Karolinska Institutet, Stockholm, Sweden

Synthetic Studies of Heterocyclic Natural Products

Jeffrey John Mason

Karolinska Institutet

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ABSTRACT

The methods that were developed for the synthesis of the heterocyclic natural products luotonin A, ophiuroidine, cephalandole B, and the indol-3-yl-benzoxazinones of cephalandole A are described in this thesis. The reoccurrence of the 4(3*H*)-quinazolinone structure is a common theme throughout these studies, and an overview of some quinazoline chemistry is introduced in the first chapter.

The second chapter describes the progression of a series of 9-oxo-pyrroloquinazolinone compounds which led to a new synthetic method of the natural product luotonin A, isolated from *Peganum nigellasterum*, and some analogues thereof (Paper I). This synthetic strategy proved to be an efficient route, and the new analogues of luotonin A that were created predominantly had substitutions at the 14th position.

The third chapter details the synthesis of cephalandoles A and B, isolated from the orchid *Cephalantheropsis gracilis*, as well as a revision to the structure of cephalandole A (Paper II). The proposed structures for cephalandole A was reported as 2-(1*H*-indol-2-yl-carbonyl)-4*H*(3,1)benzoxazinone, and cephalandole B as methyl 2-(1*H*-indole-3-carboxamido)benzoate; both compounds had never previously been synthesized. Upon preparation of the target molecule, inconsistencies between the data of the synthesized compound and that of the reported structure prompted further investigations. The revised structure was found to be 3-(1*H*-indol-3-yl)-2*H*(1,4)-benzoxazinone, a compound previously synthesized by Moffett in 1966. From the 2-(1*H*-indol-2-yl-carbonyl)-4*H*(3,1)-benzoxazinone, the corresponding 4(3*H*)-quinazolinone was prepared. It was also found that cephalandole B could be prepared from 2-(1*H*-indol-2-yl-carbonyl)-4*H*(3,1)benzoxazinone, suggesting cephalandole B may be an artefact of the original extraction process.

The fourth chapter features the development of synthetic strategies towards preparation of the marine natural product ophiuroidine (Paper III), isolated from the brittle star *Ophiocoma resii*. The 4,8,9-trihydroxytryptanthrin was prepared starting from 5,6-dimethoxyisatin and 8-methoxyisatoic anhydride, and the corresponding 4,8,9-trimethoxytryptanthrin was demethylated using pyridinium hydrochloride in *N*-methylpyrrolidone, with the use of microwave heating. A section discussing isatins and their preparation is included in this chapter. A new method for the preparation of several methoxyisatins is described, starting from *m*-methoxyanilines, which was an improvement over Sandmeyer's isonitrosoacetanilide protocol. The isatins generated from this work led to the preparation of several other methoxytryptanthrins.

As an extension of the ophiuroidine project, investigations of the demethylation of aryl ethers with pyridinium hydrochloride was undertaken with the use of microwave heating and the inclusion of the solvent *N*-methylpyrrolidone (Paper IV). This modification to the previously known procedure is described, as well as the limitations of the reaction. A dozen compounds containing aryl methyl ethers were assessed using this modification, including two substituted 2-styryl-4(3*H*)-quinazolinones, which were found not only to undergo demethylation but also dehalogenation under the described reaction conditions.

LIST OF PAPERS

This thesis is based on the following articles:

I. "Total Synthesis of Luotonin A and 14-substituted Analogues"
Jeffrey J. Mason and Jan Bergman.
Org. Biomol. Chem. **2007**, *5*, 2486-2490.

II. "Synthetic Studies of Cephalandole Alkaloids and the Revised Structure of Cephalandole A"
Jeffrey J. Mason, Jan Bergman and Tomasz Janosik.
J. Nat. Prod. **2008**, *71*, 1447-1450.

III. "Synthesis of Ophiuroidine and Other Hydroxytryptanthrins"
Jeffrey J. Mason, Tomasz Janosik and Jan Bergman.
Synthesis **2009**, Advanced online publication 08.09.2009
DOI 10.55/s-0029-1217004; Art ID: P08309SS.

IV. "Investigation of the Microwave Assisted Deprotection of Aryl Ethers with Pyridinium Hydrochloride in NMP"
Jeffrey J. Mason, Tomasz Janosik and Jan Bergman.
Manuscript

CONTENTS

1 Introduction to Natural Products and Natural Products Chemistry

Traditional medicines and remedies play an important role in many societies. Ever since human beings have understood that certain plants, animals, and fungi contain substances that can have an affect on our bodies in specific ways, chemists have studied these compounds through the process of isolation and identification, as well as confirmation by total synthesis, to better understand their function. Such compounds are called natural products.

A natural product by definition is *"A chemical substance produced by a living organism; - a term used commonly in reference to chemical substances found in nature that have distinctive pharmacological effects. Such a substance is considered a natural product even if it can be prepared by total synthesis."*[1] It can also be described as naturally occurring compounds that are end products of secondary metabolism; often they are unique for particular organisms or classes of organisms.

The study of natural products is called natural product chemistry and is defined as; *"That branch of chemistry which deals with the isolation, identification, structure, and study of the chemical characteristics of chemical substances produced by living organisms."*[1]

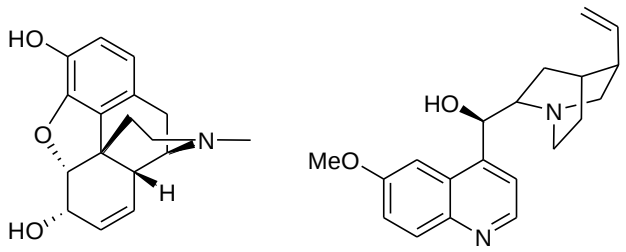
Synthetic natural product chemists work to provide strategies for total synthesis of the proposed elucidated structures, in part, as a final confirmation. One such chemist Meissner, in 1819, proposed the classification *"Alkaloid"* for weakly basic (alkali-like) chemical compounds isolated from nature. The term was inspired from investigations of the plant genus *Veratrum* where some isolates presented these properties.[2] Chemical compounds were proposed to be classed as an alkaloid as long as they met three criteria:

- The structure was unknown
- The compound displayed some degree of physiological activity
- The compound gave an alkali-like response

It was not until the late 1800's that the term alkaloid was widely accepted and used, but the term has, over time, undergone several revisions.[3] The most current version states: "*An alkaloid is a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms*."[4]

Two of the most widely known alkaloids would be morphine (an isoquinoline alkaloid) from the opium poppy (*Papaver somniferum*),[5] having analgesic properties and used in the treatment of severe pain; and quinine (a quinoline alkaloid) from the cinchona tree (*Cinchona sp.*), which has been found to display analgesic, anti-pyretic (fever reducing), anti-inflammatory and anti-malarial properties, and has been used in the treatment of lupus, nocturnal leg cramps, arthritis, and malaria (Figure 1).[6]

Due to their long histories and potent biological activities these compounds were first used as remedies from crude extracts as infusions; known as opium and laudanum for the crude extract of *P. somniferum*, containing morphine; and the Cinchona extracts containing quinine were known as Jesuits' powder. Morphine was isolated from the crude extracts, as its hydrochloride salt, in 1805 by Sertürner,[7] while quinine was isolated in 1820 by Pelletier and Caventou.[8] Being structurally complex compounds identification and confirmation by total synthesis was laborious work. Morphine was finally reported by Gates and Tschudi in 1952, almost 150 years after its isolation,[9] as a result of demand spurring from the Second World War (WWII). The demand for synthetic quinine was realized after the First World War, and with the outbreak of WWII this demand became paramount. The claims to the total synthesis of quinine has seen a hotly contested series of debates by two factions emerge.[10] The currently accepted perception is that the work of Rabe and Kindler can be considered the first total synthesis,[10a] while Woodward and Doering's contribution is though of as a formal synthesis of quinine.[10c, 10d]

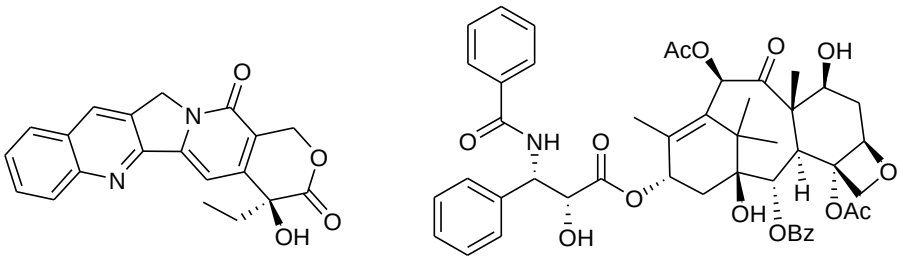


morphine (-)-quinine

Figure 1 Structures of morphine and quinine.

From the mid 1950's through to the 1970's activity of pharmaceutical chemistry and natural products chemistry was booming, partly due to the end of the WWII.[11] The total synthesis of compounds such as penicillin V,[12] reserpine,[13] and psilocybin,[14] just to name a few; as well as advancements in the understanding of the functions behind their pharmacological activities, created a push in chemistry that increased the focus on the study of natural products in various medical fields.

The two major discoveries made in the 60's by Munroe Wall and Manusk Wani, were the natural products camptothecin,[15] another quinoline alkaloid; and paclitaxel (commercially known as Taxol®)[16] (Figure 2), which have been found to be active against various tumours.[17] The significance of camptothecin with regard to this thesis is its association with the natural product luotonin A, which is discussed in the second chapter.



20-(*S*)-camptothecin paclitaxel

Figure 2 The structures of 20-(*S*)-camptothecin and paclitaxel.

The work described in this thesis covers a range of heterocyclic systems that can be considered the building blocks of many alkaloids; these include quinazolinones, indoles, isatins, and benzoxazinones, but the main recurring theme across these studies are preparations involving 4(3*H*)-quinazolinone heterocycles.

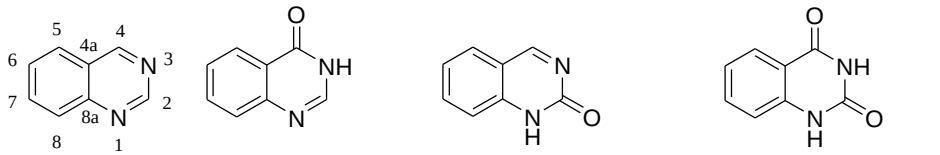
1.1 Quinazolines and Quinazolinones

The quinazolines are a group within the quinoline alkaloids. The scaffold that defines a quinazoline consists of a fused benzene and pyrmidine bicyclic structure (Figure 3). These compounds have been described as privileged structures,[18] as they provide various points of attachment for a diverse array of structural elements that can be used to target receptor agonists or antagonists.[19] The pharmacological activities that have been discovered amongst the quinazolines include the treatment categories of:[20]

> Anticonvulsant; hypnotic; anti-Parkinson's; sedative; analgesic and muscle relaxant; enzyme inhibiting; antiviral; antitumour; anti-inflammatory; cardiovascular; antibacterial; antifungal; antimicrobial; antihelminthic (infestation of parasitic worms such as tapeworm or hookworm); antimalarial; antimycobacterial (tuberculosis); antihistaminic; anti-ulcer; and hypoglycaemic activities.

Quinazolinones are the oxidized form of quinazolines, and as such are also part of the quinoline alkaloids. Both naturally occurring and synthetic quinazolines and quinazolinones have attracted widespread attention due to the diverse range of pharmacological activities, included in the above mentioned treatment categories. These structures are defined by the location of the oxygen and the hydrogen on the nitrogen (NH), and the commonly accepted numbering for quinazolines and quinazolinones is described using the quinazoline structure (Figure 3). The major sub-classes of quinazolinones fall into the categories of:

- 4(3*H*)-quinazolinone (3*H*-1,3-quinazolin-4-one)
- 2(1*H*)-quinazolinone (1*H*-1,3-quinazolin-2-one)
- 2,4(1*H*,3*H*)-quinazolinedione (1*H*,3*H*-1,3-quinazoline-2,4-dione)



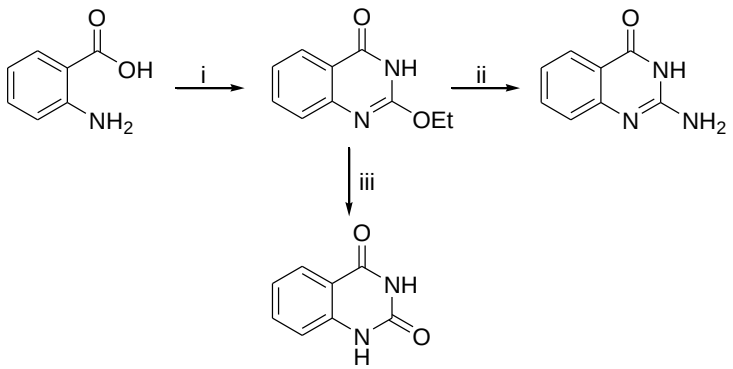
quinazoline 4(3*H*)-quinazolinone 2(1*H*)-quinazolinone 2,4(1*H*,3*H*)-quinazolinedione

Figure 3 Core structures of quinazoline and quinazolinone alkaloids.

Of the three quinazolinone structures the 4(3*H*)-quinazolinones are most prevalent, either as intermediates, or as natural products in many proposed biosynthetic pathways.[21] This is partly due to the structure being derived from the anthranilates (anthranilic acid or various esters, isatoic anhydride, anthranilamide and anthranilonitrile) while the 2(1*H*)-quinazolinone is predominantly a product of anthranilonitrile, or benzamides with nitriles.[22] Through the process of auto-oxidation, quinazoline precursors can be converted to the corresponding 4(3*H*)-quinazolinone; an example of this is given in the next section.

Although quinazolinone chemistry is considered an established area, newer and more complex variants of the quinazolinone structure are still being discovered.[23] The first reported synthesis of a quinazolinone occurred in 1869, which was prepared from anthranilic acid and cyanide in ethanol, creating 2-ethoxy-4(3*H*)-quinazolinone.[24] These findings were confirmed by the preparation of the derivatives 2-amino-4(3*H*)-quinazolinone and 2,4(1*H*,3*H*)-quinazolinedione by reactions with ammonia and water, respectively (Scheme 1).

Another quinazolinone compound, methaqualone (Figure 4),[25] has been of significance due to its potent sedative and hypnotic effects,[26] ease of preparation,[27] and history of abuse - "luding out".[28] The procedure described by Klosa is one of the most efficient preparations of methaqualone,[27a] comprising of a one pot approach using anthranilic acid, acetic acid, *o*-toluidine, and phosphorus pentachloride. Many analogues of methaqualone have been prepared and tested for similar sedative effects.[29]



Scheme 1 Griess' syntheses of 4(3*H*)-quinazolinones and 2,4(1*H*,3*H*)-quinazolinedione from anthranilic acid. *Reagents and conditions:* i) HCN, EtOH; ii) NH_4OH, reflux; iii) H_2O.

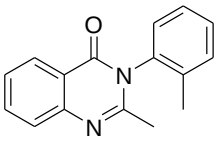


Figure 4 Structure of the commercial drug methaqualone.

Investigations of quinazolinones show there is a strong lactam-lactim tautomeric interaction,[30] and examples of other tautomeric effects which were observed as a result of the studies undertaken are mentioned at various points in the thesis. The significance of this tautomeric interaction can also be seen when a 4(3*H*)-quinazolinone containing a methyl in the 3-position is subjected to chlorination with $POCl_3$, the methyl group is lost and chlorination proceeds;[31] and when the methyl group is present in the 2-position, the tautomeric effect is extended generating an exomethylene carbon, this compound can be condensed with aldehydes producing 2-styryl-4(3*H*)-quinazolinones. This particular reaction was performed to create two halogenated 2-styryl-4(3*H*)-quinazolinones for the demethylation study presented in Chapter 4. The significance of these extended tautomeric effects is that they enhance reactivity of the substituted 4(3*H*)-quinazolinones (Figure 5). [32]

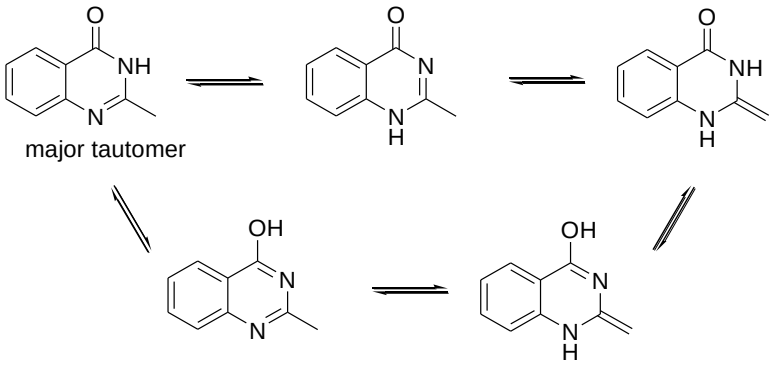


Figure 5 Diagram of the tautomeric states of 2-methyl-4(3*H*)-quinazolinone.

2 Investigations of Luotonin A and Pyrroloquinazolinones

2.1 Introduction to the pyrroloquinazolinones

Pyrroloquinazolinones have been found as components of several natural products such as vasicinone, tryptanthrins, and luotonins, and many pyrroloquinazolinones have been prepared; inspired from natural products vasicine and vasicinone (Figure 6).

Vasicine[33] and its corresponding keto-analogue,[34] vasicinone have been discovered as natural products in several plant species (*Adhatoda vasica*, *Peganum harmala*, and *P. nigellasterum*). Before the discovery of the therapeutic value of these compounds by "Western" scientific methodologies (Allopathic Medicine),[35] these plants were used as traditional herbal remedies throughout Asia, particularly India, in the treatment of various respiratory disorders as well as other ailments (Ayurvedic medicine).[35,36] Administration of these remedies was through various approaches; the leaves were dried and then smoked; the roots were dried and used as an infusion; and the essential oils were either applied topically, or consumed. The bronchiodilatory properties of vasicine and vasicinone have been clinically assessed,[37] and were found to have similar efficacy (20 mg/Kg, oral, rat)[38] as the alkaloid theophylline, a component of tea (Figure 6).[39]

The biosynthetic pathway of vasicinone was elucidated by Gröger *et al.*,[40] when ^{14}C labelled anthranilic acid was fed into the plant and labelled vasicinone was isolated. Vasicine and vasicinone were initially considered a racemic mixture, but the absolute configuration was later identified and proposed as the *R* isomer,[41] but this assignment was reversed through the use of X-ray crystallographic studies.[42] Of the two stereoisomers, the (-)-form (*S* configuration) was found to be the natural isolate of both compounds.

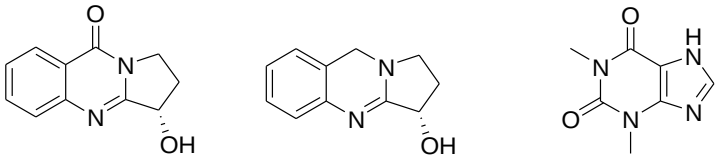
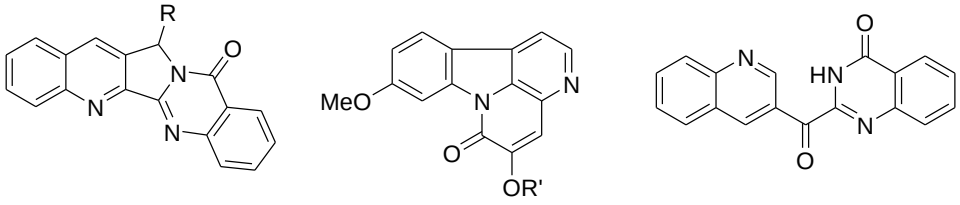


Figure 6 Structures of (-)-vasicinone, (-)-vasicine, and theophylline.

2.2 The Luotonins

The first luotonin compounds, luotonin A and B, were isolated in 1997 by Ma and Nomura, from aerial parts of the plant *Peganum nigellasterum* (Zygophyllaceae) Bunge.[43] The Chinese name "骆驼蒿 – luòtuohāo"[44] gives the name-sake of this alkaloid group. The plant is endemic to the north-west, semi-arid areas of China, and has been used as a traditional remedy for several ailments including rheumatism and inflammation.[45] Luotonin A (**1**) and luotonin B were correctly identified to contain quinazoline and quinoline structures (Figure 7). The other new compounds that were later isolated, and their structures elucidated, from this plant were appropriately labelled luotonins C-F (Figure 7),[46] several other alkaloids were also isolated from this plant including vasicinone.[45]

Luotonin A was shown to first display activity in murine leukaemia P-388 cell lines (IC_{50} 1.8 μg/mL), and in intact cells (IC_{50} 5.7-12.6 μg/mL),[45] thus making it a useful lead compound for chemotherapeutic agents, due to the simplicity of preparing this structure. Since its discovery close associations have been made between luotonin A and another, more complex anti-tumour agent, camptothecin (CPT) (Figure 8).



R = H luotonin A (**1**)
R = OH luotonin B
R = OMe luotonin E

R' = Me luotonin C
R' = Et luotonin D

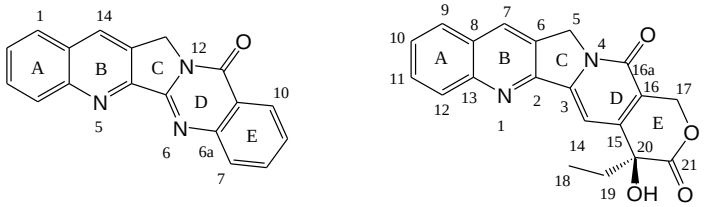
luotonin F

Figure 7 Structures of luotonins A-F.

Camptothecin was found in the crude samples taken from the tree *Camptotheca acuminata*, in the late 1950's as part of a survey for anti-cancer activity. Initially the compound was dropped from the program as it displayed poor solubility, but it continued as a side project. Its structure was elucidated characterized in the laboratories of Wall and Wani,[15] who championed its potential as a chemotherapeutic agent as it had shown excellent anti-tumour activity in rodent assays. The structure of this compound was another particular point of interest, as it represented new challenges of total synthesis of this new system of heterocyclic rings. The first total synthesis of CPT was reported by Stork and Schultz,[47] closely followed (9 weeks later) by Danishefsky

et al.[48] Both pathways utilized a Friedländer condensation as the key step, and produced CPT as a racemic mixture. Other significant total syntheses of CPT include those by Rapoport,[49] whose total yield (~15%, 15 steps) was significantly higher than the two previously mentioned; and Corey's method,[50] as this was the first stereospecific synthesis of the optically active compound, 20-(*S*)-camptothecin. Biosynthetic studies of camptothecin as well as other natural products of *C. acuminata* were confirmed through feeding experiments using ^{14}C labelled compounds.[51]

The most notable similarities of CPT and luotonin A are their "boomerang-like" pentacyclic structures, comprising of a pyrroloquinoline ring system creating the first three rings (A-C), while differences are observed in the D- and E-rings. The D-ring of CPT lacks the nitrogen of luotonin A, while the E-ring contains a lactone, and a stereogenic centre (Figure 8). Several studies involving luotonin A or analogues thereof use CPT as a standard to reference efficacy of the new compounds, since the chemistry behind CPT has a longer history of development, and greater efficacy as a cytotoxic topoisomerase I (top I)-DNA inhibitor by a factor of ten compared to luotonin A.[52] The crystal structure of a CPT analogue (Topotecan), and its ternary DNA-top I complex was reported by Stewart *et al.*, showing the interaction of the E-ring lactone with the DNA.[53] This stabilization has been found to generate double strand breaks causing apoptosis. While luotonin A does not contain the lactone, it still displays the same topoisomerase activity, but to a lower extent,[52] and luotonin A has also been discovered to also interact with DNA-Topoisomerase-II.[54] No such elucidations into the method of inhibition based on the crystal structures of luotonin A-DNA-top I have as yet been reported, but many structure activity relationship calculations for luotonin A are based on the existing CPT models.[55] Before the discovery of luotonin A, the commonly accepted perception was that the E-lactone ring was necessary for top I inhibition,[51,56] this perception was later questioned through the studies of Hecht *et al.*,[52] when luotonin A was shown to have a similar Topoisomerase I activity as CPT.



luotonin A 20-(*S*)-camptothecin

Figure 8 Numbered structures of luotonin A and 20-(*S*)-camptothecin.

Both of these compounds (luotonin A and CPT) also share the similar, and unfortunate quality of having poor water solubility, thus affecting both of their respective efficacies,[57] but improvements of camptothecin analogues has led to two compounds which are now used as therapeutic agents against cancers (Figure 9); topotecan® which is used in relapsed second stage ovarian, cervical, and small-cell lung cancers;[58] while irinotecan™ (CPT-11)[59] is used as a chemotherapy against first- and second-stage colo-rectal cancer. Both compounds have been described as having undesirable side effects, such as nausea, diarrhoea, and neutropaenia. The biologically active metabolite of irinotecan is the phenolic derivative SN-38,[60] but SN-38 (Figure 9) has been described as also suffering from poor bioavailability and is excreted.[61] Currently clinical trials of a combinatorial therapy involving hyaluronic acid and CTP-11 are underway which may improve uptake.[62]

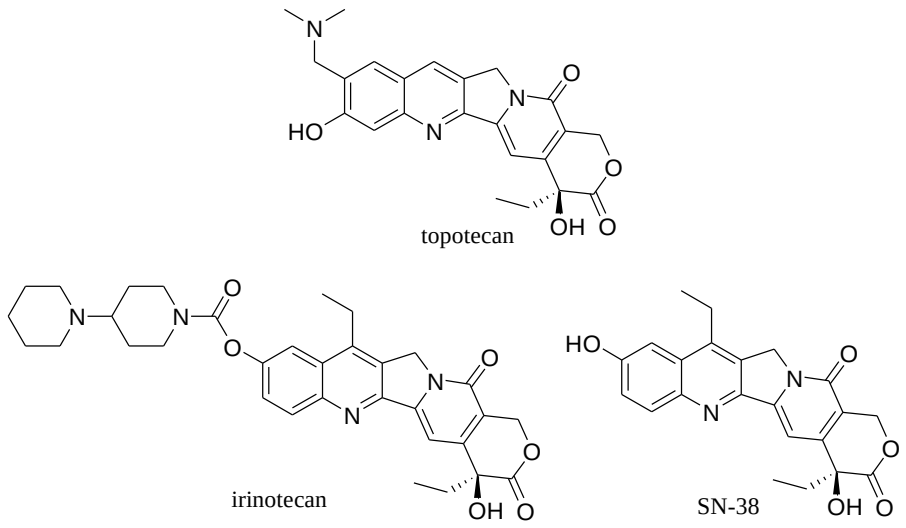
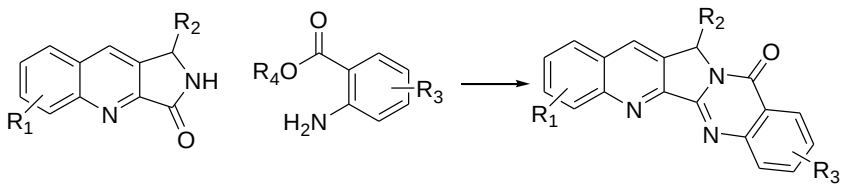


Figure 9 Structures of therapeutically approved camptothecin derivatives topotecan, irinotecan, and SN-38.

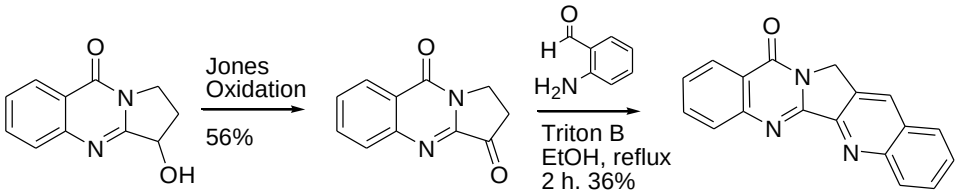
2.3 Preparation of Luotonin A

Due to the moderate anti-tumour activity of luotonin A, and the promise of discovering greater biological activity from analogues, there have been a steady growing number of reported procedures described for total synthesis or analogues of luotonin A. The most efficient of these methods utilizes the Kametani's amide condensation,[63] by reacting the appropriate pyrroloquinolines with anthranilates. This method has been used to create the broadest range of luotonin A analogues (Scheme 2).[64]



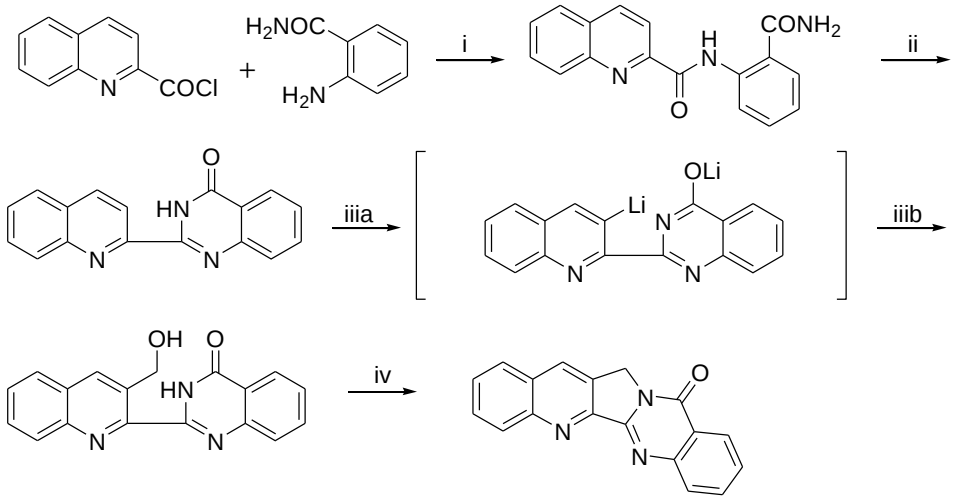
Scheme 2 General application of Kametani's amide condensation in luotonin A synthesis.

The biosynthesis of luotonin A was proposed by Ma *et al*., comprising of components of vasicinone and anthranilic acid.[45] This postulation was supported by the findings of Kelly *et al.* (Scheme 3),[65a] and several months later by Ma *et al.*,[65b] where luotonin A was prepared using a Friedländer condensation of vasicinone and anthranilamide, both procedures gave moderate yields (20% over 2 steps,[65a] and 23.5% over 3 steps[65b]). To date, no reports of feeding studies using isotope labelled precursors have been performed to confirm these inferences.



Scheme 3 Preparation of luotonin A using the Friedländer condensation by Kelly *et al.*[65a]

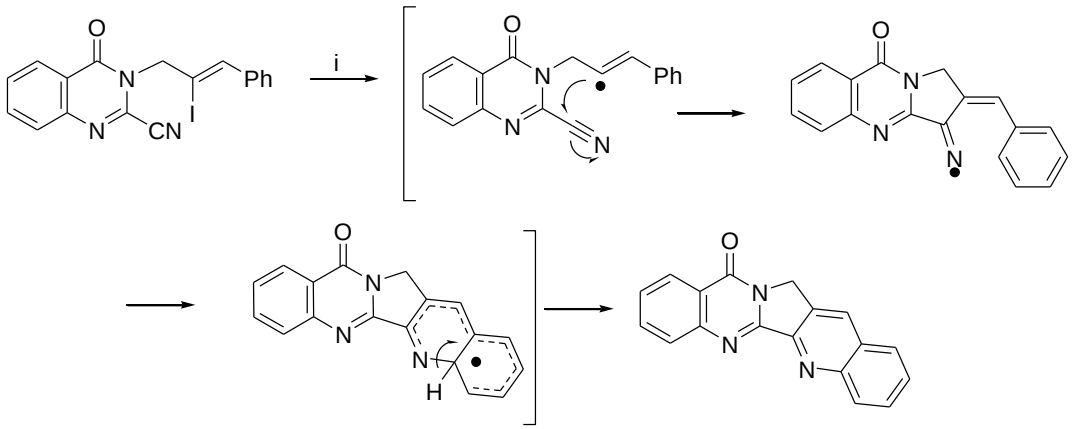
One of the best yielding methods reported would be the procedure by Mhaske and Argade (total yield 77% in 4 steps) from anthranilamide and quinoline-2-carbonyl chloride.[66] This method utilized the Mitsunobu reaction in the last step to form the C-ring (Scheme 4).



Scheme 4 Mhaske and Argade's synthesis of luotonin A.[66] *Reagents and conditions:* i) Et_3N, THF, rt, 3 h (96%). ii) 5% aq. KOH, EtOH, reflux, 5 min (98%). iiia) mesityllithium, -78 °C, 30 min to -20 °C; iiib) HCHO in THF, -20 °C, satd. aq. NH_4Cl (86%). iv) PPh_3, DEAD, THF, rt, 1 h (95%).

A review by Ma, Hano and Nomura covers the total synthetic methods for luotonin A from its discovery in 1997 to early 2005, as well as insights of biological activity of luotonin A and analogues relating to camptothecin.[67] Since this review there have been a further dozen strategies for total synthesis,[68] including the procedure that is described as part of this thesis.[68g]

Of this group of methods developed after above mentioned review,[67] the procedure by Bowman *et al.* reports a method where a radical cascade was used to create luotonin A.[68a] This procedure was a new approach, but involved several low yielding steps to achieve the desired intermediate for radical cyclization (Scheme 5).



Scheme 5 Radical cascade cyclization by Bowman.[68a] *Reagents and conditions:* i) $(Me_3Sn)_2$, *t*-BuPh, *hv*.

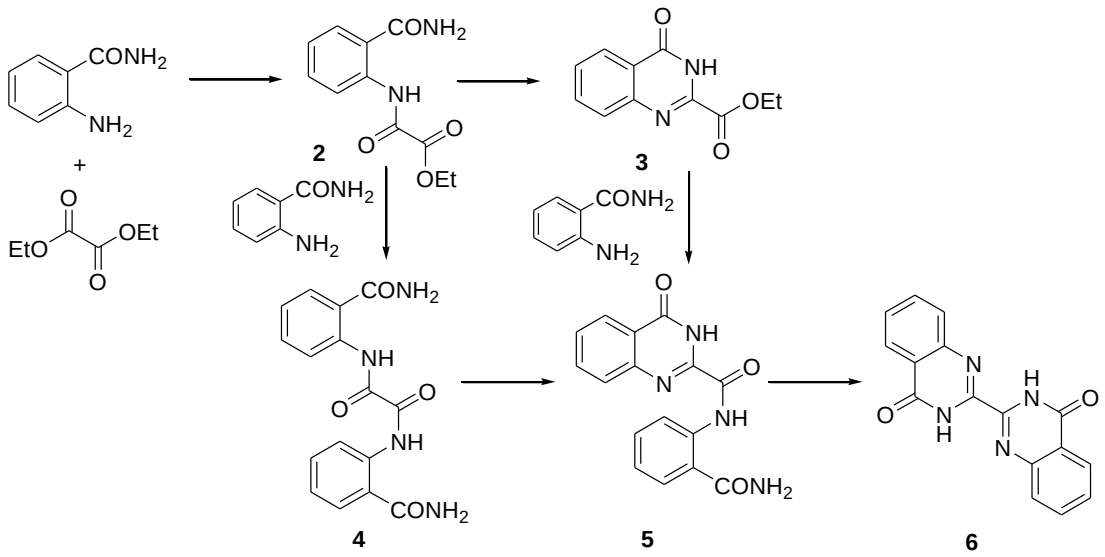
2.4 Investigations of Luotonin A and Pyrroloquinazolinones

The aim of this project can be summarized as: From simple starting materials provide an efficient and new method for the total synthesis of luotonin A (**1**).

2.4.1 Preparation of the quinazolinone core

The reaction of diethyl oxalate with anthranilamide was found to give a series of compounds, the first intermediate being ethyl (2-carbamoylphenylcarbamoyl)formate (**2**). Using a three fold excess of diethyl oxalate, at reflux for several hours, was found to be optimal for the preparation of ethyl 4-oxo-3,4-dihydroquinazoline-2-carboxylate (**3**) (92%). Analysis of the mother liquor by TLC and LCMS showed minor side-products, presumed to be structures proposed as **4**-**6**, but upon recrystallization in ethanol **3** was found to be pure (Scheme 6). When the reaction was performed in DMF or DMA with less than 3 equivalents of diethyl oxalate, larger proportions of **2** and **4**-**6** could be observed, along with **3** as the major product.

Another procedure has been described for the formation of **3** that utilizes sodium ethoxide in ethanol with the same starting components as in scheme 6.[69] This procedure has many desirable qualities for larger scale production; shorter times of reaction, less waste of reagent and near quantitative yields were reported, with no indications of compounds **4**-**6**.

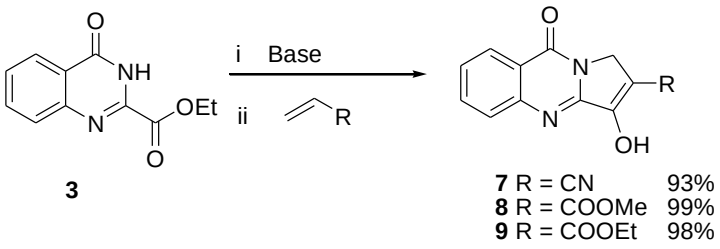


Scheme 6 Components of thermal condensation of anthranilamide and diethyl oxalate. *Optimal conditions for 4-oxo-3,4-dihydroquinazoline-2-carboxylate* (**3**): Anthranilamide (melt), diethyl oxalate (3 eq), reflux 8 h (92%).

Formation of **2**, in its own right, has been previously reported using ethyloxalyl chloride with anthranilamide.[70] Compound **2** can easily be converted to **3** by either of the methods mentioned above.

2.4.2 Studies of 9-oxo-pyrrolo[2,1-*b*]-quinazolinones

Deprotonation of **3**, with either *t*-BuOK or NaH, in DMF, and subsequent reaction with acrylonitrile was found to proceed efficiently. Workup with aqueous acid gave the tricyclic product 1,9-dihydro-3-hydroxy-9-oxo-pyrrolo[2,1-*b*]-quinazoline-carbonitrile (**7**). The use of methyl acrylate and ethyl acrylate under the described conditions afforded the corresponding methyl (**8**) and ethyl (**9**) esters in good yield (Scheme 7). A limitation to this procedure, thus far, occurred when the reaction conditions were tested using acrolein, where no product was obtained. The product of the reaction using methyl acrylate, **8**, was found to have poor solubility, compared to either **7** or **9** which were easier to recrystallize from ethanol.



Scheme 7 Preparation of pyrroloquinazolinones **7**-**9** using vinyl reactants. *Reagents and conditions:* i) *t*-BuOK/NaH, DMF, 60 °C, 30 min; ii) vinyl reactant, 1 h, 60 °C; H^+/H_2O workup.

To confirm the structure of these products a 2D-NMR study (HMBC) was performed on compound **7**. The resulting spectrum indicated correlations (J_3 coupling) between the pyrrole CH_2 singlet to both the quinazolinone carbonyl and the quaternary carbon of the enol, confirming the structure of **7** as the linear cyclized form, rather than the alternately proposed structure 1,5-dihydro-3-hydroxy-5-oxopyrrolo[1,2-*a*]quinazoline-2-carbonitrile (**10**) (Figure 10).

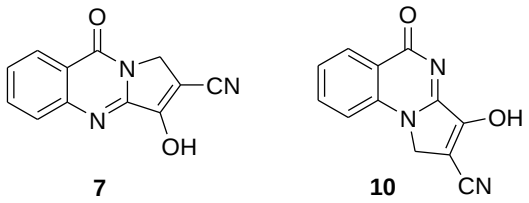
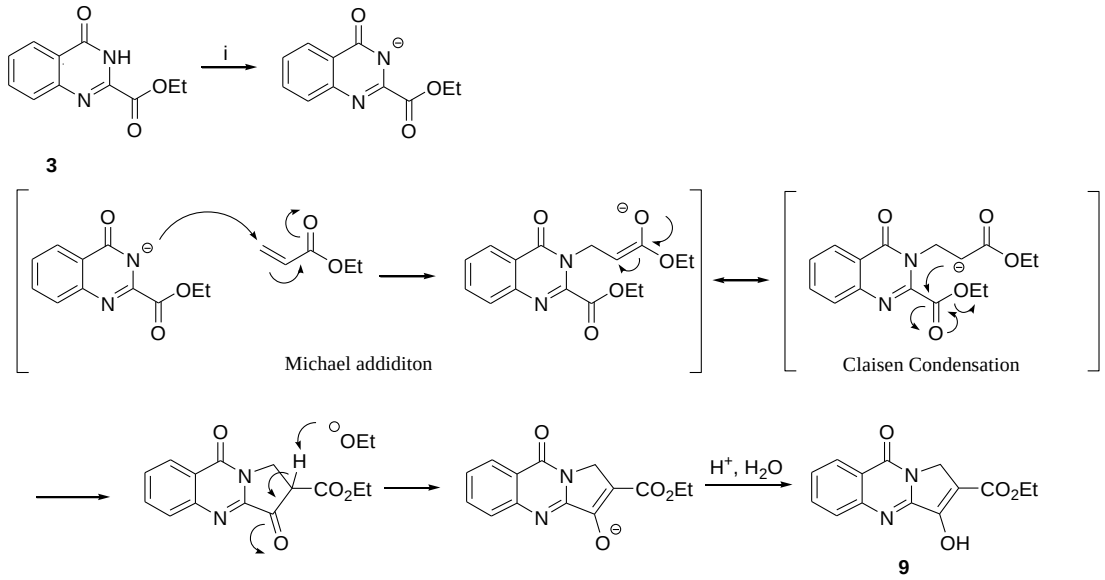


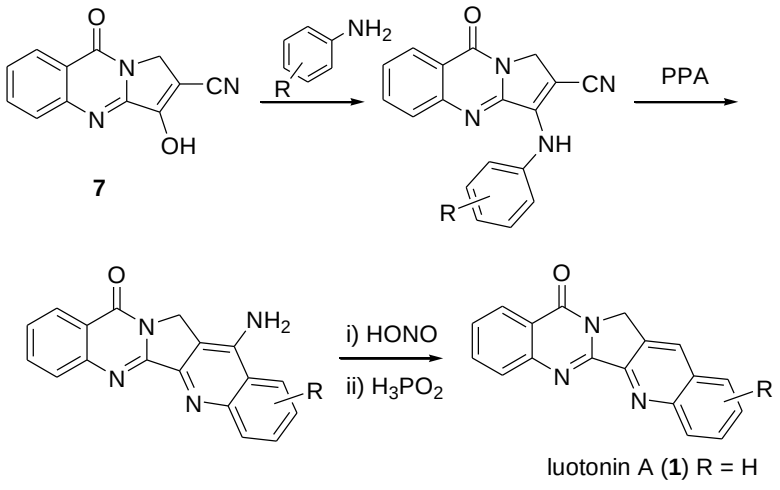
Figure 10 Possible structures from the reaction between **3** and acrylonitrile.

The proposed mechanism of this reaction is suggested to comprise of two steps, after deprotonation of **3** in the 3-position. Firstly a Michael addition of the vinyl reactant with the deprotonated quinazolinone **3** occurs, followed by a Claisen condensation of the ethyl ester, creating the pyrrole. Isolation of the enol was achieved by workup with aqueous hydrochloric acid (Scheme 8).



Scheme 8 Proposed mechanism for the formation of the pyrroloquinazolinones, an example using ethyl acrylate as the vinyl reactant.

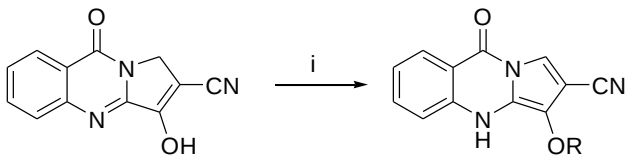
The initial design for the preparation of luotonin A, was to substitute the pyrroloquinazolinones **7**-**9** with various anilines generating a small library of compounds that could be condensed using PPA to leading to substitutions on the A ring, and a site for further transformation in the 14-position (B ring). Reduction of the aminoquinoline portion to **1** was envisioned through dediazotisation, forming the diazonium salt, and reduction with hypophosphorus acid (H_3PO_2) (Scheme 9).[71]



Scheme 9 Proposed synthetic pathway for luotonin A.

Attempts to substitute the enol of compounds **7**-**9** with ammonia, aniline, or aniline derivatives failed to give any reaction. This was considered an unusual result as one would expect a condensation reaction from either the enol, or the esters of **8** or **9** to occur. This failure to substitute with amines may be due to the formation of salts between the amine and the enol. It was later discovered that decomposition of **8** could be achieved by heating in DMSO giving an intractable mixture of products. Chlorination attempts directed at the enol, using oxalyl chloride, $POCl_3$ or $SOCl_2$ resulted in the formation of complex, and intractable mixtures. Limited success was achieved with the use of an acetyl protecting group (using Ac_2O or AcCl) where the initial reaction afforded a product visible on TLC, but quickly decomposed to an intractable compound upon concentration under vacuum, and exposure to air on the TLC plate. Similar substances were formed with the application of BOC protecting strategies, also giving these intractable mixtures. At this point we had affectionately coined the phrase regarding these unknown products as the "Blue stuff" due to colour of the final product for this group of compounds being a deep blue. This change in colour was attributed to the possible formation of dimers or an auto-oxidation process, but was not followed through with as it did not give the desired compound. Another reason why these compounds were not characterized further was due to problems with solubility, making any attempts for purification a futile process.

Further progress, with the substituted reaction products of **7** was achieved with the use of tosyl (**11**) and mesyl (**12**) protecting groups. These compounds were isolated but were found to have a short shelf life, leading to the previously mentioned (blue stuff) intractable mixtures (Scheme 10).

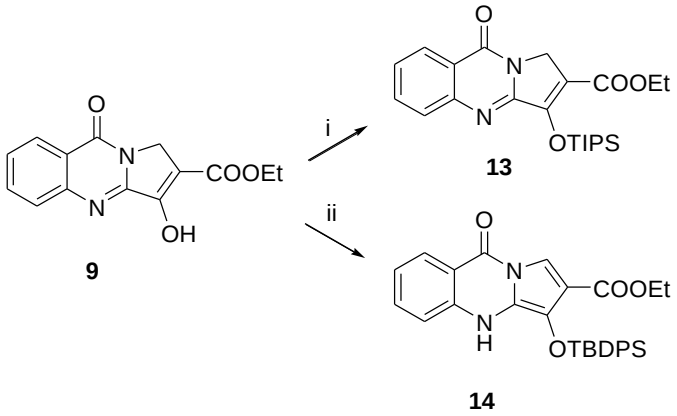


7

11 R = Ts 61%
12 R = Ms 68%

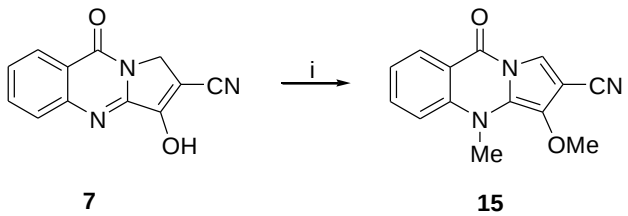
Scheme 10 *Reagents and conditions:* i) DMF, TsCl/MsCl, rt, 1 h.

A breakthrough with regard to stable isolable compounds was found when the silylating reagents, TIPSCl and TBDPSCl, were reacted with **9**, generating the *O*-silyl compounds **13** and **14** (Scheme 11). These stable compounds led to the discovery of a tautomeric rearrangement when the reaction was heated, as was the case for compound **14**. No longer did the signature singlet CH_2 peak appear in the 5.5-4.5 ppm region but an additional aromatic singlet and an exchangeable, broad NH signal were now present in the ^1H NMR spectra. Attempts to add protecting groups such as acetyl or BOC to react the 1*H*-position of **13** or **14** resulted in the complex and intractable mixtures similar to the reactions mentioned previously for these protecting strategies (more blue stuff).



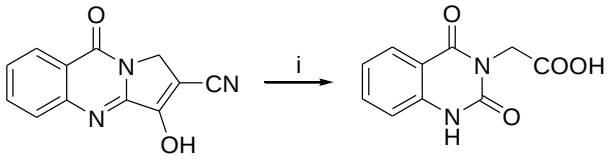
Scheme 11 Silylations of **9**. *Reagents and conditions:* i) TIPSCl, DIPEA, THF, rt, 1 h (87%). ii) TBDPSCl, DIPEA, THF, reflux, 24 h (73%).

When compound **7** was reacted with excess methyl iodide the resulting methylated product **15** was formed (Scheme 12). The same tautomeric rearrangement was observed, now with *O*- and *N*-methyl substitutions. Attempts to benzylate **7**, using similar conditions for the methylations resulted in a mixture of products which showed some indications of being the desired compound, by NMR, but as a mixture and was not able to be purified.



Scheme 12 *Reagents and conditions:* THF, MeI (excess), K_2CO_3, rt, 4 h (72%).

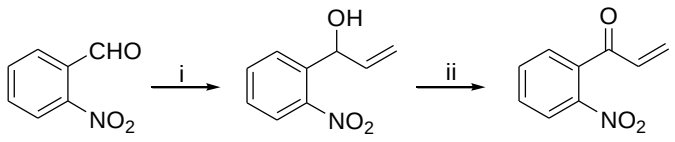
Finally, reacting **7** with hydrogen peroxide (30% solution) gave a previously described compound, 1,4-dihydro-2,4-dioxo-3(2*H*)-quinazoline acetic acid (**16**) (Scheme 13).[72] Upon establishing the structure of **16**, the logical assumption was that **7** had undergone a series of decarboxylations, giving further indications as to the reactive nature of these pyrroloquinazolinone compounds.



7 **16**

Scheme 13 Oxidative decarboxylation of **7** in 30% hydrogen peroxide solution. *Reagents and conditions:* i) H_2O_2 (30%), reflux, 4 h (73%).

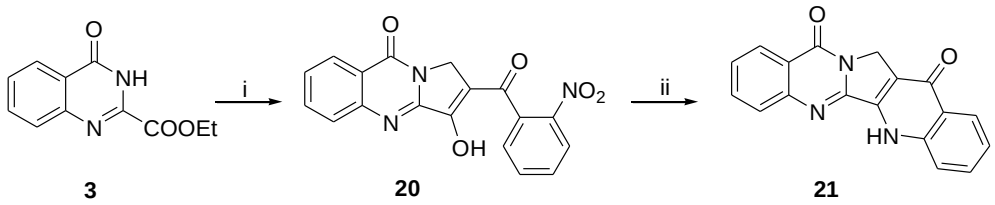
After establishing that the reactions of the pyrroloquinazolinones were not leading towards the target molecule, luotonin A, the strategy was revised, and a vinyl reactant that already contained the desired components was selected, thus temporarily circumventing the problems of substitution at the enol carbon. The proposed compound of choice, for this particular case, was the vinyl ketone 1-(2-nitrophenyl)prop-2-en-1-one (**17**). This was prepared following Danishefsky's method, reacting vinylmagnesium bromide with 2-nitrobenzaldehyde (**18**).[73] Eventually, ideal preparative scale conditions were established to this protocol with the use of the entire 100 ml (1.0 M solution in THF) of vinylmagnesium bromide, in one reaction via cannula, under argon. The transfer was performed slowly, and with careful monitoring of the temperature, to a solution of **18** in THF at -78 °C. The reaction proceeded flawlessly and was complete within 1 hour, giving the vinyl alcohol (**19**), which was efficiently oxidized to the ketone (**17**) using the Jones reagent (Scheme 14).[74] Uncannily, the procedure described by Daniskefsky's group was devised for the use in the total synthesis of camptothecin, but not in the same way we had intended its use.



18 **19** **17**

Scheme 14 Preparation of 1-(2-nitrophenyl)prop-2-en-1-one. *Reagents and conditions:* i) vinylmagnesium bromide, THF, -78 °C. ii) CrO_3, H_2SO_4, acetone (86%, 2 steps).

Addition of the vinyl reactant, **17**, to the deprotonated quinazolinone, **3**, produced the 3-(2-nitrophenyl)-pyrroloquinazolinomethyl ketone (**20**), which upon catalytic hydrogenation with Pd/C gave the cyclized quinolone compound 5*H*,12*H*-5,6,11*a*-triazadibenzo[*b*,*h*]fluoren-11,14-dione (**21**) (Scheme 15).

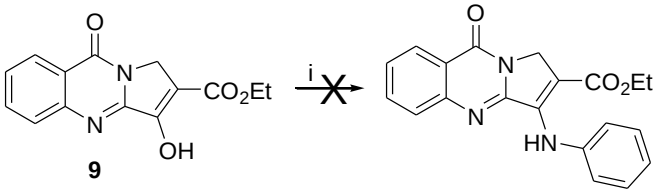


3 **20** **21**

Scheme 15 *Reagents and conditions:* i) *t*-BuOK, DMF, **17** (83%). ii) Pd/C 10%, H_2, EtOH (92%).

With a renewed sense of purpose, after the successful insertion of the aniline during catalytic hydrogenation (Scheme 15), attempts to react nitrobenzene with **9** under the above described conditions using catalytic hydrogenation failed to generate the desired product (Scheme 16), and only **9** and aniline were observed. This suggested, as with the aniline reactions, that the enol is more labile in **20** than the nitrile and ester substituted pyrroloquinazolinones (**7**-**9**). Strengthening the hypothesis, that a salt between the newly formed aniline and the enol of **7**-**9** is generated, blocking the reaction from substitution.

An alternative, and more speculative, suggestion could be that the reactivity of the hydroxyl group is more phenol-like in nature and is masked by an affinity of the adjacent nitrogen. This hydrogen bonding would stabilize the pyrrole as the observed tautomer in **7**-**9** and **20**, giving the characteristic 4-6 ppm CH_2 signal in the ^{1}H NMR spectra. In the cases of **7** and **9** when the proton is removed the alternative tautomer is established as observed with compounds **11**, **12**, **14**, and **15**. In the case of **20**, hydrogenation of the nitro group and the associated intermolecular cyclization, forming the quinolone **21**, the formation of the 6-membered ring and resonance stabilization could be the driving forces behind this reaction.

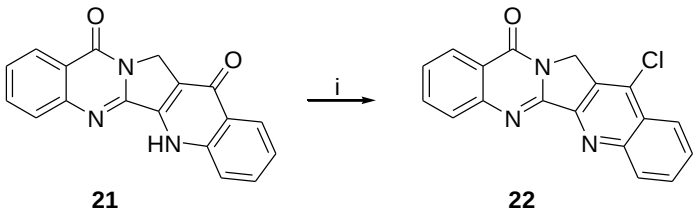


9

Scheme 16 *Reagents and conditions:* i) nitrobenzene, Pd/C 10%, H_2, EtOH.

2.4.3 Formation of the luotonin A core and diversification

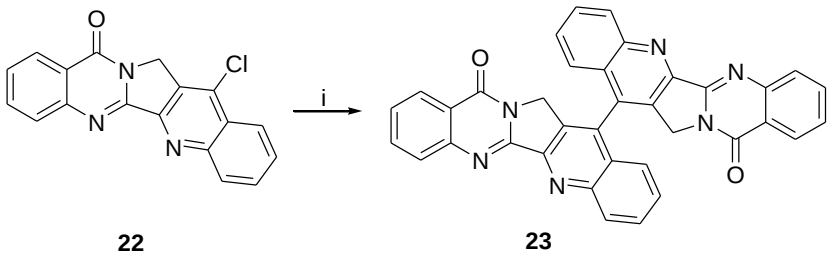
The key compound 14-chloroluotonin A (**22**) was then prepared by reaction of **21** in neat refluxing $POCl_3$ (Scheme 17). From here, the diversity of **22** was explored by performing substitutions at the chlorinated position.



21 **22**

Scheme 17 *Reagents and conditions:* i) $POCl_3$, reflux, 1 h (99%).

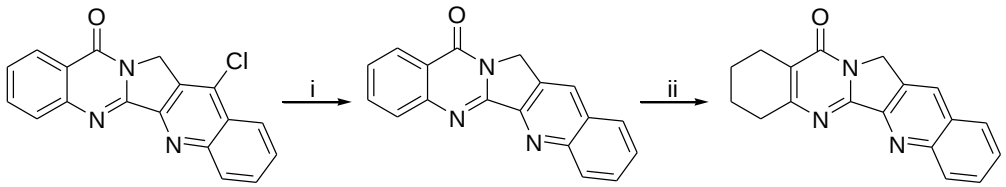
To achieve the natural product **1**, it was envisioned that reduction of **22** would produce the target compound. Catalytic hydrogenation was attempted first, with Pd/C in acetic acid. The product when analyzed by ^{1}H NMR gave a spectrum that indicated this compound was definitely not luotonin A (**1**). Several significant differences from luotonin A included; the absence of the final aromatic hydrogen; an intricate series of couplings in the aromatic region; and two sets of peaks between 5.5 to 4.5 ppm, where a pair of coupled signals were detected. This was also very different from the spectrum given by **22**, where only a singlet CH_2 signal was given at 5.3 ppm. The ^{13}C NMR APT experiment produced the same type of carbon signals as for **22**, but the shifts were further upfield. This conundrum was quickly rectified upon mass analysis, giving a mass of 569 [M+H], and the structure proposed as 14,14′-bisluotonin A (**23**). This was an unexpected result, but upon further consideration it was apparent that a coupling reaction had occurred, the reaction was believed to be a Wurtz-type coupling.[75] Typically the Wurtz reaction requires sodium as a catalyst for it to generate biphenyls, but the palladium used in the reaction must have caused a coupling process to occur generating the dimeric structure (Scheme 18).



22 **23**

Scheme 18 *Reagents and conditions:* i) Pd/C 10%, AcOH, 40 °C, 3 h (100%).

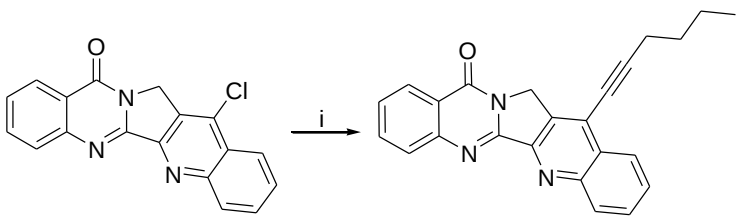
Reduction of **22** with Raney Nickel in dioxane was found to produce the target molecule **1**, with careful monitoring of the reaction. It was found that after 1 hour at room temperature the reaction had achieved the desired product. If the reaction was left for several hours, or heated to reflux, the hydrogenated compound 7,8,9,10-tetrahydroluotonin A (**24**) was exclusively obtained (Scheme 19). This compound was previously reported using a different method by Hecht *et al.*, using the numbering system of camptothecin, and accordingly named 16,17,18,19-tetrahydroluotonin A, with a yield of 6%.[51]



22 **1** **24**

Scheme 19 *Reagents and conditions:* i**)** Raney nickel, dioxane, rt, 1 h (84%). ii) Raney nickel, dioxane, reflux, 1 h (94%), or rt, >4 h (92%).

With the use of a copper free Sonogashira reaction,[76] it was possible to introduce hexyne to **22** forming 14-(hex-15-ynyl)luotonin A (**25**) with the use of palladium acetate, potassium carbonate, and *rac*-BINAP (Scheme 20). This reaction was performed, in part, to show that palladium acetate would not generate **23**, but also to increase the synthetic diversity of the substrate. Addition of alkynyl groups can be a useful strategy in synthetic development, as it allows for a range of reactions that introduces various functionality to a molecule. Examples include; carbonyl groups (formation of the ketone or carboxylic acid); selective generation of the *cis* or *trans* alkenes; transformation to the alkane.[77] Unfortunately, time did not permit further exploration of the reactions mentioned.

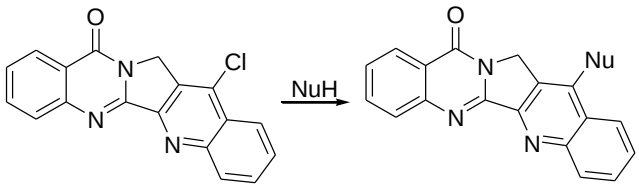


22 **25**

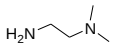
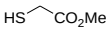
Scheme 20 *Reagents and conditions:* i) $Pd(OAc)_2$, *rac*-BINAP, K_2CO_3, benzene, reflux, 18 h (87%).

A small group of substitutions were also performed using **22** as the starting substrate. Excess *N*,*N*-dimethylpropylamine or piperidine were heated neat with **22** to give the respective 14-substituted derivatives **26** and **27**. While it was found that benzylamine or methyl mercaptoacetate when reacted with **22**, the use of DMF and K_2CO_3 gave the best results producing **28** and **29**, respectively (Table 1).

Table 1 Nucleophilic substitutions of 14-chloroluotonin A.



22 **26-29**

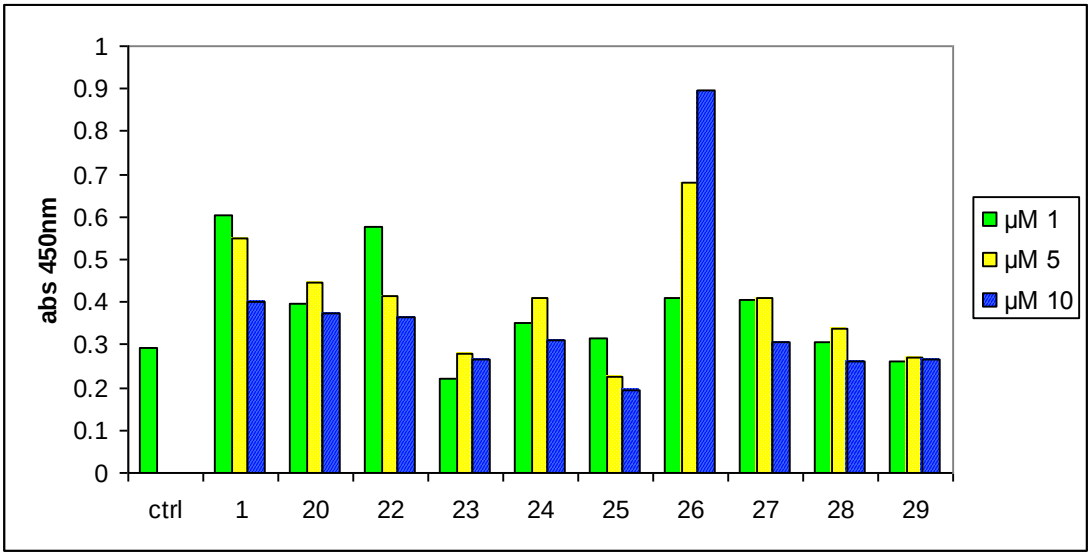
NuH	Conditions	Product	Yield (%)
	Neat, excess amine, sealed tube 145 °C, 2 h	**26**	81
	Neat, excess amine, reflux, 4 h	**27**	79
	DMF, K_2CO_3, reflux, 18 h	**28**	87
	DMF, K_2CO_3, reflux, 18 h	**29**	86

2.4.4 The M30 Assay: A quick biological test for apoptosis

An M30-ELISA was conducted on this small library of compounds (**1**, **20**, **22**-**29**), which is a useful screen for identifying pro-drug candidates for cell apoptosis (Figure 11). This service was kindly performed by the group of Professor Stig Linder (Cancercentrum Karolinska). This assay measures the release of cytokeratin-18 after cleavage at the aspartic acid residue 396 (CK18Asp396), which is expressed in many carcinoma forms after apoptosis.[78] The M30 antibody recognizes the CK18Asp396 neo-epitope exposed after cleavage by capsases, a process of apoptosis. The acronym ELISA stands for enzyme-linked immunosorbent assay which means the M30 antibody, acting as an indicator, interacts with the CK18Asp396 fragments which are captured at the terminal end (the other end of the M30 antibody) by the linked enzyme (antigen).

This study was performed to test the potential of these compounds as chemotherapeutic agents through their ability to initiate apoptosis; DMSO was used as the control (Figure 10). No other studies were conducted on these compounds, so any information regarding topoisomerase I inhibition cannot be directly drawn from the results of the M30 assay. Compound **26** was found to give the highest response of all compounds, including luotonin A. Whether this effect was due to improved solubility of **26** is unknown, but it should be noted that irinotecan and topotecan both have similar alkyl substituents in the A and B rings, which are contributing factors to their activities. The other luotonin A based studies have tested the activity of their compounds using the leukaemia P-388,[45] and colon H-460 carcinoma cell lines,[55b] as well as *Saccharomyces cerevisiae* (yeast), and using CPT as a standard from which their results were established.[52,55a]

Figure 11 M30 Apoptosis response to luotonin analogues



3 Studies of cephalandoles A and B

In 2006 the structures of cephalandole A (**30**) and B (**31**) were claimed, by Wu *et al.*, as isolates from *Cephalantheropsis gracilis*, a Taiwanese orchid.[79] The crude extract of this plant displayed activity against breast (MCF-7, IC_{50} 7.57 µM), lung (NCI-H460, IC_{50} 7.8 µM) and CNS (SF-268, IC_{50} 12.2 µM) carcinoma cell lines. The newly proposed structures were given the names cephalandoles A-C (**30**-**32**), and cephalinones A-D (**33**-**36**) (Figure 12), derived from the name of the plant genus. Eight previously described compounds were also discovered including derivatives of indole, as well as the compounds indigo and isatin. The previously unsynthesized structures of **30** (2-(1*H*-Indol-3-yl)-4*H*(3,1)-benzoxazinone) and **31** (methyl 2-(1*H*-indole-3-carboxamido)benzoate) were considered possible synthetic precursors to quinazolinones, and as such attracted our interest as suitable targets for preparation.

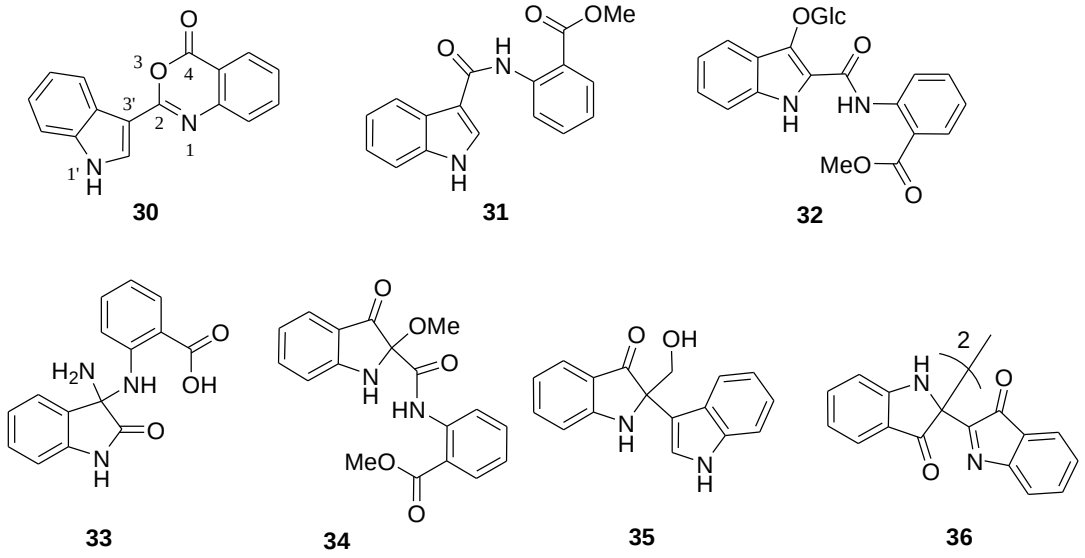
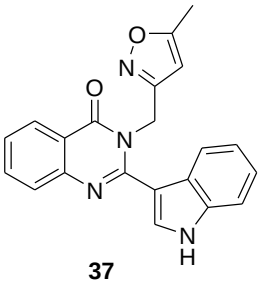


Figure 12 Structures proposed as cephalandoles A-C, and cephalinones A-D by Wu *et al.*[79]

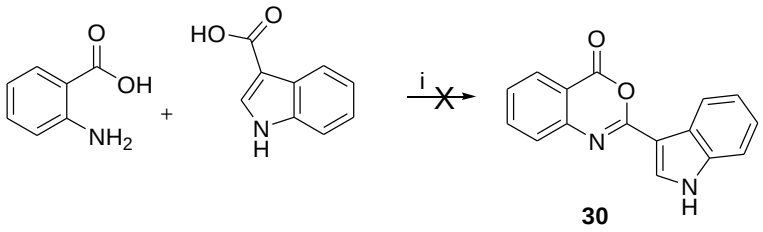
Previous to the report of the cephalandoles, a one-pot reaction for the preparation of 2-(1*H*-indol-3-yl)-3-((5-methylisoxazol-3-yl)methyl)-4(3*H*)-quinazolinone (**37**) was reported, using a combinatorial chemistry approach, creating a series of 2,3-substituted quinazolinones (Figure 13).[80] The procedure for the preparation of **37** described a two-step approach starting from anthranilic acid, and various substituted carboxylic acids (including indole-3-carboxylic acid), in pyridine with a slight excess of triphenyl phosphite, under microwave conditions. The respective amines were added after the initial reaction, and were reheated to give the final products.



37

Figure 13 A reported compound 2-(1*H*-indol-3-yl)-3-((5-methylisoxazol-3-yl)methyl)-4(3*H*)-quinazolinone similar to 2-(1*H*-indol-3-yl)-4*H*(3,1)-benzoxazinone.

Compound **30** was never isolated as a reaction intermediate, as compounds were directly converted to the corresponding 4(3*H*)-quinazolinones, since the amines were subsequently added. Attempts to obtain the benzoxazinone directly, from anthranilic acid and indole-3-carboxylic acid with triphenyl phosphite in pyridine, using the described procedure failed (Scheme 21), so alternative routes were investigated.



30

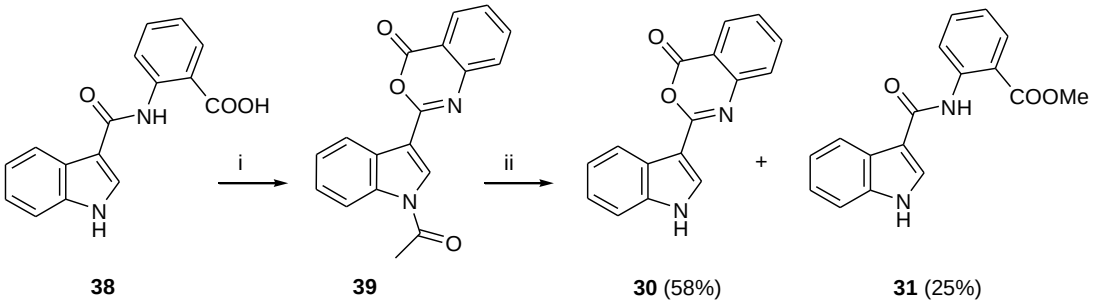
Scheme 21 Microwave assisted trial to directly achieve 2-(1*H*-indol-3-yl)-4*H*(3,1)-benzoxazinone. *Reagents and conditions:* i) $P(OPh)_3$, pyridine, MW, 150 °C, 10 min.

The proposed structure for **30** was reported to have a ^{13}C NMR value at 153 ppm corresponding to the carbonyl of the benzoxazinone at C-4 (Figure 12). This was considered a low value for the 4*H*(3,1)-benzoxazinone structures, which would normally give a spectral value closer to 160 ppm. This low chemical shift value for such bicyclic structures at the carbonyl C-4, for either 4*H*(3,1)-benzoxazinones or 4(3*H*)-quinazolinones, usually indicates the presence of groups at C-2 that are strongly shielding, such as CF_3.[81]

3.1.1 Preparations of benzoxazinones and cephalandole B

The first approach for the preparation of **30** and **31** involved treating 2-(1*H*-indol-3-ylcarbonyl)aminobenzoic acid (**38**)[82] in acetic anhydride, giving the *N*-acetyl analogue of **30**, 2-(*N*-acetyl-indol-3-yl)-4*H*(3,1)-benzoxazinone (**39**). The ^{13}C NMR spectra for **39** gave a signal at 158.7 ppm corresponding to the carbonyl C-4. This result by itself

did not disprove the reported structure of **30**, but further confirmed the trend to what was expected for the ^{13}C NMR C-4 signal of such molecules. Hydrolysis of the *N*-acetyl group with sodium hydroxide in aqueous methanol produced a mixture of **30** and compound **31** in a ratio of approximately 7:3 (Scheme 22).

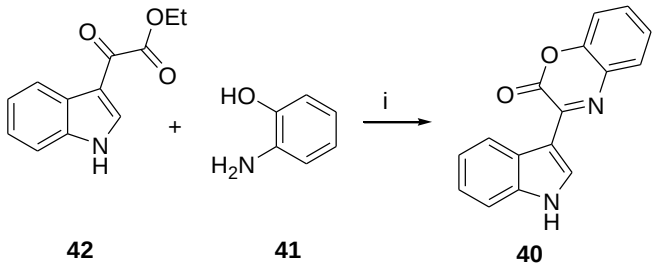


38 **39** **30** (58%) **31** (25%)

Scheme 22 *Reagents and conditions:* i) Ac_2O, neat, reflux, 2 h (87%). ii) NaOH, MeOH, H_2O.

With a sample of **30** now isolated, the ^{13}C NMR data showed that the C-4 carbonyl value was at 160.2 ppm, which did not agree with the previously reported value of 153 ppm.[79] This result prompted us to begin investigations as to the true structure of cephalandole A, as well as exploring other methods to exclusively prepare **30** and **31**.

A previously reported isomer of **30**, 3-(1*H*-indol-3-yl)-2*H*(1,4)-benzoxazinone (**40**), prepared by Moffett,[83] was considered a possible candidate as the true structure of cephalandole A. Unfortunately, the analytical data consisted of the results from elemental analysis, the physical description of "yellow crystals" and the melting point (246.5-247 °C). These were sufficient with the analytical techniques of the time, but obviously lacked NMR data to corroborate what was suspected. The significance of the physical description as yellow crystals, although not a quantitative analysis by modern scientific methods was our strongest lead, as this description was also used by Wu *et al*,[81] while the prepared substance **30** was an off-white powder. The procedure described the condensation of methyl 3-indolylglyoxalate and 2-aminophenol (**41**), at reflux, in DMF, and then recrystallized from ethanol to give **40** in a yield of 33%. Our attempt of this procedure gave similar yields from **41** and ethyl 3-indolylglyoxalate (**42**) (Scheme 23). The spectral data of **40** was an exact match with the reported values for cephalandole A.

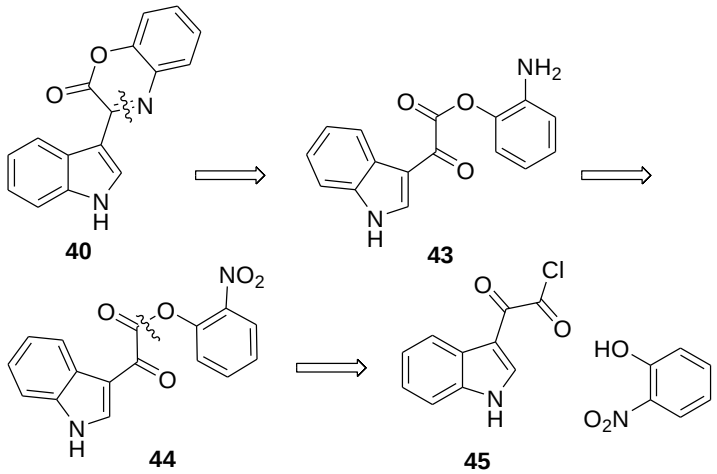


42 **41** **40**

Scheme 23 *Conditions and yields:* i) DMF, reflux, 6 h (40%).

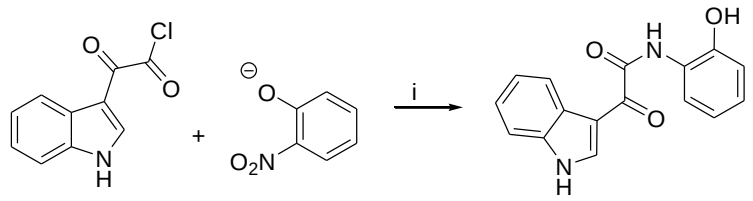
With the hope of improving the yield of **40** another similar route was conceived by retrosynthetic design. The condensation of 2-anilino 2-(1*H*-indol-3-yl)-2-oxoacetate (**43**) was considered a plausible compound from which the desired product could be obtained. The preparation of compound **43**, or as the intermediate, was proposed by reduction of 2-nitrophenyl 2-(1*H*-indol-3-yl)-2-oxoacetate (**44**), starting from 2-nitrophenol and α-oxo-(1*H*)-indole-3-acetyl chloride (**45**) (Scheme 24).

When **45** was prepared, from indole and oxalyl chloride, and heated with the sodium salt of 2-nitrophenol under various conditions no reaction took place, and only the starting materials were observed. This reaction showed that 2-nitrophenol, even when deprotonated, was not sufficiently nucleophilic to react with **45**. Although, the reaction mixture when reduced, using catalytic hydrogenation (10% Pd/C in DMF), the resulting product was found to be *N*-(2-hydroxyphenyl)-2-(1*H*-indol-3-yl)-2-oxoacetamide (**46**). This reaction indicated that when 2-aminophenol was generated in-situ, it was sufficiently nucleophilic to react with **45** (Scheme 25). These reactions led us to the conclusion that a 40% yield of **40**, from Moffet's one-pot method was sufficient as the envisioned procedure offered no apparent advantages over the existing method.



40 **43** **44** **45**

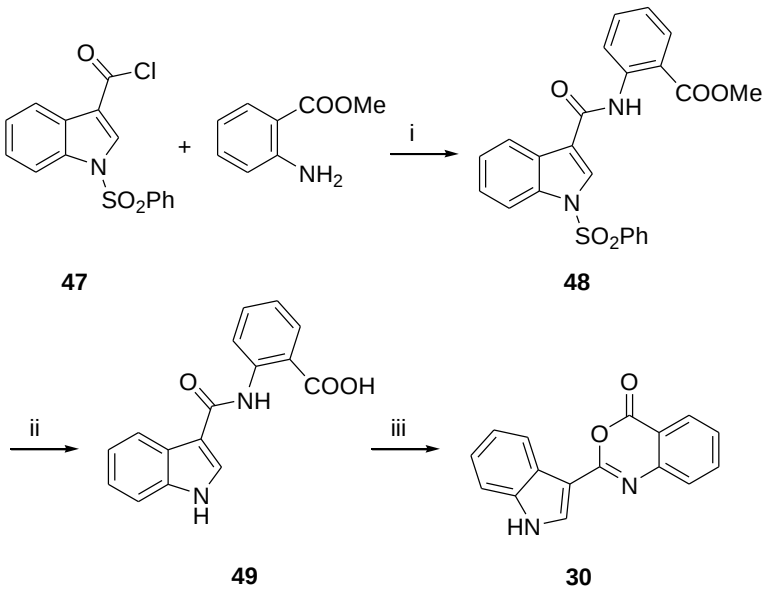
Scheme 24 Proposed retrosynthesis of **40**.



45 **46**

Scheme 25 *Reagents and conditions:* i) Pd/C 10%, H_2, DMF, 80 °C, 1 h (74%).

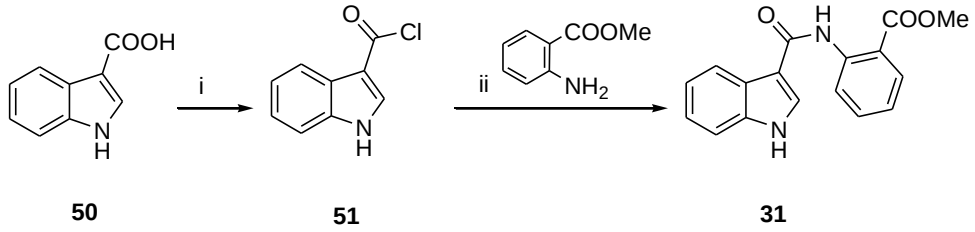
With the true structure of cephalandole A now established as 3-(1*H*-indol-3-yl)-2*H*(1,4)-benzoxazinone (**40**), the focus returned to investigate methods for the exclusive preparations of **30** and **31**. Applying Gribble's procedures for the preparation of *N*-phenylsulfonyl-indol-3-yl-carboxylic acid chloride (**47**) from indole,[84,85] and reacting this with methyl anthranilate in-situ produced methyl 2-(*N*-phenylsulfonyl-indole-3-carboxyamido)benzoate (**48**). Several attempts to obtain **31** selectively by hydrolysis of the *N*-phenylsulfonyl group failed, and 2-(indole-3-carboxyamido)benzoic acid (**49**) was repeatedly obtained under various basic conditions; aqueous hydroxide in methanol; K_2CO_3 in THF; or aqueous $NaHCO_3$. By heating **49** in $SOCl_2$ compound **30** was obtained exclusively (Scheme 26).



47 **48** **49** **30**

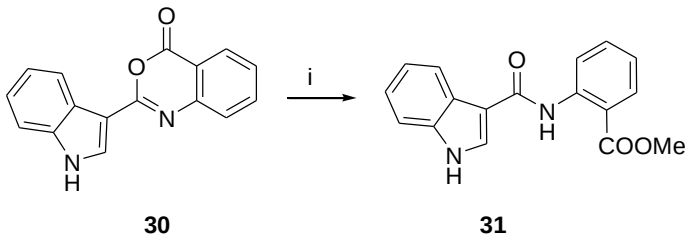
Scheme 26 *Reagents and conditions:* i) CH_2Cl_2, rt, 18 h (69%); ii) NaOH, CH_2Cl_2, MeOH, H_2O, 50 °C, 2 h (100%); iii) $SOCl_2$, neat, reflux, 2 h (70%).

The preparation of **31** was accomplished via a similar route where indole-3-carboxylic acid (**50**) was converted to the corresponding acid chloride (**51**) quantitatively.[86] This was achieved by first dissolving of **50** in CH_2Cl_2, and then treating with oxalyl chloride and a catalytic amount of DMF. Addition of **51** as a solution, in THF, to excess methyl anthranilate in THF produced the target compound **31** (Scheme 27).



Scheme 27 *Reagents and conditions:* i) $(COCl)_2$, CH_2Cl_2, DMF (cat.), rt, 2 h; ii) THF, rt, 18 h (58%, over 2 steps).

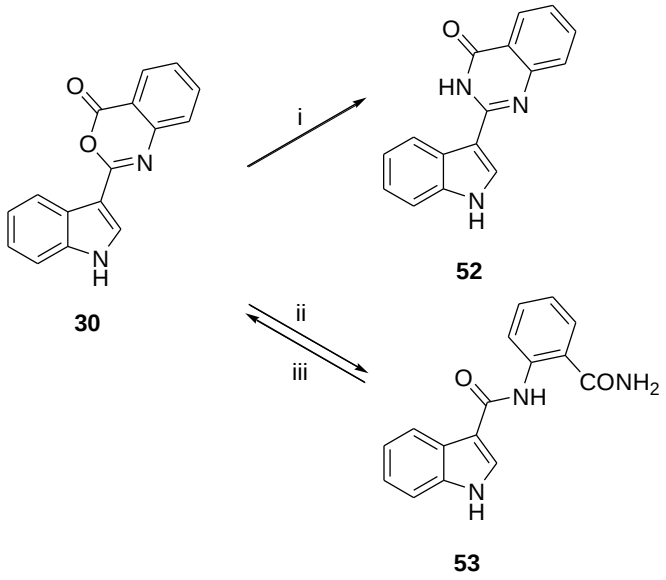
As it was now confirmed that the structure of cephalandole A was **40**, and not the previously reported structure **30**, and considering the conversion of **39** led to a mixture of **30** and **31**, see Scheme 23, the next logical step was to test whether compound **30** could be directly converted to **31**, by imitating the reported extraction process from Wu *et al.*.[79] When compound **30** was heated at reflux in methanol it was discovered that the ring opened derivative **31** was generated (Scheme 28). This result suggests that the reported structure of **31** is most likely an artefact of the extraction process and the true structure of cephalandole B might actually be **30**.



Scheme 28 *Reagents and conditions:* i) MeOH, reflux, 4 h (99%).

Further experiments of substituting the oxygen of the benzoxazinone of **30** were investigated to establish the conditions that would produce the corresponding 2-(indol-3-yl)-4(3*H*)-quinazolinone (**52**), as analogous structures have previously been described by Liu *et al.*[80] The previously unreported compound **52** was prepared from **30**, assisted by microwave heating in formamide. The ring opened compound, 2-[(1H-indol-3-ylcarbonyl)amino]benzamide (**53**), could be prepared by heating **30** in DMF with

ammonium acetate, which reverted back to **30** by heating in xylenes at reflux in the presence of a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH). This was proposed to occur as a result of thermal elimination of ammonia (Scheme 29).

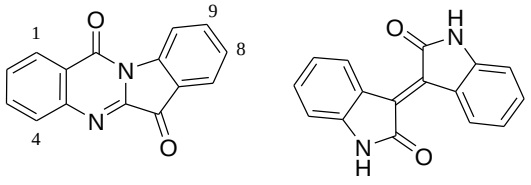


Scheme 29 *Reagents and conditions:* i) formamide (neat), 200 °C, MW, 10 min (71%); ii) NH_4OAc, DMF, 70 °C, 2 h (88%); iii) *p*-TsOH (cat.), xylenes, reflux, 24 h (88%).

4 Studies of Methoxyisatins and Hydroxytryptanthrins

Tryptanthrin is another compound containing a quinazolinone core, and has a history prior to the discovery of the quinazolines themselves. Reports of its preparation have been cited as far back as 1822 (Dumas),[87] preceding its discovery as a natural product by more than a century.[88] Tryptanthrin has re-emerged in several areas of research; in 1892 by Tafel,[89] and in 1915 by Friedländer and Roschdestwensky,[90] as a result of the synthetic dye industry, being a decomposition product of indigo (Figure 14).[91] It has also been discovered in several plant species,[92] and used as a traditional remedy for fungal infections.[93] Recently, tryptanthrin and its analogues have again attracted attention due to the discovery of biological activities in the areas of COX-2 (cyclooxgenase),[94] anti-bacterial (including anti-tubercular),[95] anti-inflammatory, anti-parasitic, in the cases of human African trypanosomiasis (*sleeping sickness*),[96] as well as anti-malarial.[97]

The recent discovery of 4,8,9-trihydroxytryptanthrin (**54**), the first tryptanthrin derived compound from the animal kingdom (*Ophiocoma riisei*), was given the name ophiuroidine (Figure 15).[98] No NMR spectral data for **54** could be given, due to issues with insolubility, so the trimethoxy derivative 4,8,9-trimethoxytryptanthrin (**55**) was prepared and characterized. The assignment of the natural product was inferred from these results. *Ophiocoma riisei* is from a group of echinoderms commonly known as brittle stars (Class Ophiuroidea). The most notable difference from typical starfish (Class *Asteroidea*) is the long, brittle looking arms that extend from the central discoid body. Other physiological differences separate this family (Fam. *Ophiocomadae*) from other echinoderms; one such example is the centralization of the reproductive organs to the disc in the *Ophicomadae*, while in the *Asteroidea* the gonads can be found extending into the arms.[99]



tryptanthrin indigo

Figure 14 Structures of tryptanthrin and indigo.

Previous reports of the preparation of tryptanthrin and analogues include; heating the isatin and isatoic anhydride components in pyridine;[88] in toluene with triethylamine;[100] or with the use of coupling reagents such as DCC (dicyclohexylcarbodiimide) or DIC (disopropylcarbodiimide).[88] Eguchi *et al.* have also described an intramolecular aza-Wittig reaction for the preparation of tryptanthrins starting from 2-azidobenzoyl chloride with either isatin or oxindole, but this procedure was found to give low yields.[101]

The strategy for the preparation of **54** was via the reverse pathway described by Utkina *et al.*, by first creating 4,8,9-trimethoxytryptanthrin (**55**), from 5,6-dimethoxyisatin (**56**) and 6-methoxyisatoic anhydride (**57**) (Figure 15). An established procedure for the preparation of **57** already existed and when repeated gave the desired isatoic anhydride.[102] The next step was the preparation of the isatin starting material.

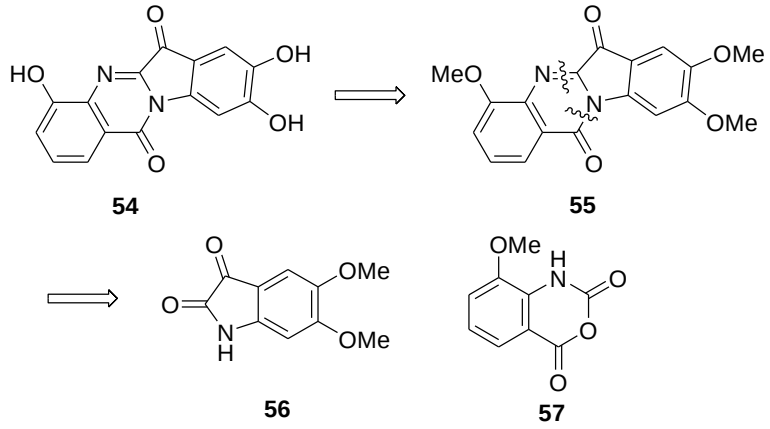


Figure 15 Retrosynthetic analysis of ophiuroidine.

4.1 Introduction to Isatin Synthesis

Isatins can be found in many plants and animals as an intermediate of various metabolic pathways. Several reviews effectively cover the range of different isatin analogues and methods over the years and procedures in greater detail than what is achievable through the scope of this thesis, so only a short summary of the main isatin procedures shall be given.[103]

Sandmeyer's procedure for the preparation of isatins via an isonitrosoacetanilide intermediate is by far the most versatile method.[104a] The typical procedure describes the preparation of an isonitrosoacetanilide, by adding in sequential order to a solution of chloral hydrate in water; 1) sodium sulfate; 2) the desired aniline substrate dissolved in

10:1 aqueous HCl solution; 3) a solution of hydroxylamine hydrochloride. The solution is then heated at reflux for several minutes, and upon cooling the crystalline product precipitates and can be collected.[104b] In the second step of this procedure condensation of the isonitrosoacetanilide in sulfuric acid under mild heating gives the isatin upon addition onto ice/water (Figure 16). A useful modification to this procedure is the inclusion of ethanol as a co-solvent, which improves the solubility of lipophilic substituents that would normally form tars.[105]

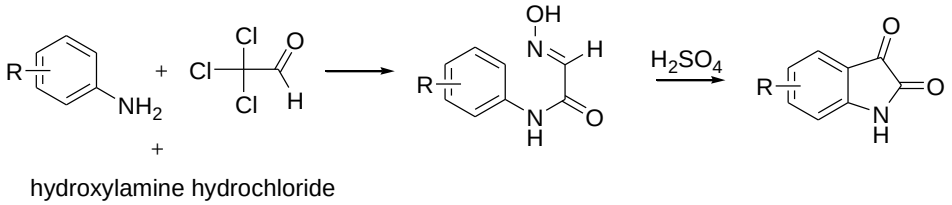


Figure 16 An example of Sandmeyer's isonitrosoacetanilide procedure for isatins.

Martinet's preparation of isatin involves the condensation of methyl or ethyl malonates with substituted anilines. Treatment with alkaline solutions in the presence of air generates the isatin (Figure 17).[106]

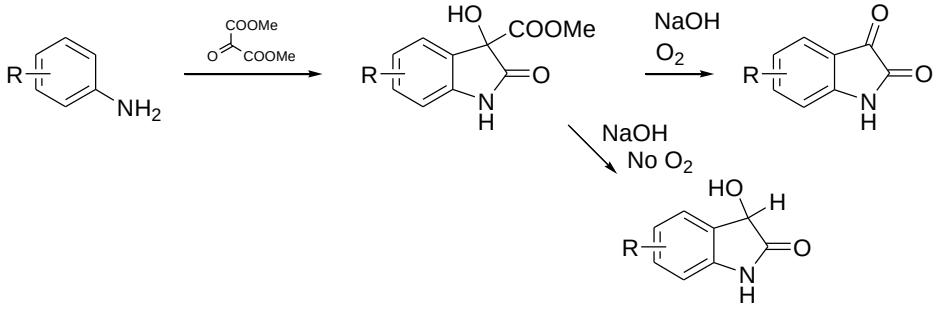


Figure 17 Martinet's isatin procedure.

Stollé's procedure for the preparation of isatins utilizes the reagent oxalyl chloride with hydrochloride salts of the respective anilines, with cyclization achieved with $AlCl_3$. Hydrolysis in water produces the isatin.[107] This method normally requires that the starting aniline is a secondary amine, so the resulting amide does not contain a proton ($R' \neq H$, Figure 18).

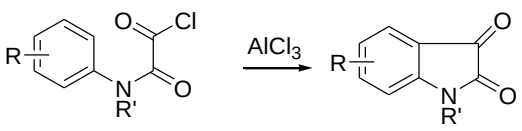


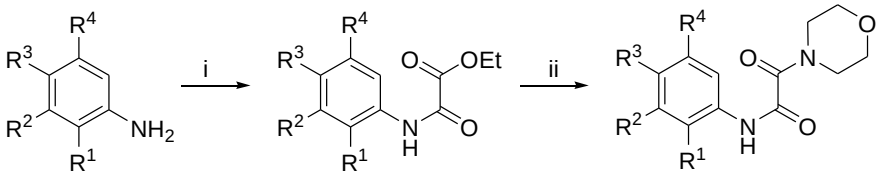
Figure 18 Stollé's isatin procedure.

4.2 Preparation of Methoxyisatins

Initial attempts to prepare 6-methoxyisatin (**58**) from *m*-anisidine using Sandmeyer's procedure failed to provide sufficient yields of product (~*ca.* 10 %), the same was found for the synthesis of 5,6-dimethoxisatin (**56**) from the corresponding dimethoxyaniline, so other avenues were investigated.

Inspiration struck when comparing papers by Meth-Cohn et al.,[108] of umpoled Vilsmeier reactions on *N*-substituted formanilides, to procedures by Bauer,[109] and Stollé, it was postulated that similar tertiary *N*-(methoxyphenyl)amido-2-oxoacetamides could generate isatins. Since aryl methoxy esters were being used in the reactant, the procedure had to exclude the use of $AlCl_3$, as this reagent is known to demethylate aryl methyl ethers to the corresponding phenols. [110]

Understanding that aryl methoxy substituents are strongly *para*- directing, the proposed approach utilized this feature. Reacting the respective anisidine, and poly-methoxyaniline compounds, with ethyloxalyl chloride the corresponding ethyl 2-(methoxyphenyl)amino-2-oxoacetates (**59a**-**e**) were prepared. Heating in neat morpholine generated the *N*-(methoxyphenyl)-2-morpholino-2-oxoacetamides (**60a**-**e**) in good yields over the two steps (Scheme 30). The use of a morpholino group was preferred over other amines, such as dimethyl or diethylamine, purely from the perspective of ease of substitution both displacing the ethyl ester and as an effective leaving group.



59a (98%)
b (99%)
c (93%)
d (99%)
e (95%)

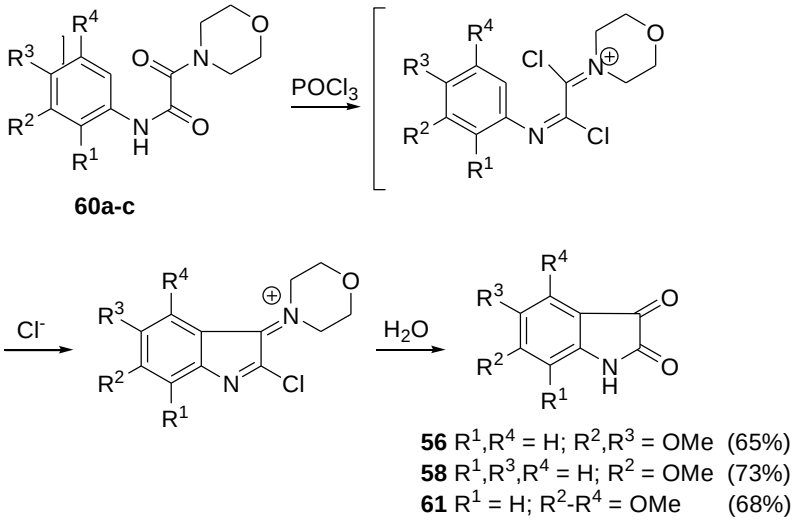
60a R^1,R^4 = H; R^2,R^3 = OMe (95%)
b R^1,R^3,R^4 = H; R^2 = OMe (93%)
c R^1 = H; R^2-R^4 = OMe (98%)
d R^1 = OMe; R^2-R^4 = H (90%)
e R^1,R^3 = OMe; R^2 = H (96%)

Scheme 30 *Reagents and conditions:* i) $EtO(CO)_2Cl$, diethyl ether, rt, 18 h; ii) morpholine, neat, reflux, 4 h.

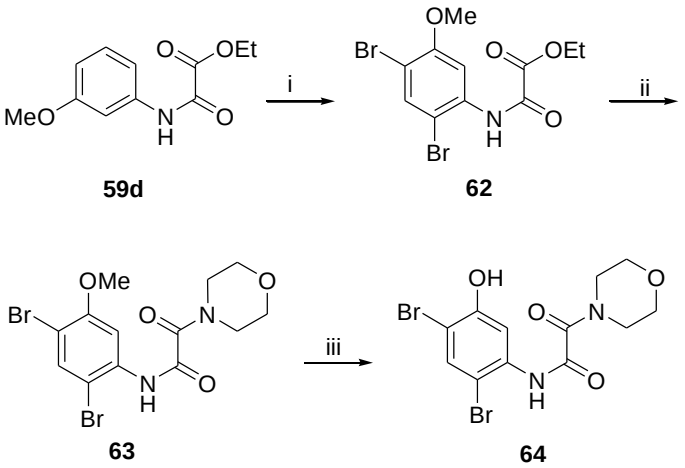
Reacting the *N*-(methoxyphenyl)-2-morpholino-2-oxoacetamides in neat $POCl_3$, created a Vilsmeier intermediate, and after quenching the reaction with ice water the respective methoxyisatins were obtained. In the case of the preparation of 6-methoxyisatin (**58**) the alternate isomer 4-methoxyisatin was not isolated, although this has been shown before by Sadler,[111] using Sandmeyer's isonitrosoacetanilide method.

The proposed mechanism can be considered a modification of the Stollé procedure, but uses the milder reagent $POCl_3$ (Scheme 31). In the cases of **60d** and **60e** the reaction failed to give the desired isatin as there was no *meta*-methoxy substituent on the aniline to guide the reaction in that direction. Observations by LCMS of the reaction products of **60d** and **60e** gave indications of polymeric compounds.

In an attempt to generate 4-methoxyisatin analogues, a strategy was conceived that ensured the exclusive formation of this isomer, by blocking the alternative site with bromine. Bromination of **59d** (ethyl 2-[2-(methoxyphenyl)amino]-2-oxoacete) with NBS (2 equivalents or more) produced the 2,4-dibromo derivative (**62**) which was then converted to the corresponding morpholino compound (**63**) in the same manner as described for Scheme 30 (Scheme 32).



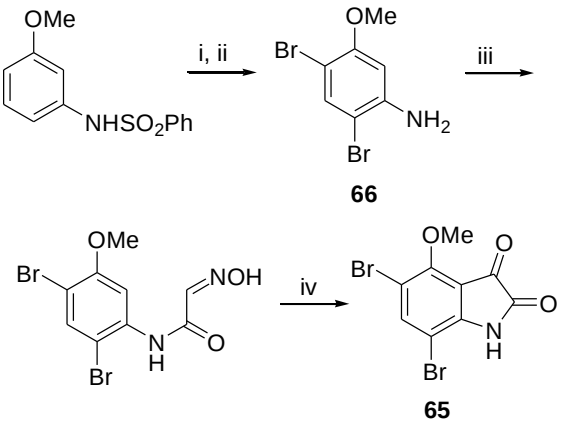
Scheme 31 *Reagents and conditions:* i) $POCl_3$, neat, 65 °C, 4 h.



Scheme 32 *Reagents and conditions:* i) NBS (>2eq.), MeCN, 90 °C, 1 h (98%); ii) morpholine, neat, reflux, 4 h (95%); iii) H_2SO_4, 100 °C, 4 h (85%).

When compound **63** was heated in $POCl_3$ no reaction was observed, but when **63** was heated in sulfuric acid it was found that demethylation had occurred producing the phenol derivative **64** (Scheme 32). This reaction clearly demonstrated the importance of the methoxy substituent in the position *para* to the site of insertion on the mexthoxyphenyl-2-morpholino-oxoacetanilides to drive the formation of these isatins.

As a final attempt for the preparation of the 5,7-dibromo-4-methoxyisatin (**65**) Sandmeyer's isonitrosoacetanilide method was again tried starting from 2,4-dibromo-5-methoxyaniline (**66**), this time with success. From *N*-phenylsulfonyl *m*-anisidine the same bromination procedure, as for the formation of **63** was used, forming *N*-phenylsulfonyl 2,4,-dibromo-5-methoxyaniline. The *N*-phenlsulfonyl group was then removed in aqueous base to give **66**, which was reacted using Sandmeyer's isonitrosoacetanilide procedure. After treatment with H_2SO_4, and worked up, the desired isatin **65** was obtained (Scheme 33).

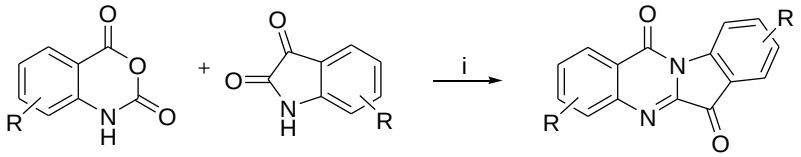


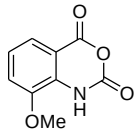
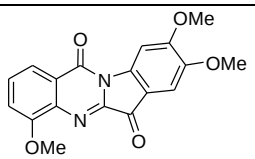
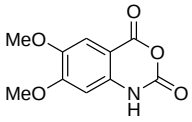
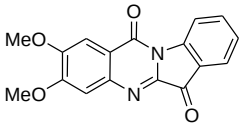
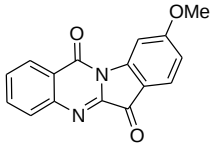
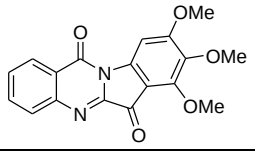
Scheme 33 *Reagents and conditions:* i) NBS (>2eq.), MeCN, 90 °C, 1 h (89%). ii) NaOH, MeOH, H_2O (98%); iii) Chloral hydrate, hydroxylamine hydrochloride, Na_2SO_4. iv) H_2SO_4 (40% over 2 steps).

4.3 Preparation of Methoxytryptanthrins

With the isatins now prepared, the condensation of **56** with **57** in xylenes with triethylamine was attempted, after 24 h only the starting materials were observed. So a literature procedure using the same conditions was attempted,[100] which reported the successful thermal condensation of isatin and 5,6-dimethoxyisatoic anhydride, but this also failed to produce 2,3-dimethoxyisatin (**67**). The same was observed when pyridine, at reflux, was used as the solvent. However, when the coupling reagent DIC was used, in conjunction with *N*-methylpiperidine in pyridine, compound **67** was generated in good yield (Table 2). In addition to compound **55** a small collection of other methoxytryptanthrins (**67**-**69**) were prepared using this technique, previously described by Bergman *et al.*,[88] which was originally used in the formation of the parent molecule, tryptanthrin. To achieve the natural product ophiuroidine (**54**) the aryl methyl ethers needed to be demethylated. In nature this can occur from enzymatic systems such as cytochrome P-450,[112] and other methyltransferases.[113] Several options for demethylation were available including various Lewis acids such as BBr_3,[114] $AlCl_3$,[110] or the one selected, namely pyridinium hydrochloride.[115]

Table 2 Preparation of methoxytryptanthrins.



Isatoic anhydride component	Isatin component	Product		Yield (%)
	**56**		**55**	89
	Isatin		**67**	86
Isatoic anhydride	**58**		**68**	61
Isatoic anhydride	**61**		**69**	65

Reagents and conditions: i) DIC, *N*-methylpiperidine, pyridine, 60 °C, 30 min. 60 →100 °C, 30 min. 100 °C, 1 h.

4.4 Studies of Demethylations using Pyridinium Hydrochloride

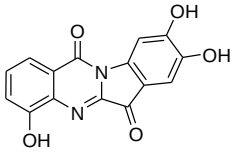
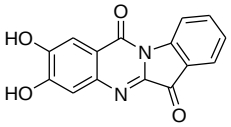
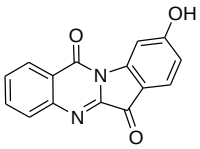
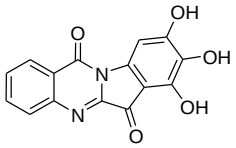
The use of pyridinium hydrochloride (py·HCl) in the demethylation of aryl methyl ethers has been known for a very long time. The first reports by Prey,[115] of using molten py·HCl at temperatures of 180 to 200 °C in demethylation reactions, has also given the reaction the name of Prey demethylation. It has been described as a mild technique, and due to the simplicity of preparing the reagent it has been used on industrial scales. There are other conflicting reports of this technique being unsuccessful, forming dark intractable mixtures, on substrates that should respond well to this procedure.[116] Reports of addition of solvents to the Prey demethylation is somewhat of a newer modification to the technique, solvents such as quinoline, *t*-butylmethyl ether and 2-ethoxyethanol have been successfully reported, but these reports include long reaction times.[117] Ethyl acetate has been reported to decompose when used as a solvent forming ethyl chloride and acetic acid.[117b]

Microwave (MW) heating is a more recent adaptation of the Prey demethylation, first described by Kulkarni *et al.*[118] This modification allows for rapid heating of the salt, enabling it to melt faster, since heating occurs within the container it does not have to first transfer heat through the glass. This technique was successful using microwave instruments that were less sophisticated than their modern counterparts, which now have included safety features. These newer models halt the heating of the sample if a rapid temperature increase is detected. This is commonly the case when dealing with melts of salts which have very high microwave absorption properties, including py·HCl. By using a suitable solvent the unscheduled instrument shutdowns can be avoided.

From the previous literature of non-microwave methods of the Prey demethylation, the use of 2-ethoxyethanol has been reported,[117] but use of this solvent was rejected as it is a hazardous and potentially dangerous substance. Screening for a suitable solvent to carry out the Prey demethylation started with DMF, DMA, NMP and DMSO. While DMF and DMA worked reasonably well, for the demethylation of 4-methoxyacetophenone (**70**) with py·HCl, it was however found that NMP outperformed the other three solvents, as its stability and boiling point had desirable qualities at higher temperatures (max. 250 °C).

It was observed when the methoxy tryptanthrins **55**, **67** and **69** were demethylated in py·HCl, initially mixtures of hydroxy and methyoxy substituents could be identified. With further heating these were fully converted to the target compounds, ophiuroidine (**54**) as well as the other hydroxytryptanthrins **71**-**73** (Table 3).

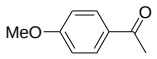
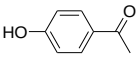
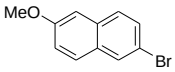
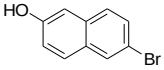
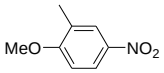
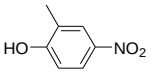
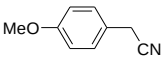
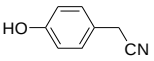
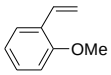
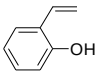
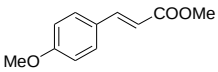
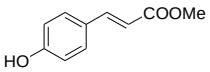
Table 3 Conversion of methoxytryptanthrins with py·HCl.

Substrate	Time (min)	Product		Yield (%)
55	7		**54**	98
67	5		**71**	98
68	3		**72**	99
69	7		**73**	98

Reagents and conditions: i) py·HCl, NMP, MW, 240 °C.

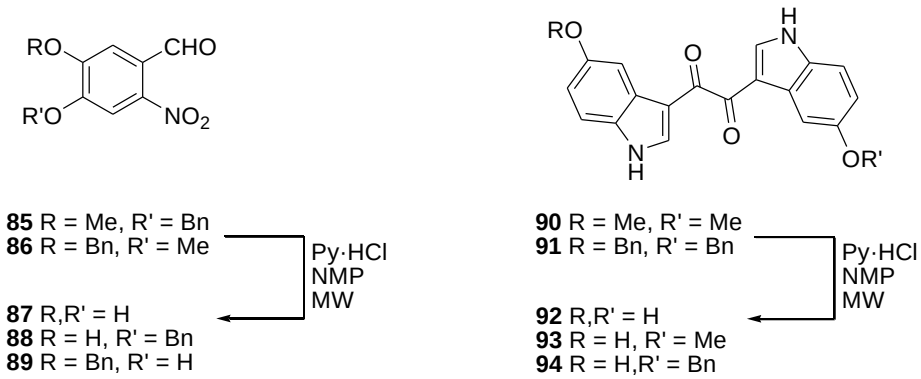
With the successful demethylation of the methoxytryptanthrins, the capabilities of this new modification were tested against known commercial compounds containing simple aryl methyl ether structures (**70**, **74**-**78**). All of which were found to convert to the corresponding phenols (**79**-**84**). It was also found that the amount of py·HCl used in the reaction could be minimized, from 1.5 to 3 equivalents (Table 4). Typically large excesses of py·HCl were previously reported as a solvent free method, where the reagent took the place of the solvent.

Table 4 Demethylation of commercially available compounds containing aryl methyl ethers using py·HCl in NMP.

	Substrate	Time (Min)	Temp (°C)	Product		Yield[a]
70[b]		20	240		**79**	92[d]
74[b]		15	240		**80**	86[d]
75[b]		12	240		**81**	87[d]
76[b]		20	240		**82**	77[d]
77[c]		15	180		**83**	65[e]
78[c]		20	220		**84**	78[e]

[a] *Reagents and conditions:* i) py·HCl, NMP, MW; [b] 3 equivalents py·HCl used; [c] 1.5 equivalents py·HCl used; [d] Isolated after purification by silica gel chromatography; [e] Isolated from preparative HPLC.

Next, two benzyloxy-methoxy-nitrobenzaldehydes (**85** and **86**) were prepared, from vanillin and 3-benzyloxy-4-methoxybenzaldehyde, respectively using known procedures (Scheme 34).[119] These compounds when treated with py·HCl (1.5 eq./ aryl ether) in NMP were found to convert to the corresponding *o*-diphenol compound (**87**). By adjusting the amount of reagent, temperature, and reaction time the benzyloxy-hydroxy-nitrobenzaldehyde intermediates, **88** and **89**, were observed by LCMS (Table 5). The same stepwise demethylation was also observed when bis-(5-methoxyindol-3-yl)-1,2-ethane (**90**), and bis-(5-benzyloxyindol-3-yl)-1,2-ethane (**91**) were converted to the natural product hyrtiosin B (**92**);[120] indicating this method of demethylation can be mild when used with the conditions optimized for the reactant. Although the intermediate compounds of **90** and **91** (**93** and **94**, respectively) were observed in the reaction mixture by LCMS, they were not isolated and characterized, due to time restrictions (Table 5). Demethylation of compounds **90** and **91** had previously been attempted using the typical Prey conditions and were described as failing to give product.[116b]



Scheme 34 Demethylations of dialkoxy aryl ethers. For conditions see table 5.

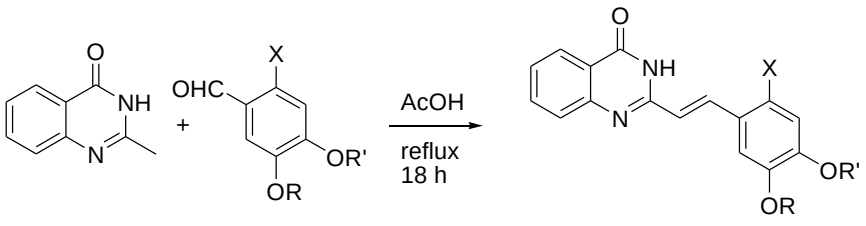
Table 5 Conditions for deprotections of aryl methyl and aryl benzyl ethers.

Substrate	**py·HCl (equiv)**	**Time (min)**	**Product/s** [b]	**Yield** [b] **(%)**
85 [ai]	3	10	**87**	85
85 [aii]	1.5	7	**88**, **87**	68, 14
86 [ai]	3	10	**87**	83
86 [aii]	1.5	7	**89**, **87**	78, 9
90 [ai]	3	15	**92**	91
91 [ai]	3	15	**92**	89

[a] *Reagents and conditions*: i) py·HCl, NMP, MW, 200 °C ;
ii) py·HCl, NMP, MW, 180 °C.

[b] Results analyzed using HPLC and LCMS.

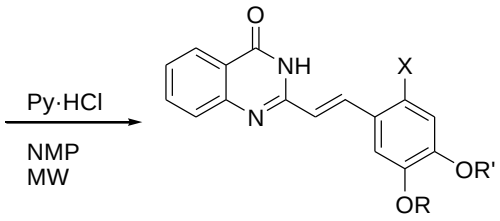
Finally two halogenated methoxy styrylquinazolinones were synthesized and tested. These were prepared through the condensation of 2-bromo-4,5-dimethylbenzaldehyde (**95**)[121] and 4-hydroxy-2-iodo-5-methoxybenzaldehyde (**96**) with 2-methyl-4(3*H*)-quinazolinone (**97**)[31] in acetic acid giving 2-(2-bromo-4,5-dimethoxy styryl)-4(3*H*)-quinazolinone (**98**) and 2-(2-iodo-4-hydroxy-5-methoxystyryl)-4(3*H*)-quinazolinone (**99**), respectively (Scheme 35).



97

95 X = Br; R,R' = Me
96 X = I; R =Me; R' = H

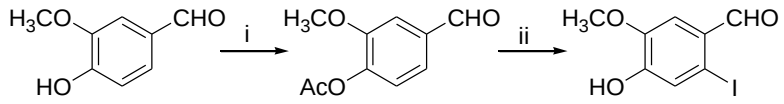
98 X = Br; R,R' = Me
99 X = I; R = Me; R' = H



100 X = Br; R = Me; R' = H
101 X = Br; R, R' = H
102 X = I; R,R' = H
103 X = R, R' = H

Scheme 35 *Reagents and conditions:* i) AcOH, reflux, 18 h; ii) Py·HCl, NMP, MW.

The preparation of **96** was adapted from the iodination conditions described by Hathaway *et al.*,[122] upon acetoxy-vanillin (Scheme 36).[123] A side effect of this method was that the acetyl group was simultaneously deprotected. The same iodination conditions when performed on vanillin were described to give 3-iodovanillin; while it was found that iodination in the 2 position had occurred from acetoxy-vanillin. This was confirmed using an HMBC NMR experiment where only one J_3 correlation between the aryl hydrogen and the aldehyde carbon could be found. The –OMEM protected vanillin has also been shown to iodinate in the 2 position.[123]



96

Scheme 36 *Reagents and conditions:* i) Ac_2O, pyridine, rt, 18 h (78%);
ii) I_2, $AgNO_3$, MeOH, rt, 18 h (70%).

When reacted with py·HCl in NMP **98** and **99** proceeded to demethylate as expected. The monomethoxy intermediate of **98**, compound **100**, was observed by LCMS as a transient product, and 2-(2-bromo-4,5-dihydroxystyryl)-4(3*H*)-quinazolinone (**101**) as the final product (Scheme 35). With compound **99** it was discovered that demethylation occurred, and 2-(4,3-dihydroxy-6-iodostyryl)-4(3*H*)-quinazolinone (**102**) could be

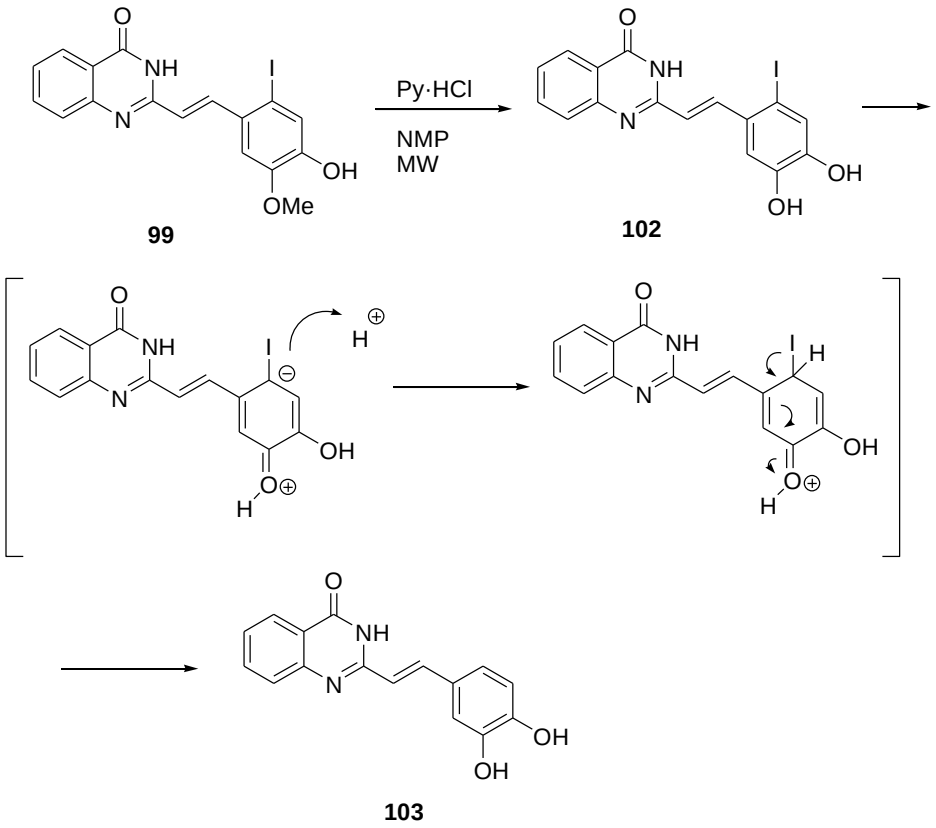
detected by LCMS, although the major product was found to be 2-(3,4-dihydroxystyryl)-4(3*H*)-quinazolinone (**103**), showing that dehalogenation had occurred. Extending the heating time, or increasing the temperature, for the reaction of **98** it was discovered that debromination could be induced, giving **103** (Table 6). This was an unexpected result as 2-bromo-6-methoxynaphthalene (**74**) was successfully converted to 6-bromonaphthol (**80**) without dehalogenation occurring.

Table 6 Results of demethylation results of styrylquinazolinones in NMP with Py·HCl.

Substrate	Py·HCl (equiv)	Temp. (°C)	Time (min)	Product	Yield (%)
98	3	180	15	**100**, **101**	70, 10[a]
98	3	200	15	**101**	82[a]
98	3	220	15	**103**	87[a]
99	1.5	180	30	**102**	64[a]
99	1.5	200	15	**102**, **103**	68,18[a]
99	3	220	15	**103**	83[a]
99	3	240	20	**103**	32[a]

[a] Yield established from HPLC.

An explanation for this observed effect would be the strong tautomeric effects of this particular system, and the formation of a protonated semi-quinone species. The py·HCl protonates the Meisenheimer complex, and results in the elimination of the halide giving rise to the product **103** (Scheme 37). This proposal is supported by the results of a similar reaction where it was observed that 5,7-dibromo-8-hydroxyquinoline was substituted with chlorine when heated in py·HCl.[124] The corresponding 5,7-diiodo-8-hydroxyquinoline was found to give mixtures of the dichlorinated, mono-chlorinated hydroxyquinolines and 8-hydroxyquinoline. Other compounds such as 2-hydroxypyridine, 2- and 4-bromophenols, and mono substituted variants of the 8-hydroxyquinolines were discussed giving similar results. The chloro-substituted variants of **98** and **99** were not observed suggesting these particular compounds favour dehalogenation over halogen substitution.



Scheme 37 Proposed dehalogenation of **99**.

5 Conclusions

The heterocyclic natural products, luotonin A, ophiuroidine, and cephalandole B, as well as the 2-(indol-3-yl)-benzoxazinone structures of cephalandole A, have been achieved through the use of various synthetic techniques. Some derivatives, and or analogous structures have been developed in parallel with these target compounds. These new preparations of the described compounds can lead to a greater range of analogues, which could be further developed. Ideally biological testing in various trials against carcinoma cell lines, malaria, tuberculosis, and other bacteria would be a desirable ending to the work described above. Unfortunately, the moderate activities of the luotonins, and tryptanthrins described in the broader literature, as well as their reduced solubility, will probably make any progress go at a slower pace in favour of more potent substances.

The pyrroloquinazolinone series of compounds provided a challenge in structural interpretations of tautomerism. The two tautomeric forms discovered for the O-substituted pyrroloquinazolinones gave indications as to the reactive nature of these molecules. This series of compounds could be further investigated, as well as establishing the structures of the unknown blue substances that were generated on many occasions. One idea conceived, but never acted upon, as the other projects had taken precedence, was the investigation of Grignard reactions at the nitrile on the silyl protected derivatives of **7**. This reaction, if successful, could lead to keto- and imino-compounds analogous to **20**, which has shown to be reactive for cyclization, and could provide a more cost effective route to luotonin A structures.

The new route for luotonin A and analogues is a versatile, high yielding and cost effective method. The total yield for luotonin A over 5 steps, from anthranilamide was 62%, while the total yield for either 14-chloroluotonin A (**22**) or its precursor **21** was 73% showing this method could be a plausible route for analogues with substitutions available on the A, B and E rings from suitable precursors.

The discovery and subsequent revision of the natural product cephalandole A is now confirmed as being the previously known compound 3-(1*H*-indol-3-yl)-2*H*(1,4)-benzoxazinone (**40**). A reinvestigation of the compounds extracted from *Cephalantheropsis gracilis* should be undertaken to verify the isolates reported as

cephalandole B (**31**), cephalandole C (**32**), and cephalinone B (**34**), as all these proposed structures contain methoxy subunits which could be artefacts of the methanolic extraction process. The previous report by Liu *et al.*[80] of 2-(indol-3-yl)-4(3*H*)-quinazolinones does not define whether biological activity was found for these particular compounds, so a greater range of 3-substituted 2-(indol-3-yl)-4(3*H*)-quinazolinones could be prepared and tested, using **30** or **31** as starting substrates.

The preparation and confirmation of ophiuroidine (**54**) from the corresponding trimethoxytryptanthrin **55** was achieved through demethylation using a new modification of the Prey demethylation, where NMP was used as a solvent, minimizing the amount of pyridinium hydrochloride used in the microwave assisted reaction. The use of the coupling reagent diisopropylcarbodiimide was effective on 5,6-dimethoxyisatin (**56**) and 7-methoxyisatoic anhydride (**57**) to create **55** when thermal condensation in xylenes and triethylamine, or heated neat in pyridine, was not sufficient to generate a reaction.

A new modification for the preparation of methoxyisatins was described which could be further investigated using different electron donating groups in the 3 position of aniline. As observed with the 2,4-dibromo-5-methoxyaniline derivative, **63**, this particular compound did not respond to the conditions of this new modification, and demethylation was found to be favoured over cyclization in H_2SO_4.

The final series of microwave assisted demethylations using pyridinium hydrochloride in NMP showed that many compounds previously considered sensitive to this method could be preformed and produce reasonable yields. The use of NMP in the Prey demethylation under normal heating conditions could be useful, as the amount of reagent required is minimized. The dehalogenation of the halo 2-styryl-4(3*H*)-quinazolinone compounds **98** and **99** to **103** could be further studied to see if this trend holds true for chlorine as well.

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To “Ollie” - Daddy loves you.

7 Supplementary Material

General Methods: All reagents and solvents were obtained from commercial sources and used as received, with the exception of THF, which was distilled from sodium and benzophenone. Melting points were taken using a Büchi Melting Point B-545 capillary apparatus and were uncorrected. NMR data was recorded using a Bruker Avance 300 at frequencies of 300.1 MHz for ^{1}H and 75.5 MHz for ^{13}C, the residual solvent signal was used as the reference. Thin-layer chromatography (TLC) was performed with aluminium plates coated with silica gel and chromatography was performed using silica gel (40-63 µm). Procedures and data for all other relevant compounds can be found in their respective papers.

General description for the preparation of pyrroloquinazolinones (7-9)

Compound **3** (10.92 g, 50 mmol) was dissolved in DMF (400 mL) at 50 °C, under an atmosphere of argon, once all material had dissolved, the flask was removed from warming and allowed to cool. A suspension of *t*-BuOK (6.73 g, 60 mmol) in DMF (50 mL) was prepared and slowly transferred to the reaction vessel. Additional DMF (2 ×10 mL) was used to transfer the last of the *t*-BuOK. This solution was stirred for 1 h, followed by addition of the acrylic reagent (1.05 mmol) to the reaction mixture, and stirred for 1 h at rt. Removal of the solvent (~300 mL) under vacuum produced a thick solution and was transferred into ice water (600 mL) with stirring. Any solid that formed was filtered away and the aqueous portion was neutralized using 1 M HCl, and was stirred for one hour and filtered collecting a small amount of starting material. Finally the pH was adjusted to pH 3-4, and the milky suspension the formed was stirred overnight. The product was collected by filtration and the aqueous portion was evaporated under vacuum to ~200 ml and again acidified to pH 2, more of the product was collected. Combination of the product was recrystallized twice from ethanol giving pure product, as a series of crystal crops.

1,9-Dihydro-3-hydroxy-9-oxopyrrolo[2,1-*b*]quinazoline-2-carbonitrile (7) – White crystals (93%). NMR (300 MHz, DMSO-d_6) δ: 9.4 (br s, 1H), 8.17 (d, *J* = 7.9 Hz, 1H), 7.90-7.83 (m, 1H), 7.76 (d, *J* = 7.9 Hz, 1H), 4.65 (s, 2H). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 158.6, 158.3, 151.2, 141.1, 134.5, 127.3, 125.9, 120.3, 114.0, 87.5, 46.5.

Methyl 1,9-dihydro-3-hydroxy-9-oxopyrrolo[2,1-*b*]quinazoline-2-carboxylate (8) – Off white solid (99%, from 1 recrystallization attempt). NMR (300 MHz, DMSO-d_6) δ: 8.21 (d, *J* = 8.0Hz, 1H), 7.9-7.76 (m, 2H), 7.56 (dd, *J* = 6.7, 1.4 Hz, 1H), 4.54 (s, 2H), 3.70 (s, 3H).

Ethyl 1,9-dihydro-3-hydroxy-9-oxopyrrolo[2,1-*b*]quinazoline-2-carboxylate (9) – White crystals (97%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.17 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.80-7.75 (m, 2H), 7.52-7.43 (m, 1H), 4.43 (s, 2H), 4.10 (q, *J* = 7.1 Hz, 2H), 1.23 (t, *J* = 7.1 Hz, 3H).
^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 167.0, 164.9, 159.2, 157.7, 149.3, 133.3, 127.3, 125.3, 120.5, 92.4, 56.9, 45.1, 14.5.

2-Cyano-4,9-dihydro-9-oxopyrrolo[2,1-*b*]quinazolin-3-yl 4-methylbenzenesulfonate (11) – Under an nitrogen atmosphere the substrate **7** (0.225g, 1.0 mmol) was suspended in DMF (1.0 mL) and stirred. To this, *p*-toluenesulfonyl chloride (0.210 mg, 1.1 mmol) was added and stirred for 1 h at rt. The solution was poured onto ice-water washed (20 mL) and extracted with diethyl ether. The organic phase was washed with satd. aq. NH_4Cl, and brine, then dried over Na_2SO_4, filtered and evaporated to a concentrated solution. The product was purified using silica chromatography giving **11** (231 mg, 61%) as a yellow-green solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.53 (br s, 1H), 8.34 (s, 1H), 8.14 (dd, *J* = 8.0, 1.2 Hz, 1H), 8.07 (d, *J* = 8.3 Hz, 1H), 7.97 (d, *J* = 8.3 Hz, 2H), 7.88-7.79 (m, 1H), 7.45 (d, *J* = 7.1 Hz, 2H), 7.38-7.31 (m, 1H), 2.37 (s, 3H).
^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 155.6, 144.2, 140.1, 139.7, 135.7, 135.4, 130.1, 128.0, 127.2, 126.3, 122.9, 117.8, 113.1, 111.5, 99.3, 94.9, 21.0.

2-Cyano-4,9-dihydro-9-oxopyrrolo[2,1-*b*]quinazolin-3-yl methanesulfonate (12) – This was performed in a similar fashion as the prepatation of **11**. Yellow solid, which changes to green (103 mg, 68%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.20 (s, 1H), 8.14 (s, 1H),8.12 (d, *J* = 8.0 Hz, 1H), 7.8-7.2 (m, 1H), 7.48 (d, *J* = 8.1 Hz, 1H), 7.26-7.17 (m, 1H), 3.51 (s, 3H).
^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 155.8, 140.7, 135.6, 127.6, 125.0, 121.1, 116.1, 113.4, 112.0, 111.7, 109.5, 93.7, 36.7.

Ethyl 1,9-dihydro-3-triisopropylsiloxy-9-oxopyrrolo[2,1-*b*]quinazoline-2-carboxylate (13) – The substrate (0.272g, 1.0 mmol) was suspended in THF (30 mL) at rt. To this suspension TIPSCl (0.3 mL, 1.4 mmol) and DIPEA (0.4 mL, 2.3 mmol) were added stirred at rt for 4 h. The solution was diluted with EtOAc (20 mL) and transferred to a separatory funnel. The solution was made up to 100 mL with the addition of EtOAc (50 mL) and washed with 0.5M HCl solution (2 × 20 mL), and brine (2 × 20 mL). The organic phase was dried over Na_2SO_4, filtered, and evaporated to dryness. Purification by silica chromatography gave the pure product as a white solid (0.374 g, 87%).
^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.38 (d, *J* = Hz, 1H), 7.82-7.73 (m, 2H), 7.58-7.47 (m, 1H), 4.74 (s, 2H), 4.34 (q, *J* = Hz, 2H), 1.64-1.47 (m, 3H), 1.19-1.15 (m, 18H).
^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 162.4, 159.9, 153.1, 152.0, 149.2, 134.3, 127.9, 127.2, 126.7, 121.1, 114.5, 60.8, 46.9, 18.1, 14.2, 12.4.

Ethyl 4,9-dihydro-3-*t*-butyldiphenylsiloxy-9-oxopyrrolo[2,1-*b*]quinazoline-2-carboxylate (14) – To the substrate (1.361 g, 5.0 mmol) suspended in THF (100 mL), DIPEA (3.0 mL, 17 mmol) and TBDPSCl (2.6 mL, 10.0 mmol) were added. The solution was heated at reflux for 24 h. The solution was diluted with EtOAc (100 mL) and washed with 0.5 M HCl (3 × 20 mL), and brine (2 × 20 mL). The aqueous portions were back extracted with EtOAc (2 × 50 mL) and the organic phases combined, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified using silica chromatography giving a white solid (1.868 g, 73 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.65 (br s, 1H), 8.42-8.34 (m, 1H), 8.17 (d, *J* = 8.4, 1H), 7.96- 7.88 (m, 1H), 7.88-7.81 (m, 4H), 7.76-7.68 (m, 1H), 7.47-7.31 (m, 6H), 4.37 (q, *J* = 7.2 Hz, 2H), 1.31 (t, *J* = 7.2 Hz, 3H), 1.29 (s, 9H).
^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 165.8, 162.9, 151.8, 151.3, 135.6, 134.4, 132.1, 130.3, 129.5, 127.8, 124.3, 118.3, 92.0, 62.2, 27.7, 20.0, 14.4.

4,9-Dihydro-3-methoxy-4-methyl-9-oxopyrrolo[2,1-*b*]quinazoline-2-carbonitrile (15) – The substrate (0.226 g, 1.0 mmol) was dissolved in DMF (20 mL) by warming to 60 °C. The solution was removed from warming and allowed to cool (~10min). While stirring vigorously, K_2CO_3 (0.4 g) was added to the reaction mixture, followed by addition of MeI (0.62 mL, 5 mmol). The solution was stirred for 1 h at rt then quenched with water. The solution was evaporated to dryness, taken up in water, and neutralized with NH_4Cl solution. The product **15** was collected by filtration and purified by silica chromatography as a yellow solid (0.183 g, 72%).
^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.11 (d, *J* = 7.8 Hz, 1H), 7.916 (s, 1H), 7.77 (t, *J* = 7.7, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 3.92 (s, 3H), 3.83 (s, 3H).
^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 155.5, 141.8, 135.8, 128.1, 126.0, 120.5, 114.0, 113.3, 110.8, 109.8, 93.3, 64.0, 32.4.

1,4-Dihydro-2,4-dioxo-3(2*H*)-quinazoline acetic acid (16) – The substrate **7** (0.450 g, 2.0 mmol) was added to hydrogenperoxide solution (20 mL) and heated at reflux for 4 h. The compound was then extracted into EtOAc, and washed with satd. $NaHCO_3$, aq. HCl (0.5M), water, and brine. The organic phase was dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure giving **16** (0.281 g, 73%) as a white powder. ^{1}H and ^{13}C NMR spectral data agrees with literature (Reference 64).

Preparation of 2-(*N*-acetyl-indol-3-yl)-1,3-benzoxazin-4-one (39) – A suspension of **38** (1.4 g, 5.0 mmol) in AC_2O (20 mL) was heated at reflux for 2 h. Upon cooling to rt the product (**39**) crystallized, which was collected via filtration, washed with diethyl ether. The beige crystals (1.32 g, 87%) were dried under high vacuum. The analytical sample was recrystallized once from DMSO-d_6, giving colorless crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.57 (s, 1H), 8.55-8.50 (m, 1H), 8.430-8.35 (m, 1H), 8.13(d, *J* = 7.9 Hz, 1H), 7.98-7.90 (m, 1H), 7.75 (d, *J* = 7.9 Hz, 1H), 7.63-7.55 (m, 1H), 7.50-7.42 (m, 2H), 2.80 (s, 3H). ^{13}C NMR (75.5 MHz, DMSO-d_6, @ 25 °C) δ: 170.15, 158.7, 146.5, 136.9, 135.7, 131.9, 131.7, 128.2, 128.1, 126.7, 126.3, 125.9, 124.5, 121.8, 116.9, 116.1, 111.9, 23.9. ^{13}C NMR (75.5 MHz, DMSO-d_6, @ 120 °C) δ: 168.8, 157.8, 153.4, 146.2, 136.1, 135.4, 130.5, 127.5, 127.5, 126.1, 125.6, 125.1, 123.8, 121.2, 116.3, 115.4, 111.8, 23.1.

Preparation of *N*-(2-hydroxyphenyl)-2-(1*H*-indol-3-yl)-2-oxoacetamide (46) – To a solution of **45** (0.207 g, 1.0 mmol) and 2-nitrophenol (0.160 g, 1.0 mmol) in DMF (15 mL), Pd/C 10% (30 mg) was added to the reaction vessel which was then bubbled with H_2 for 1 h at rt. This was poured onto ice water and stirred, allowing a precipitate to form, which was collected by filtration and dried giving **46** (0.207 g, 74%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.39 (br s, 1H), 10.32 (br s, 1H), 9.88 (s, 1H), 9.03 (s, 1H), 8.32-8.26 (m, 1H), 8.24 (d, *J* = 8.0Hz, 1H), 7.6-7.54 (m, 1H), 7.34-7.26 (m, 2H), 7.06-6.92 (m, 2H), 6.91-6.83 (m, 1H).^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 180.2, 160.0, 147.2, 139.3, 136.2, 126.5, 125.3, 124.9, 123.7, 122.8, 121.4, 119.8, 119.2, 115.0, 112.7, 111.7.

Preparation of 5,7-dibromo-4-methoxyisatin (65) –To a beaker of H_2O warmed to 35 °C, chloral hydrate (4.55 g) was added, followed by Na_2SO_4 (31.2 g). A solution containing **66** (7.03 g, 25 mmol), conc. HCl (5 mL), and H_2O (30 mL) was added to the chloral hydrate solution. A solution containing hydroxylamine hydrochloride (5.2g, 75 mmol), in warm water (40 mL) was added to the previously combined mixture and heated at 80-90 °C for 2 h. The solution was allowed to cool and the precipitate filtered (8.6 g). This was taken through without further purification to the next step. A solution containing conc. H_2SO_4 (10 mL) and H_2O (2 mL) was prepared and allowed to reach room temperature with stirring. The isonitroso-2-(2,4-dibromo-3-methoxyphenyl)-oxoacetamide (7.1 g) was added in portions to the diluted H_2SO_4 solution. Once addition was complete the vessel was heated to 60 °C for 1 h and then at 90 °C for 30 min. This was poured onto crushed ice and stirred. The precipitate was filtered and purified with chromatography, and recrystallized from acetonitrile giving 5,7-dibromo-4-methoxyisatin (2.68 g, 40%) as red needles, mp. 237-239 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.85 (s, 1H), 7.81 (br s, 1H), 4.25 (s, 3H).

Preparation of 2,4-dibromo-5-methoxyaniline (66) – *N*-Bromo succinimide (18g, 102 mmol) was added to *N*-(3-methoxyphenyl)benzylsulfonylamide (13.165 g, 50 mmol), dissolved in in acetonitrile (150 mL) and heated at reflux for 2 h. The solvent was removed under vacuum and taken up into EtOAc (300 mL). This was washed with HCl (2M 3 100 mL), H_2O (100 mL) and brine (100 mL), and the organic phase was dried over $MgSO_4$, filtered and evaporated to dryness. Giving *N*-(2,4-dibromo-5-methoxyphenyl)benzylsulfonamide (18.6 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.90-7.65 (m, 2H), 7.63-7.35 (m, 4H), 7.29 (s, 1H), 6.92 (br, s, 1H), 3.90 (s, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 156.2, 138.7, 135.6, 134.9, 133.7, 129.3, 127.4, 108.6, 106.7, 56.8.

N-(2,4-Dibromo-5-methoxyphenyl)benzylsulfonamide (10.53 g, 25 mmol) was added to a mixture of aqueous methanol (1:1v/v 150 mL) and NaOH (1.2 g, 30 mmol) and heated 60 °C for 30 min. This was evaporated to dryness and taken up into EtOAc (100 ml). The organic phase was treated with satd aq. NH_4Cl, bicarbonate, water and brine, and then dried over $MgSO_4$. The contents were filtered and evaporated to dryness giving 2,4-dibromo-5-methoxyaniline (**66**) as an oil (6.94 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.51 (s, 1H), 6.32 (s, 1H), 4.08 (br s, 2H), 3.82 (s, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 156.0, 143.9, 134.9, 100.0, 99.0, 55.8.

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