Graduate Research School

RP

Research Proposal Coversheet for Candidates in

Research Higher Degrees

EXAMPLE OF RESEARCH PROPOSAL SUBMISSION

RESEARCH AREA: CHEMISTRY

DEGREE: PhD

Please note that all identifying information has been removed from this research proposal and replaced with XXX.

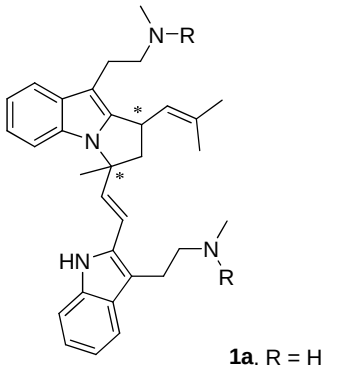
PhD Research Proposal

A. Proposed Study

Proposed title: The Total Synthesis of the Antimalarial Natural Products, Flinderoles.

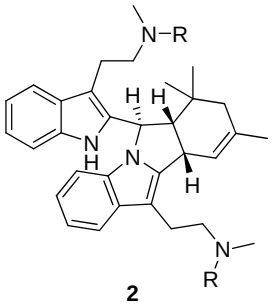
The aim of this project is to develop an efficient synthetic route to the recently isolated class of antimalarial compounds termed the flinderoles.[1] The unique chemical structure of this series of compounds, which contain a *bis*-indole based carbocyclic skeleton, and their significant biological activity make them a highly attractive target for this study.

Nitrogen-containing heterocycles have been used as medicinal compounds for centuries, and form the basis for many common drugs such as Morphine (analgesic), Captopril (treatment of hypertension) and Vincristine (cancer chemotherapy). The chemical structure of the flinderoles (**1**) is based on the nitrogen-containing indole ring system; however, these compounds have a novel structure not reported in the literature, due to the attachment of the two indole rings. The flinderoles (**1**) are related to the borreverine compounds, such as isoborreverine (**2**), but as a consequence of their more 'open' structure, the development of a new synthetic methodology for their preparation is required. Thus the aim of this project is to develop novel synthetic methodology for this ring-system, to synthesise the flinderoles for further testing, and, if successful, to apply this methodology to the synthesis of other related heterocycles which may show similar or improved bioactivity.



1a. R = H
1b. R = CH_3

1



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B. Research Direction

The aims of this study are to develop a methodology for the total synthesis of the novel bis-indole alkaloid ring-system found in the recently isolated natural products, the flinderoles; and to further investigate these compounds and derivatives for biological activity. The novel structure of the compound makes it a desirable synthetic target, as a successful synthetic route will allow the investigation of related heterocylic compounds which may show improved levels of bioactivity. These studies could also lead to a structure activity relationship (SAR) study of these compounds.

The flinderoles were identified as having good antimalarial activity against the *Plasmodium falciparum* parasite after a recent (2008) screening program of natural product extracts and compounds from Australian and Papua New Guinean plants,[2] and as such are a good lead target for new antimalarial drugs.

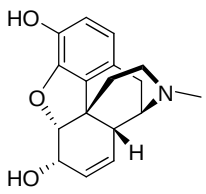
Malaria is the most common parasitic disease in the tropic and sub-tropic regions of the world today. The World Health Organisation's 2008 'World Malaria Report' estimates that in 2006, 247 million cases of malaria were reported, causing nearly one million deaths, mostly of children under five years old. Approximately forty percent of the world's total population lives in areas where malaria is endemic, although eighty percent of cases occur in sub-Saharan Africa, with a majority of the remaining cases in South-East Asia and South America.[3]

A protozoan parasitic disease, malaria is transmitted to humans through the bites of infected, female *Anopheles* mosquitoes. Four strains of the parasite are responsible for malaria in humans, *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. *Plasmodium vivax* is the most common cause of malaria; but the most life-threatening and drug-resistant cases are due to infection by *P. falciparum*.[4] Malaria commonly causes recurrent flu-like symptoms such as fever, shivering, aches and vomiting; however in cases of 'severe malaria' caused by *P. falciparum*, the nervous, respiratory and renal systems are often affected, and hypoglycemia is a common complication.

Given the severity of disease caused by the *P. falciparum* parasite, several key research programs, such as that of the Leiden Malaria Research Group,[5] have been established for

targeting this parasite and its mode of action; and the continual development of parasite resistance to drugs means that the search for new antimalarials is ongoing.

Natural products and natural product derivatives have been a good source of new, biologically active compounds for centuries. Plant material has historically been made into teas and tonics for the treatment of ailments by indigenous societies, while nowadays active compounds in Western society are initially isolated from biota and become the basis for synthetic drugs. Well-known examples of drugs derived from natural products include morphine (**3**) (from the opium poppy *Papaver somniferum*), penicillin G (**4**) (*Penicillium* fungi), and the common anti-cancer agent Taxol (**5**) (or paclitaxel, from the Pacific yew tree *Taxus brevifolia*). Taxol has been incredibly successful for the treatment of lung, ovarian, breast, head and neck cancers, and remains one of the blockbuster drugs of the past few decades.



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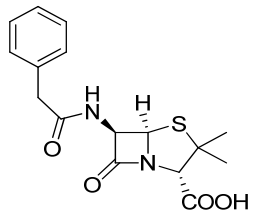

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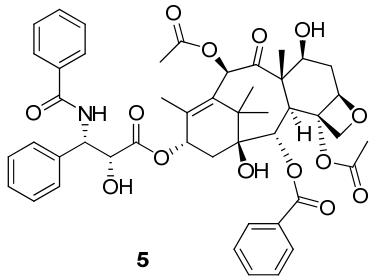

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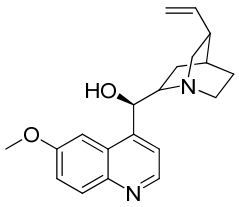
The first effective treatment for malaria was the natural product quinine (**6**), an alkaloid isolated from the bark of the South American *Cinchona* tree in 1817. Until the production of the synthetic drug Chloroquine, this was the only effective remedy, but shortages of the drug during WWI and WWII prompted the search for new treatments or drug candidates. Chloroquine (**7**), a simpler structural analogue of quinine, was synthesised in the 1940s; however, parasite resistance to the drug developed relatively quickly, with evidence of resistant strains appearing on the Thai-Cambodian border (historically a source of emerging antimalarial drug resistance) as soon as the late 1950s, possibly due to both parasite migration and new indigenous mutations.[6]



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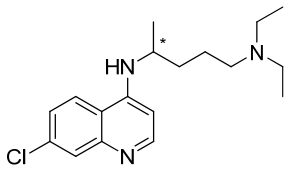

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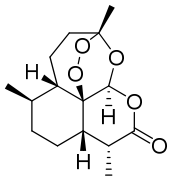

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Another natural product, Artemisinin (**8**), isolated from the Chinese herb *Artemisia annua*, is currently the best treatment for malaria, but resistance to this drug is already starting to appear in South-East Asia.[7] In order to slow the development of parasite resistance to artemisinin-derived drugs, these are generally administered in combination with other antimalarial compounds and their use as a monotherapy has been discouraged.[8]

There are few effective alternatives to artemisinin-based drugs, and with artemisinin-resistant or tolerant cases already being reported, the search for new antimalarial drugs is again of even greater significance. With the historic potential of natural products as excellent candidates for potential drugs, the identification of the flinderoles' antimalarial activity is significant. Their novel ring structure means that they differ significantly from existing anti-malarial drugs and hence may become a lead for the preparation of new drugs to which malaria parasites are not yet resistant.

Organic synthesis and Methodology

Often, natural products of interest are found only in low concentrations when isolated from their natural source. Developing new synthetic strategies and methods towards such compounds is therefore an attractive option for obtaining larger quantities of the natural product without putting strain on the natural resource. As well as obtaining the natural product in greater quantities, a total synthesis of a natural product also allows biological assay of fragments or analogues of the target compound. Consequently, it is of significant interest and importance to develop total syntheses of biologically active compounds in the search for new drugs.

As the flinderoles have a general *bis*-indole alkaloid ring system linked by an alkene chain, it is synthetically more efficient to prepare the two related indole fragments individually; and in the latter stages of the synthesis to attach (couple) these to give the target compound. This convergent approach is commonly used in large pharmaceutical companies and natural product synthetic research programs for economic reasons, and to optimise overall yields.

Two options for coupling the fragments together have been proposed (Figure 1); a metathesis reaction between two terminal olefins, or a Wittig reaction between a phosphine and a carbonyl group on the respective fragments. The olefin metathesis reaction for the formation of C-C bonds, developed by Robert Grubbs, is gaining popularity as a reliable synthetic transformation, particularly following the award of the 2005 Nobel Prize in Chemistry for this work. Similarly, the Wittig reaction has been widely used for the preparation of alkenes since its discovery in 1954 by Georg Wittig.

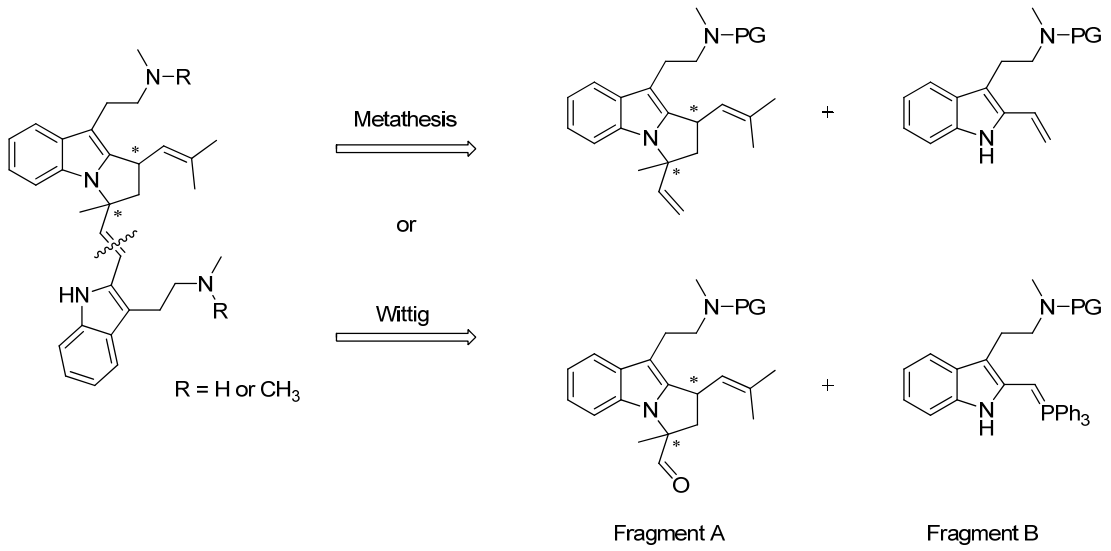
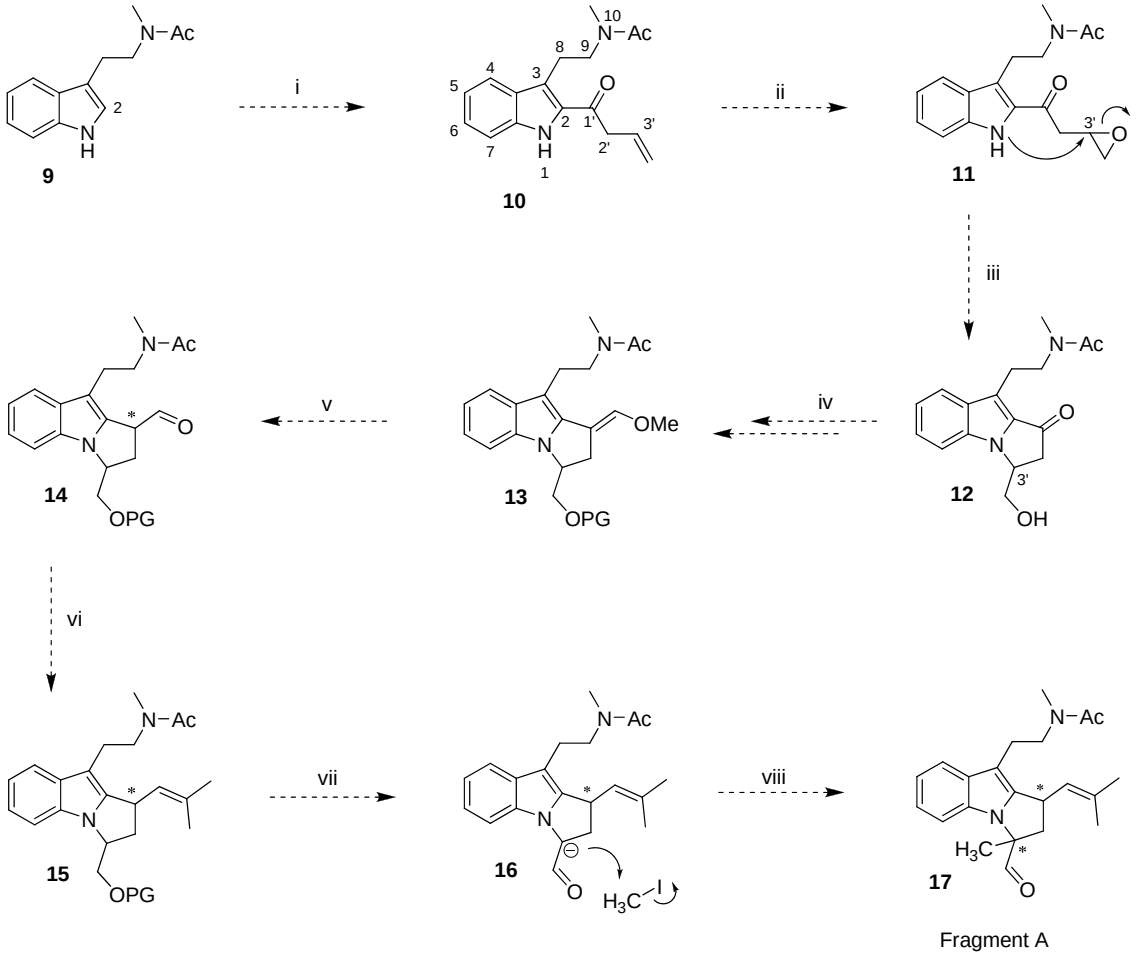


Figure 1. Proposed routes for coupling Fragments A and B.

A synthesis has been designed wherein both ring fragments will be accessed from commercially available tryptamine. The starting indole (**9**, Scheme 1) is prepared by protection of the reactive amine of tryptamine with a methyl group (required in the final product) and an acetate group, masking the reactivity of that position, and later allowing modification of the flinderoles for the investigation of further analogues. The flinderoles are chiral compounds, with two stereocentres, but an initial racemic synthesis will be investigated before the development of an asymmetric synthesis.



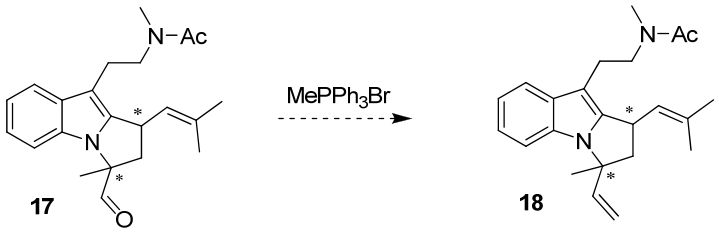
Scheme 1. Proposed synthesis of Fragment A. Reagents: (i) $ClCOCH_2CHCH_2$, Lewis acid; (ii) *m*-CPBA or DMDO (iii) Lewis acid; (iv) $MeOCH_2PPh_3Cl$; alcohol protection; (v) *p*-TsOH ; (vi) $(CH_3)_2CHPPh_3Cl$; (vii) deprotection; NaHMDS; (viii) CH_3I

The synthesis of Fragment A requires the generation of an additional five-membered ring on the indole nitrogen (Scheme 1). A Lewis acid-catalysed acylation reaction[9] at C2 of compound **9** will provide a side chain containing a terminal olefin required for cyclisation, as well as a synthetic handle to install the C1' side-chain. The reaction conditions for the acylation step, including the type of Lewis acid, solvent and substrate to be used must be investigated. If a reagent containing a terminal alkene such as 3-butenoyl chloride can be used, epoxidation of that alkene in compound **10** with *m*-chloroperoxybenzoic acid or dimethyldioxirane will allow, in the subsequent reaction, the nucleophilic N^1 nitrogen to attack at the newly electrophilic carbon 3' atom of **11**. The product from this latter transformation is expected to contain the required five-membered ring and a terminal, primary alcohol moiety (**12**), which can be used in later steps.

The *iso*-butyl side chain at C1' will be inserted after protection of this new terminal alcohol. A Wittig reaction of the existing C1' carbonyl group with methoxymethyl triphenylphosphonium chloride will provide the enol ether **13**, and further acid-catalysed hydrolysis of this group with a strong acid, such as *p*-toluenesulfonic acid, will then produce the aldehyde **14**. A second Wittig reaction between *iso*-propyl triphenylphosphonium chloride and the highly reactive aldehyde moiety found in compound **14** should then result in the desired side chain at C1' (**15**).

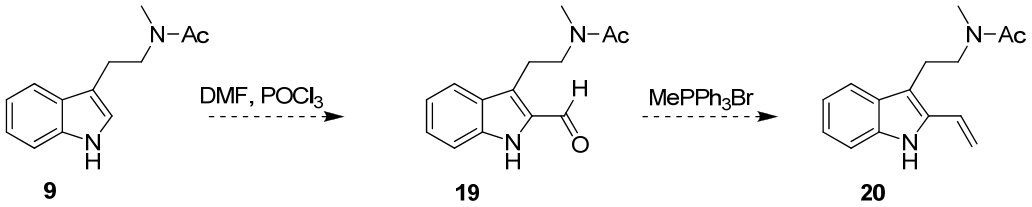
The quaternary methyl group at C3' could then be provided by iodomethane. Thus deprotection and subsequent oxidation of the terminal alcohol in compound **15** allows the formation of an enolate at C3' (**16**), which can then be methylated using iodomethane to produce Fragment A (**17**).

Following the successful synthesis of the two flinderole ring fragments, the reaction conditions required for coupling the two together will be investigated. For a metathesis reaction, each of the fragments requires a terminal alkene, accessible through a Wittig reaction of a carbonyl group with methyl triphenylphosphonium bromide. Fragment A will be reacted with this Wittig reagent to give the alkene **18**, as shown in Scheme 2.



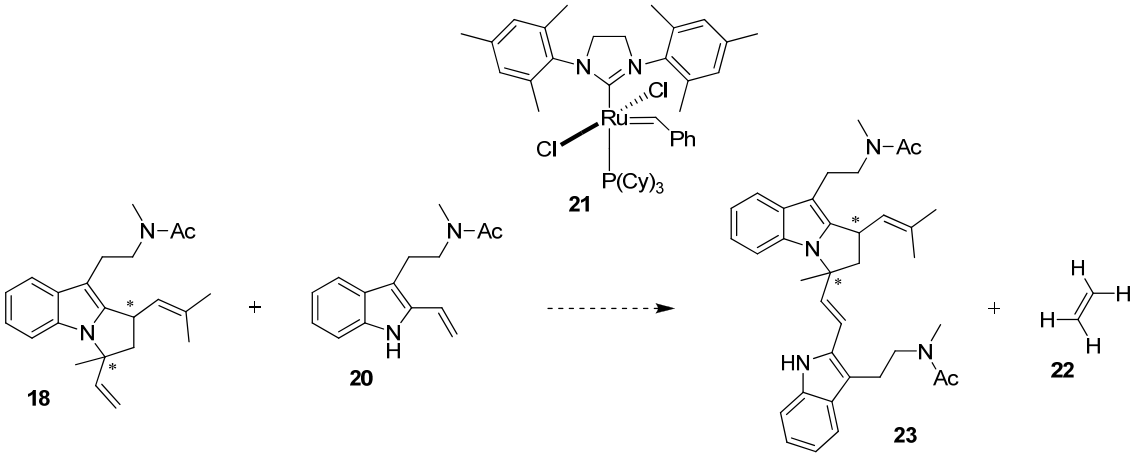
Scheme 2. Formation of a terminal olefin on Fragment A.

The Fragment B required for a metathesis reaction is proposed to be synthesised from the same starting indole **9** as Fragment A (Scheme 3). A Vilsmeier reaction between the indole and dimethylformamide in the presence of phosphorous oxychloride should provide an aldehyde at C2 (**19**). This could then be treated with methyl triphenylphosphonium bromide, as with compound **17**, to produce Fragment B containing a terminal olefin (**20**).



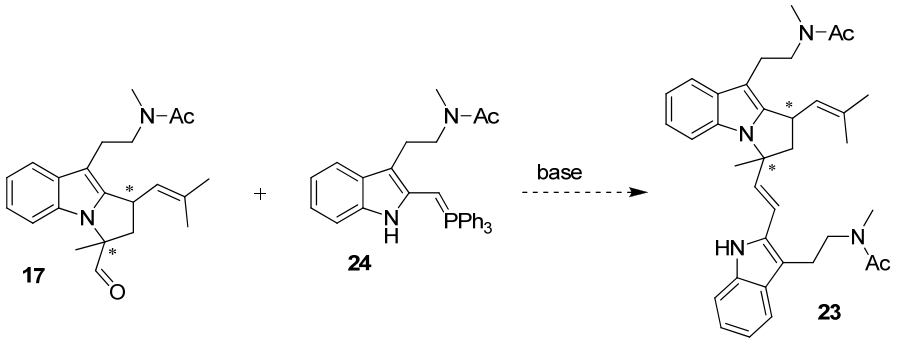
Scheme 3. Synthesis of Fragment B as precursor for a metathesis reaction.

A metathesis reaction would then be catalysed by an organometallic catalyst such as a Grubbs catalyst (**21**) to give the flinderoles, and the easy removal of the gaseous ethene byproduct (**22**) drives the reaction to completion (Scheme 4).



Scheme 4. Olefin metathesis attachment of Fragments A and B.

If the metathesis reaction is unsuccessful in attaching the two fragments, a Wittig reaction will be investigated. For this reaction, one of the ring fragments must be an ylide or phosphorane reagent. With an aldehyde already present in the desired position in Fragment A, it is more convenient to create a Wittig reagent from Fragment B and treat this phosphonium salt (**24**) with the aldehyde in the presence of a base such as sodium hexamethyldisilazane (Scheme 5).



Scheme 5. Wittig reaction to attach Fragments A and B.

Medicinal Chemistry

As no synthetic route to the target flinderoles yet exists, there is no way of identifying which part of the compound is responsible for its biological activity (the pharmacophore), or whether in fact the whole carbocyclic ring system is essential. Developing a total synthetic route to a natural product enables the formation of intermediate compounds and provides a means of developing further derivative compounds which may demonstrate improved biological activity. In this study, a number of the proposed intermediate compounds will be tested for antimalarial activity in an effort to initially identifiy a pharmacophore. It may be the case that only one of the two flinderole indole ring fragments is necessary for bioactivity; if Fragment A is sufficient for a good level of activity, the length and difficulty of the synthetic route will be reduced.

Further derivatives of the flinderoles (Figure 2) are also accessible with the development of a total synthesis. As the N^{10} position carries a protecting group through the entirety of the proposed synthesis, the removal of that group in the final step and substitution with various chemical moieties may provide analogues with improved bioactivity, solubility or bioavailability. The formation of the hydrochloride salt of the target compound may also improve its solubility in a biological system.

The C1’ side-chain of Fragment A can also be modified through the use of different Wittig phosphines, and the linking alkyl chain between the fragments could be extended. The size of the introduced third ring in Fragment A can also be increased if the starting indole **9** is initially substituted with a longer terminal alkene chain.

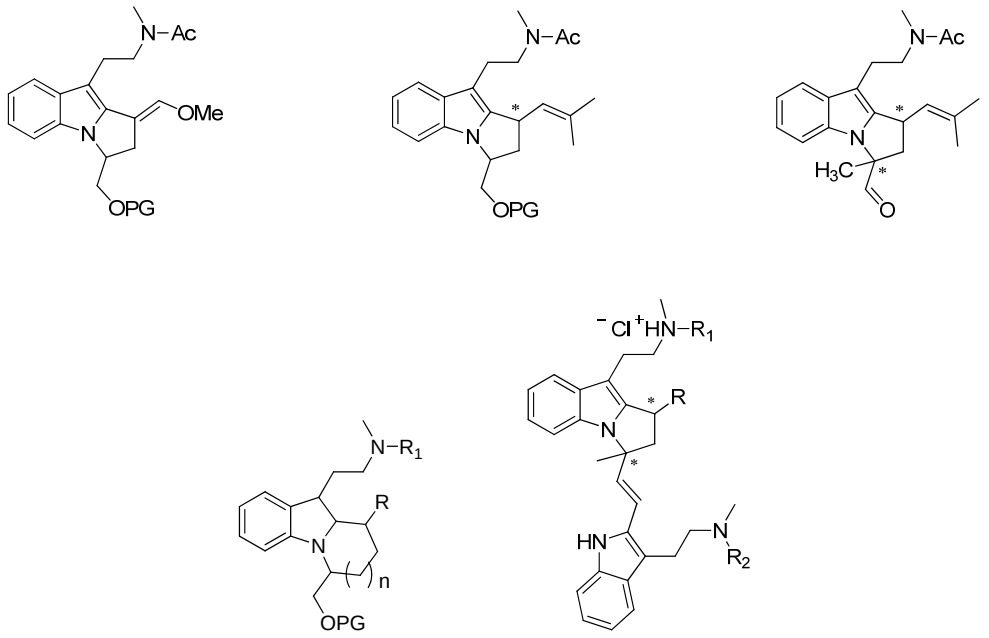


Figure 2. Intermediate compounds or flinderole derivatives to be tested for biological activity.

As the flinderoles are also reported to show activity against the HEK-293 mammalian cell line, biological testing to determine whether the compound shows any cytotoxicity will be investigated. A number of projects within the group involve collaboration with Professor XXX and Professor XXX of the School of XXX for cell testing, thus an initial screening for cytotoxicity of compounds produced in this study can be carried out within the School. The cytotoxic assay will be performed using Jurkat or T-lymphocyte cell lines, with cell death measured as the percentage of cells showing a decrease in cell size and granularity (forward and side scatter) in flow cytometric analysis. Analysis will be completed using a FACSCalibur 4-colour Flow Cytometer (Becton, Dickinson and Company, New Jersey, USA). Data analysis can be performed using FlowJo software (TreeStar, Ashland, OR, USA). Viable and non-viable cells are measured following either solvent (DMSO) alone or compound treatment for 24 hours.

Professor XXX has been approached regarding antimalarial testing; which will be carried out using a colorimetric plasmodium lactate dehydrogenase assay and the tritiated hypoxanthine (isotopic) method to assess parasite growth in response to dilutions of the test compounds.
If the target flinderole compounds are not successfully prepared by the proposed method or an alternative route in the given time period, intermediate compounds can be tested for biological activity. Many of the proposed intermediates of this synthetic scheme have not yet

been reported in the literature, and thus may have novel biological activities worth investigating. The development of synthetic methodology towards the novel *bis*-indole ring system and the three-ring system of Fragment A is also publishable, and will allow the investigation of related natural-product based heterocycles.

C. Candidature Plan

Research timeline

See page 15.

Methods and Skills

All methods used in this project will be those standard to synthetic chemistry. Chemicals will be synthesised in batch reactors (glass flasks), then subjected to the appropriate purification technique, for example chromatography on silica or alumina supports, distillation, or recrystallisation. All air and moisture sensitive reactions will be undertaken in flame dried glassware under an inert gas (argon or nitrogen) atmosphere. Reactions will be monitored using thin layer chromatography on commercially available silica or alumina plates. Solvents will be dried and purified following standard procedures in organic chemistry. Compound structures will be elucidated and characterised using a variety of spectroscopic techniques, such as Nuclear Magnetic Resonance (NMR) spectroscopy, Mass Spectrometry, Infrared spectroscopy and X-Ray crystallography, as standard within organic chemistry.

D. Facilities

A variety of equipment and techniques will be employed for the elucidation and characterisation of the flinderoles and any intermediates and derivatives produced; including Nuclear Magnetic Resonance spectroscopy, Mass Spectrometry, Infrared spectroscopy and X-Ray Crystallography. The specialist equipment required for the above, including various high field NMR spectrometers (Varian 300, Varian 400, Bruker AX-500, and Bruker AV-600), a VG Autospec Mass Spectrometer, and Perkin Elmer 69557 Spectrum One Infrared Spectrometer are accessible within the School XXX. A number of expert staff are employed within the school to assist in the operation, acquisition and analysis of data obtained from these techniques, namely Dr XXX (NMR), Dr XXX (mass spectrometry, GC-MS), Mr XXX (Infrared) and Dr XXX (X-Ray Crystallography). The School also provides a scientific glass

blower for the fabrication and repair of glassware, and a mechanical workshop for the maintenance and repair of other laboratory equipment.

Testing of compounds will be carried out in collaboration with Professor XXX or Professor XXX of the School of XXX, and Professor XXX of XXX.

The science library at the University of Western Australia provides an extensive range of chemistry reference texts and major journals. The library also subscribes to the abstracting service Scifinder Scholar, and the online journal database Science Direct, which provide access to a worldwide range of scientific research publications. The UWA library also provides a loan system for requesting material held outside the university.

E. Estimated Costs

The cost of the research is estimated at $8 000 per annum, approximately $24 000 over a three year period. Part of this sum ($1 500 per annum) will be made available by the school. The remainder of the sum will come out of research grants to Dr XXX. Potential grants will subsidise the cost of using the instruments including the Nuclear Magnetic Resonance spectrometers, Mass Spectrometer, and Infrared Spectrometer are currently covered by the School of XXX.

Fine chemicals	7 000
Solvents	6 000
Glassware	1 000
NMR consumables	1 000
Chromatography supplies	4 000
Testing	3 000
Other consumables	2 000
Total (over 3 years)	**24 000**

F. Fieldwork

No field work is required for this project.

G. Supervisors

Coordinating supervisor: Dr XXX 50 %

Dr XXX will be responsible for ensuring that all administrating and reporting requirements of the supervisors are met. He will provide research advice and respond to queries relating to organic synthetic chemistry, natural products chemistry, and interpretation of spectral data. Dr XXX will also participate in weekly meetings regarding research.

Co-supervisor: Dr XXX 25 %

Dr XXX will be present at monthly group meetings and will respond to queries and provide advice regarding organic synthesis, chromatography and spectral data.

External supervisor: Professor XXX 25 %

Professor XXX will act as an offshore supervisor and will provide advice on the synthetic planning and overall progress in the synthesis of new natural products. His expertise in synthetic chemistry spans 30 years, in which he has over 140 publications.

H. Confidentiality and Intellectual Property

No IP related issues are currently connected with this project.

I. Approvals

No special approvals are required for this project.

Bibliography

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Research timeline	2009				2010				2011				2012	
	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June
Reviewing literature														
Synthesis of Fragment A														
Submit research proposal														
PhD proposal presentation														
Confirmation of candidature due														
Annual report due														
Synthesis of Fragment B														
Attaching fragments														
Biological testing and analogue preparation														
Annual report due														
Thesis writing														