindol-2-yl)-4-methylthiazole [186]

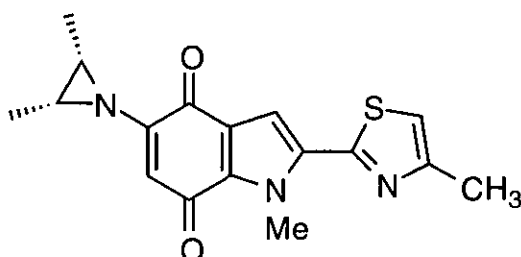
Ethyl 2-(5-methoxy-1-methyl-4,7-dioxoindol-2-yl)thiazole-4-carboxylate [165] (25.6 mg, 0.074 mmol), aziridine (0.5 ml) and methanol (3 ml) were stirred at rt for 16 h. The reaction mixture was evaporated under reduced pressure, **CAUTION**. Flash column chromatography (dichloromethane : ethyl acetate elution) followed by size exclusion chromatography (sephadex LH 20 gel filtration dichloromethane : methanol elution) gave the *title compound* [186] (11.2 mg, 42%) as a red solid; (Found M^+ , 299.0732. $C_{15}H_{13}N_3O_2S$ requires M , 299.0728); ν_{max} ($CHCl_3$)/ cm^{-1} 1676, 1640, 1587, 1480, 1351, 1203 and 782; δ_H (250 MHz; $CDCl_3$)

2.24 (4H, s, N(CH₂)₂), 2.50 (3H, s, CH₃), 4.35 (3H, s, NCH₃), 5.88 (1H, s, H₆), 6.97 (1H, s) and 6.99 (1H, s); δ_C (62.5 MHz; CDCl₃) 17.66, 28.11, 35.41, 110.31, 114.48, 118.57, 125.25, 131.49, 133.72, 154.51, 157.61, 157.86, 178.99 and 179.43; m/z 299 (M⁺, 100%), 272 (20), 244 (10), 215 (10), 176 (10), 71 (20) and 45 (15).



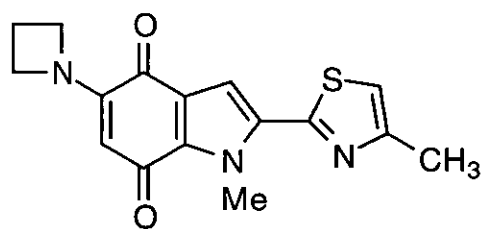
2-(1-Methyl-5-(2-methylaziridin-1-yl)-4,7-dioxindol-2-yl)-4-methylthiazole [187]

As a red solid; (Found M⁺, 313.0891. C₁₆H₁₅N₃O₂S requires M, 313.0885); ν_{\max} (CHCl₃)/cm⁻¹ 1669, 1594, 1552, 1503, 1393, 1258 and 753; δ_H (400 MHz; CDCl₃) 1.48 (3H, d, *J* 6.8 Hz, CHCH₃), 2.46 (3H, s, CH₃), 3.29 (2H, m, CH₂), 4.16 (1H, m, CH), 4.29 (3H, NCH₃), 5.81 (1H, s, H₆), 6.82 (1H, s) and 6.89 (1H, s); δ_C (100 MHz; CDCl₃) 17.93, 23.71, 36.89, 47.28, 49.32, 110.17, 114.22, 129.23, 132.99, 133.54, 135.70, 147.10, 154.48, 158.06, 178.04 and 179.31; m/z 313 (M⁺, 50%), 286 (100), 176 (15), 142 (15), 71 (70) and 45 (60).



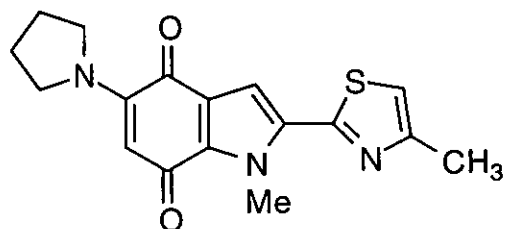
2-(5-(2,3-cis-Dimethylaziridin-1-yl)-1-methyl-4,7-dioxindol-2-yl)-4-methylthiazole [188]

As a red solid; (Found M⁺, 327.1039. C₁₇H₁₇N₃O₂S requires M, 327.1041); ν_{\max} (CHCl₃)/cm⁻¹ 1669, 1642, 1586, 1552, 1395 and 783; δ_H (400 MHz; CDCl₃) 1.32 (6H, d, *J* 6.6 Hz, (CH₃)₂), 2.15 (2H, m, (CH)₂), 3.89 (3H, s, CH₃), 4.37 (3H, s, NCH₃), 5.81 (1H, s, H₆), 6.95 (1H, s) and 8.11 (1H, s); δ_C (100 MHz; CDCl₃) 13.13, 35.49, 40.57, 52.89, 111.08, 117.07, 125.14, 127.83, 132.16, 148.20, 158.64, 159.41, 161.85, 179.02 and 179.62; m/z 327 (M⁺, > 5%), 270 (10), 70 (20), 57 (15), 41 (100) and 27 (15).



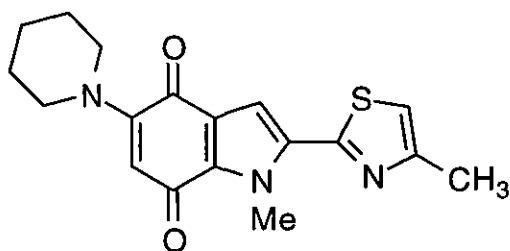
2-(5-(Azetidin-1-yl)-1-methyl-4,7-dioxindol-2-yl)-4-methylthiazole [189]

As a purple solid; (Found M^+ , 313.0887. $C_{16}H_{15}N_3O_2S$ requires M , 313.0885); ν_{max} ($CHCl_3$)/ cm^{-1} 1663, 1622, 1576, 1549, 1384, 1258 and 777; δ_H (400 MHz; $CDCl_3$) 2.11 (3H, s, CH_3), 2.86 (6H, m), 4.73 (3H, s, NCH_3), 5.72 (1H, s, H_6), 7.24 (1H, s) and 7.63 (1H, s); δ_C (62.5 MHz; $CDCl_3$) 17.27, 17.76, 29.71, 34.87, 109.36, 113.49, 122.75, 128.30, 131.93, 133.60, 148.78, 153.91, 157.94, 177.44 and 179.40; m/z 313 (M^+ , 100%), 284 (30), 257 (20), 229 (20), 176 (20), 71 (45), 53 (20), 45 (35) and 28 (30).



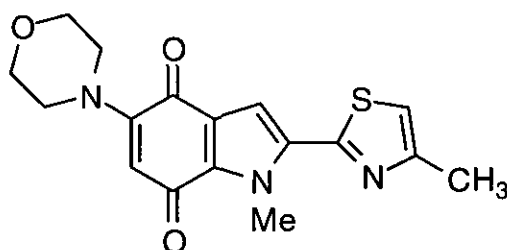
2-(1-Methyl-5-(pyrrolidin-1-yl)-4,7-dioxindol-2-yl)-4-methylthiazole [190]

Ethyl 2-(5-methoxy-1-methyl-4,7-dioxindol-2-yl)thiazole-4-carboxylate [165] (10 mg, 0.052 mmol), pyrrolidine (0.5 ml) and methanol (3 ml) were stirred at rt for 16 h. Water (10 ml) was added and the mixture extracted with dichloromethane (3 x 10 ml). The extracts were combined, washed with hydrochloric acid (2M, 3 x 10 ml), brine (10 ml), dried (Na_2SO_4) and evaporated under reduced pressure. Flash column chromatography (dichloromethane : ethyl acetate elution) followed by size exclusion chromatography (sephadex LH 20 gel filtration dichloromethane : methanol elution) gave the *title compound* [190] (7.1 mg, 63%) as a purple solid; (Found M^+ , 327.1048. $C_{17}H_{17}N_3O_2S$ requires M , 327.1042); ν_{max} ($CHCl_3$)/ cm^{-1} 1663, 1606, 1541, 1383, 1274 and 773; δ_C (100 MHz; $CDCl_3$) 17.61, 35.52, 51.52, 53.76, 103.40, 110.16, 113.77, 123.79, 132.28, 133.19, 149.32, 154.24, 158.39, 178.18 and 180.16; m/z 327 (M^+ , 100%), 284 (15), 229 (10), 155 (20), 113 (15), 70 (30), 51 (40) and 28 (90).



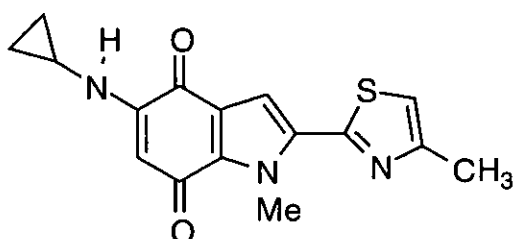
2-(1-Methyl-5-(piperidin-1-yl)-4,7-dioxindol-2-yl)-4-methylthiazole [191]

As a purple solid; (Found M^+ , 341.1204. $C_{18}H_{19}N_3O_2S$ requires M , 341.1198); ν_{max} ($CHCl_3$)/ cm^{-1} 1663, 1632, 1547, 1385, 1244 and 782; δ_H (250 MHz; $CDCl_3$) 1.69 (6H, m), 2.49 (3H, s, CH_3), 3.44 (4H, m, $N(CH_2)_2$), 4.35 (3H, s, NCH_3), 5.59 (1H, s, H_6), 6.88 (1H, s) and 6.92 (1H, s); δ_C (100 MHz; $CDCl_3$) 17.61, 24.68, 26.20, 35.16, 51.27, 110.0, 110.42, 113.98, 125.27, 132.18, 132.82, 154.32, 154.34, 158.21, 178.97 and 180.29; m/z 341 (M^+ , 100%), 284 (10), 257 (10), 229 (10), 205 (10), 84 (30), 55 (10), 41 (25) and 28 (30).



2-(1-Methyl-5-(morpholin-1-yl)-4,7-dioxindol-2-yl)-4-methylthiazole [192]

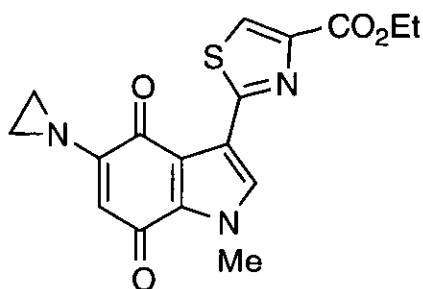
As a purple solid; ν_{max} ($CHCl_3$)/ cm^{-1} 1664, 1629, 1547, 1386 and 778; δ_H (250 MHz; $CDCl_3$) 2.51 (3H, s, CH_3), 3.46 (4H, m, $N(CH_2)_2$), 3.85 (4H, m, $O(CH_2)_2$), 4.35 (3H, s, NCH_3), 5.59 (1H, s, H_6), 6.91 (1H, s) and 6.94 (1H, s).



2-(5-Cyclopropylamino-1-methyl-4,7-dioxindol-2-yl)-4-methylthiazole [193]

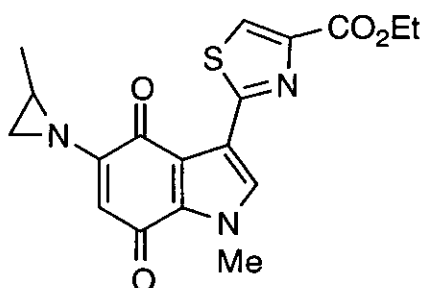
As a purple solid; (Found M^+ , 313.0892. $C_{16}H_{15}N_3O_2S$ requires M , 313.0885); ν_{max} ($CHCl_3$)/ cm^{-1} 1664, 1585, 1551, 1496, 1394, 1215 and 753; δ_H (400 MHz; $CDCl_3$) 0.54 (2H, m), 0.77 (2H, m), 2.37 (1H, m, CH), 2.38 (3H, s, CH_3), 4.30 (3H, s, NCH_3), 5.56 (1H, s, H_6), 5.85 (1H, br, NH), 6.81 (1H, s) and 6.86 (1H, s); δ_C (100 MHz; $CDCl_3$) 6.55,

16.72, 23.72, 34.52, 99.82, 109.06, 113.13, 121.80, 131.79, 132.85, 148.65, 153.46, 157.20, 177.61 and 180.31; m/z 313 (M^+ , 100%), 296 (30), 284 (35), 71 (25) and 51 (50).



Ethyl 2-(5-(aziridin-1-yl)-1-methyl-4,7-dioxindol-3-yl)thiazole-4-carboxylate [194]

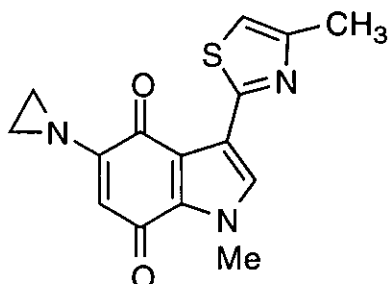
Ethyl 2-(5-methoxy-1-methyl-4,7-dioxindol-3-yl)thiazole-4-carboxylate [146] (15.2 mg, 0.044 mmol), aziridine (0.5 ml) and methanol (3 ml) were stirred at rt for 16 h. The reaction mixture was evaporated under reduced pressure, **CAUTION**. Flash column chromatography (dichloromethane : ethyl acetate elution) followed by size exclusion chromatography (sephadex LH 20 gel filtration dichloromethane : methanol elution) gave the *title compound* [194] (14.3 mg, 95%) as a red solid; (Found M^+ , 357.0783. $C_{17}H_{15}N_3O_4S$ requires M , 357.0783); ν_{max} ($CHCl_3$)/ cm^{-1} 1711, 1664, 1632, 1592, 1400, 1215 and 755; δ_H (250 MHz; $CDCl_3$) 1.35 (3H, t, J 7.2 Hz, OCH_2CH_3), 2.21 (4H, s, $N(CH_2)_2$), 3.95 (3H, s, NCH_3), 4.34 (2H, q, J 7.2 Hz, OCH_2CH_3), 5.82 (1H, s, H_6), 7.75 (1H, s) and 8.09 (1H, s); δ_C (100 MHz; $CDCl_3$) 14.39, 27.86, 36.79, 61.39, 106.79, 117.89, 120.12, 127.62, 130.30, 131.20, 146.66, 157.75, 160.01, 161.60, 178.75 and 179.06; m/z 357 (M^+ , 55%), 284 (25), 274 (30), 217 (20), 155 (15), 113 (20), 84 (30), 57 (25), 51 (60), 42 (100) and 29 (60).



Ethyl 2-(1-methyl-5-(2-methylaziridin-1-yl)-4,7-dioxindol-3-yl)thiazole-4-carboxylate [195]

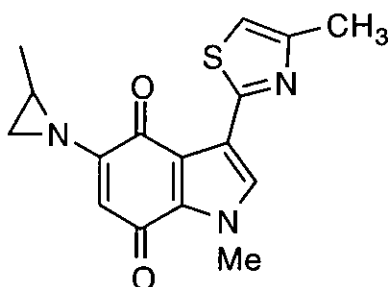
As a red solid; (Found M^+ , 371.0938. $C_{18}H_{17}N_3O_4S$ requires M , 371.0939); ν_{max} ($CHCl_3$)/ cm^{-1} 1731, 1659, 1632, 1590, 1396, 1210 and 753; δ_H (400 MHz; $CDCl_3$) 1.36 (3H, t, J 7.2 Hz, OCH_2CH_3), 1.39 (3H, d, J 6.8 Hz, $CHCH_3$), 2.07 (2H, m, $N(CH_2)_2$), 2.28 (1H, m, CH), 4.33 (3H, s, NCH_3), 4.39 (2H, q, J 7.2 Hz, OCH_2CH_3), 5.89 (1H, s,

*H*6), 7.74 (1H, s) and 8.11 (1H, s); δ_C (100 MHz; $CDCl_3$) 14.78, 28.29, 30.10, 37.25, 57.18, 61.81, 107.14, 117.20, 119.73, 120.40, 128.08, 130.32, 131.4, 146.90, 160.30, 161.93, 179.03 and 179.5; m/z 371 (M^+ , 5%), 229 (15), 172(50), 127 (60), 101 (65), 58 (100) and 44 (55).



2-(5-(Aziridin-1-yl)-1-methyl-4,7-dioxindol-3-yl)-4-methylthiazole [197]

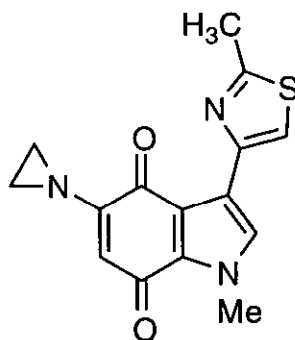
2-(5-Methoxy-1-methyl-4,7-dioxindol-2-yl)-4-methylthiazole [196] (10.2 mg, 0.034 mmol), aziridine (0.5 ml) and methanol (3 ml) were stirred at rt for 16 h. The reaction mixture was evaporated under reduced pressure, **CAUTION**. Flash column chromatography (dichloromethane : ethyl acetate elution) followed by size exclusion chromatography (sephadex LH 20 gel filtration dichloromethane : methanol elution) gave the *title compound* [197] (9.3 mg, 92%) as a red solid; (Found M^+ , 299.0727. $C_{15}H_{13}N_3O_2S$ requires M , 299.0728); δ_H (250 MHz; $CDCl_3$) 2.25 (4H, s, $N(CH_2)_2$), 2.47 (3H, s, CH_3), 4.01 (3H, s, NCH_3), 5.88 (1H, s, *H*6), 6.93 (1H, s) and 7.62 (1H, s); δ_C (100 MHz; $CDCl_3$) 17.08, 27.84, 36.73, 114.54, 116.72, 119.02, 119.95, 129.96, 130.19, 152.52, 157.88, 158.58, 178.76 and 176.86; m/z 299 (M^+ , 100%), 288 (30), 272 (25), 259 (10), 244 (10), 230 (10), 215 (10), 203 (10), 174 (10), 161 (5), 91 (20) and 42 (30).



2-(1-Methyl-5-(2-methylaziridin-1-yl)-4,7-dioxindol-3-yl)-4-methylthiazole [198]

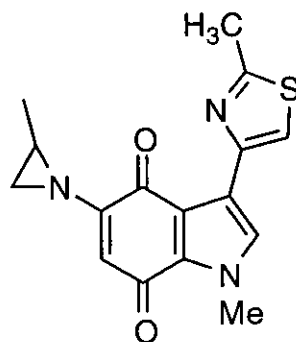
As a red solid; (Found M^+ , 313.0885. $C_{16}H_{15}N_3O_2S$ requires M , 313.0885); ν_{max} ($CHCl_3$)/ cm^{-1} 1661, 1634, 1589, 1500, 1397, 1216 and 753; δ_H (400 MHz; $CDCl_3$) 1.38 (3H, d, J 5.5 Hz, $CHCH_3$), 2.08 (2H, m, $N(CH_2)$), 2.23 (1H, m, CH), 2.33 (3H, s, CH_3), 3.96 (3H, s, NCH_3), 5.70 (1H, s, *H*6), 6.83 (1H, s) and 7.52 (1H, s); δ_C (100 MHz;

CDCl₃) 15.97, 18.09, 35.12, 36.61, 37.12, 106.97, 114.89, 116.39, 119.20, 130.17, 130.59, 152.80, 158.21, 160.75, 179.13 and 179.27; *m/z* 313 (M⁺, 25%), 267 (30), 217 (30), 155 (60), 144 (70), 51 (90) and 31 (100).



4-(5-(Aziridin-1-yl)-1-methyl-4,7-dioxindol-2-yl)-2-methylthiazole [200]

4-(5-Methoxy-1-methyl-4,7-dioxindol-3-yl)-2-methylthiazole [199] (17.2 mg, 0.059 mmol), aziridine (0.5 ml) and methanol (3 ml) were stirred at rt for 16 h. The reaction mixture was evaporated under reduced pressure, **CAUTION**. Flash column chromatography (dichloromethane : ethyl acetate elution) followed by size exclusion chromatography (sephadex LH 20 gel filtration dichloromethane : methanol elution) gave the *title compound* [200] (14 mg, 87%) as a red solid; ν_{\max} (CHCl₃)/cm⁻¹ 1636, 1588, 1490, 1389, 1215 and 755; δ_{H} (250 MHz; CDCl₃) 2.24 (4H, s, N(CH₂)₂), 2.72 (3H, s, CH₃), 4.0 (3H, s, NCH₃), 5.85 (1H, s, H₆), 7.52 (1H, s) and 8.61 (1H, s); δ_{C} (100 MHz; CDCl₃) 19.45, 28.17, 37.03, 106.76, 117.01, 117.40, 120.52, 130.78, 147.13, 158.63, 161.17, 165.11, 179.13 and 179.37.

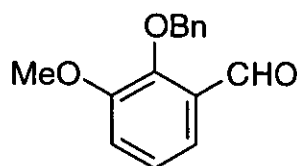


4-(1-Methyl-5-(2-methylaziridin-1-yl)-4,7-dioxindol-2-yl)-2-methylthiazole [201]

As a red solid; (Found M⁺, 313.0889. C₁₆H₁₅N₃O₂S requires M, 313.0884); ν_{\max} (CHCl₃)/cm⁻¹ 1658, 1633, 1589, 1490, 1387, 1172 and 752; δ_{H} (400 MHz; CDCl₃) 1.38 (3H, d, *J* 5.7 Hz, CHCH₃), 2.05 (2H, m, N(CH₂)), 2.25 (1H, m, N(CH)), 2.60 (3H, s, CH₃), 3.93 (3H, s, NCH₃), 5.80 (1H, s, H₆), 7.37 (1H, s) and 8.60 (1H, s); δ_{C} (100 MHz;

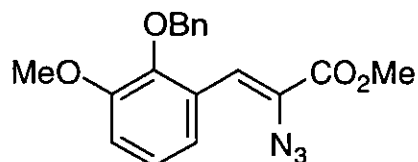
CDCl₃) 18.11, 19.51, 34.50, 35.06, 36.68, 116.34, 117.37, 120.43, 120.51, 130.63, 130.96, 147.35, 158.68, 165.14, 179.25 and 179.49; *m/z* 313 (M⁺, > 5%), 249 (10), 125 (20), 98 (10), 84 (50), 70 (25), 56 (30), 41 (100) and 27 (35).

7.5. Chapter 4 experimental



2-Benzyloxy-3-methoxybenzaldehyde [207]

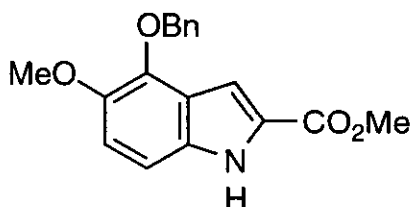
Potassium hydroxide (16 g, 286 mmol) was added to stirred solution of *ortho*-vanillin [206] (40 g, 264 mmol) in ethanol (240 ml). The solution was stirred for 5 min then benzyl chloride (36.04 g 286 mmol) added. The mixture was heated under reflux for 14 h. Water (200 ml) was added and the mixture extracted with diethyl ether (3 x 300 ml), the extracts were combined, washed with water (2 x 100 ml), potassium hydroxide (5 x 200 ml, of a 2M solution), water (2 x 200 ml), brine (200 ml), dried (MgSO₄) and evaporated under reduced pressure. Trituration (hexane) gave the *title compound* [207] (47 g, 74%) as an off white solid; m.p. 44-46°C; (Found M⁺, 242.0950. C₁₅H₁₄O₃ requires M, 242.0943); ν_{\max} (nujol)/cm⁻¹ 1694, 1480, 1367 and 1062; δ_{H} (250 MHz; CDCl₃) 3.95 (3H, s, OCH₃), 5.17 (2H, s, OCH₂Ar), 7.13 (2H, m), 7.35 (6H, m) and 10.23 (1H, s, CHO); δ_{C} (62.5 MHz; CDCl₃) 56.05 (OCH₃), 76.27 (OCH₂Ar), 118.01, 119.02, 124.17, 128.44, 128.52, 128.58, 130.28, 136.35, 151.29, 153.01 and 190.03; m/z 242 (M⁺, 7%), 213 (5), 150 (10), 91 (100) and 65 (10).



Methyl 2-azido-3-(2-benzyloxy-3-methoxyphenyl)propenoate [208]

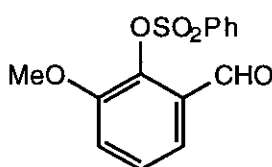
Sodium metal (6.07 g, 261 mmol) was added to methanol (144 ml), under a nitrogen atmosphere. The mixture was cooled to -15°C then a solution of methyl azidoacetate [205] (30.4 g, 201 mmol), 2-benzyloxy-3-methoxybenzaldehyde [207] (15.98 g, 65.52 mmol) in methanol (12 ml) was added dropwise. The mixture was stirred at -15°C for 3 h then at 4°C for 14 h. Water (2 x 300 ml) was cautiously added and the mixture was extracted with ethyl acetate (500 ml). The extracts were washed with water (2 x 300 ml) and the aqueous layers combined and extracted with ethyl acetate (200 ml). The extracts were combined, washed with brine (300 ml), dried (MgSO₄) and evaporated under reduced pressure. The crude yellow solid was recrystallised (diethyl ether) to give (16.2 g) and the mother liquors were purified by flash column chromatography (light petroleum : diethyl ether elution) to give (1.4 g), total yield of the *title compound* [208] (17.6 g, 82%), as a yellow solid; m.p. 69-71°C; ν_{\max} (nujol)/cm⁻¹ 2114, 1709 and 1452; δ_{H} (250 MHz; CDCl₃) 3.89 (3H, s, CO₂CH₃), 3.93 (3H, s, OCH₃),

5.07 (2H, s, OCH₂Ar), 6.93 (1H, d, *J* 8.19 Hz), 7.09 (1H, t, *J* 8.12 Hz), 7.30 (1H, s), 7.35 (5H, m) and 7.79 (1H, d, *J* 7.82); δ_C (62.5 MHz; CDCl₃); 52.78 (CO₂CH₃), 55.83 (OCH₃), 75.68 (OCH₂Ar), 113.45, 119.58, 121.99, 122.03, 123.93, 125.53, 127.81, 128.14, 128.75, 137.03, 146.69, 152.62 and 162.03; *m/z* 311 (M⁺ -N₂, 10%), 220 (20), 189 (10), 91 (100) and 65 (15).



Methyl 4-benzyloxy-5-methoxyindole-2-carboxylate [154]

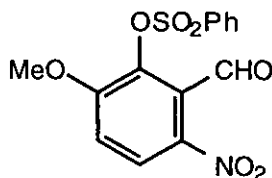
A solution of methyl-2-azido-3-(2-benzyloxy-3-methoxyphenyl)propenoate [208] (5 g, 15.4 mmol) in xylene (160 ml) was added dropwise to refluxing xylene (500 ml). After the addition was complete the mixture was refluxed for 1 h. The mixture was evaporated under reduced pressure to give an orange solid. Recrystallisation (diethyl ether) gave (1.54 g). Flash column chromatography of the mother liquors (light petroleum : diethyl ether elution) gave (1.56 g). Total yield of the *title compound* [154] (3.1 g, 66% yield) as a pale yellow solid; m.p. 117-120°C (Found M⁺, 311.1157. C₁₈H₁₇NO₄ requires M, 311.1157); ν_{\max} (nujol)/cm⁻¹ 1697, 1453 and 1258; δ_H (250 MHz; CDCl₃) 3.87 (3H, s, OCH₃), 3.91 (3H, s, CO₂CH₃), 5.25 (2H, s, OCH₂Ar), 7.02 (1H, s), 7.35 (6H, m), 7.48 (2H, m) and 9.25 (1H, s, NH); δ_C (62.5 MHz; CDCl₃) 51.96 (CO₂CH₃), 58.47 (OCH₃), 75.03 (OCH₂Ar), 106.16, 107.13, 116.36, 123.15, 127.31, 127.85, 128.01, 128.33, 128.74, 134.16, 137.90, 145.14 and 162.45; *m/z* 311 (M⁺, 40%), 220 (100), 188 (90), 91 (85) and 63 (20).



2-Benzenesulfonyloxy-3-methoxybenzaldehyde [224]

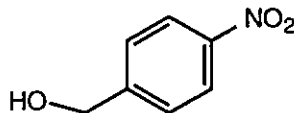
Benzenesulfonyl chloride (80 g, 450 mmol), *ortho*-vanillin [206] (68 g, 450 mmol) and sodium hydroxide (15% solution, 200 ml) were shaken at rt for 1 h. The resultant yellow solid was collected by filtration and washed with water (200 ml), saturated sodium hydrogen carbonate (2 x 200 ml), water (200 ml). Recrystallisation (methanol) gave the *title compound* [224] (95.4 g, 72%) as a pale yellow solid; m.p. 122-123°C; (Found: C, 57.51; H, 4.0. C₁₄H₁₂O₅S requires C, 57.53; H, 4.14%); δ_H (250 MHz; CDCl₃) 3.53 (3H, s, OCH₃), 7.09 (1H, d, *J* 8.15 Hz), 7.29 (1H, t, *J* 7.9 Hz), 7.52 (3H, m), 7.70 (1H, d, *J* 7.5 Hz), 7.98 (1H,

d, J 7.5 Hz) and 10.15 (1H, s, CHO); m/z 230 (M^+ , 30%), 151 (100), 108 (32), 93 (30), 65 (30), 52 (33) and 41 (20).



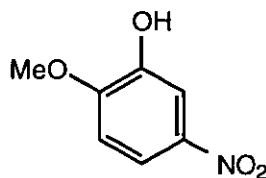
2-Benzenesulfonyloxy-3-methoxy-6-nitrobenzaldehyde [225]

2-Benzenesulfonyloxy-3-methoxybenzaldehyde [224] (52.6 g, 180 mmol) was added portion wise to fuming nitric acid (526 ml) maintaining the temperature between 5-15°C. The mixture was stirred for a further 10 min, then poured onto ice (approx. 3 kg). The precipitate was collected by filtration and recrystallised (methanol) to give the *title compound* [225] (20 g, 33%) as a pale yellow solid; m.p. 143-144°C; (Found M^+ 337.0237. $C_{14}H_{11}NO_7S$ requires M , 337.0259); δ_H (250 MHz; $CDCl_3$) 3.53 (3H, s, OCH_3), 7.09 (1H, d, J 6.7 Hz), 7.34 (1H, dd, J 8.3 7.1 Hz), 7.52 (1H, dd, J 7.1 1.6 Hz), 7.56 (1H, d, J 6.7 Hz), 7.69 (1H, m), 7.90 (2H, dd, J 8.3 1.6 Hz) and 10.16 (1H, s, CHO); δ_C (62.5 MHz; $CDCl_3$) 55.78, 117.84, 119.41, 127.92, 128.50, 128.97, 132.46, 134.39, 135.27, 140.92, 152.41 and 187.21; m/z 337 (M^+ , 10%), 294 (10), 179 (10), 141 (40), 123 (25), 77 (100) and 51 (40).



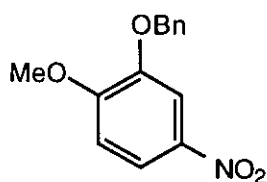
4-Nitrobenzylalcohol [226]

2-Benzyloxy-3-methoxybenzaldehyde [207] (5 g, 20.6 mmol) was added to fuming nitric acid (50 ml) maintaining the temperature between 5-15°C. The reaction mixture was stirred at 10°C for 10 min then ice water (150 ml) was added. The mixture was extracted with diethyl ether (3 x 100 ml) and the extracts were combined washed with sodium hydrogen carbonate solution (2 x 100 ml), brine (2 x 100 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [226] (1.92 g, 60%) as a yellow solid; m.p. 94-96°C (Lit., 92-94°C); δ_H (250 MHz; $CDCl_3$) 5.53 (2H, s, OCH_2Ph), 7.57 (2H, d, J 6.8 Hz) and 8.26 (2H, d, J 6.8 Hz); δ_C (62.5 Hz; $CDCl_3$) 72.75, 123.58, 129.19, 134.68 and 139.54.



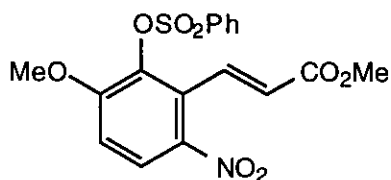
2-Methoxy-5-nitrophenol [229]

2-Benzenesulfonyloxy-3-methoxy-6-nitrobenzaldehyde [225] (1.86 g, 5.23 mmol), ethanol (50 ml) and potassium hydroxide (15% solution, 30 ml) were heated under reflux for 30 min. The reaction mixture was cooled and then made acidic by the addition of hydrochloric acid (2M). The mixture was extracted with diethyl ether (3 x 50 ml) the extracts were combined, washed with brine (2 x 50 ml), dried (MgSO₄) and evaporated under reduced pressure to give the *title compound* [229] (0.992 g, 96%) as a pale brown solid; m.p. 107-108°C (lit., 104-106°C); δ_{H} (250 MHz; CDCl₃) 4.02 (3H, s, OCH₃), 5.53 (1H, br, OH), 6.92 (1H, d, *J* 8.9 Hz), 7.77 (1H, d, *J* 2.7 Hz) and 7.85 (1H, dd, *J* 8.7 2.7 Hz); δ_{C} (62.5 MHz; CDCl₃) 56.44, 109.51, 110.0, 116.86, 117.97, 145.43 and 151.84.



3-Benzyloxy-4-methoxynitrobenzene [230]

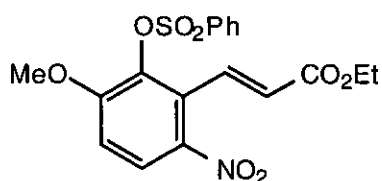
2-Methoxy-5-nitrophenol [229] (0.823 g, 4.2 mmol), ethanol (20 ml) and potassium hydroxide (0.25 g, 4.52 mmol) were stirred at rt for 10 min. Benzyl chloride (0.773 g, 4.52 mmol) was added and the mixture stirred at reflux for 16 h. The reaction mixture was cooled and water (50 ml) was added. The mixture was extracted with diethyl ether (3 x 50 ml), the extracts were combined, washed with potassium hydroxide (2M, 4 x 50 ml), brine (2 x 50 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (diethyl ether elution) gave the *title compound* [230] (1.15 g, 96%) as a pale yellow solid; m.p. 65-67°C; (Found M⁺, 259.0830. C₁₄H₁₃NO₄ requires M, 259.0844); δ_{H} (250 MHz; CDCl₃) 3.97 (3H, s, OCH₃), 5.20 (2H, s, CH₂Ar), 6.94 (1H, d, *J* 8.9 Hz), 7.41 (5H, m), 7.80 (1H, d, *J* 2.6 Hz) and 7.93 (1H, dd, *J* 8.9 2.6 Hz); δ_{C} (62.5 MHz; CDCl₃) 56.37, 71.15, 108.49, 110.09, 118.04, 125.06, 127.49, 128.30, 128.68, 136.06, 142.71 and 155.29; *m/z* 259 (M⁺, 5%), 91 (100) and 65 (10).



(*E*)-Methyl 3-(2-benzenesulfonyloxy-3-methoxy-6-nitrophenyl)prop-2-enoate [231]

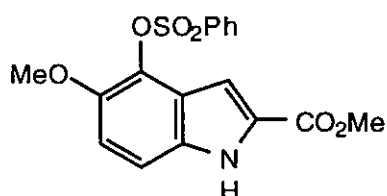
Sodium hydride (42.7 mg, 1.78 mmol) was added to dry THF (5 ml) under a nitrogen atmosphere and the mixture was left to stir for 5 min. Methyl diethyl phosphonoacetate (374 mg, 1.78 mmol) was added dropwise and the mixture was stirred for 1 h. A solution of 2-benzenesulfonyloxy-3-methoxy-6-nitrobenzaldehyde [225] (300 mg, 0.89 mmol) in THF (15

ml) was added dropwise and the mixture left to stir at rt for 2 h. Ammonium chloride solution (10 ml) was added and the mixture extracted with ethyl acetate (3 x 20 ml), the extracts were combined and washed with brine (4 x 20 ml), dried (MgSO₄) and evaporated under reduced pressure to give a brown solid. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [231] (297 mg, 85 %) as an off white solid; δ_{H} (250 MHz; CDCl₃) 3.71 (3H, s, OCH₃), 3.86 (3H, s, CO₂CH₃), 6.0 (1H, d, *J* 16.1 Hz), 7.02 (1H, d, *J* 9.3 Hz), 7.46 (1H, d, *J* 16.2 Hz), 7.53 (1H, d, *J* 7.9 Hz), 7.58 (1H, m), 7.68 (1H, m), 7.88 (1H, d, *J* 7.3 Hz) and 8.10 (1H, d, *J* 9.3 Hz); δ_{C} (62.5 MHz; CDCl₃) 55.53, 60.27, 115.70, 129.27, 129.35, 131.79, 132.01, 132.15, 132.93, 133.11, 138.20, 139.79, 144.82, 161.22 and 169.79.



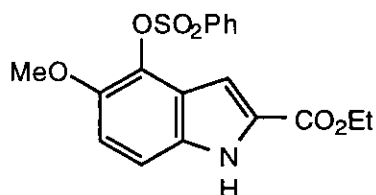
(E)-Ethyl 3-(2-benzenesulfonyloxy-3-methoxy-6-nitrophenyl)prop-2-enoate
[232]

Sodium hydride (43.6 mg, 1.80 mmol) was added to dry THF (5 ml) under a nitrogen atmosphere and the mixture was left to stir for 5 min. Triethyl phosphonoacetate (407 mg, 1.8 mmol) was added dropwise, the mixture was stirred for 1 h. A solution of 2-benzenesulfonyloxy-3-methoxy-6-nitrobenzaldehyde [225] (200 mg, 0.59 mmol) in THF (10 ml) was added dropwise and the mixture left to stir at rt for 2 h. Ammonium chloride solution (10 ml) was added and the mixture extracted with ethyl acetate (3 x 20 ml), the extracts were combined and washed with brine (4 x 20 ml), dried (MgSO₄) and evaporated under reduced pressure to give a brown solid. Recrystallisation (diethyl ether) gave the *title compound* [232] (297 mg, 85%) as a colourless solid; m.p. 104-106°C; (Found M⁺, 407.6678. C₁₈H₁₇NO₈S requires M, 407.6675); δ_{H} (250 MHz; CDCl₃) 1.20 (3H, t, *J* 7.3 Hz, OCH₂CH₃), 3.91 (3H, s, OCH₃), 4.18 (2H, q, *J* 7.7 Hz, OCH₂CH₃), 5.98 (1H, d, *J* 16.3 Hz), 7.0 (1H, d, *J* 9.3 Hz), 7.42 (1H, d, *J* 16.3 Hz), 7.55 (2H, m), 7.58 (1H, m), 7.86 (1H, m) and 8.11 (1H, d, *J* 9.3 Hz); *m/z* 407 (M⁺, 5%), 361 (25), 250 (20), 177 (30), 141 (30), 77 (100), 51 (30) and 29 (80).



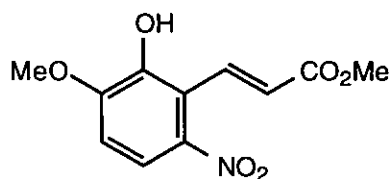
Methyl 4-benzenesulfonyloxy-5-methoxyindole-2-carboxylate [233]

Methyl 3-(2-benzenesulfonyloxy-3-methoxy-6-nitrophenyl)propenoate [231] (185 mg, 0.47 mmol) and triethyl phosphite (383 mg, 2.3 mmol, 0.4 ml) were heated in an oil bath at 170°C for 3 h. The reaction mixture was cooled and evaporated under reduced pressure to give a viscous brown oil. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [233] (112 mg, 66%) as a pale brown solid; m.p. 169-170°C; (Found: C, 56.4; H, 3.92; N, 3.88. C₁₇H₁₅NO₆S requires C, 56.50; H, 4.18; 3.88%); δ_H (250 MHz; CDCl₃) 3.55 (3H, s, OCH₃), 3.91 (3H, s, CO₂CH₃), 6.97 (1H, d, *J* 8.9 Hz), 7.02 (1H, d, *J* 9.5 Hz), 7.31 (1H, d, *J* 7.9 Hz), 7.50 (2H, m), 7.68 (1H, m), 7.95 (1H, d, *J* 7.3 Hz) and 10.34 (1H, s, NH); δ_C (62.5 MHz; CDCl₃) 51.84, 56.94, 105.53, 111.52, 113.41, 128.36, 128.64, 129.13, 129.60, 129.87, 129.93, 133.84, 136.44, 145.61 and 163.05; *m/z* 361 (M⁺, 10%), 220 (100), 188 (75), 132 (75), 132 (150, 77 (40) and 51 (30); (Found M⁺, 361.0608. C₁₇H₁₅NO₆S requires M, 361.0620).



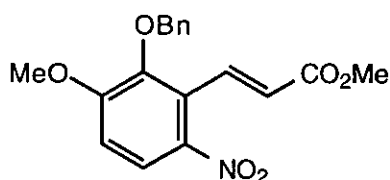
***Ethyl 4-benzenesulfonyloxy-5-methoxyindole-2-carboxylate* [234]**

Ethyl 3-(2-benzenesulfonyloxy-3-methoxy-6-nitrophenyl)propenoate [232] (96.6 mg, 0.24 mmol) and triethyl phosphite (196 mg, 1.18 mmol, 0.2 ml) were heated in an oil bath at 170°C for 3 h. The reaction mixture was cooled and evaporated under reduced pressure to give a viscous brown oil. Flash column chromatography (light petroleum : diethylether elution) gave the *title compound* [234] (69.4 mg, 79%) as a pale brown solid; m.p. 127-129°C; (Found C, 57.28; H,4.48; N, 3.72. C₁₈H₁₇NO₈S requires C, 57.59; H, 4.56; N, 3.73%); δ_H (250 MHz; CDCl₃) 1.32 (3H, t, *J* 7.2 Hz, OCH₂CH₃), 3.44 (3H, s, OCH₃), 4.29 (2H, q, *J* 7.2 Hz, OCH₂CH₃), 6.89 (1H, d, *J* 8.3 Hz), 7.18 (1H, dd, *J* 8.7 1.2 Hz), 7.43 (2H, m), 7.55 (1H, m), 7.86 (2H, m) and 9.25 (1H, br, NH); δ_C (62.5 MHz; CDCl₃) 14.26, 57.02, 61.19, 105.65, 111.30, 113.50, 121.40, 123.60, 128.53, 128.68, 129.0, 133.26, 133.82, 136.72, 145.67 and 161.80; *m/z* 375 (M⁺, 10%), 234 (100), 188 (85), 132 (20), 77 (50), 64 (27) and 51 (30); (Found M⁺, 375.0770. C₁₈H₁₇NO₆S requires M, 375.0776).



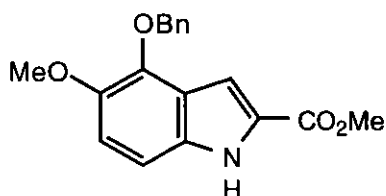
***(E)-Methyl 3-(2-hydroxy-3-methoxy-6-nitrophenyl)prop-2-enoate* [235]**

Sodium hydride (0.255 g, 10.6 mmol) was added to dry THF (10 ml) under a nitrogen atmosphere and the mixture was left to stir for 5 min. Methyl diethyl phosphonoacetate (2.24 g, 10.6 mmol) was added dropwise and the mixture was stirred for 1 h, then added to a solution of 2-benzenesulfonyloxy-3-methoxy-6-nitrobenzaldehyde [225] (3.27 g, 9.7 mmol) in THF (30 ml) and the mixture left to stir at rt for 2 h. Sodium hydride (0.255 g, 10.6 mmol) was added and the mixture left to stir for a further 2 h. Ammonium chloride solution (30 ml) was added and the mixture extracted with ethyl acetate (3 x 50 ml), the extracts were combined and washed with brine (4 x 50 ml), dried (MgSO₄) and evaporated under reduced pressure to give a yellow solid. Recrystallisation (ethanol) gave the *title compound* [235] (1.42 g, 58 %) as a pale yellow solid; m.p. 123-125°C; ν_{max} (CHCl₃)/cm⁻¹ 3400(br) and 1721; δ_{H} (250 MHz; CDCl₃) 3.75 (3H, s, CO₂CH₃), 3.96 (3H, s, OCH₃), 6.38 (1H, s, OH), 6.66 (1H, d, *J* 16.2 Hz), 6.81 (1H, d, *J* 8.9 Hz), 7.55 (1H, d, *J* 8.9 Hz) and 7.73 (1H, d, *J* 16.2 Hz); δ_{C} (62.5 MHz; CD₃OD : CDCl₃) 51.39, 55.96, 109.14, 116.04, 116.70, 124.02, 124.86, 135.96, 142.78, 151.26 and 167.82; *m/z* 253 (M⁺, 10%), 221 (20), 207 (100), 176 (30), 77 (40), 51 (55) and 29 (40).



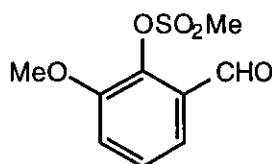
(E)-Methyl 3-(2-benzyloxy-3-methoxy-6-nitrophenyl)prop-2-enoate [236]

Methyl 3-(2-hydroxy-3-methoxy-6-nitrophenyl)prop-2-enoate [235] (5.06 g, 20.01 mmol), potassium carbonate (3.04 g, 22.01 mmol), benzyl bromide (3.48 g, 20.01 mmol) and THF (100ml) were heated under reflux for 14 h. The reaction mixture was cooled and ammonium chloride solution (100 ml) added. The mixture was extracted with diethyl ether (3 x 100 ml), the extracts were combined and washed with saturated sodium hydrogen carbonate (3 x 100 ml), brine (2 x 100 ml), dried (MgSO₄) and evaporated under reduced pressure to give a brown oil. Trituration (light petroleum) gave an orange solid which was recrystallised (diethyl ether) to give the *title compound* [236] (4.66 g, 68 %) as a pale yellow solid; m.p. 115-116°C; (Found: C, 63.1; H, 4.93; N, 4.02. C₁₈H₁₇NO₆ requires C, 63.0; H, 5.0; N, 4.1%); ν_{max} (CHCl₃)/cm⁻¹ 1701; δ_{H} (250 MHz; CDCl₃) 3.79 (3H, s, CO₂CH₃), 3.99 (3H, s, OCH₃), 4.94 (2H, s, OCH₂), 6.34 (1H, d, *J* 16.1 Hz), 6.97 (1H, d, *J* 9.0 Hz), 7.34 (5H, m), 7.71 (1H, d, *J* 16.2 Hz) and 7.88 (1H, d, *J* 9.2 Hz); δ_{C} (62.5 MHz; CDCl₃) 51.73, 56.31, 73.82, 110.90, 121.54, 121.90, 125.76, 126.44, 128.19, 128.30, 128.38, 128.52, 136.02, 141.86, 157.34 and 166.45; *m/z* 343 (M⁺, 3%), 266 (5), 91 (100) and 65 (10).



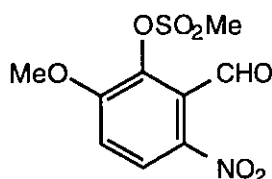
Methyl 4-benzyloxy-5-methoxyindole-2-carboxylate [154]

Methyl 3-(2-benzyloxy-3-methoxy-6-nitrophenyl)propenoate [236] (4.4 g, 12.8 mmol) and triethyl phosphite (10.68 g, 64.1 mmol, 11.15 ml) were heated in an oil bath at 170°C for 3 h. The reaction mixture was cooled and evaporated under reduced pressure to give a viscous brown oil. Flash column chromatography (light petroleum : diethyl ether elution), gave the *title compound* [154] (2.2g, 54%) as a pale yellow solid, with analytical data identical to that previously prepared.



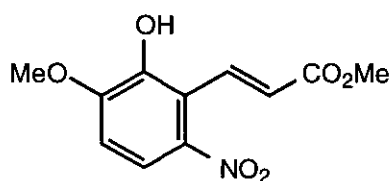
2-Methanesulfonyloxy-3-methoxybenzaldehyde [237]

Methanesulfonyl chloride (17.9 g, 156 mmol, 12.1 ml) was added to a solution of *ortho*-vanillin [206] (20 g, 131.4 mmol) in dichloromethane (300 ml) at 0°C. The mixture was left to stir for 5 min then triethylamine (33.4 g, 330 mmol, 46 ml) was added dropwise and the temperature maintained between 0-5°C. The mixture was stirred for a further 30 min at rt and the precipitate was removed by filtration. The filtrate was washed with water (200 ml), hydrochloric acid (3 x 200 ml, of a 1M solution), saturated sodium hydrogen carbonate (2 x 200 ml), brine (200 ml), dried (MgSO₄) and evaporated under reduced pressure to give the *title compound* [237] (28.65 g, 95 %) as an off white solid; m.p. 79-80°C; (Found: C, 47.16; H, 4.26. C₉H₁₀O₅S requires C, 46.95; H, 4.38%); ν_{\max} (CHCl₃)/cm⁻¹ 1701; δ_{H} (250 MHz; CDCl₃) 3.36 (3H, s, SO₂CH₃), 3.95 (3H, s, OCH₃), 7.28 (1H, dd, *J* 9.2, 1.7 Hz), 7.37 (1H, t, *J* 9.3 Hz), 7.51 (1H, dd, *J* 9.2, 1.6 Hz) and 10.35 (1H, s, CHO); δ_{C} (62.5 MHz; CDCl₃) 39.29, 56.37, 116.29, 120.09, 127.99, 131.07, 141.09, 152.02 and 188.34; *m/z* 230 (M⁺, 30%), 151 (100), 108 (32), 93 (30), 65 (30), 52 (33) and 41 (20).



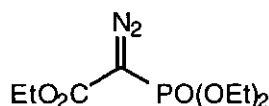
2-Methanesulfonyloxy-3-methoxy-6-nitrobenzaldehyde [238]

2-Methanesulfonyloxy-3-methoxybenzaldehyde [237] (25 g, 107.5 mmol) was added portion wise to fuming nitric acid (675 ml) maintaining the temperature between 5-15°C. The mixture was stirred for a further 10 min, then poured onto ice (approx. 2 kg) and the precipitate was collected by filtration and recrystallised (methanol) to give the *title compound* [238] (14.82 g, 50 %) as a pale yellow solid; m.p. 143-144°C; (Found: C, 39.36; H, 3.02; N, 5.02. C₉H₉NO₇S requires C, 39.28; H, 3.3; N, 5.09%); ν_{\max} (CHCl₃)/cm⁻¹ 3020, 1719, 1582, 1525, 1378, 1289, 1168 and 928; δ_{H} (250 MHz; CDCl₃) 3.34 (3H, s, SO₂CH₃), 4.06 (3H, s, OCH₃), 7.21 (1H, d, *J* 9.2 Hz), 8.2 (1H, d, *J* 8.1 Hz) and 10.28 (1H, s, CHO); δ_{C} (62.9 MHz; CDCl₃) 44.05, 62.11, 119.54, 130.10, 136.18, 140.93, 141.34, 163.28 and 192.04; *m/z* 275 (M⁺, 10%), 196 (20), 123 (100), 79 (70), 51 (80) and 30 (50).



***(E)-Methyl 3-(2-hydroxy-3-methoxy-6-nitrophenyl)prop-2-enoate* [235]**

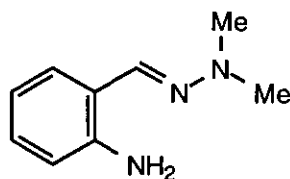
Sodium hydride (0.645 g, 27.27 mmol) was added to dry THF (40 ml) under a nitrogen atmosphere and the mixture was left to stir for 5 min. Methyl diethyl phosphonoacetate (5.73 g, 27.27 mmol) was added dropwise, the mixture was stirred for 1 h then added to a solution of 2-methanesulfonyloxy-3-methoxy-6-nitrobenzaldehyde [238] (5 g, 18.18 mmol) in THF (70 ml), and the mixture was left to stir at rt for 2 h. Sodium hydride (0.654 g, 27.27 mmol) was added and the mixture left to stir for a further 2 h. Ammonium chloride (50 ml) was added and the mixture extracted with ethyl acetate (3 x 100 ml), the extracts were combined and washed with brine (4 x 100 ml), dried (MgSO₄) and evaporated under reduced pressure to give a yellow solid. Recrystallisation (ethanol) gave the *title compound* [235] (3.34 g, 73%) as a pale yellow solid, with analytical data identical to that previously prepared.



***Triethyl diazophosphonoacetate* [240]**

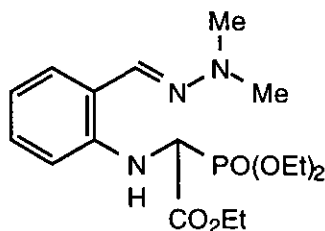
Triethylphosphonoacetate [242] (2.45 g, 10.95 mmol), caesium carbonate (3.59 g, 10.95 mmol) and THF (50 ml) were stirred under a nitrogen atmosphere for 30 min. A solution of *para*-toluenesulfonyl azide (2.16 g, 10.9 mmol) in THF (20 ml) was added and the mixture stirred under a nitrogen atmosphere for 16 h. The mixture was filtered through a plug of silica (diethyl ether elution) to give the *title compound* [240] (2.22 g, 81%) as a colourless oil; ν_{\max} (film)/cm⁻¹ 2986, 2128, 1689, 1437 and 745; δ_{H} (250 MHz; CDCl₃) 1.23 (3H, t, *J*

7Hz, CO₂CH₂CH₃), 1.29 (6H, t, *J* 6.8 Hz, P(OCH₂CH₃)₂), 4.15 (4H, dq, *J* 6.8 1.2 Hz, P(OCH₂CH₃)₂) and 4.18 (2H, q, *J* 7 Hz, CO₂CH₂CH₃).



2-Aminophenyl-*N,N*-dimethylhydrazone [243]

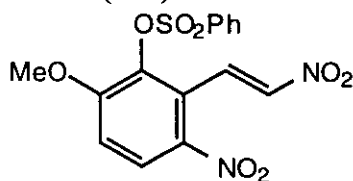
ortho-Nitrobenzaldehyde [221] (1 g, 6.6 mmol), *N,N*-dimethylhydrazine (4.16 g, 69.3 mmol) and methanol (20 ml) were stirred under reflux for 1 h. Decolourising charcoal (0.165 g) and iron(III)chloride (0.022 g, 0.088 mmol) were added and the mixture was left to stir at reflux for 16 h. The reaction mixture was cooled, filtered over a bed of celite and evaporated under reduced pressure. Flash column chromatography (light petroleum : dichloromethane elution) gave the *title compound* [243] (0.57 g, 50%) as a pale yellow oil; (Found M⁺, 163.1101. C₉H₁₃N₃ requires M, 163.1109); $\nu_{\max}(\text{film})\text{cm}^{-1}$ 3456, 3313, 1610, 1593, 1469, 1442, 1391, 1028 and 748; δ_{H} (250 MHz; CDCl₃) 2.92 (6H, s, N(CH₃)₂), 5.81 (2H, br, NH₂), 6.69 (1H, d, *J* 8.3 Hz), 6.71 (1H, d, *J* 8.3 Hz), 7.04 (2H, m) and 7.48 (1H, s); *m/z* 163 (M⁺, 100%), 133 (20), 118 (75), 104 (20), 91 (30), 65 (20), 58 (25) and 43 (55).



Triethyl (2-aminophenyl-*N,N*-dimethylhydrazone)-phosphonoacetate [244]

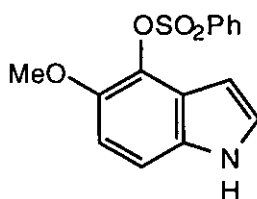
2-Aminophenyl-*N,N*-dimethylhydrazone [243] (1 g, 5.7 mmol), triethyl diazophosphonoacetate [240] (0.715 g, 2.85 mmol), toluene (20 ml) and rhodium(II)acetate (0.025 g, 0.057 mmol, 2 mol%) were stirred a reflux for 16 h. The reaction mixture was cooled and evaporated under reduced pressure. Flash column chromatography (dichloromethane : diethyl ether elution) gave the *title compound* [244] (0.67 g, 63%) as a brown oil; (Found M⁺, 385.1772. C₁₇H₂₈N₃O₅P requires M, 385.1766); $\nu_{\max}(\text{film})\text{cm}^{-1}$ 2985, 2937, 1709, 1368, 1282, 1022 and 749; δ_{H} (250 MHz; CDCl₃) 1.26 (3H, t, *J* 7.2 Hz, CO₂CH₂CH₃), 1.31 (6H, t, *J* 8.2 Hz, P(OCH₂CH₃)₂), 2.97 (6H, s, N(CH₃)₂), 4.09 (4H, m, P(OCH₂CH₃)₂), 4.17 (2H, q, *J* 7.2 Hz, CO₂CH₂CH₃), 6.49 (1H, d, *J* 8.2 Hz), 6.68 (1H, t, *J* 8.2 Hz), 7.03 (1H, m), 7.09 (1H, d, 8.2 Hz) and 7.51 (1H, s); δ_{C} (62.5 MHz; CDCl₃) 14.06 (CO₂CH₂CH₃), 16.38 (d, *J* 5.7 Hz, P(OCH₂CH₃)₂), 42.85 (N(CH₃)₂), 55.80 (d, *J* 144 Hz, P(OCH₂CH₃)₂), 61.91 (CO₂CH₂CH₃), 63.54 (NHC), 110.49, 116.77,

119.61, 128.20, 130.96, 138.38 and 168.93; m/z 385 (M^+ , 30%), 326 (30), 203 (40), 174 (30), 144 (45), 131 (50), 59 (75) and 28 (100).



(E)-2-Benzenesulfonyloxy-3-methoxy-6-nitrophenyl-nitro-2-ethene [249]

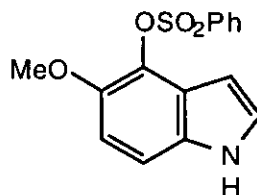
2-Benzenesulfonyloxy-3-methoxy-6-nitrobenzaldehyde [225] (2 g, 5.9 mmol), nitromethane (5 ml, 5.63 g, 92 mmol), ammonium acetate (5 g, 64.8 mmol) and glacial acetic acid (15 ml) were heated under reflux for 2h. The reaction mixture was cooled and water (40 ml) was added, the mixture was then neutralized by the addition of sodium hydrogen carbonate, **CAUTION**. The mixture was extracted with ethyl acetate (3 x 50 ml). The extracts were combined and washed with brine (2 x 30 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give a dark brown solid. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [249] (1.06 g, 47%) as a pale orange solid; m.p. 145-146°C; (Found: C, 47.24; H, 2.83; N, 7.18. $C_{15}H_{12}N_2O_2S$ requires C, 47.37; 3.18; N, 7.37%); δ_H (250 MHz; $CDCl_3$) 3.89 (3H, s, OCH_3), 7.12 (1H, d, J 9.40 Hz), 7.59 (1H, d, J 8.2 Hz), 7.72 (1H, d, J 8.2 Hz), 7.86 (5H, m) and 8.23 (1H, 9.3 Hz); δ_C (62.5 MHz; $CDCl_3$) 56.76, 112.60, 123.65, 125.72, 127.76, 128.34, 129.49, 129.80, 134.81, 136.72, 140.21, 141.60 and 157.74; m/z 380 (M^+ , 5%), 163 (10), 141 (50), 77 (100), 51 (22) and 30 (14); (Found M^+ , 380.0309. $C_{15}H_{12}N_2O_2S$ requires M, 380.0314).



4-Benzenesulfonyloxy-5-methoxyindole [250]

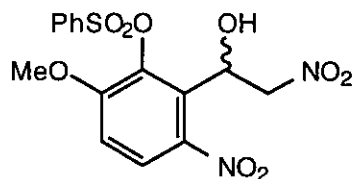
Iron powder (4.34 g, 77.7 mmol) was added to a mixture of (E)-2-benzenesulfonyloxy-3-methoxy-6-nitrophenyl-nitro-2-ethene [249] (2.17 g, 5.72 mmol), ethanol (50 ml) and glacial acetic acid (50 ml) over a 15 min period. The mixture was then heated under reflux for 1 h. Water (100 ml) was added and the mixture was extracted with diethyl ether (3 x 100 ml), the extracts were combined and washed with a saturated solution of sodium hydrogen carbonate (5 x 50 ml), brine (100 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give a brown solid. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [250] (348 mg, 20%) as a pale yellow solid; m.p. 136°C; (Found: C, 59.79; H, 4.33; N, 4.55. $C_{15}H_{13}NO_3S$ requires C, 59.4; H, 4.32; N, 4.62%); δ_H (250 MHz; $CDCl_3$) 3.43 (3H, s, OCH_3), 6.15 (1H, d, J 3.3 Hz), 6.69 (1H, d, J 8.8 Hz), 7.01 (1H, d, J

3.2 Hz), 7.14 (2H, m), 7.42 (2H, m), 7.81 (2H, m) and 8.15 (1H, br, NH); δ_C (62.5 MHz; CDCl₃) 56.94, 98.79, 109.15, 110.29, 116.03, 123.92, 126.24, 128.25, 128.57, 132.40, 133.68, 137.08 and 145.12; m/z 303 (M⁺, 20%), 162 (100), 134 (30), 77 (50), 44 (100), 28 (50) and 20 (50); (Found M⁺, 303.0528. C₁₅H₁₃NO₃S requires M, 303.0565).



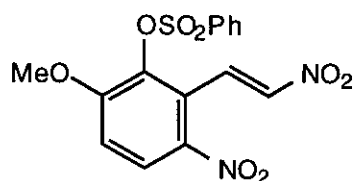
4-Benzenesulfonyloxy-5-methoxyindole [250]

(E)-(2-Benzenesulfonyloxy-3-methoxy-6-nitrophenyl)-nitro-2-ethene [249] (0.178 g, 0.47 mmol), silica gel (4.70 g), iron powder (0.39 mg, 7 mmol), glacial acetic acid (3 ml) and toluene (5 ml) were heated at reflux under a nitrogen atmosphere for 1 h. The reaction mixture was cooled and water (10 ml) added, the mixture was extracted with diethyl ether (3 x 20 ml), the extracts were combined and washed with a saturated solution of sodium hydrogen carbonate (5 x 15 ml), brine (15 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [250] (0.13 g, 92%) as a pale yellow solid, with identical data to that previously prepared.



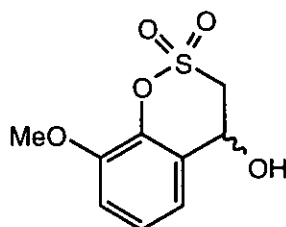
(2-Benzenesulfonyloxy-3-methoxy-6-nitrophenyl)-1-hydroxy-2-nitroethane [251]

2-Benzenesulfonyloxy-3-methoxy-6-nitrobenzaldehyde [225] (3 g, 8.9 mmol), Potassium fluoride (0.240 g, 2.54 mmol) and propan-2-ol (12 ml) were stirred at rt for 48 h. Water (50 ml) was added and the mixture extracted with dichloromethane (3 x 70 ml), the extracts were combined and washed with brine (50 ml), dried (MgSO₄) and evaporated under reduced pressure to give a pale yellow glass. Recrystallisation (methanol) gave the *title compound* [251] (2.52 g, 71%) as a pale yellow solid; m.p. 131-133°C; ν_{\max} (CHCl₃)/cm⁻¹ 1606, 1582, 1558, 1533, 1380, 1214 and 759; δ_H (250 MHz; CDCl₃) 3.47 (3H, s, OCH₃), 4.78 (1H, dd, *J* 14.3 2.5 Hz), 5.23 (1H, dd, *J* 14.4 9.6 Hz), 6.03 (1H, m), 6.98 (1H, d., *J* 9.1 Hz), 7.63 (3H, m), 7.76 (2H, m) and 8.04 (1H, d, 9.1 Hz), hydroxyl OH unobserved.



***(E)*-2-benzenesulfonyloxy-3-methoxy-6-nitrophenyl-nitroethene [249]**

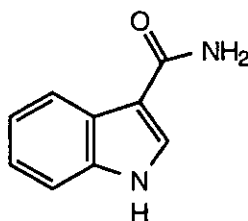
Methanesulfonyl chloride (0.68 g, 5.97 mmol) was added dropwise to a solution of (2-benzenesulfonyloxy-3-methoxy-6-nitrophenyl)-1-hydroxy-2-nitroethane [251] (2 g, 5.02 mmol) in dichloromethane (40 ml) at 0°C. The mixture was stirred at 0°C for 5 min then triethylamine (1.52 g, 15.02 mmol) was added dropwise and the mixture left to stir for 30 min. Water (40 ml) was added and the mixture was extracted with dichloromethane (3 x 50 ml), the extracts were combined and washed with brine (50 ml), dried (MgSO₄) and evaporated under reduced pressure to give a brown solid. Recrystallisation (ethanol) gave the *title compound* [249] (1.12 g, 59%) as a pale orange solid, with identical data to that previously prepared.



***Don't know what it is called* [252]**

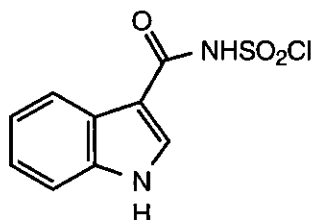
ortho-Vanillin [206] (5 g, 32.8 mmol), methanesulfonyl chloride (4.5 g, 39.8 mmol) and a sodium hydroxide (15% solution, 15 ml) were shaken at rt for 1 h. The reaction mixture was filtered and the solid was washed with sodium hydroxide (2M, 50 ml), water (50 ml) then recrystallised (methanol) to give the *title compound* [252] (2.59 g, 34%) as a colourless solid; m.p. 147°C; (Found M⁺, 230.0235. C₉H₁₀O₅S requires M, 230.0249); ν_{max}(CHCl₃)/cm⁻¹ 3416 (br), 1585, 1481, 1377, 1216 and 771; δ_H (250 MHz; CDCl₃) 3.46 (1H, dd, *J* 8.6 5.0 Hz), 3.72 (1H, m), 3.89 (3H, s, OCH₃), 5.30 (1H, dt, *J* 5.1 1.2 Hz), 6.95 (1H, dd, 8.1 1.6 Hz), 7.13 (1H, dd, *J* 8.1 1.6 Hz) and 7.27 (1H, dd, 8.2 8.0 Hz), hydroxyl OH unobserved; δ_C (62.5 MHz; CDCl₃) 51.79, 55.96, 65.37, 112.44, 119.40, 125.59, 126.17, 138.27 and 148.41; *m/z* 230 (M⁺, 50%), 151 (50), 137 (100), 77 (30), 51 (40) and 31 (35).

7.6. Chapter 5 experimental



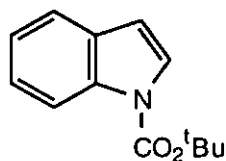
Indole-3-carboxamide [95]

A solution of CSI (1.2 g, 8.5 mmol) in acetonitrile (2 ml) was added dropwise to a solution of indole [93] (1 g, 8.5 mmol), and acetonitrile (15 ml) over a 10 min period at 0°C. The mixture was warmed to rt and stirred for 1 h. A solution of acetone (8 ml) and water (2 ml) was added and the mixture was rendered alkaline by the addition of potassium hydroxide (10% solution, approx. 10 ml). The mixture was extracted with ethyl acetate (3 x 20 ml) and the extracts were combined and washed with brine (20 ml), dried (MgSO₄) and evaporated under reduced pressure. Recrystallisation (water) gave the *title compound* [95] (0.815 g, 61%) as a colourless solid; m.p. 191-193°C; (Found M⁺, 160.0631. C₉H₈N₂O requires M, 160.0630); ν_{\max} (CHCl₃)/cm⁻¹ 3378, 1660, 1608, 1531, 1454, 1215, 1119 and 747; δ_{H} (250 MHz; CDCl₃ : CD₃OD) 7.23 (2H, m), 7.49 (1H, m), 7.84 (1H, s, H₂) and 7.97 (1H, m), indole NH and amide NH₂ unobserved; δ_{C} (62.5 MHz; CD₃OD) 107.21, 115.26, 124.19, 124.55, 125.93, 129.31, 132.55, 141.91 and 173.91; m/z 160 (M⁺, 80%) 144 (100), 116 (20) and 89 (20).



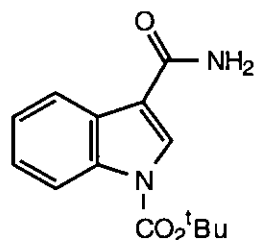
Indole-3-chlorosulfonylanide [94]

CSI (2.75 g, 19.4 mmol) was added dropwise to a solution of indole [93] (0.57 g, 4.9 mmol) in diethyl ether (30 ml) at 0°C. The mixture was stirred at 0°C for 30 min then at rt for 1 h. The resultant precipitate was filtered to give the *title compound* [94] (0.994 g, 78%) as a cream solid; m.p. 155-157°C; (Found M⁺, 259.9821. C₉H₇³⁷CIN₂O₃S requires M, 259.9836; 257.9831. C₉H₇³⁵CIN₂O₃S requires M, 257.9864); ν_{\max} (nujol)/cm⁻¹ 1678, 1459, 1422 and 1377; δ_{H} (250 MHz; CD₃OD) 7.21 (2H, m), 7.49 (1H, m), 8.14 (1H, m) and 8.23 (1H, m), indole NH and sulfonyl amide NH unobserved; δ_{C} (62.5 MHz; CD₃OD) 111.55, 112.92, 120.95, 121.49, 122.01, 122.75, 126.42, 130.32 and 171.61; m/z 260 (M⁺, 5%) 196 (20), 142 (100), 91 (30), 80 (40) and 36 (80).



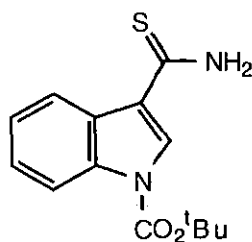
tert-Butyl indole-1-carboxylate [282]

Indole [93] (5 g, 42.5 mmol), di-*tert*-butyl dicarbonate (11.15 g, 51 mmol), DMAP (515 mg, 4.25 mmol) and acetonitrile (115 ml) were stirred at rt for 1 h. Saturated sodium hydrogen carbonate solution (50 ml) was added and the mixture stirred for a further 30 min. The mixture was extracted with diethyl ether (2 x 50 ml) the extracts were combined washed with brine (2 x 50 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [282] (8.89 g, 96%) as a colourless oil; (Found M⁺, 217.1094. C₁₃H₁₅NO₂ requires M, 217.1103); ν_{\max} (film)/cm⁻¹ 1735 and 1225; δ_{H} (250 MHz; CDCl₃) 1.69 (9H, s, (CH₃)₃) 6.57 (1H, m), 7.23 (2H, m), 7.60 (2H, m) and 8.13 (1H, m); δ_{C} (62.5 MHz; CDCl₃) 28.16 ((CH₃)₃), 84.72 (C(CH₃)₃), 107.29, 115.19, 120.95, 122.65, 124.20, 125.86, 130.85, 135.12 and 150.21; *m/z* 217 (M⁺, 10%), 161 (20), 117 (60), 57 (100), 41 (40) and 29 (28).



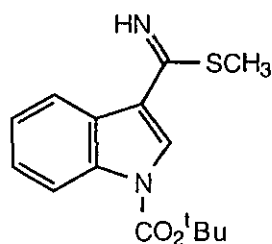
tert-Butyl 3-carboxamideindole-1-carboxylate [100]

CSI (7 g, 40.8 mmol) was added dropwise to a solution of *tert*-Butyl indole-1-carboxylate [282] (8.89 g, 40.8 mmol), and acetonitrile (50 ml) over a 10 min period at 0°C. The mixture was warmed to rt and stirred for 1 h. A solution of acetone (40 ml) and water (5 ml) was added and the solution was rendered alkaline by the addition of potassium hydroxide (10% solution, approx. 100 ml). The mixture was extracted with ethyl acetate (3 x 50 ml) and the extracts were combined and washed with brine (50 ml), dried (MgSO₄) and evaporated under reduced pressure. Recrystallisation (dichloromethane) gave the *title compound* [100] (6.85 g, 64%) as a colourless solid; m.p. 163-164°C; (Found M⁺, 260.1180. C₁₄H₁₆N₂O₃ requires M, 260.1161); ν_{\max} (CHCl₃)/cm⁻¹ 1740, 1666, 1450, 1215, 1153 and 768; δ_{H} (250 MHz; CDCl₃) 1.69 (9H, s, (CH₃)₃), 5.80 (2H, br, NH₂), 7.36 (2H, m), 8.07 (1H, d, *J* 8.2 Hz), 8.13 (1H, m) and 8.21 (1H, d, *J* 8.2 Hz); δ_{C} (62.5 MHz; CDCl₃) 31.61 ((CH₃)₃), 88.90 (C(CH₃)₃), 118.86, 125.14, 127.30, 127.50, 128.84, 131.49, 132.62, 139.09, 153.71 and 171.81; *m/z* 260 (M⁺, 10%), 204 (20), 160 (30), 144 (30), 57 (100) and 41 (30).



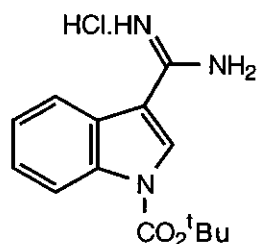
tert-Butyl 3-thiocarboxamideindole-1-carboxylate [101]

tert-Butyl 3-carboxamideindole-1-carboxylate [100] (2 g, 7.69 mmol), Lawesson's reagent [99] (1.55 g, 3.84 mmol) and benzene (20 ml) were heated at reflux for 1 h. The reaction mixture was cooled and evaporated under reduced pressure to give a yellow solid. Flash column chromatography (dichloromethane : diethyl ether elution) gave the *title compound [101]* (1.88 g, 90%) as a yellow solid; m.p. 98-99°C; (Found M^+ , 276.0922. $C_{14}H_{16}N_2O_2S$ requires M , 276.0932); ν_{max} ($CHCl_3$)/ cm^{-1} 1742, 1598; 1372, 1215 and 755; δ_H (250 MHz; $CDCl_3$) 1.68 (9H, s, $(CH_3)_3$), 7.34 (2H, m), 7.86 (2H, br, NH_2), 8.03 (1H, d, J 8.2 Hz), 8.19 (1H, d, J 8.2 Hz) and 8.25 (1H, s); δ_C (62.5 MHz; $CDCl_3$) 28.02, 85.27, 115.59, 120.41, 121.06, 124.0, 125.25, 125.98, 128.54, 129.70, 135.83 and 159.92; m/z 276 (M^+ , 5%), 142 (20), 84 (65), 57 (30), 49 (100), 41 (20) and 31 (20).



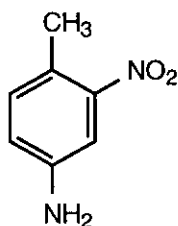
tert-Butyl 3-thiocarboximidateindole-1-carboxylate [283]

tert-Butyl 3-thiocarboxamideindole-1-carboxylate [101] (1.2 g, 4.34 mmol), methyl iodide (1.86 g, 13.2 mmol) and dichloromethane (10 ml) were stirred at rt for 48 h under a nitrogen atmosphere. Dichloromethane (30 ml) was added and the mixture was washed with a saturated solution of sodium hydrogen carbonate (2 x 20 ml), brine (2 x 20 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give the *title compound [283]* (0.93 g, 74% crude) as yellow solid which was used in the next step without further purification. **CARE** the compound is extremely **SMELLY**, the reaction must be carried out in an efficient fume cupboard; m.p. 207-208°C; (Found M^+ , 290.1087. $C_{15}H_{18}N_2O_2S$ requires M , 290.1056); ν_{max} ($CHCl_3$)/ cm^{-1} 1741, 1653, 1451, 1215 and 757; δ_H (250 MHz; $CDCl_3$) 1.69 (9H, s, $(CH_3)_3$), 2.46 (3H, s, SCH_3), 3.50 (1H, br, NH), 7.34 (2H, m), 8.07 (1H, s) and 8.17 (2H, m); δ_C (62.5 MHz; $CDCl_3$) 12.37 (SCH_3), 28.28 ($(CH_3)_3$), 84.88 ($C(CH_3)_3$), 112.51, 115.31, 120.0, 121.85, 123.89, 125.39, 127.41, 135.81, 149.38 and 166.97; m/z 290 (M^+ , 5%), 142 (100), 115 (40), 57 (60) and 27 (20).



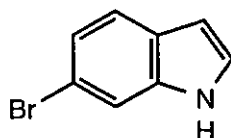
tert-Butyl 3-carbamidinylindole-1-carboxylate hydrochloride [284]

tert-Butyl 3-thiocarboximidateindole-1-carboxylate [283] (1.13 g, 3.9 mmol), ammonium chloride (2.17 g, 4.12 mmol) and methanol (8 ml) were heated under reflux for 3 h. **CARE**, during the reaction methyl mercaptam is liberated and so a potassium permanganate bubbler was attached to the reflux condenser. The reaction mixture was cooled and ethyl acetate (100 ml) was added. The mixture was washed with brine (2 x 30 ml), dried (MgSO₄) and evaporated under reduced pressure to give a pale brown solid. Recrystallisation (ethanol) gave the *title compound [284]* (0.714 g, 62%) as a pale brown solid; m.p. 207-208°C; δ_{H} (250 MHz; CDCl₃) 1.61 (9H, s, C(CH₃)₃), 7.26 (2H, m), 7.47 (1H, m), 8.03 (1H, m), 8.39 (2H, br, NH₂), 8.78 (1H, s) and 9.31 (1H, br, NH).



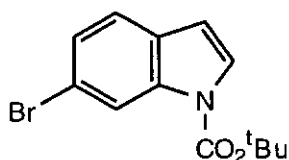
4-Bromo-2-nitrotoluene [286]

4-Amino-2-nitrotoluene [285] (25 g, 164 mmol) was added to water (208 ml) and the mixture was heated to reflux. Hydrobromic acid (48%, 83 ml) was added and the mixture was stirred at reflux for 20 min. The mixture was cooled to 0°C and a solution of sodium nitrate (11.29 g, 163.5 mmol) in water (57 ml) was added with rapid stirring, such that the temperature did not exceed 5°C. The solution was stirred at 0°C for a further 15 min then it was added dropwise to a solution of copper(I)bromide (27.03 g, 188.4 mmol) in hydrobromic acid (48%, 55.2 ml) and water (145 ml). The resultant thick suspension was stirred at rt for 30 min then at reflux for 30 min. The mixture was then left to stand at rt for 16 h. The mixture was steam distilled to give the *title compound [286]* (31.1 g, 88%) as a pale yellow solid; m.p. 44-45°C; (Found M⁺, 214.9589. C₇H₆NO₂Br requires M, 214.9563); ν_{max} (CHCl₃)/cm⁻¹ 1529; δ_{H} (250 MHz; CDCl₃) 2.56 (3H, s, CH₃), 7.25 (1H, d, *J* 8.2 Hz), 7.63 (1H, dd, *J* 8.2 2.1 Hz) and 8.11 (1H, d, *J* 8.2 Hz); δ_{C} (62.5 MHz; CDCl₃) 19.2, 119.53, 127.37, 132.45, 134.04, 135.86 and 159.92; *m/z* 217 (MH⁺, 10%) 215 (10), 198 (40), 90 (30), 78 (30), 63 (60), 39 (30) and 30 (20).



6-Bromoindole [287]

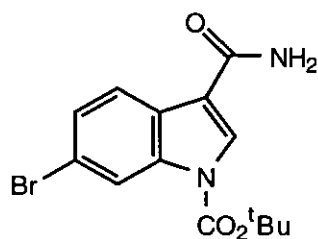
4-Bromo-2-nitrotoluene [286] (10g, 46.2 mmol), DMF (90 ml), *N,N*-dimethylformamide dimethyl acetal (16.29 g, 137 mmol) and pyrrolidine (3.24 g, 45.6 mmol) were stirred at 110°C for 3 h. The reaction mixture was cooled and water (200 ml) was added. The mixture was extracted with diethyl ether (3 x 100 ml), the extracts were combined and washed with water (5 x 100 ml), brine (100 ml), dried (MgSO₄) and evaporated under reduced pressure to give a dark red oil. Acetic acid (80%, 300 ml) was added and the mixture stirred at 75°C. Zinc dust (25.8 g, 395 mmol) was added over a 1 h period and the mixture was then stirred at 85°C for a further 2 h. The mixture was cooled, filtered, the filtrate was washed with diethyl ether (400 ml) and the washings were combined with the filtrate and washed with saturated sodium hydrogen carbonate solution (4 x 200 ml), water (2 x 200 ml), brine (2 x 200 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (light petroleum : dichloromethane elution) gave the *title compound* [287] (5.03g, 56%) as a gun metal solid; m.p. 94-95°C; (Found M⁺, 194.9697. C₈H₆NBr requires M, 194.9684); ν_{\max} (CHCl₃)/cm⁻¹ 1522, 1423, 1215 and 929; δ_{H} (250 MHz; CDCl₃) 6.54 (1H, m), 7.21 (2H, m), 7.49 (1H, m) and 8.16 (1H, br, NH); δ_{C} (62.5 MHz; CDCl₃) 102.73, 114.04, 115.40, 121.98, 123.12, 123.87, 125.0 and 126.73; m/z 195 (M⁺, 100%) 116 (90), 89 (45), 63 (20), 51 (30) and 31 (20).



tert-Butyl 6-bromoindole-1-carboxylate [288]

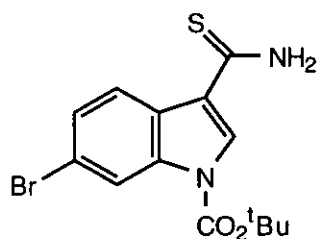
6-Bromoindole [287] (2 g, 10.25 mmol), di-*tert*-butyl dicarbonate (2.46 g, 11.28 mmol), DMAP (122 mg, 1 mmol) and acetonitrile (28 ml) were stirred at rt for 1 h. Saturated sodium hydrogen carbonate solution (20 ml) was added and the mixture stirred for a further 30 min. The mixture was extracted with diethyl ether (2 x 20 ml) the extracts were combined washed with brine (2 x 20 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [288] (2.34 g, 77%) as a colourless solid; m.p. 60-62°C; (Found M⁺, 295.0208. C₁₃H₁₄NBrO₂ requires M, 295.0216); ν_{\max} (CHCl₃)/cm⁻¹ 1732, 1434, 1377, 1215, and 761; δ_{H} (250 MHz; CDCl₃) 1.69 (9H, s, (CH₃)₃), 6.54 (1H, d, *J* 3.7 Hz), 7.33 (1H, dd, *J* 8.3 1.7 Hz), 7.41

(1H, d, J 8.3 Hz), 7.56 (1H, d, J 3.7) and 8.37 (1H, br); δ_C (62.5 MHz; $CDCl_3$) 28.06, 84.91, 106.97, 118.01, 118.31, 121.88, 125.79, 126.26, 129.10, 135.87 and 159.42; m/z 295 (M^+ , 5%) 239 (15), 195 (20), 115 (10), 57 (100), 41 (40) and 29 (20).



tert-Butyl 6-bromo-3-carboxamideindole-1-carboxylate [289]

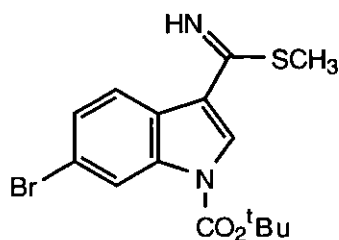
CSI (2.24 g, 15.84 mmol) was added dropwise to a solution of *tert*-Butyl 6-bromoindole-1-carboxylate [288] (2.33 g, 7.92 mmol), and acetonitrile (18 ml) over a 10 min period at 0°C. The mixture was warmed to rt and stirred for 1 h. A solution of acetone (10 ml) and water (2 ml) was added and the solution was rendered alkaline by the addition of potassium hydroxide (10% solution). The mixture was extracted with dichloromethane (3 x 20 ml) and the extracts were combined and washed with brine (20 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (light petroleum : dichloromethane elution) gave the *title compound* [289] (1.69 g, 64%) as a colourless solid; m.p. 149-151°C; (Found M^+ , 338.0246. $C_{14}H_{15}N_2BrO_3$ requires M , 338.0266); ν_{max} ($CHCl_3$)/ cm^{-1} 1743, 1667, 1371, 1215 and 759; δ_H (250 MHz; $CDCl_3$) 1.68 (9H, s, $(CH_3)_3$), 5.93 (2H, br, NH_2), 7.42 (1H, dd, J 8.5 1.6 Hz), 7.97 (1H, d, J 8.5 Hz), 8.04 (1H, s) and 8.36 (1H, d, J 1.5 Hz); δ_C (62.5 MHz; $CDCl_3$) 27.73 ($(CH_3)_3$), 85.55 ($C(CH_3)_3$), 114.92, 118.16, 118.78, 122.43, 126.36, 126.85, 128.55, 136.20, 148.33 and 166.21; m/z 338 (M^+ , 5%) 282 (10), 222 (30), 114 (15), 57 (80), 44 (90) and 28 (100).



tert-Butyl 3-thiocarboxamideindole-1-carboxylate [290]

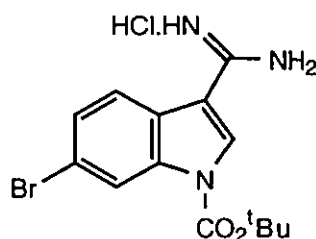
tert-Butyl 6-bromo-3-carboxamideindole-1-carboxylate [289] (300 mg, 0.88 mmol), Lawesson's reagent [99] (179 mg, 0.44 mmol) and benzene (5 ml) were heated at reflux for 1 h. The reaction mixture was cooled and evaporated under reduced pressure to give a yellow solid. Flash column chromatography (dichloromethane : diethyl ether elution) gave the *title compound* [290] (153 mg, 49%) as a yellow solid; m.p. 150-151°C; (Found M^+ , 354.0013. $C_{14}H_{15}BrNO_2S$ requires M , 354.0038); δ_H (250 MHz; $CDCl_3$) 1.69 (9H, s, $(CH_3)_3$), 7.24

(2H, br, NH_2), 7.46 (1H, dd, J 8.6 1.7 Hz), 8.05 (1H, d, J 8.6 Hz), 8.16 (1H, s) and 8.42 (1H, d, J 1.7 Hz); m/z 354 (M^+ , 50%) 220 (80), 155 (30), 141 (45), 114 (45) and 41 (100).



tert-Butyl 6-bromo-3-thiocarboximidateindole-1-carboxylate [291]

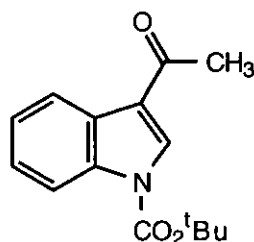
tert-Butyl 6-bromo-3-thiocarboxamideindole-1-carboxylate [290] (246 mg, .7 mmol), methyl iodide (0.88 g, 6.2 mmol) and dichloromethane (2 ml) were stirred at rt for 48 h under a nitrogen atmosphere. Dichloromethane (10 ml) was added and the mixture was washed with a saturated solution of sodium hydrogen carbonate (2 x 10 ml), brine (2 x 10 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow gum (237 mg, 91% crude) which was used in the next step without further purification. **CARE** the compound is extremely **SMELLY**, the reaction must be carried out in an efficient fume cupboard; (Found M^+ , 368.0199. $C_{15}H_{17}BrN_2O_2S$ requires M , 368.0195); ν_{max} ($CHCl_3$)/ cm^{-1} 1742, 1653, 1371, 1215 and 756; δ_H (250 MHz; $CDCl_3$) 1.68 (9H, s, $(CH_3)_3$), 2.43 (3H, s, SCH_3), 7.40 (1H, dd, J 8.4 1.7 Hz), 8.01 (1H, S), 8.09 (1H, d, J 8.5 Hz) and 8.35 (1H, d, J 1.6 Hz); δ_C (62.5 MHz; $CDCl_3$) 11.42 (SCH_3), 27.21 ($(CH_3)_3$), 85.11 ($C(CH_3)_3$), 117.91, 119.67, 121.61, 122.24, 124.54, 126.33, 127.21, 128.28, 145.31 and 149.26; m/z 368 (M^+ , 5%) 222 (30), 141 (20), 114 (20), 57 (100) and 41 (30).



tert-Butyl 6-bromo-3-carbamidinyndole-1-carboxylate hydrochloride [292]

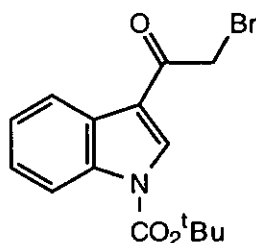
tert-Butyl 6-bromo-3-thiocarboximidateindole-1-carboxylate [291] (239 mg, 0.65 mmol), ammonium chloride (40 mg, 0.7 mmol) and methanol (5 ml) were heated under reflux for 3 h. **CARE**, during the reaction methyl mercaptam is liberated and so a potassium permanganate bubbler was attached to the reflux condenser. The reaction mixture was cooled and ethyl acetate (100 ml) was added the mixture was washed with brine (2 x 30 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give a pale brown solid. Recrystallisation (ethanol) gave the *title compound [292]* (109 g, 45%) as a pale yellow solid; m.p. $>300^\circ C$; (Found M^+ , 372.9832. $C_{14}H_{17}BrClN_3O_2$ requires M , 373.0193); ν_{max} ($CHCl_3$)/ cm^{-1} 1746, 1715,

1428, 1372, 1215 and 759; m/z 368 (M^+ , 5%) 297 (10), 253 (30), 224 (30), 114 (10), 57 (100) and 28 (40).



tert-Butyl 3-acetylidole-1-carboxylate [295]

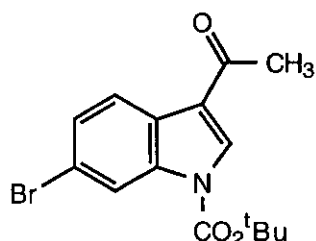
3-Acetylidole [294] (1 g, 6.3 mmol), di-*tert*-butyl dicarbonate (1.5 g, 6.9 mmol), DMAP (75 mg, 0.61 mmol) and acetonitrile (17 ml) were stirred at rt for 1 h. Saturated sodium hydrogen carbonate solution (20 ml) was added and the mixture stirred for a further 30 min. The mixture was extracted with diethyl ether (2 x 40 ml) the extracts were combined washed with brine (2 x 20 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [295] (1.47 g, 90%) as a colourless solid; m.p. 148-149°C; (Found M^+ , 259.1201. $C_{15}H_{17}NO_3$ requires M , 259.1208); ν_{max} ($CHCl_3$)/ cm^{-1} 1740, 1664, 1546, 1450, 1371, 1215 and 755; δ_H (250 MHz; $CDCl_3$) 1.72 (9H, s, $(CH_3)_3$), 2.57 (3H, s, CH_3), 7.36 (2H, m), 8.10 (1H, m), 8.23 (1H, s) and 8.37 (1H, m); δ_C (62.5 MHz; $CDCl_3$) 27.83 ($(CH_3)_3$), 28.03, (CH_3) 85.31 ($C(CH_3)_3$), 114.86, 120.05, 122.62, 124.28, 125.39, 127.41, 132.31, 135.73, 149.39 and 196.21; m/z 259 (M^+ , 5%) 217 (5), 144 (10), 57 (10), 41 (100) and 28 (20).



tert-Butyl 3-(2-bromoacetyl)indole-1-carboxylate [296]

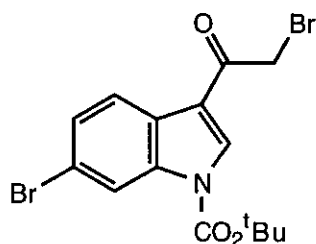
tert-Butyl 3-acetylidole-1-carboxylate [295] (500 mg, 1.93 mmol), chloroform (2 ml) and ethyl acetate (2 ml) were stirred at 75°C under a nitrogen atmosphere for 20 min. Copper(II)bromide (800 mg, 3.7 mmol) was added and the mixture was stirred at 75°C for a further 3 h. The mixture was cooled and filtered, washed with ethyl acetate (30 ml), the filtrate and washings were combined washed with brine (20 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (light petroleum : dichloromethane elution) gave the *title compound* [296] (293 mg, 45%) as a pale yellow solid; m.p. 142-143°C; (Found M^+ , 337.0302. $C_{15}H_{16}BrNO_3$ requires M , 337.0314); ν_{max} ($CHCl_3$)/ cm^{-1} 1743, 1665,

1543, 1450, 1372, 1215 and 756; δ_{H} (250 MHz; CDCl_3) 1.73 (9H, s, $(\text{CH}_3)_3$), 4.37 (2H, s, CH_2Br), 7.39 (2H, m), 8.12 (1H, m), 8.34 (1H, s) and 8.36 (1H, m); δ_{C} (62.5 MHz; CDCl_3) 28.01 ($(\text{CH}_3)_3$), 31.54, (CH_2Br) 85.83 ($\text{C}(\text{CH}_3)_3$), 114.98, 116.31, 122.45, 122.52, 124.59, 125.80, 132.30, 132.81, 159.0 and 186.37; m/z 337 (M^+ , 5%) 237 (10), 144 (80), 89 (10), 57 (100), 41 (50) and 29 (20).



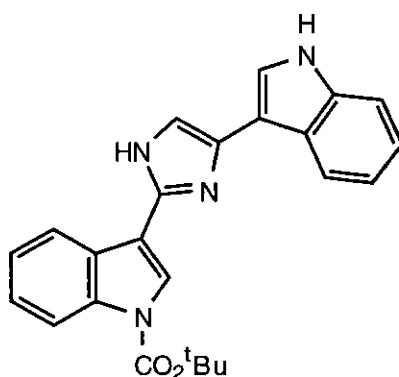
tert-Butyl 3-acetyl-6-bromoindole-1-carboxylate [298]

N,N-Dimethylacetamide (0.8 ml, 8.4 mmol) was added dropwise to solution of phosphorous oxychloride (0.78 ml, 8.4 mmol) and chloroform (10 ml) maintaining the temperature below 10°C. The mixture was stirred for 10 min then a solution of 6-bromoindole [287] (500 mg, 2.56 mmol) in chloroform (10 ml) was added dropwise over a 30 min period, maintaining the temperature below 10°C. The mixture was then stirred at reflux for 3h. The mixture was cooled and potassium hydroxide (40% solution, 50 ml) was added. The mixture was extracted with dichloromethane (3 x 50 ml), the extracts were combined and washed with brine (50 ml), dried (MgSO_4) and evaporated under reduced pressure to give a dark brown solid. A solution of di-*tert*-butyl dicarbonate (408 mg, 2.8 mmol), DMAP (30 mg, 0.24 mmol) and acetonitrile (10 ml) was added and the mixture stirred at rt for 1 h. Saturated sodium hydrogen carbonate solution (20 ml) was added and the mixture stirred for a further 30 min. The mixture was extracted with diethyl ether (2 x 40 ml) the extracts were combined washed with brine (2 x 20 ml), dried (MgSO_4) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [298] (345 mg, 40%) as a colourless solid; m.p. 150-152°C; (Found M^+ , 337.0063. $\text{C}_{15}\text{H}_{16}\text{BrNO}_3$ requires M , 337.0314); ν_{max} (CHCl_3)/ cm^{-1} 1745, 1668, 1432, 1215 and 756; δ_{H} (250 MHz; CDCl_3) 1.72 (9H, s, $(\text{CH}_3)_3$), 2.56 (3H, s, CH_3), 7.46 (1H, dd, J 8.5 1.8 Hz), 8.17 (1H, s), 8.24 (1H, d, J 8.5 Hz) and 8.36 (1H, d, J 1.8 Hz); δ_{C} (62.5 MHz; CDCl_3) 27.56 (CH_3), 27.98 ($(\text{CH}_3)_3$), 86.01 ($\text{C}(\text{CH}_3)_3$), 118.14, 119.31, 120.93, 123.81, 126.50, 127.59, 132.34, 142.86, 159.38 and 182.76; m/z 337 (M^+ , 5%) 203 (20), 159 (30), 144 (60), 57 (100), 41 (30) and 28 (90).



tert-Butyl 6-bromo-3-(2-bromoacetyl)indole-1-carboxylate [299]

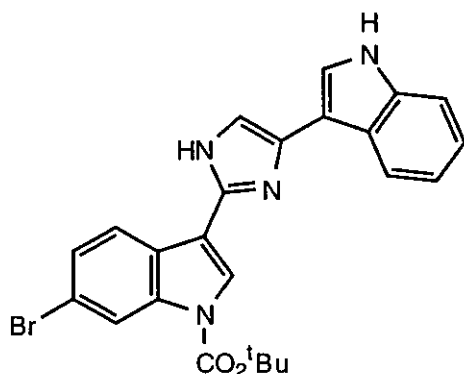
tert-Butyl 6-bromo-3-acetylindole-1-carboxylate [298] (254 mg, 0.75 mmol), chloroform (2 ml) and ethyl acetate (2 ml) were stirred at 75°C under a nitrogen atmosphere for 20 min. Copper(II)bromide (311 mg, 1.4 mmol) was added and the mixture was stirred at 75°C for a further 3 h. The mixture was cooled and filtered, washed with ethyl acetate (30 ml), the filtrate and washings were combined washed with brine (20 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (light petroleum : dichloromethane elution) gave the *title compound [299]* (140 mg, 45%) as a pale yellow solid; m.p. 180-181°C; (Found M⁺, 414.9442. C₁₅H₁₅Br₂NO₃ requires M, 414.9442); ν_{\max} (CHCl₃)/cm⁻¹ 1742, 1666, 1543, 1371, 1215 and 759; δ_{H} (250 MHz; CDCl₃) 1.73 (9H, s, (CH₃)₃), 4.33 (2H, s, CH₂Br), 7.45 (1H, dd, *J* 8.5 1.7 Hz), 8.17 (1H, d, *J* 8.5 Hz), 8.26 (1H, s) and 8.31 (1H, d, *J* 1.7 Hz); δ_{C} (62.5 MHz; CDCl₃) 28.43 ((CH₃)₃), 31.79 (CH₂Br), 86.92 (C(CH₃)₃), 117.29, 118.69, 120.11, 124.10, 126.52, 132.82, 136.43, 137.94, 148.75 and 187.36; *m/z* 416 (M⁺, 5%) 317 (10), 224 (20), 155 (20), 113 (20), 57 (100), 41 (35) and 28 (80).



2-(1-tert-Butyl(indol-3-yl)carboxylate)-4(5)-(1-(H)-indol-3-yl)imidazole [300]

tert-Butyl 3-carbamidinylindole-1-carboxylate hydrochloride [284] (92 mg, 0.31 mmol), 3-bromoacetylindole [123] (75 mg, 0.31 mmol), potassium carbonate (43 mg, 0.31 mmol) and acetonitrile (10 ml) were heated under reflux for 7 h. The reaction mixture was cooled and water (10 ml) added. The mixture was extracted with dichloromethane (2 x 10 ml), the extracts were combined, washed with brine (10 ml), dried (MgSO₄) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound [300]* (51 mg, 41%) as an amber solid; m.p. 112-114°C; (Found M⁺, 398.1734.

$C_{24}H_{22}N_4O_2$ requires M, 398.1743); ν_{max} ($CHCl_3$)/ cm^{-1} 2330 (br), 1741, 1427, 1353, 1215 and 756; δ_H (250 MHz; $CDCl_3$) 1.55 (9H, s, $C(CH_3)_3$), 7.17 (4H, m), 7.23 (3H, m), 7.46 (1H, br, NH), 7.76 (1H, d, J 8.5 Hz), 7.92 (1H, s), 8.09 (1H, dd, J 8.5 2.3 Hz) and 8.68 (1H, br, NH); δ_C (62.5 MHz; $CDCl_3$) 27.95, 84.13, 108.42, 111.43, 111.66, 115.04, 119.63, 120.06, 120.82, 121.85, 122.21, 123.16, 123.56, 124.85, 125.11, 125.17, 127.55, 135.19, 136.34, 141.15, 149.40 and 161.32; m/z 398 (M^+ , >5%) 217 (10), 186 (10), 155 (15), 113 (15), 70 (10), 56 (50) and 41 (100).



2-(1-tert-Butyl(-6-bromoindol-3-yl)carboxylate)-4(5)-(1-(H)-indol-3-yl)imidazole [301]

tert-Butyl 6-bromo-3-carbamidinyndole-1-carboxylate hydrochloride [292] (56 mg, 0.15 mmol), 3-bromoacetylindole [123] (36 mg, 0.15 mmol), potassium carbonate (21 mg, 0.15 mmol) and acetonitrile (5 ml) were heated under reflux for 14 h. The reaction mixture was cooled and water (10 ml) added. The mixture was extracted with dichloromethane (2 x 10 ml), the extracts were combined, washed with brine (10 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (light petroleum : diethyl ether elution) gave the *title compound* [301] (24 mg, 34%) as a pale brown solid, which turned dark brown over a few days; m.p. 145-147°C; (Found M^+ , 478.0823. $C_{24}H_{21}^{81}BrN_4O_4$ requires M, 478.0828, 476.0848. $C_{24}H_{21}^{79}BrN_4O_2$ requires 478.0828); ν_{max} ($CHCl_3$)/ cm^{-1} 3230 (br), 1739, 1427, 1371, 1310, 1215 and 756; δ_H (250 MHz; $CDCl_3$) 1.59 (9H, s, $C(CH_3)_3$), 7.21 (4H, m), 7.43 (1H, s), 4.46 (1H, d, J 3.4 Hz), 7.58 (1H, br, NH), 7.87 (1H, s), 7.92 (1H, dd, J 8.5 3.2 Hz), 8.09 (1H, d, J 8.5 Hz), 8.33 (1H, s) and 8.51 (1H, br, NH); m/z 478 ($M^+(^{81}Br)$, >5%), 479 ($M^+(^{79}Br)$, >5), 267 (20), 217 (20), 186 (20), 155 (40), 113 (40), 70 (25), 51 (100) and 31 (60).

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