Design, synthesis and evaluation of spirocyclic chromanes, dihydropyridines, and naphthoquinones as antimalarial agents

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Dedication

This work is dedicated to God Almighty. Your divine grace and mercy was evident in my entire degree program.
Acknowledgements

Firstly, I will like to acknowledge my advisor, Roman Manetsch. You have been a mentor, a friend and a huge support. Without you, I would not have accomplished what I have. Thank you Roman.

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Words cannot really express my gratitude to God. It has been You all the way.
Abstract of Dissertation

Malaria is a mosquito-borne infectious disease, which remains a public health challenge globally. About half of the world population is at risk with 214 million new cases and approximately 438,000 deaths in 2015. The emergence of resistance to all known antimalarials especially to the current standard treatments artemisinin and artemisinin combination therapy (ACT) is a major cause of concern, necessitating the need for development of new antimalarial chemotypes. The Manetsch laboratory has been involved in the discovery of novel orally bioavailable antimalarials to mitigate its menace with special interest in the multi-drug resistant malaria.

Screening of a library of synthetic compounds containing drug/natural product-based motifs against a chloroquine sensitive strain (Dd2) and a chloroquine resistant strain (3D7) led to the identification of a spirocyclic chromane (SPC) \(2.1\) with promising antimalarial activity and excellent physicochemical properties. Systematic structure-activity relationship and structure-property relationship (SAR and SPR) studies were carried out to identify the essential structural features and improve antimalarial activity and physicochemical properties. The spirocyclic chromane core was identified to be relevant for its activity, whereas amine \(2.5a\) without the \(N\)-substituent of the piperidine ring was only 2-fold less active than the original compound. Attempts to rigidify the entire molecule by substitution of an amide bond for the alkyl linker revealed that flexibility was an essential part of the molecule. With the intention of improving the bioavailability, compounds with a higher saturation index (Fsp\(^3\)) were designed by a variety of changes to the naphthalene core. Changing the angle of trajectory of the piperidine ring substituent displayed a clear pattern of activity with a scaffold possessing a chiral quaternary carbon being marginally more potent than the initial compound.
Another screening using a chemical genetic approach identified a class of asymmetric dihydropyridines (DHPs) displaying synergy with mefloquine, a known antimalarial.\textsuperscript{4} Extensive hit-to-lead optimization revealed the most potent DHP to be the trans enantiomer with (4\textit{R},7\textit{S}) configuration. Furthermore, the besylate salt of an amino-containing DHP was demonstrated to possess significantly improved oral bioavailability. A synthetic route was optimized to provide large amounts of compound required for detailed in vivo efficacy and pharmacokinetic studies. The amino-substituted DHP was found to be orally bioavailable displaying with moderate killing profile like pyrimethamine. It eliminated \textit{Plasmodium falciparum} from peripheral blood of mice upon oral administration of three daily doses.

As the major threat to eradication of malaria is the constant emergence of resistance to antimalarials, an understanding of how the parasites develop resistance will be useful in developing a formidable weapon in the fight against malaria. The Manetsch laboratory in collaboration with the Kyle laboratory believes that menoctone is an interesting compound to study the process of resistance induction and emergence. Menoctone is a hydroxynaphthoquinone, which is difficult to synthesize\textsuperscript{5} as existing synthetic methods are long with over 10 steps involving expensive reagents. Most of the steps are tedious and low yielding.\textsuperscript{6} We designed, developed and optimized a shorter synthetic route (7 steps) with cheaper starting materials using modern and classical chemical transformations. On induction of resistance with menoctone, the resistant parasite demonstrated cytochrome b mutation M133I located outside of the Qo site, the same resistance mutation for atovaquone. A study to access the efficacy of menoctone against liver stages and gametocytes of menoctone resistant \textit{P. berghei} is currently ongoing.
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<th>Meaning</th>
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<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>ACN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>AcOH</td>
<td>acetic acid</td>
</tr>
<tr>
<td>ACT</td>
<td>artemisinin-based combination therapy</td>
</tr>
<tr>
<td>AIDS</td>
<td>acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>Ar</td>
<td>aryl</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butyloxycarbonyl</td>
</tr>
<tr>
<td>Bu</td>
<td>butyl</td>
</tr>
<tr>
<td>BuLi</td>
<td>butyllithium</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]-undec-7-ene</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DHA</td>
<td>dihydroartemisinin</td>
</tr>
<tr>
<td>DHFR</td>
<td>dihydrofolate reductase</td>
</tr>
<tr>
<td>DHPS</td>
<td>dihydropteroate synthase</td>
</tr>
<tr>
<td>DIA</td>
<td>diisopropylamine</td>
</tr>
<tr>
<td>Dibal</td>
<td>diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift (ppm)</td>
</tr>
<tr>
<td>EC50</td>
<td>half maximal effective concentration</td>
</tr>
<tr>
<td>ee</td>
<td>enantiomeric excess</td>
</tr>
<tr>
<td>equiv.</td>
<td>equivalent</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionization</td>
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<tr>
<td>Et</td>
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<td>EtOAc</td>
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<td>ethanol</td>
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<td>Et2O</td>
<td>diethyl ether</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FVB</td>
<td>Friend leukemia virus B</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
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<tr>
<td>HRMS</td>
<td>high-resolution mass spectrometry</td>
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<tr>
<td>HTS</td>
<td>high throughput screening</td>
</tr>
<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>IC50</td>
<td>half maximal inhibitory concentration</td>
</tr>
<tr>
<td>iPr</td>
<td>isopropyl</td>
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</table>
iPrOH isopropanol
LC-MS liquid chromatography-mass spectrometry
LDA lithium diisopropylamide
Me methyl
MHz megahertz
MeOH methanol
MIC minimum inhibitory concentration
min minutes(s)
MMV Medicines for Malaria Venture
NaBH₄ sodium borohydride
NaOH sodium hydroxide
NBS N-bromosuccinimide
ND not determined
NMR nuclear magnetic resonance
p.o. oral administration (per os)
PABA para-aminobenzoic acid
PEG polyethylene glycol
Ph phenyl
PK pharmacokinetics
PPh₃ triphenylphosphine
Pr propyl
prep-HPLC preparative high-pressure liquid chromatography
pTSA para-toluenesulfonic acid
Py pyridine
PyBr₃ pyridinium tribromide
pyr pyrimethamine
RP reverse phase
RT room temperature
SAR structure activity relationship
SE staphylococcal enterotoxin
SI selectivity index
SPR structure property relationship
TBAF tertrabutylammonium fluoride
TBS tributyl silyl
tBu tert-butyl
t-BuOH tert-butanol
TEA triethylamine
TFA trifluoroacetic acid
THF tetrahydrofuran
TLC thin layer chromatography
TMS trimethyl silyl
UCF University of Central Florida
USF University of South Florida
WHO World Health Organization
WRAIR Walter Reed Army Institute of Research
Chapter 1. Introduction to Malaria

1.1 Malaria Overview

Malaria continues to be a global public health challenge, resulting in 214 million new cases and approximately 438,000 deaths, with 306,000 children under the age of 5, in 2015 alone. Half of the world’s population is at risk for malaria with Africa accounting for 88% of the cases and 90% of the deaths. Malaria is caused by protozoa of the genus *Plasmodium*, which destroy infected red blood cells. Of the five *Plasmodium* species known to cause the human disease, *Plasmodium falciparum* and *Plasmodium vivax* are the most prevalent. *P. falciparum* is most prevalent on the African continent, and is responsible for most deaths from malaria. *P. vivax*, which is more common in many areas outside Africa, has a wider geographic distribution because it can develop in the *Anopheles* mosquito vector at lower temperatures, and can survive at higher altitudes and cooler climates. In humans, it has a dormant liver stage, which can activate months after initial infection thereby causing a relapse of symptoms.1-2

The past decade has witnessed significant progress in reduction of malaria through decreasing the number of deaths by about half. This success is due to renewed efforts to control and kill mosquito populations by indoor residual spraying (IRS), preventing transmission by insecticide-treated nets (ITNs), and improved accessibility to effective medicines.3 However, the recent reports of emergence of resistance to artemisinin and artemisinin combination therapy (ACT), the current standard treatments, and all previously known antimalarials is a major concern.4 In order to sustain and improve the success achieved in the elimination and eradication of malaria, it is paramount to conduct research possibly leading to new antimalarial chemotypes that will have potential to be developed into drug therapies. An ideal therapy will have a single-dose efficacy against the blood
stages of multiple drug resistant malaria, block transmission of infectious gametocytes to mosquitoes, and eradicate the liver stage infections in the dormant form.

1.2 Drug Design and Oral Bioavailability

The rule-of-five (RO5), a physical property guideline for drug bioavailability was published almost twenty years ago.\(^5\) Derived from a database of a library of clinical drug candidates reaching Phase II trials or higher, RO5 predicts that compounds with poor absorption and permeability are more likely those that the number of hydrogen-bond donors (OH plus NH count) is \(> 5\); hydrogen bond acceptors (O plus N) is \(> 10\); molecular weight is \(> 500\) Daltons (Da); the calculated Log P (CLogP) is \(> 5\).\(^6\) The RO5’s introduction has increased the cognizance of physical properties of potential drug candidates in the medicinal chemistry community. It’s the simplicity of the concept and easy calculations that have made RO5 the leading measure of drug-likeness with the original article having more than 4,280 literature citations. Subsequent studies on drug absorption have identified additional properties like polar surface area (PSA) and rotatable bonds that play a significant role in the success of drug development.\(^7\) Physiochemical profiles of orally bioavailable drugs are consistent with the rule-of-five but oral bioavailability does not just depend only on absorption but also on dissolution, gut transit time and first pass metabolism.\(^8\)

Despite the advancements and increase in the pharmaceutical research and development, the number of new drugs approved annually has steadily declined over the past decade. One of the main causes of this is that many compounds synthesized by medicinal chemists are not drug-like when measured by criteria like the RO5.\(^9\) Many researchers have carried out analyses of the property of compounds under clinical trial and marketed drugs. Leeson and co-workers analyzed the difference between oral drugs launched before 1983 and those launched between 1984 and
2004 and found out that though the ClogP, polar surface area, and the number of hydrogen bond donors did not display statistical differences. Nevertheless, the number of hydrogen bond acceptors, the number of rings and rotatable bonds have increased over time.\textsuperscript{10} In a later study conducted in 2011, they found that the molecular weight, number of hydrogen bond acceptors and CLogP have increased over time\textsuperscript{11}. While comparing pharmaceutical patents and approved drugs, they found that compounds in pharmaceutical patents are larger, more lipophilic and less three dimensional than oral drugs.\textsuperscript{12} A statistical study of chemical compounds published in \textit{Journal of Medicinal Chemistry} between 1959 and 2009 by Walters and co-workers also revealed that properties such as lipophilicity and three-dimensionality are moving away from ranges found in successful drugs.

Another property that relates to aqueous solubility and drug-likeness of a molecule is its saturation index or fractional sp\textsuperscript{3} (Fsp\textsuperscript{3}) character. It was originally defined as the ratio of the number of sp\textsuperscript{3} carbons to the total number of carbon atoms.\textsuperscript{13}

\[ Fsp^3 = \frac{\text{number of sp}^3\text{carbons}}{\text{Total number of carbons}} \]

Fsp\textsuperscript{3} is directly related to aromatic character of drug-like molecules and indirectly related to the three-dimensional shape. A study by Lovering and co-workers on chemical compounds at different stages of clinical development revealed that saturation index which closely correlates with solubility is an important property for success in drug discovery as the average Fsp\textsuperscript{3} increases consistently with the stages of drug development from 0.36 for discovery compounds to 0.47 for drugs. The higher the saturation index, the higher the solubility and the lower the melting point. It also showed that complexity, as measured by saturation, has the capacity to access larger chemical
space. Another property closely related to the saturation index is the three-dimensional shape of the compound. The higher the saturation index and the more the chiral and quaternary carbon centers, the more three-dimensional in shape a compound will be. The molecular shape could allow for improved target interactions, improved potency and specificity of the drug candidate. However, despite the relevance of high saturation index to the success of a chemical compound in drug discovery, there have been a steady decline in the level of saturation in most synthesized compounds partly because of the advances in sp\(^2\)-sp\(^2\) couplings and other sp\(^2\) couplings.\(^{14}\) It is generally believed that ignorance or deliberately ignoring these essential qualities of chemical compounds have partly been the reason for failure in drug discovery including malaria.

1.3 Development of Antimalarial Drugs

Medicines for Malaria Venture (MMV) is a nonprofit organization established in Switzerland in 1999 with a mission to reduce the malaria burden in endemic countries through the discovery, development and delivery of novel, effective and affordable antimalarial drugs. Its ultimate goal is to help in eradicating malaria.

The malaria parasite cycle is complex involving successful infection of both the female *Anopheles* mosquitoes and human hosts (figure 1.1). When taking a blood meal to nourish her eggs, the female *Anopheles* mosquito injects sporozoites into the host bloodstream where they are taken in by the liver developing to the hypnozoites or liver-stage parasites. In *Plasmodium vivax* and *Plasmodium ovale* malaria species, some hypnozoites remain dormant for months or even years and can initiate the life cycle in the absence of a new mosquito bite, thereby causing the clinical symptoms of malaria (relapsing malaria). In all *Plasmodium* species, the parasite develops into schizonts from which several merozoites develop. Upon rupturing of the liver cells,
the mereozites are released into the blood stream where they begin rapid invasion of the red blood cells. These blood-stage parasites rapidly attain a high parasitic burden by asexual replication, which leads to the clinical symptoms of malaria.

**Figure 1.1: Life cycle of malaria parasite**

In the blood, some mereozites differentiate into male and female gametocytes which when taken up by the female *Anopheles* mosquito as a blood meal, continue the cycle of transmission in the mosquito.  

All current available antimalarials act on the blood-stage cycle of the malaria life cycle. Though this is the symptomatic stage of the disease, for complete eradication, drugs targeting the asymptomatic stages like the dormant liver stage causing malaria relapse or drugs that prevent the gametocytes formation thereby preventing transmission to mosquitoes need to be designed and
developed. A drug that alters the parasite life cycle in the mosquito and/or prevents its development into the sporozoites will also be essential to prevent the transmission from mosquitoes to man. These issues in combination with the report of confirmed cases of emergence and spread of resistance to all the current antimalarials including artemisinin combination therapy (ACT) make the need of replacements for the current antimalarials urgent. In a combination therapy, ideally, at least one component need to be as fast-acting as the artemisinin derivatives to provide rapid symptom relief and as affordable as chloroquine when used as a first-line treatment. Other desired qualities in antimalarials include oral bioavailability with single dose cure, no interaction risks with other medicines for treating co-infections, chemical stability in Zone IV climate conditions (hot and humid) for a long duration and safe for sensitive patient groups, including pregnant women\textsuperscript{16} and infants.\textsuperscript{17}

In the quest for malaria eradication, MMV’s database reflects the progress in the development of a new orally bioavailable antimalarial in over the last decade. A handful of interesting compounds under development will be discussed.

### 1.3.1 Dihydroisoquinolone SJ733

SJ733, a dihydroisoquinolone (DHIQ), is a clinical antimalarial candidate discovered by an international research collaboration led by St. Jude Children’s Research Hospital.\textsuperscript{18} Using a chemical genetic screening approach, a library containing 309,474 unique compounds covering diverse and extensive bioactive space was screened against \textit{Plasmodium falciparum} chloroquine-sensitive 3D7 strain and the chloroquine-resistant K1 strain. After subsequent \textit{in vitro} and \textit{in vivo} screening to identify molecules that block the proliferation of \textit{Plasmodium falciparum} in cocultures with human erythrocytes, three high-priority series were identified which included
DHIQs.\textsuperscript{19} The structure-activity and structure-property, pharmacological and chemical optimization of the dihydroisoquinolone series led to the selection of (+)-SJ733 for development as a fast clearance component for a single-exposure radical cure and prophylaxis (SERCaP) drug.\textsuperscript{3} A look at the chemical features of (+)-SJ733 (figure 1.2) shows it has two stereogenic carbons, a characteristic of a three-dimensionality of a molecule. It also has five hydrogen bond acceptors, one hydrogen bond donor and Fsp\textsuperscript{3} value of 0.16.

**Figure 1.2: Structure of SJ733**

\begin{center}
\includegraphics[width=0.3\textwidth]{sj733.png}
\end{center}

SJ733

When tested *in vivo* against various strains of *P.falciparum*, including those resistant to other antimalarial drugs, (+)-SJ733 was highly potent (EC\textsubscript{50} range 10-60 nM) and it was also potent against all erythrocytic life cycle stages. The (+)-SJ733 enantiomer was significantly more potent than the (-)-SJ733 enantiomer; however, it was about 10-fold less potent against *Plasmodium berghei* and other rodent malarias *ex vivo*. Interestingly, (+)-SJ733 was highly potent and efficacious *in vivo* in a NOD-scid IL2R\gamma\textsuperscript{null} mouse model when administered in four sequential daily oral doses.\textsuperscript{20} It exhibits impressive *in vivo* pharmacokinetics in all preclinical species, including mouse, rat and dog with excellent exposure and reasonable clearance with an oral bioavailability of >65\% in both rats and dogs. A study of the mechanism of action and tendency
of resistance acquisition showed that (+)-SJ733 targets ATP4; a cation-transporting ATPase responsible for maintaining low intracellular Na$^+$ levels in the parasite. It slowly mutates a gene PFL0590c encoding the putative Na$^+$-ATPase PfATP4 by pressurizing the erythrocytic coculture with (+)-SJ733.$^{21}$ Two resistant strains (P.falciparum ATP4L350H and ATP4P966T) tested in a competition assay were found to be substantially less fit compared to their parent strains in the absence of drug pressure, so suggesting that the mutations that confer resistance to (+)-SJ733 carry a high fitness cost. Another important activity of (+)-SJ733 is its rapid parasite clearance, reducing the parasite burden by 80% within the first 24 hours and completely clearing the parasite by 48 hours in treatment of P. falciparum-infected NOD-scid IL2R$^\gamma$null mice, P. falciparum-infected splenectomized NOD-scid IL2R$^\gamma$null or normal mice infected with rodent malarias.$^{18}$ However, in in vitro analysis of infected erythrocytes, growth fully stopped within 24 hours but it required 96 hours to fully kill the parasite implying that (+)-SJ733 is about fourfold faster in vivo than in vitro. This suggests that by targeting ATP4, it induces physical changes in the infected erythrocyte that allows for recognition and clearance of treated, infected cells by the host. (+)-SJ733 also does possess transmission blocking activity.$^{15}$

1.3.2 Imidazolopiperazine KAF156/GNF156

In a bid to identify novel chemotypes inhibiting Plasmodium falciparum, Novartis Institute for Tropical Diseases in Singapore in collaboration with Genomics Institute of the Novartis research foundation (GNF), the Biomedical Primate Research Centre (BPRC), and the Swiss Tropical Institute with support from Wellcome Trust and Medicine for Malaria Venture extensively screened a library of approximately 2 million compounds using a cell proliferation assay of 3D7 strain Plasmodium falciparum. Five thousand of them were disclosed in a public malaria database (https://www.ebi.ac.uk/chembldb/index.php) after multiple confirmations of the hits. Using
criteria such as IC\textsubscript{50} values less than 1 µM against a wild type and a drug resistant strain, a safety index greater than 20-fold, synthetic tractability, as well as confirmation of multiple active hits within the scaffold, a class of imidazolopiperazines was identified as an attractive hit series. Advantages of this compound series include the novelty of their structures compared to currently known antimalarial drugs such as aminoquinolones and endo-peroxides, their activity against wild type and resistant strains, high selectivity index (>20-fold), high solubility of one of the compounds and its non-interaction with cytochrome P450 isoforms.\textsuperscript{22} However, the series was a moderate hERG substrate and had poor plasma oral exposure indicating metabolic instability or poor permeability, which necessitated initial structure-activity and structure-property relationship (SAR and SPR) studies to improve potency and remove metabolically vulnerable functionality in the compounds.

**Figure 1.3: Optimization of hit to KAF156**

An extensive SAR study on the three vulnerable moieties in the hit compound led to analogue 1.1 (figure 1.3) with good \textit{in vitro} and \textit{in vivo} antimalarial activities as well as good pharmacokinetic and physiochemical properties with double-digit nanomolar antimalarial activity and moderate oral exposure in mice. The unsubstituted piperazine possessed chemical and metabolic liabilities as \textit{in vitro} metabolic stability in murine microsomes results revealed that biologically inactive
metabolites were formed from the oxidation of the piperazine ring. On optimizing the piperazine ring by substituting all possible positions (positions 5, 6 and 8), the preclinical candidate KAF156 with improved mouse oral exposure, reduced hERG risk, improved efficacy over chloroquine and artemesunate was discovered.

**Figure 1.4: Structure of KAF156**

![KAF156](image)

Structurally, while the hydrogen bond acceptor count was reduced from 7 in the hit to 5 in the preclinical candidate KAF156, the number of hydrogen bond donors remained the same (figure 1.4). During the hit to lead optimization, the Fsp$^3$ character increased from 0.23 to 0.27, a possible contributor to the improved oral bioavailability.

The nanomolar activity of KAF156 in laboratory-adapted *P. falciparum* strains cultured in human erythrocytes was maintained over a variety of strains resistant to one or more current antimalarial drugs. It was also active against clinical isolates of *P. vivax* and *P. falciparum* collected from malaria patients in the Thai-Myanmar border region and Papua Indonesia where resistance of *P. vivax* and *P. falciparum* have been reported. Drug resistance development has been a big issue in drug development for malaria. To study the potential for developing resistance to KAF156 as well as its mechanism of action, *P. falciparum* clones were selected. After analysis of the resistant clones, all the resistance lines acquired single nucleotide polymorphism (SNP) in a single gene *pfcarl* (PlasmoDB ID PF0970w), which encodes a protein of unknown function with seven
putative transmembrane domains. Interestingly, current antimalarial drugs were found to be active against the KAF156-resistant strains and the mutations in *Pfcarl* are specific to imidazolopiperazines and do not result in resistance to other classes of drugs making it an interesting combination therapy treatment. When the frequency of drug resistance mutations was studied, resistant parasites emerged only from cultures containing more than 10⁸ parasites with a frequency of about 1 per 10⁸ parasites with Dd2 *P. falciparum* strain.

Imidazolopiperazines also inhibited the growth of exo-erythrocytic forms and intrahepatic schizonts *in vitro* using CD81-expressing HepG2 heptoma cells infected with rodent *P. yoelli* liver-stage parasites. In a causal prophylactic rodent malaria model study, mice were given a single oral dose of 10 mg/kg of KAF156 two hours before being intravenously infected with liver targeting *P. berghei* sporozoites. The compound was fully protective when mice were monitored for over 30 days. KAF156 also inhibits gametocytogenesis and blocks transmission.²⁴ When treated with early-stage gametocytes, the compound inhibits gametocyte maturation in vitro even at concentrations as low as 50 nM and when fed to mosquitoes, through a standard membrane-feeding assay (SMFA), there was no maturation to oocysts. Also, when fully matured gametocytes were incubated for about 15 minutes with KAF156 and fed to the mosquitoes, there was a drastic reduction of the oocysts formed. When infected mice were treated with a single oral dose of 100 mg/kg, they were not able to infect *Anopheles* mosquitoes feeding on their blood.²⁵

### 1.3.3 Spiroindolone KAE609/NITD609

KAE609, also known as cipargamin and formerly known as NITD609 is a new synthetic antimalarial drug belonging to the spiroindolone class of compounds. It was developed at Novartis Institute for Tropical Diseases in Singapore in collaboration with the Genomics Institute of the Novartis research foundation (GNF), the Biomedical Primate Research Centre and the Swiss
Tropical Institute. It was awarded MMV project of the Year 2009. Spiroindolone KAE609 was discovered by screening a Novartis library of about 12,000 pure natural products and synthetic compounds with natural product features. Initial screening identified 275 hits with submicromolar activity against *Plasmodium falciparum*. Screening against multidrug-resistant parasites and cytotoxicity led to 17 viable compounds including a compound from the spiroazepineindole class with favorable pharmacological profile and a starting point for a lead optimization. Synthesis and optimization to address its metabolic liabilities leading to improved stability and exposure levels yielded spirotetrahydro-β-carboline (or sproindolone) compound NITD609.

**Figure 1.5: Structure of KAE609**

![Structure of KAE609](image)

It is a compound with interesting and unique structural features including two chiral centers, one of which is quaternary, conferring much three-dimensionality to it. KAE609 has three hydrogen bond donors, one hydrogen bond acceptor and Fsp$^3$ value of 0.21 (figure 1.5). It is synthesized in eight steps, including chiral separation of an active enantiomer with the possibility of large-scale production. NITD609 displayed single-digit nanomolar antimalarial blood stage activity *in vitro* in the *P. falciparum* strain and it maintains similar activity against drug resistant strains. On testing in *ex vivo* assays with *P. falciparum* and *P. vivax* isolated from patients on the Thai-Burmese border, a place known for chloroquine resistance, NITD609 was as effective as artesunate as it displayed nanomolar activity against both species of *plasmodium*. In similarity to artesunate, it
has the ability to kill both mature trophozoite and immature *P. vivax* ring stages. In spite of their stage of development, all NITD609 treated parasites showed obvious signs of dying parasites.  

**1.3.4 Dihydrofolate Reductase Inhibitor P218**

P218 was discovered by Yongyuth Yuthavong and coworkers at National Science and Technology Development Agency/National Center for Genetic Engineering and Biotechnology (NSTDA/BIOTEC), together with MMV, Monash University and the London School of Hygiene and Tropical Medicine. Its design was based on 3-dimentional structures of malaria protein targets with the aim of avoiding mutations that led to resistance development to pyrimethamine, a safe and previously effective drug for malaria treatment. There are few well-defined clinically validated targets for antimalarial drug discovery and the best known of such target is *P. falciparum* dihydrofolate reductase (DHFR), which the antimalarial drugs pyrimethamine and cycloguanil inhibit. In *Plasmodia*, DHFR and thymidylate synthase (TS) coexist as a single chain bifunctional enzyme (DHFR-TS). The crystal structures of the wild-type bifunctional DHFR-TS and the pyrimethamine-resistant quadruple mutant (QM) enzyme from *P. falciparum* have been studied and reported. A comprehensive investigation of antimalarial inhibitors (pyrimethamine and cycloguanil) binding to the wild-type and mutant forms of PfDHFR-TS revealed a structural basis for reduced binding of the inhibitors to mutated PfDHFR-TS.  

A lot is known about antifolate-based malaria. Rieckmann in 1973 reported WR99210 with potent *in vitro* antimalarial activity in the nano- to picomolar range, but was not developed further due to its low bioavailability and extreme gastrointestinal toxicity.
Structurally, WR92210 has seven hydrogen bond acceptors, two hydrogen bond donors and Fsp \(^3\) value of 0.42 (figure 1.6).

Using the information from the crystal structure of the binding pockets of wild-type DHFR-TS and the pyrimethamine-resistant QM, Yongyuth Yuthavong and coworkers optimized WR92210 to inhibit pyrimethamine-resistant DHFR by focusing on structural changes to avoid steric clashes with known mutations, improve hydrogen bonding and bioavailability. Their analogue P218 was studied to compare how it binds to PfDHFR-TS and human DHFR by cocrystallization and interestingly, it binds differently with no direct interaction with human DHFR.\(^3^4\)

**Figure 1.7: Structure of P218**

P218 has eight hydrogen bond acceptors, three hydrogen bond donors and Fsp \(^3\) value of 0.38 (figure 1.7). It is highly active against pyrimethamine-resistant *P. falciparum* with quadruple mutant PfDHFR *in vitro*.\(^3^5\) In *in vivo* studies against both *Plasmodium chabaudi* in CD-1 mice and quadruple mutant *P. falciparum* in SCID mice, the compound was highly active and efficacious. In rats at 30 mg/kg, P218 showed good oral bioavailability and a reasonable half-life with minimal
brain distribution. It doesn’t induce gene mutation, thereby making it less susceptible to drug resistance compared to pyrimethamine. P218 is a safe, potent and selective inhibitor of wild-type and resistant malaria with good drug-like physiochemical properties and pharmacokinetic profile. It is currently undergoing preclinical evaluation.\(^{30}\)

1.3.5 Artefenomel (OZ439)

The recommended first-line antimalarial drugs are the semisynthetic artemisinin’s artemether and artesunate usually employed with partner drugs in artemisinin combination therapies (ACTs). Artemisinin derivatives are clinically effective because they are fast acting against all erythrocytic stages of malaria parasite.\(^{36}\) However, they have short \textit{in vivo} half-lives and so require a three-day treatment plan in combination with longer-acting antimalarial drugs so as to maximize cure rates.\(^{37}\) The plant source remains the only means of isolating artemisinin, the starting material for its derivative, thereby affecting its production.\(^{38}\) Also recently, there have been reports of ACT treatment failures at the Thai-Cambodian border as well as parasitic resistance, thereby making it necessary to explore other peroxides that could overcome its limitations. The first synthetic ozonide to be clinically evaluated is Arterolane (OZ277) and is now in Phase III clinical trial in the form of an arterolane maleate/piperaquine phosphate combination.\(^{39}\)

\textbf{Figure 1.8: Structure and optimization of OZ277 to OZ439}
OZ277 has five hydrogen bond acceptors, two hydrogen bond donors and a remarkably high Fsp\(^3\) value of 0.95. OZ277, like Artemisinin has a peroxide bond, which is responsible for its activity. It exhibits antimalarial activity against all asexual blood stages of \textit{P. falciparum} with a fast acting mechanism. However, its half-life is relatively short and it displayed reduced plasma exposure in clinical trials. In order to overcome the \textit{in vivo} half-life and blood exposure profile limitation so as to achieve a single-dose oral cure, OZ277 was synthetically modified to reduce clearance and thereby increase the half-life and overall exposure profile.\(^{40}\) Structural modification of the \textit{cis}-8’-alkyl group to a \textit{cis}-8’ phenyl substituent (OZ439) drastically increased the stability to Fe(II)-mediated degradation. OZ439 has six hydrogen bond acceptors and no hydrogen bond donor. It also has a reduced Fsp\(^3\) value of 0.78 (figure 1.8). When both ozonides were administered to rats at an oral dose of 3 mg/kg, OZ439 showed significant clearance reduction, improved bioavailability and a half-life of over 20 hours compared to 1 hour for OZ277.\(^{41}\) The half-life was consistent with female FVB mice, male Sprague-Dawley rats and male beagle dogs when used for the study. In an antimalarial efficacy study with \textit{P. berghei} model, complete cure was observed at a single dose of 20 mg/kg or three daily oral doses of 5 mg/kg of OZ439. This level of efficacy is superior to OZ277 and all reported semisynthetic artemisinin derivatives. To test its rapidity of action, OZ439 was orally administered to \textit{P. berghei} infected mice orally 3 days post-infection. Between 7 to 8 days after treatment, parasitaemia was reduced to below detection limit (<0.1%) and there was no recrudescence and no detectable parasites at 30 days post infection. When orally administered 48 hours before infection, OZ439 was completely protective thereby demonstrating exceptional prophylactic activity. In a toxicity study in mice, OZ439 was orally administered in doses as high as 300 mg/kg and only minimal side effects where observed and the effects were
completely reversible. It was found to be nongenotoxic and is currently in phase IIb clinical trial in combination with piperaquine as a single-exposure cure. 42

1.4 Identification of New Antimalarials

Despite the advancements that have been made in the design and development of novel antimalarials, there is currently no drug or drug candidate that tackles all the concerns about malaria and possesses the desired qualities in antimalarials which include single oral dose cure, fast acting and/or long lasting. This underlines the imperativeness of new medicines as weapons in the fight against malaria. In a quest to discover new antimalarial agents, efforts have been made in different projects to identify, design and develop chemical agents that will help in addressing some issues like resistance and bioavailability in antimalarial development. A novel class of compound having a spirocyclic chromane (SPC) core with antiplasmodial activity was identified after screening of a unique set of natural-product-like compounds enriched with oxygen and saturated rings. The compound was fast-acting against the blood-stage of malaria parasite development. The inherent feature of the SPC showed that it is in close compliance with the Lipinski’s rule-of-five. Another class of compounds comprising of dihydropyridines (DHPs) was identified using a chemical genetic approach screening for compounds with antimalarial activity. Interestingly, this compound showed synergy with mefloquine, a known antimalarial drug. This synergistic effect is essential in developing a drug as a combination therapy. Furthermore, menoctone, an old compound in the naphthoquinones class that was identified as potent against malaria parasite but not developed for reasons associated with its bioavailability and synthetic difficulties was found to be useful in both antimalarial drug development and parasite resistant induction evaluation. The potential of these three chemotypes motivated us to (a) conduct
structure-activity and structure-property relationship studies to further optimize the SPC and DHP compound series, and (b) to develop a new synthetic route for menoctone and to prepare large amounts of compound required for resistance inductions studies.

1.5 References


Chapter 2. Spirocycic Chromanes as Antimalarials

2.1 Introduction

Malaria remains a global and deadly infectious disease, with over 200 million annual cases and about half of the world’s population at risk.\textsuperscript{1-3} The heaviest burden of the disease is in sub-Saharan Africa, accounting for about 90\% of all deaths predominantly children under five years.\textsuperscript{3} Malaria is caused by protozoa of the genus \textit{Plasmodium}, which destroy infected red blood cells. \textit{Plasmodium falciparum} and \textit{Plasmodium vivax} are the most prevalent of the five \textit{Plasmodium} species known to cause the human disease.\textsuperscript{4,5} The development of multi-drug resistant parasites has particularly made current available drugs less effective. Most drugs used for the treatment of malaria were either developed over 30 years ago or derived from older drugs, which makes the search for a novel line of therapy imperative.

Natural products (NPs) and their derivatives or mimics have been a productive source of most of the active ingredients in drug development.\textsuperscript{6-8} Besides their diversity, they are known to occupy biologically important chemical space.\textsuperscript{6,7} Interestingly, researches have shown that the higher the Fsp\textsuperscript{3} character (saturation index) of a chemical compound, the more bioavailable it will be.\textsuperscript{9-10} However, despite the advantages and success of NPs, their use in drug discovery have decreased due to structural complexity, limited accessibility and supply, challenges working with NPs, and concerns about intellectual property rights.\textsuperscript{6-7} With a view to identify novel antimalarial scaffolds that could overcome the problem of drug resistance, screening of a set of compounds with drug/natural product-based substructural motif led to the discovery of a unique spirocyclic chromane 2.1 (fig. 2.1) with promising antimalarial activity against the chloroquine-resistant Dd2 strain and the chloroquine sensitive 3D7 strains of the parasite. The scaffold exhibited excellent
potency with a 50% effective concentration (EC$_{50}$) of 350 nM against the chloroquine-resistant Dd2 strain and a selectivity over 50 against human liver HepG2 cells. A close analysis of the physiochemical properties showed close compliance with Lipinski’s parameters with an acceptable physiochemical profile. The biological evaluation of 2.1 indicated that it is early-acting in blocking parasite development at all intraerythrocytic stages including ring, trophozoite and schizont development as well as merozoite invasion. Interestingly, it exhibits a cellular mechanism of action distinct from current antimalarials.\textsuperscript{11}

**Figure 2.1. Structure of spirocyclic chromane**

2.2 Results and Discussion

2.2.1 Synthetic Chemistry

The synthesis of 2.1 from α-naphthol was first reported by Bracken et al.\textsuperscript{11,12} Boc-deprotection of tert-butyl 4-oxo-3,4-dihydrospiro[benzo[h]chromene-2,4’-piperidine]-1’-carboxylate 2.2 using TFA in DCM afforded 2.2a which was alkylated with various alkyl halides to give 2.2b-h, 2.2j-k in moderate to good yields. Sodium borohydride reduction of the corresponding alkylated carbonyl compounds yielded the hydroxy analogues 2.3a-h, 2.3j-k. For the preparation of compounds 2.2h-i, the corresponding epoxide, which was prepared from the sulfur ylide epoxidation of their aldehyde, was refluxed with 2.2a in ethanol which was subsequently reduced to afford analogues 2.3h-i in good yields. The bischlorinated analogues 2.2i, 2.2k, 2.3i and 2.3k were inspired by the studies carried out by Medicine for Malaria Ventures (MMV) and GlaxoSmithKline (GSK)\textsuperscript{14}. The
essence of flexibility was also evaluated by altering the linker length between the spirocyclic and bisfluoro moiety. Due to lack of commercial availability of bisfluoro phenyl bromides, commercially available bromides with linker length 1-3 were used in alkylation **2.2a** to **2.2c, 2.2d, 2.2f** which were then reduced to afford their hydroxy analogues **2.3c, 2.3d, 2.3f**. The rigidified analogues were also prepared from an EDCI coupling of **2.2a** with 2-(3,4-difluorophenyl)acetic acid to afford the amide **2.2l** which was then reduced to its hydroxy analogue **2.3l** (scheme 2.1).

**Scheme 2.1: Synthesis of analogues with different oxidation state of C-4 and the N-substituent of the piperidine ring**

Reactions and conditions: (i) bromoethane, K$_2$CO$_3$, DMF (ii) 4-(2-bromoethyl)-1,2-difluorobenzene, K$_2$CO$_3$, DMF (iii) (bromomethyl)benzene, K$_2$CO$_3$, DMF (iv) 2-bromoethynylethynyl, K$_2$CO$_3$, DMF (v) (2-bromoethyl)cyclohexane, K$_2$CO$_3$, DMF (vi) (3-bromopropyl)benzene, K$_2$CO$_3$, DMF (vii) 4-(2-bromoethyl)-1,2-dichlorobenzene, K$_2$CO$_3$, DMF (viii) 2-bromo-1-(3,4-difluorophenyl)ethanone, Et$_3$N, DCM (ix) 2-(3,4-difluorophenyl)oxirane, EtOH, reflux (x) 2-(3,4-dichlorophenyl)oxirane, EtOH, reflux (xi) EDCI, DMAP, DCM.

All direct hydrogenolysis attempts on **2.3a-2.3l** using Pd/C, Pd/BaSO$_4$ or Raney Nickel to afford the dehydroxylated product were not successful. An alternate approach consisting of an initial reduction of the carbonyl group in tert-butyl 4-oxo-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidine]-1'-carboxylate **2.2**, with sodium borohydride and subsequent hydrogenolysis of the
hydroxyl group using Pd/C in ethanol afforded the methylene analogue tert-butyl 3,4-
dihydrospiro[benzo[h]chromene-2,4'-piperidine]-1'-carboxylate 2.4, in good yields. However, a
slightly more non-polar compound was also always formed albeit in low yields. Surprisingly, upon
isolation and characterization, it was identified to be an analogue with the complete reduction of
the outer aromatic ring of the naphthalene moiety 2.17, which was considered to be an interesting
analogue in studying the effect of increasing the Fsp³ by partial reduction of the naphthalene ring.
Boc-deprotection of tert-butyl carboxylate 2.4 and the subsequent alkylation of the resultant amine
2.5a, afforded the alkylated amines 2.5b-2.5h in good yields. Treating the amine 2.5a with 2-(3,4-
difluorophenyl)oxirane and 2-(3,4-difluorophenyl)acetic acid afforded the alcohol 2.5i and amide
2.5j respectively (scheme 2.2).

**Scheme 2.2: Synthesis of dehydroxylated analogues**

Reactions and conditions: (i) bromoethane, K₂CO₃, DMF (ii) 4-(2-bromoethyl)-1,2-
difluorobenzene, K₂CO₃, DMF (iii) (bromomethyl)benzene, K₂CO₃, DMF (iv) (2-
bromoethyl)benzene, K₂CO₃, DMF (v) 2-bromo-1-(3,4-difluorophenyl)cyclohexane, K₂CO₃, DMF (vi) (3-
bromopropyl)benzene, K₂CO₃, DMF (vii) 2-bromo-1-(3,4-difluorophenyl)ethanone, Et₃N, DCM
(viii) 2-(3,4-difluorophenyl)oxirane, EtOH, reflux (ix) EDCI, DMAP, DCM.

Deprotection of tert-butyl carboxylate 2.17 with TFA in DCM and then alkylation afforded the
alkylated analogues 2.5m-2.5o (scheme 2.3).
Scheme 2.3: Synthesis of complete reduction of naphthalene ring analogues

Reactions and conditions: (i) bromoethane, K$_2$CO$_3$, DMF (ii) 4-(2-bromoethyl)-1,2-difluorobenzene, K$_2$CO$_3$, DMF

In order to study the effect of an extended conjugation of the naphthalene ring, the carbonyl 2.2 was converted to tert-butyl spiro[benzo[h]chromene-2,4'-piperidine]-1'-carboxylate 2.18 via the PTSA catalyzed dehydration of the intermediate alcohol recovered from the reduction. Boc-deprotection followed by alkylation afforded the analogues 2.5p-2.5r in good yields (scheme 2.4).

Scheme 2.4: Synthesis of dehydrated analogues

Reactions and conditions: (i) bromoethane, K$_2$CO$_3$, DMF (ii) 4-(2-bromoethyl)-1,2-difluorobenzene, K$_2$CO$_3$, DMF

The spirocyclic piperidine linkage was examined to determine whether the current angle of 180° between the benzo[h]chromane plane and the trajectory of the N-substituent of the piperidine ring was optimal. Aldol condensation and cyclization of 1-(1-hydroxynaphthalen-2-yl)ethan-1-one and tert-butyl 3-oxopiperidine-1-carboxylate in methanol using pyrrolidine afforded the racemic tert-butyl carboxylate 2.6 in moderate yield which was then deprotected and finally amidated with a
chiral auxiliary \((R)-2\text{-acetoxy-2-phenylacetic acid}\) to generate the diastereomeric amides 2.7 in good yield.\(^{15}\)

**Scheme 2.5: Synthesis of chiral chromanes**

Reagents and conditions: (i) \((R)-2\text{-acetoxy-2-phenylacetic acid}, EDCI, DMAP, DCM\); (ii) bromoethane, \(K_2CO_3, DMF\) (iii) 4-(2-bromoethyl)-1,2-difluorobenzene, \(K_2CO_3, DMF\).

The diastereomeric separation of the amide using flash column afforded both clean diastereomers 2.8 and 2.9. The first spot from the flash column was crystallized using vapor diffusion with dimethyl sulfoxide (DMSO) as the solution solvent and hexanes as the diffusion solvent. X-ray crystallography showed that its quaternary carbon was the \(S\) enantiomer (figure 2.2).
Removal of the chiral auxiliary by refluxing in a 1:1 mixture of ethanol in hydrochloric acid afforded the enantiomerically pure amines 2.10a and 2.10d that were then alkylated with selected alkyl bromides to 2.10b-2.10c and 2.10e-2.10f. The sodium borohydride reduction of the carbonyl group yielded the hydroxy analogues 2.11a-2.11d in good yields (scheme 2.5).

Next, the enantiopure isomers of the original compound 2.1 were synthesized. The enantiopure bisfluoro epoxides 2.14a and 2.14b was prepared in a 3 step synthesis starting from 1-(3,4-difluorophenyl)ethan-1-one which was brominated using cupric bromide in a mixture of chloroform and ethyl acetate to afford halide 2.12 in good yield. Reduction with (+)-DIP-chloride™ or (-)-DIP-chloride™ as the asymmetrical reducing reagents at -25 °C in THF for 60 hours afforded the R and S enantiomers of the alcohol 2.13 respectively (scheme 2.6) with excellent enantiomeric excess (figure 2.3).
Figure 2.3: SFC trace analysis of the enantiomeric purity of 2.13

Conditions: CO$_2$ : MeOH (80 : 20) 4 mL/min
Phenomenex Lux 5μ Cellulose-4 column (250 X 4.60 mm)
The alcohols were cyclized to their respective epoxides 2.14 by treatment with 15% NaOH in ethanol.\textsuperscript{16}

Scheme 2.6. Synthesis of chiral epoxide

\[
\begin{align*}
\text{2.12} & \xrightarrow{i} \text{2.13a, 2.13b} & \xrightarrow{ii} \text{2.14a, 2.14b} \\
\end{align*}
\]

Reactions and Conditions: (i) CuBr$_2$, CH$_3$Cl/EA, reflux 5 h; (ii) (-) - DIP-Cl (R) or (+)-DIP-Cl (S), THF, -25 °C, 60 h; (iii) 15% NaOH/EtOH (1:1), rt, 2 h
Scheme 2.7: Synthesis of enantiopure analogues

Reactions and Conditions: (iv) EtOH, reflux (v) Noyori (R, R) or Noyori (S, S), FA/TEA (5:2), 28°C, 48 h

Epoxide ring opening of both enantiopure epoxides with amine 2.2a yielded the respective enantiopure analogues of 2.2m which retained the excellent enantiomeric excess despite the base-catalyzed epoxidation and the subsequent epoxide ring opening reaction (figure 2.4).

Figure 2.4: SFC trace analysis of the enantiomeric purity of 2.2m

Conditions: CO₂ : EtOH (93 : 7) 3 mL/min. Phenomenex Lux 5µ Cellulose-4 column (250 X 4.60 mm)

Initial attempts to stereoselectively reduce the carbonyl 2.2m using (+)-DIP-chloride™ or (-)-DIP-chloride™ as the asymmetrical reducing reagents were not successful. Ruthenium (II) catalyzed asymmetric transfer hydrogenation using formic acid and trimethylamine azeotropic mixture was
used in converting the carbonyl group to the required hydroxyl group. To a mixture of the corresponding ketone in a formic acid-trimethylamine azeotrope was added (S)-RuCl[(1S,2S)-p-TsNCH(C₆H₅)CH(C₆H₅)NH₂][ƞ⁶-mesitylene] and (R)-RuCl[(1R,2R)-p-TsNCH(C₆H₅)CH(C₆H₅)NH₂][ƞ⁶-mesitylene] respectively.

The solution was warmed to 28 °C and was maintained at that temperature for 48 h to afford the alcohol 2.3m in excellent enantiomeric excess (scheme 2.7 and figure 2.5).\textsuperscript{17,18}

**Figure 2.5: SFC trace analysis of the enantiomeric purity of 2.3m**

![SFC trace analysis](image)

Conditions: CO\textsubscript{2} : EtOH with 0.1\% DEA (85 : 15) 3 mL/min

Phenomenex Lux 5μ Cellulose-4 column (250 X 4.60 mm)

Lastly, SAR studies on the naphthalene ring were explored. To study the effect on an increased Fsp\textsuperscript{3} character, the naphthalene ring was substituted with a benzene ring. Aldol condensation and cyclization of ethanone 2.15a with tert-butyl 4-oxopiperidine-1-carboxylate afforded the boc-protected spirocyclic chromane 2.16a which was subsequently deprotected and alkylated to yield
the alkylated analogues 2.2aa-2.2ac in good yields. The hydroxy analogue of this scaffold was synthesized by reduction of 2.2ac to afford 2.3ac. The ethanone 2.15b was synthesized via a 3-step synthesis starting from β-naphthol. It was then subjected to aldol condensation and cyclization with tert-butyl 4-oxopiperidine-1-carboxylate to 2.16b which was deprotected and alkylated to yield the spirocyclic chromanes 2.2ba-bc. The spirocyclic chromane 2.2bd was obtained by the epoxide ring opening of 2-(3,4-difluorophenyl)oxirane with the amine 2.16b in ethanol. The reduction of the carbonyl analogues of this scaffold afforded the hydroxy analogues 2.3bb-bd in good yields. Boc-protected chromane 2.16c was prepared from commercially available ethanone 2.15c in excellent yields which was then alkylated to the chromanes 2.2ca-2.2cc. 1’-(2-(3,4-difluorophenyl)-2-hydroxyethyl)spiro[benzo[f]chromene-3,4’-piperidin]-1(2H)-one 2.2cd was obtained by refluxing the amine 2.16c with 2-(3,4-difluorophenyl)oxirane in ethanol. The reduction of the carbonyl analogues of this scaffold afforded the hydroxy analogues 2.3cb-2.3cd in good yields (scheme 2.8).

Scheme 2.8: Synthesis of analogues with different orientation of the spirocyclic chromane

Reactions and conditions: (i) bromoethane, K₂CO₃, DMF (ii) 4-(2-bromoethyl)-1,2-difluorobenzene, K₂CO₃, DMF (iii) 2-(3,4-difluorophenyl)oxirane, EtOH, reflux
2.3 Antimalarial Activity

All synthesized compounds were tested, as previously reported, against the clinically relevant drug resistance *Plasmodium falciparum* Dd2 (chloroquine resistant) and 3D7 (chloroquine sensitive). *Plasmodium falciparum* Dd2 and 3D7 strains were maintained at 37 °C in 5% CO₂ and 95% air in RPMI media with L-glutamine and supplemented with 25 mM HEPES, 26 mM NaHCO₃, 2% dextrose, 15 mg/L hypoxanthine, 25 mg/L gentamicin, and 0.5% Albumax II. Different dilutions of the compound in RPMI 1640 from a 10 mM stock in dimethyl sulfoxide (DMSO) were added to the culture at a 1% parasitemia and 2% hematocrit in 96-well black plates. Chloroquine at 1 µM was used as a positive control to determine the baseline value. Following 72 h incubation at 37 °C, the ability of the compounds to inhibit growth of the parasite was determined by a SYBR green I-based DNA quantification assay. EC₅₀ was calculated (n = 3) from a dose response curve that was generated from a concentration range of 0-20 µM using GraphPad Prism v5.0.¹¹

2.4 Structure-Activity Relationship (SAR) Studies

The initial SAR study focused on determining the importance of the spirocyclic chromane moiety to its activity while also exploring the essence of the benzylic alcohols. This study was considered to deduce if stereocenters were integral for the activity of the spirocyclic chromane. Furthermore, as bioavailability is very important, the study covered the essence of increasing the Fsp³ and thereby bioavailability while improving antimalarial potency and addressing physiochemical liabilities.

The first sub-series of analogues were designed to probe the importance of the N-substituent of the piperidine ring and to test for the need of the hydroxyl group (Table 2.1). The spirocyclic chromanes without a substituent 2.2a, 2.3a and 2.5a were 2-fold more potent than those in which
the N-substituent was substituted with an ethyl group 2.2b, 2.3b and 2.5b in all the class of molecules. On substituting the hydroxyl group on the N-substituent with a carbonyl group 2.3g, there was over 10-fold loss in potency while substituting with methylene group 2.3j showed less than 2-fold loss in potency. Replacing the bisfluoro on the N-substituent with bischloro 2.3i and 2.3k also did not display any improve in potency. Though substituting the bisfluoro group on the N-substituent with a cyclohexyl group 2.3e did not improve the potency, it displayed similar potency in comparison to reference compound 2.1. The rigid analogue 2.3l synthesized by making an amide bond with the N- of the piperidine ring lacked antimalarial activity. This was consistent with other amides made. The study on the C-4 position showed that the hydroxyl group 2.3e-2.3l was more potent than the carbonyl group 2.2e-2.2l in all the analogues made in this sub-series. In contrast, there was no clear pattern of potency evident on substituting the hydroxyl group with a methylene group in compound 2.5a-2.5i. However, it was evident that the hydroxy improved selectivity as 2.3a and 2.3h displayed the highest selectivity of > 53 against human liver HepG2 cells in this series of compounds tested. In summary, though no compound showed better potency that the reference compound 2.3h with EC_{50} value of 0.40 µM, compounds 2.2e and 2.3e were equipotent at 0.43 µM and 0.42 µM respectively.
Table 2.1: Sequential changing of the oxidation state of C-4 and the N-substituent of the piperidine ring

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>R</th>
<th>Dd2 EC_{50} (µM)</th>
<th>HepG2 EC_{50} (µM)</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2a</td>
<td>CO</td>
<td>H</td>
<td>2.49</td>
<td>7</td>
<td>3.2</td>
</tr>
<tr>
<td>2.2b</td>
<td>CO</td>
<td>CH₂CH₃</td>
<td>3.52</td>
<td>18</td>
<td>4.7</td>
</tr>
<tr>
<td>2.2e</td>
<td>CO</td>
<td>(CH₂)₂cyclohexyl</td>
<td>0.43</td>
<td>16</td>
<td>31.2</td>
</tr>
<tr>
<td>2.2g</td>
<td>CO</td>
<td>CH₂CO(3,4-diF)Ph</td>
<td>1.82</td>
<td>4</td>
<td>2.7</td>
</tr>
<tr>
<td>2.2h</td>
<td>CO</td>
<td>CH₂CHOH(3,4-diF)Ph</td>
<td>3.32</td>
<td>&gt;20</td>
<td>&gt;6.1</td>
</tr>
<tr>
<td>2.2i</td>
<td>CO</td>
<td>CH₂CHOH(3,4-diCl)Ph</td>
<td>3.29</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2.2j</td>
<td>CO</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>&gt;5</td>
<td>&gt;20</td>
<td>4.0</td>
</tr>
<tr>
<td>2.2k</td>
<td>CO</td>
<td>(CH₂)₂(3,4-diCl)Ph</td>
<td>&gt;5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2.2l</td>
<td>CO</td>
<td>COCH₂(3,4-diF)Ph</td>
<td>&gt;5</td>
<td>18</td>
<td>&lt;3.6</td>
</tr>
<tr>
<td>2.3a</td>
<td>CHOH</td>
<td>H</td>
<td>2.36</td>
<td>&gt;20</td>
<td>&gt;54.5</td>
</tr>
<tr>
<td>2.3b</td>
<td>CHOH</td>
<td>CH₂CH₃</td>
<td>&gt;5</td>
<td>16</td>
<td>3.2</td>
</tr>
<tr>
<td>2.3e</td>
<td>CHOH</td>
<td>(CH₂)₂cyclohexyl</td>
<td>0.42</td>
<td>14</td>
<td>35.1</td>
</tr>
<tr>
<td>2.3g</td>
<td>CHOH</td>
<td>CH₂CO(3,4-diF)Ph</td>
<td>&gt;5</td>
<td>33</td>
<td>&lt;6.7</td>
</tr>
<tr>
<td>2.3h</td>
<td>CHOH</td>
<td>CH₂CHOH(3,4-diF)Ph</td>
<td>0.40</td>
<td>&gt;20</td>
<td>&gt;53.3</td>
</tr>
<tr>
<td>2.3i</td>
<td>CHOH</td>
<td>CH₂CHOH(3,4-diCl)Ph</td>
<td>1.83</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2.3j</td>
<td>CHOH</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>0.63</td>
<td>&gt;20</td>
<td>&gt;32.5</td>
</tr>
<tr>
<td>2.3k</td>
<td>CHOH</td>
<td>(CH₂)₂(3,4-diCl)Ph</td>
<td>2.18</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2.3l</td>
<td>CHOH</td>
<td>COCH₂(3,4-diF)Ph</td>
<td>&gt;5</td>
<td>5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2.5a</td>
<td>CH₂</td>
<td>H</td>
<td>0.84</td>
<td>&gt;20</td>
<td>4.0</td>
</tr>
<tr>
<td>2.5b</td>
<td>CH₂</td>
<td>CH₂CH₃</td>
<td>1.73</td>
<td>&gt;20</td>
<td>&gt;52.1</td>
</tr>
<tr>
<td>2.5e</td>
<td>CH₂</td>
<td>(CH₂)₂cyclohexyl</td>
<td>1.18</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>2.5g</td>
<td>CH₂</td>
<td>CH₂CO(3,4-diF)Ph</td>
<td>3.44</td>
<td>&gt;20</td>
<td>&gt;5.5</td>
</tr>
<tr>
<td>2.5h</td>
<td>CH₂</td>
<td>CH₂CHOH(3,4-diF)Ph</td>
<td>2.02</td>
<td>&gt;20</td>
<td>4.0</td>
</tr>
<tr>
<td>2.5i</td>
<td>CH₂</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>0.53</td>
<td>&gt;20</td>
<td>&gt;10.4</td>
</tr>
<tr>
<td>2.5j</td>
<td>CH₂</td>
<td>COCH₂(3,4-diF)Ph</td>
<td>&gt;5</td>
<td>5</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

The next sub-series of spirocyclic chromanes 2.2c-2.2f, 2.3c-2.3f and 2.5c-2.5f were synthesized to study the flexibility of the spirocyclic chromane core and the N-substituent of the piperidine.

We anticipated that altering the linker length between the spirocyclic chromane and N-substituent...
of the piperidine with between 1 to 3 methylene units would display a pattern of potency. There was a clear trend of potency of 2 methylene units > 1 methylene unit > 3 methylene units in the compounds with a hydroxyl group at the 4- position \textbf{2.3c-2.3f}. Though the pattern was not definite for compounds with a carbonyl or methylene groups at the C-4 position, compounds with 1 methylene unit linker \textbf{2.2c} and \textbf{2.5c} were least potent in the series. The most active compound \textbf{2.3d} with an EC$_{50}$ of 0.30 µM in this series was slightly more potent than the reference compound. It also displays a slightly higher selectivity (Table 2.2).

Table 2.2: Optimal aliphatic chain length

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>n</th>
<th>Dd2 EC$_{50}$ (µM)</th>
<th>HepG2 EC$_{50}$ (µM)</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textbf{2.2c}</td>
<td>CO</td>
<td>1</td>
<td>&gt;5</td>
<td>11</td>
<td>4.0</td>
</tr>
<tr>
<td>\textbf{2.2d}</td>
<td>CO</td>
<td>2</td>
<td>0.59</td>
<td>8</td>
<td>14.0</td>
</tr>
<tr>
<td>\textbf{2.2f}</td>
<td>CO</td>
<td>3</td>
<td>0.57</td>
<td>&gt;20</td>
<td>&gt;36.5</td>
</tr>
<tr>
<td>\textbf{2.3c}</td>
<td>CHOH</td>
<td>1</td>
<td>1.27</td>
<td>6</td>
<td>5.7</td>
</tr>
<tr>
<td>\textbf{2.3d}</td>
<td>CHOH</td>
<td>2</td>
<td>0.30</td>
<td>&gt;20</td>
<td>&gt;68.9</td>
</tr>
<tr>
<td>\textbf{2.3f}</td>
<td>CHOH</td>
<td>3</td>
<td>0.52</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>\textbf{2.5c}</td>
<td>CH$_2$</td>
<td>1</td>
<td>&gt;5</td>
<td>&gt;20</td>
<td>&gt;9.6</td>
</tr>
<tr>
<td>\textbf{2.5d}</td>
<td>CH$_2$</td>
<td>2</td>
<td>2.54</td>
<td>&gt;20</td>
<td>&gt;45.6</td>
</tr>
<tr>
<td>\textbf{2.5f}</td>
<td>CH$_2$</td>
<td>3</td>
<td>1.21</td>
<td>&gt;20</td>
<td>&gt;45.6</td>
</tr>
</tbody>
</table>

We next synthesized and tested a sub-series of spirocyclic chromanes (\textbf{2.2a-2.2c}, \textbf{2.3a}, \textbf{2.5m-2.5o} and \textbf{2.2a-2.2b}) with increased Fsp$^3$ character by reducing the level of unsaturation on the naphthalene core. Compounds \textbf{2.2a-2.2c}, \textbf{2.3a} and \textbf{2.2a-2.2b} lacked the outer ring in the reference compound. This sub-series displayed reduced potency and selectivity. The analogues with the carbonyl group on the C-4 lacked activity altogether in this class of compounds. On
reduction of the outer ring C-7 to C-10 in compounds 2.5m-2.5o, the antiplasmodial potency was lost against the Dd2 strain (Table 2.3).

**Table 2.3: Structural Activity Relationship of the naphthalene ring**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>R’</th>
<th>Dd2 EC50 (µM)</th>
<th>HepG2 EC50 (µM)</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2aa</td>
<td>H</td>
<td>CO</td>
<td>H</td>
<td>&gt;5</td>
<td>6</td>
<td>1.2</td>
</tr>
<tr>
<td>2.2ab</td>
<td>H</td>
<td>CO</td>
<td>CH2CH3</td>
<td>&gt;5</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>2.2ac</td>
<td>H</td>
<td>CO</td>
<td>(CH2)2(3,4-diF)Ph</td>
<td>&gt;5</td>
<td>18</td>
<td>3.6</td>
</tr>
<tr>
<td>2.3ac</td>
<td>H</td>
<td>CHOH</td>
<td>(CH2)2(3,4-diF)Ph</td>
<td>3.27</td>
<td>&gt;20</td>
<td>&gt;7.0</td>
</tr>
<tr>
<td>2.22a</td>
<td>H</td>
<td>CH2</td>
<td>H</td>
<td>1.36</td>
<td>10</td>
<td>6.8</td>
</tr>
<tr>
<td>2.22b</td>
<td>H</td>
<td>CH2</td>
<td>(CH2)2(3,4-diF)Ph</td>
<td>1.48</td>
<td>&gt;20</td>
<td>&gt;14.1</td>
</tr>
<tr>
<td>2.5m</td>
<td>2,3-cyclohexane</td>
<td>CH2</td>
<td>H</td>
<td>&gt;5</td>
<td>&gt;20</td>
<td>4.0</td>
</tr>
<tr>
<td>2.5n</td>
<td>2,3-cyclohexane</td>
<td>CH2</td>
<td>CH2CH3</td>
<td>&gt;5</td>
<td>5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2.5o</td>
<td>2,3-cyclohexane</td>
<td>CH2</td>
<td>(CH2)2(3,4-diF)Ph</td>
<td>&gt;5</td>
<td>&gt;20</td>
<td>4.0</td>
</tr>
</tbody>
</table>

A set of compounds (2.10a-2.10f, 2.11a-2.11d, Table 2.4 and 2.5) with a different angle of the trajectory of the N-substituent of the piperidine ring compared to the reference compound 2.1 were prepared and tested. The S-enantiomer showed a clear pattern of at least a 3-fold higher potency than the corresponding R-enantiomer in all the sets of analogues made. Compound 2.10c, the most potent compound in this sub-series with EC50 of 0.36 µM is over 5-fold more potent than its enantiomer 2.10f with EC50 of 1.85 µM. Besides potency, the S-enantiomer is also more selective and less toxic compared to its R-enantiomer. In contrast to previously observed pattern, the
analagues with a carbonyl at the C-4 position 2.10c and 2.10f were showed about 2-fold more potency than those with a hydroxyl group in the C-4 position.

Table 2.4: Optimal angle of the trajectory of the N-substituent of the piperidine ring

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>R</th>
<th>Dd2 EC50 (µM)</th>
<th>HepG2 EC50 (µM)</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.10a</td>
<td>CO</td>
<td>H</td>
<td>0.54</td>
<td>5</td>
<td>9.0</td>
</tr>
<tr>
<td>2.10b</td>
<td>CO</td>
<td>CH₂CH₃</td>
<td>3.69</td>
<td>8</td>
<td>&lt;1.6</td>
</tr>
<tr>
<td>2.10c</td>
<td>CO</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>0.36</td>
<td>14</td>
<td>34.2</td>
</tr>
<tr>
<td>2.11a</td>
<td>CHOH</td>
<td>CH₂CH₃</td>
<td>0.64</td>
<td>&gt;20</td>
<td>&gt;28.8</td>
</tr>
<tr>
<td>2.11b</td>
<td>CHOH</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>0.66</td>
<td>&gt;20</td>
<td>&gt;34.5</td>
</tr>
<tr>
<td>2.7a*</td>
<td>CO</td>
<td>H</td>
<td>0.49</td>
<td>5</td>
<td>10.2</td>
</tr>
</tbody>
</table>

* = racemic

Table 2.5: Optimal angle of the trajectory of the N-substituent of the piperidine ring

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>R</th>
<th>Dd2 EC50 (µM)</th>
<th>HepG2 EC50 (µM)</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.10d</td>
<td>CO</td>
<td>H</td>
<td>1.55</td>
<td>4</td>
<td>2.4</td>
</tr>
<tr>
<td>2.10e</td>
<td>CO</td>
<td>CH₂CH₃</td>
<td>&gt;5</td>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td>2.10f</td>
<td>CO</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>&gt;5</td>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td>2.11c</td>
<td>CHOH</td>
<td>CH₂CH₃</td>
<td>1.85</td>
<td>10</td>
<td>5.8</td>
</tr>
<tr>
<td>2.11d</td>
<td>CHOH</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>2.34</td>
<td>&gt;20</td>
<td>&gt;7.6</td>
</tr>
<tr>
<td>2.7a*</td>
<td>CO</td>
<td>H</td>
<td>3.04</td>
<td>6</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* = racemic
A few compounds (2.5p-2.5r, Table 6) in which the hydroxyl group in C-4 was dehydrated were prepared and tested. Compound 2.5p with no N-substituent on the piperidine ring was the best in this series. It was as equipotent as the original compound 2.1 and showed over 2-fold better potency than other analogues 2.5q and 2.5r with substituents on the N-substituent on the piperidine ring with EC\textsubscript{50} of 1.23 µM and 1.43 µM respectively. Compound 2.5p also showed the best selectivity and lowest cytotoxicity in this sub-series of compounds.

**Table 2.6: Dehydration of the C-4 alcohol**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Dd2 EC\textsubscript{50} (µM)</th>
<th>HepG2 EC\textsubscript{50} (µM)</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5p</td>
<td>H</td>
<td>0.42</td>
<td>&gt;20</td>
<td>&gt;45.6</td>
</tr>
<tr>
<td>2.5q</td>
<td>CH\textsubscript{2}CH\textsubscript{3}</td>
<td>1.23</td>
<td>12</td>
<td>9.7</td>
</tr>
<tr>
<td>2.5r</td>
<td>(CH\textsubscript{2})\textsubscript{2}(3,4-diF)Ph</td>
<td>1.43</td>
<td>13</td>
<td>7.2</td>
</tr>
</tbody>
</table>

A sub-series of compounds with different vector of the naphthalene ring and spirocyclic core (Table 2.7) thereby providing different geometry were synthesized and tested. Compounds 2.3bd and 2.3cd with structural similarity to 2.1 were marginally less potent than the reference compound. However, 2.2ba and 2.2ca with structural similarity to 2.2a has EC\textsubscript{50} of 1.54 µM and 0.30 µM respectively reflecting about 2-fold and 8-fold higher potency.
Table 2.7: Optimal vector of the naphthalene ring

![Optimal vector of the naphthalene ring](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>R'</th>
<th>Dd2 EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2ba</td>
<td>3,4-benzene</td>
<td>CO</td>
<td>H</td>
<td>1.54</td>
</tr>
<tr>
<td>2.2bb</td>
<td>3,4-benzene</td>
<td>CO</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.45</td>
</tr>
<tr>
<td>2.2bc</td>
<td>3,4-benzene</td>
<td>CO</td>
<td>(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;(3,4-diF)Ph</td>
<td>0.76</td>
</tr>
<tr>
<td>2.2bd</td>
<td>3,4-benzene</td>
<td>CO</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CHOH(3,4-diF)Ph</td>
<td>0.67</td>
</tr>
<tr>
<td>2.2bb</td>
<td>3,4-benzene</td>
<td>CHOH</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3.13</td>
</tr>
<tr>
<td>2.3bc</td>
<td>3,4-benzene</td>
<td>CHOH</td>
<td>(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;(3,4-diF)Ph</td>
<td>1.36</td>
</tr>
<tr>
<td>2.3bd</td>
<td>3,4-benzene</td>
<td>CHOH</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CHOH(3,4-diF)Ph</td>
<td>0.55</td>
</tr>
<tr>
<td>2.2ca</td>
<td>4,5-benzene</td>
<td>CO</td>
<td>H</td>
<td>0.30</td>
</tr>
<tr>
<td>2.2cb</td>
<td>4,5-benzene</td>
<td>CO</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.43</td>
</tr>
<tr>
<td>2.2cc</td>
<td>4,5-benzene</td>
<td>CO</td>
<td>(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;(3,4-diF)Ph</td>
<td>0.84</td>
</tr>
<tr>
<td>2.2cd</td>
<td>4,5-benzene</td>
<td>CO</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CHOH(3,4-diF)Ph</td>
<td>1.09</td>
</tr>
<tr>
<td>2.3cb</td>
<td>4,5-benzene</td>
<td>CHOH</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.44</td>
</tr>
<tr>
<td>2.3cc</td>
<td>4,5-benzene</td>
<td>CHOH</td>
<td>(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;(3,4-diF)Ph</td>
<td>0.48</td>
</tr>
<tr>
<td>2.3cd</td>
<td>4,5-benzene</td>
<td>CHOH</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CHOH(3,4-diF)Ph</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Finally, a set of enantiopure spirocyclic chromanes (2.3ma-2.3mb, Table 2.8) were prepared and tested to probe the essentiality of hydrogen bonding to the potency of the compound series. The cis analogue 2.3ma with the R-configuration at both chiral centers was the most potent with EC<sub>50</sub> of 0.95 µM. However, it is still over 2-fold less potent compared to the reference compound 2.1 with EC<sub>50</sub> of 0.40 µM. The enantiomer with the S-configuration at both chiral centers 2.3md was about 2-fold less potent compared to its enantiomer but over 4-fold less potent than the reference compound. This is an uncommon scenario but does imply the possibility of a synergistic effect of the enantiomers or the possibility of hitting multiple targets.
Table 2.8: Enantiopure analogues

<table>
<thead>
<tr>
<th>Compound</th>
<th>*</th>
<th>**</th>
<th>Dd2 EC\textsubscript{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3ma</td>
<td>R</td>
<td>R</td>
<td>0.95</td>
</tr>
<tr>
<td>2.3mb</td>
<td>R</td>
<td>S</td>
<td>3.02</td>
</tr>
<tr>
<td>2.3mc</td>
<td>S</td>
<td>R</td>
<td>1.48</td>
</tr>
<tr>
<td>2.3md</td>
<td>S</td>
<td>S</td>
<td>1.81</td>
</tr>
</tbody>
</table>

A few of the promising compounds in addition to the reference compound 2.1 were picked and tested against another chloroquine sensitive D6 and Artemisinin resistant ARC08-022 Plasmodium falciparum strains. The original compound 2.1 had an EC\textsubscript{50} of 1.48 µM and 1.81 µM with D6 and ARC08-022 respectively. This represents at least a 3-fold loss of potency with these strains. Analogue 2.3cd with similar structural features showed improved potency over compound 2.1 with a factor of five and nine with D6 and ARC08-022 strains respectively. Compounds with N-substituent on the piperidine ring were more promising than those without substituents and those with a small substituent like an ethyl group. Spiro cyclic chromanes 2.2h and 2.5i with a hydroxyl group at the N-substituent on the piperidine were more potent with 0.26 µM and 0.31 µM respectively against the D6 strain regardless of the group on the C-4 position (Table 2.9).
Table 2.9: Potency against other *Plasmodium* strains

![Diagram of a molecule]

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>R'</th>
<th>D6 EC₅₀ (µM)</th>
<th>ARC08-022 EC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2h</td>
<td>2,3-benzene</td>
<td>CO</td>
<td>CH₂CHOH(3,4-diF)Ph</td>
<td>0.26</td>
<td>0.86</td>
</tr>
<tr>
<td>2.3h</td>
<td>2,3-benzene</td>
<td>CHOH</td>
<td>CH₂CHOH(3,4-diCl)Ph</td>
<td>1.48</td>
<td>1.81</td>
</tr>
<tr>
<td>2.5i</td>
<td>2,3-benzene</td>
<td>CH₂</td>
<td>CH₂CHOH(3,4-diF)Ph</td>
<td>0.31</td>
<td>0.97</td>
</tr>
<tr>
<td>2.3e</td>
<td>2,3-benzene</td>
<td>CHOH</td>
<td>(CH₂)₂cyclohexyl</td>
<td>0.50</td>
<td>0.63</td>
</tr>
<tr>
<td>2.3i</td>
<td>2,3-benzene</td>
<td>CHOH</td>
<td>CH₂CHOH(3,4-diCl)Ph</td>
<td>1.32</td>
<td>2.01</td>
</tr>
<tr>
<td>2.3cc</td>
<td>4,5-benzene</td>
<td>CHOH</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>0.63</td>
<td>0.42</td>
</tr>
<tr>
<td>2.3cd</td>
<td>4,5-benzene</td>
<td>CHOH</td>
<td>CH₂CHOH(3,4-diF)Ph</td>
<td>0.34</td>
<td>0.21</td>
</tr>
</tbody>
</table>

2.5 Structure-Property Relationship (SPR) Studies

In parallel to the testing of the compounds for in vitro antimalarial activity, a structure-property relationship (SPR) study focusing aqueous solubility and partition coefficient (log *D*) were determined to identify compounds with potential physicochemical liabilities (Table 2.10a-c). The log *D₃.₀* and log *D₇.₄*, the distribution coefficient between octanol and water at pH 3.0 and pH 7.4, were experimentally determined via a previously described HPLC-based method. Solubility at pH 7.4 was determined using Biomek FX lab automation workstation with pION µSOL evolution software as reported previously and at pH 2.0 using an in-house HPLC assay based on UV absorption. The spirocyclic chromanes displayed a pH dependence aqueous solubility and distribution coefficient log *D* (Table 10). The compounds have impressive solubility possible due to its hetero atoms. However, the solubility is slightly better at lower pH ranges due to the basic
amine, but the distribution coefficient is enhanced at higher pH ranges. Only a very few of the analogues display solubility below the accepted range of < 20 µM. As the aqueous solubility and the distribution coefficient of the potent compounds are well within the acceptable ranges (solubility of >20 µM, 1 < log $D < 4$), the spirocyclic chromane compound series is considered to be suitable for the development of bioavailable antimalarial compounds.
Table 2.10a: Solubility and Log $D$

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>R</th>
<th>Log $D$</th>
<th>Solubility$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pH 3.0</td>
<td>pH 7.4</td>
</tr>
<tr>
<td>2.2a</td>
<td>CO</td>
<td>H</td>
<td>1.48</td>
<td>1.78</td>
</tr>
<tr>
<td>2.2b</td>
<td>CO</td>
<td>CH$_2$CH$_3$</td>
<td>1.65</td>
<td>2.19</td>
</tr>
<tr>
<td>2.2e</td>
<td>CO</td>
<td>(CH$_2$)$_2$-cyclohexyl</td>
<td>2.99</td>
<td>4.50</td>
</tr>
<tr>
<td>2.2g</td>
<td>CO</td>
<td>CH$_2$CO(3,4-diF)Ph</td>
<td>1.52</td>
<td>1.82</td>
</tr>
<tr>
<td>2.2h</td>
<td>CO</td>
<td>CH$_2$CHOH(3,4-diF)Ph</td>
<td>2.56</td>
<td>3.81</td>
</tr>
<tr>
<td>2.2j</td>
<td>CO</td>
<td>(CH$_2$)$_2$-cyclohexyl</td>
<td>2.79</td>
<td>4.51</td>
</tr>
<tr>
<td>2.2l</td>
<td>CO</td>
<td>COCH$_2$(3,4-diF)Ph</td>
<td>4.00</td>
<td>4.03</td>
</tr>
<tr>
<td>2.3a</td>
<td>CHOH</td>
<td>H</td>
<td>2.04</td>
<td>2.46</td>
</tr>
<tr>
<td>2.3b</td>
<td>CHOH</td>
<td>CH$_2$CH$_3$</td>
<td>1.51</td>
<td>1.88</td>
</tr>
<tr>
<td>2.3e</td>
<td>CHOH</td>
<td>(CH$_2$)$_2$-cyclohexyl</td>
<td>2.97</td>
<td>3.54</td>
</tr>
<tr>
<td>2.3g</td>
<td>CHOH</td>
<td>CH$_2$CO(3,4-diF)Ph</td>
<td>1.53</td>
<td>0.89</td>
</tr>
<tr>
<td>2.3h</td>
<td>CHOH</td>
<td>CH$_2$CHOH(3,4-diF)Ph</td>
<td>2.62</td>
<td>3.15</td>
</tr>
<tr>
<td>2.3j</td>
<td>CHOH</td>
<td>(CH$_2$)$_2$(3,4-diF)Ph</td>
<td>2.79</td>
<td>4.51</td>
</tr>
<tr>
<td>2.3l</td>
<td>CHOH</td>
<td>COCH$_2$(3,4-diF)Ph</td>
<td>2.04</td>
<td>2.46</td>
</tr>
<tr>
<td>2.5a</td>
<td>CH$_2$</td>
<td>H</td>
<td>1.97</td>
<td>2.33</td>
</tr>
<tr>
<td>2.5b</td>
<td>CH$_2$</td>
<td>CH$_2$CH$_3$</td>
<td>2.17</td>
<td>2.88</td>
</tr>
<tr>
<td>2.5e</td>
<td>CH$_2$</td>
<td>(CH$_2$)$_2$-cyclohexyl</td>
<td>ND</td>
<td>4.74</td>
</tr>
<tr>
<td>2.5g</td>
<td>CH$_2$</td>
<td>CH$_2$CO(3,4-diF)Ph</td>
<td>ND</td>
<td>2.51</td>
</tr>
<tr>
<td>2.5h</td>
<td>CH$_2$</td>
<td>CH$_2$CHOH(3,4-diF)Ph</td>
<td>3.29</td>
<td>4.51</td>
</tr>
<tr>
<td>2.5i</td>
<td>CH$_2$</td>
<td>(CH$_2$)$_2$(3,4-diF)Ph</td>
<td>3.05</td>
<td>4.00</td>
</tr>
<tr>
<td>2.5j</td>
<td>CH$_2$</td>
<td>COCH$_2$(3,4-diF)Ph</td>
<td>4.02</td>
<td>4.04</td>
</tr>
</tbody>
</table>

$^a$(*): For solubility $\leq$ 10 µM,

(**): For 10 µM < solubility $\leq$ 20 µM,

(***): For 20 µM < solubility $\leq$ 40 µM,

(****): For 40 µM < solubility $\leq$ 80 µM,

(*****): For solubility $\geq$ 80 µM.
Table 2.10b: Solubility and Log D

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>R’</th>
<th>Log D</th>
<th>Solubility(^a) (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pH 3.0</td>
<td>pH 7.4</td>
</tr>
<tr>
<td>2.22a</td>
<td>H</td>
<td>CO</td>
<td>H</td>
<td>0.53</td>
<td>0.95</td>
</tr>
<tr>
<td>2.22b</td>
<td>H</td>
<td>CO</td>
<td>CH₂CH₃</td>
<td>0.73</td>
<td>0.96</td>
</tr>
<tr>
<td>2.22c</td>
<td>H</td>
<td>CO</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>1.82</td>
<td>3.46</td>
</tr>
<tr>
<td>2.32c</td>
<td>H</td>
<td>CHOH</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>2.15</td>
<td>2.68</td>
</tr>
<tr>
<td>2.22a</td>
<td>H</td>
<td>CH₂</td>
<td>H</td>
<td>ND</td>
<td>1.62</td>
</tr>
<tr>
<td>2.22b</td>
<td>H</td>
<td>CH₂</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>ND</td>
<td>3.79</td>
</tr>
<tr>
<td>2.5m</td>
<td>2,3-cyclohexane</td>
<td>CH₂</td>
<td>H</td>
<td>1.48</td>
<td>1.78</td>
</tr>
<tr>
<td>2.5n</td>
<td>2,3-cyclohexane</td>
<td>CH₂</td>
<td>CH₂CH₃</td>
<td>2.04</td>
<td>2.46</td>
</tr>
<tr>
<td>2.5o</td>
<td>2,3-cyclohexane</td>
<td>CH₂</td>
<td>(CH₂)₂(3,4-diF)Ph</td>
<td>1.97</td>
<td>2.33</td>
</tr>
</tbody>
</table>

\(^a\) For solubility ≤ 10 µM.

(**) For 10 µM < solubility ≤ 20 µM.

(*** ) For 20 µM < solubility ≤ 40 µM.

(****) For 40 µM < solubility ≤ 80 µM.

(***** ) For solubility ≥ 80 µM.
Table 2.10c: Solubility and Log D

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>n</th>
<th>Log D pH 3.0</th>
<th>Log D pH 7.4</th>
<th>Solubility&lt;sup&gt;a&lt;/sup&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pH 2.0</td>
<td>pH 6.5</td>
<td></td>
</tr>
<tr>
<td>2.2c</td>
<td>CO</td>
<td>1</td>
<td>2.37</td>
<td>4.31</td>
<td>*****  ****</td>
</tr>
<tr>
<td>2.2d</td>
<td>CO</td>
<td>2</td>
<td>2.61</td>
<td>4.12</td>
<td>*****  ****</td>
</tr>
<tr>
<td>2.2f</td>
<td>CO</td>
<td>3</td>
<td>2.74</td>
<td>4.00</td>
<td>****   ***</td>
</tr>
<tr>
<td>2.3c</td>
<td>CHOH</td>
<td>1</td>
<td>2.27</td>
<td>2.97</td>
<td>****   ****</td>
</tr>
<tr>
<td>2.3d</td>
<td>CHOH</td>
<td>2</td>
<td>2.52</td>
<td>3.11</td>
<td>****   ****</td>
</tr>
<tr>
<td>2.3f</td>
<td>CHOH</td>
<td>3</td>
<td>2.71</td>
<td>3.19</td>
<td>*****  *****</td>
</tr>
<tr>
<td>2.5c</td>
<td>CH₂</td>
<td>1</td>
<td>ND</td>
<td>4.49</td>
<td>***    ND</td>
</tr>
</tbody>
</table>

<sup>a</sup>For solubility ≤ 10 µM.

(**) For 10 µM < solubility ≤ 20 µM.

(*** For 20 µM < solubility ≤ 40 µM.

(****) For 40 µM < solubility ≤ 80 µM.

(***** For solubility ≥ 80 µM.

2.6 Cytotoxicity

Compounds were evaluated for cytotoxicity using HepG2 human hepatoma cells in a clear bottom well-plate which was seeded with 2500 cells/well and incubated for 24 h. Serial dilutions (0-40 µM concentration range) of the compound were added to the plate followed by incubation for an additional 48 h. Viability of cells were assessed by MTS [(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell proliferation assay. An important quality of antimalarial drug compounds is their selectivity to inhibit parasite growth over mammalian cells. Generally, a majority of the spirocyclic chromanes exhibited EC<sub>50</sub> of 20 µM or
higher against HepG2 (Table 10). Spirocyclic chromanes with methylene group at the C-4 position were generally less toxic than those with hydroxyl groups. The compounds displaying potent antimalarial activity especially 2.2e, 2.3d, 2.5i, 2.5p, 2.10c, were shown to have appreciable selectivity index values of >10 indicating that they are relatively selective, nontoxic chemotypes.

2.7 Discussion

Spirocyclic chromane 2.1 was identified when a compound collection containing NP-like structural motifs were screened with the intention of identifying novel antimalarial compound which interact with cellular targets different from those of current antimalarials. Its potency against the chloroquine resistant Dd2 strain underlines the possibility of its development to overcome the problem of drug-resistant malaria. The good physiochemical properties and compliance with Lipinski’s parameters makes it a good candidate for a hit to lead development for an orally bioavailable antimalarial. The SAR study revealed that the C-4 is essential for both activity and its physiochemical properties. A larger N-substituent on the piperidine ring improved its potency. Though no clear deductions regarding activity can be made from the effect of the hydroxyl group on the stereogenic carbons, these groups did have an effect in the solubility and log D of the compound series. Flexibility was identified as essential for the activity of the spirocyclic chromanes. Antimalarial potency was completely lost in compounds 2.2l, 2.3l and 2.5j that were rigidified by amidation. However, the optimum linker length was 2 methylene units as the activity began to decrease with longer linker length. Attempts to increase the Fsp$^3$ character by either substituting the naphthalene moiety with benzene or reducing one of the rings on naphthalene did not show any improvement in potency. Changing the angle of trajectory of the N-substituent on the piperidine ring helped to identify the more potent enantiomer, however, these analogues were
at best equipotent with the original compound. Altering the vector of the naphthalene ring with the spirocyclic core thereby changing the geometry of the compounds revealed some interesting properties and activity patterns. Analogues 2.2ca–2.2cd and 2.3cb–2.3cd showed a higher activity pattern than 2.2ba–2.2bd and 2.3bb–2.3bd. Curiously, the enantiopure spirocyclic chromanes were not as potent as their diastereomeric mixtures. This is an uncommon trend that would require exploration of their mode of action to understand the reason for the synergy.

2.8 Conclusion

Spirocyclic chromane 2.1 was found to display antiplasmodial activity in both the chloroquine resistant Dd2 and chloroquine sensitive 3D7 strains. Inspired by encouraging potency and physiochemical properties, a library of 74 analogues bearing a variety of \(N\)-substituents on the piperidine ring, varying angles of the trajectory of the \(N\)-substituent of the piperidine ring, different degree of oxidation of \(C-4\) and different level of flexibility. The library of compounds was also subjected to physiochemical property testing for solubility and log \(D\) in order to predict their potential for bioavailability and in vivo efficacy. The essential features necessary for activity were identified. The spirocyclic chromane motif was required for potency as analogues without substituents on the \(N\)-substituent on the piperidine ring were potent. Flexibility was also essential for the antiplasmodial activity of the compound as its potency was lost on rigidifying it. A few of the compounds 2.3d, 2.10c and 2.2ca were slightly more potent than the original compound. Interestingly, 2.3d was also more selective and less toxic than 2.1. Most of the analogues have impressive solubility and log \(D\) with appreciable \(Fsp^3\) indicating that spirocyclic chromanes have potential as orally bioavailable antimalarial drug candidates.
2.9 Experimental

**General.** All reagents and solvents were obtained from Aldrich Chemical Co. and used without further purification. NMR spectra were recorded at ambient temperature on a 400 MHz or 500 MHz Varian NMR spectrometer in the solvent indicated. All $^1$H NMR experiments are reported in δ units, parts per million (ppm) downfield of TMS, and were measured relative to the signals of chloroform (7.26 ppm) and dimethylsulfoxide (2.50 ppm) with $^1$H decoupled observation. Data for $^1$H NMR are reported as follows: chemicals shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, sept = septet, oct = octet, m = multiplet), integration and coupling constant (Hz) whereas $^{13}$C NMR analyses were reported in terms of chemical shift. NMR data was analyzed by using MestReNova Software version 6.0.2-5475. The purity of the final compounds was determined to be \( \geq 95\% \) by high-performance liquid chromatography (HPLC) using an Agilent 1100 LC/MSD-VL with electrospray ionization. Low-resolution mass spectra were performed on an Agilent 1100 LC/MSD-VL with electrospray ionization. High-resolution mass spectra (HRMS) were performed on an Agilent LC/MSD TOF system G3250AA. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 precoated plates (0.25 mm) from EMD Chemical Inc., and components were visualized by ultraviolet light (254 nm). EMD silica gel 230-400 (particle size 40-63 µm) mesh was used for all flash column chromatography.

**General procedure A: Aldol condensation and cyclization.** To a solution of the ethanone (1 equiv.) and carboxylate (1 equiv.) in absolute methanol (2.5 mL / mmol) was added pyrrolidine (2.6 equiv.) and heated to reflux for 8 h under N\(_2\). The methanol was removed under reduced pressure and the residue was purified by flash chromatography.
General procedure B: Deprotection of Boc group. A stirred solution of the protected carboxylate (1 equiv.) in DCM (1.0 M) at 0 °C was added TFA (1 equiv.) drop wise and the reaction mixture was allowed to warm up to room temperature. After 2 h, the reaction was diluted with water and extracted with DCM (2X). The aqueous layer was basified to pH 9.0 with 10% NaOH and then extracted with DCM (3X). The organic layer was combined, dried with sodium sulfate and concentrated under reduced pressure.

General procedure C: Epoxidation. To a solution of benzaldehyde (1 equiv.) and trimethylsulfonium iodide (Me₃SI) (1.5 equiv.) in acetonitrile (2 mL) and water (5 µL) was added potassium hydroxide pellets (2 equiv.). The mixture was stirred at 80 °C for 4 h and then cooled; the acetonitrile was removed by rotatory evaporator. The residue was treated with a solution of water (5 mL) and sodium hypochlorite or bleach (1.5 mL) followed by extraction with diethyl ether (5 mL X 3). The organic layer was combined, dried with sodium sulfate and the solvent was removed under reduced pressure and then purified by flash chromatography.

General procedure D: Epoxide ring opening. A solution of the amine (1 equiv.) and the epoxide (1 equiv.) in ethanol (0.1 M relative to the epoxide) was heated to reflux for 18 h. The ethanol was removed under reduced pressure and the residue was purified by flash chromatography.

General procedure E: Reduction of carbonyl group. To a solution of the carbonyl (1 equiv.) in absolute ethanol (0.1 M relative to the carbonyl) was added sodium borohydride (3 equiv.). The reaction mixture was heated at reflux overnight and then cooled. The ethanol was removed under reduced pressure, the residue was diluted with water (1X the volume of ethanol) and extracted with ethyl acetate (5X the volume of ethanol). The combined organic layer was dried with sodium sulfate, the solvent was evaporated and the residue was purified by flash chromatography.

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**General procedure F: alkylation of amine.** To a solution of the amine (1 equiv.) and halide (1 equiv.) in DMF (0.1 M relative to the halide) was added potassium carbonate (2 equiv.). The reaction mixture was stirred at room temperature until the starting halide was judged consumed by TLC analysis (8 h unless otherwise stated). The reaction was diluted with water (3X the volume of DMF) and extracted with ethyl acetate (5X the volume of DMF) thrice. The combine organic layer was washed with brine (5X the volume of DMF), dried with sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by flash chromatography.

**General procedure G: dehydroxylation.** To a solution of the SPC (1 equiv.) in ethanol (0.1 M relative to the SPC) was added Pd/C (0.5 equiv.) and stirred overnight under hydrogen balloon. The reaction mixture was filtered through a pad of celite, rinsed with ethyl acetate (2X the volume of ethanol). The solvent was removed under reduced pressure and the residue was purified by flash chromatography.

**General procedure H: Amidation.** To a solution of the amine (1 equiv.) and the acid (1 equiv.) in DCM (0.1M relative to the amine) was added EDCI (2 equiv.) and DMAP (0.2 equiv.). The reaction mixture was stirred at room temperature until the starting material was judged consumed by TLC analysis (6 h unless otherwise stated). The reaction was quenched with water (2X the volume of DCM) and extracted with DCM thrice. The combine organic layer was dried with sodium sulfate, the solvent was removed under reduced pressure and the residue was purified by flash chromatography.

*tert*-butyl 4-oxo-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidine]-1'-carboxylate (2.2) :

General procedure A was used in the reaction of 1-(1-hydroxynaphthalen-2-yl)ethanone (500 mg, 2.7 mmol), *tert*-butyl 4-oxopiperidine-1-carboxylate (536 mg, 2.7 mmol) and pyrrolidine (517 mg, 7.2 mmol) in absolute methanol (5 mL). It was purified by flash chromatography.
(hexanes:EtOAc = 7:1) to afford 2.2 (908 mg, 92%) as a yellow solid. R_f (hexanes:EtOAc = 5:1) = 0.37.

**spiro[benzo[h]chromene-2,4'-piperidin]-4(3H)-one (2.2a):** General procedure B was used to deprotect 2 (1.5 g, 4.0 mmol) in DCM (40 mL) and TFA (13 mL) to afford 2.2a (1.05 g, 97%) as a brown solid. R_f (DCM:MeOH = 10:1) = 0.21

**1'-ethylspiro[benzo[h]chromene-2,4'-piperidin]-4(3H)-one (2.2b):** General procedure F was used to alkylate 2.2 (100 mg, 0.37 mmol) with bromoethane (40 mg, 0.37 mmol) using potassium carbonate (102 mg, 0.74 mmol) in DMF (4 mL). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.2b (83 mg, 75%) as white solid. R_f (DCM:MeOH = 10:1) = 0.45

1H NMR (600 MHz, CDCl₃) δ 8.35 (d, J = 8.3 Hz, 1H), 7.86 – 7.83 (m, 1H), 7.79 (t, J = 8.0 Hz, 1H), 7.64 – 7.60 (m, 1H), 7.57 – 7.52 (m, 1H), 7.40 – 7.37 (m, 1H), 2.83 – 2.74 (m, 4H), 2.54 – 2.47 (m, 4H), 2.26 – 2.19 (m, 2H), 1.87 (t, J = 10.7 Hz, 2H), 1.12 (t, 3H). 13C NMR (151 MHz, CDCl₃) δ 191.69, 157.34, 137.90, 129.63, 128.09, 126.34, 125.56, 124.6, 121.64, 120.7, 115.24, 79.19, 52.53, 48.89, 47.79, 34.56, 12.31.

**1'-benzylspiro[benzo[h]chromene-2,4'-piperidin]-4(3H)-one (2.2c):** General procedure F was used to alkylate 2.2a (300 mg, 1.12 mmol) with (bromomethyl)benzene (191 mg, 1.12 mmol) using potassium carbonate (310 mg, 2.24 mmol) in DMF (8 mL) for 1 h. It was purified by flash chromatography (Hexanes:EtOAc = 1:1) to afford 2.2c (375 mg, 94%) as yellow solid. R_f = 0.35 in EtOAc 1H NMR (400 MHz, CDCl₃) δ 8.31 (d, J = 8.3 Hz, 1H), 7.84 (d, J = 8.7 Hz, 1H), 7.78 (d, J = 8.1 Hz, 1H), 7.64 – 7.61 (m, 1H), 7.57 – 7.51 (m, 1H), 7.38 (s, 1H), 7.36 – 7.31 (m, 4H), 7.29 – 7.24 (m, 1H), 3.60 (s, 2H), 2.80 (s, 2H), 2.71 (d, J = 11.6 Hz, 2H), 2.61 – 2.53 (m, 2H), 2.18 (d, J = 12.3 Hz, 2H), 1.89 – 1.79 (m, 2H). 13C NMR (101 MHz, CDCl₃) δ 191.65, 157.31,
1'-phenethylspiro[benzo[h]chromene-2,4'-piperidin]-4(3H)-one (2.2d): General procedure F was used to alkylate 2.2a (200 mg, 0.75 mmol) with (2-bromoethyl)benzene (138 mg, 0.75 mmol) using potassium carbonate (207 mg, 1.5 mmol) in DMF (5 mL). It was purified by flash chromatography (Hexanes:EtOAc = 1:1) to afford 2.2d (224 mg, 80%) as yellow solid. Rf = 0.35 in EtOAc. ¹H NMR (400 MHz, CDCl₃) δ 8.41 – 8.31 (m, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.83 – 7.76 (m, 1H), 7.67 – 7.60 (m, 1H), 7.59 – 7.52 (m, 1H), 7.39 (d, J = 8.7 Hz, 1H), 7.35 – 7.27 (m, 2H), 7.25 – 7.15 (m, 3H), 2.84 (d, J = 8.0 Hz, 6H), 2.77 – 2.67 (m, 2H), 2.67 – 2.54 (m, 2H), 2.29 – 2.15 (m, 2H), 1.96 – 1.76 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 191.66, 157.32, 140.37, 137.88, 129.62, 128.79, 128.53, 128.07, 126.34, 126.21, 125.54, 123.45, 121.62, 120.70, 115.23, 79.12, 60.70, 49.28, 47.79, 34.62, 33.98.

1'-(2-cyclohexylethyl)spiro[benzo[h]chromene-2,4'-piperidin]-4(3H)-one (2.2e): General procedure F was used to alkylate 2.2a (300 mg, 1.12 mmol) with (2-bromoethyl)cyclohexane (214 mg, 1.12 mmol) using potassium carbonate (310 mg, 2.24 mmol) in DMF (8 mL). It was purified by flash chromatography (Hexanes:EtOAc = 2:1) to afford 2.2e (260 mg, 61%) as yellow solid. Rf = 0.35 in EtOAc. ¹H NMR (400 MHz, CDCl₃) δ 8.43 – 8.24 (m, 1H), 7.84 (d, J = 8.7 Hz, 1H), 7.78 (d, J = 8.1 Hz, 1H), 7.66 – 7.58 (m, 1H), 7.59 – 7.50 (m, 1H), 7.37 (d, J = 8.7 Hz, 1H), 2.80 (s, 2H), 2.77 – 2.63 (m, 2H), 2.56 – 2.37 (m, 4H), 2.26 – 2.13 (m, 2H), 1.91 – 1.78 (m, 2H), 1.75 – 1.56 (m, 5H), 1.47 – 1.35 (m, 2H), 1.33 – 1.10 (m, 4H), 1.01 – 0.83 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 191.77, 157.40, 137.88, 129.60, 128.06, 126.31, 125.57, 123.52, 121.63, 120.64, 115.23, 79.28, 56.83, 49.44, 47.80, 36.50, 34.95, 34.66, 33.63, 26.73, 26.45.
1'-{(3-phenylpropyl)spiro[benzo[h]chromene-2,4'-piperidin]-4(3H)-one  (2.2f): General procedure F was used to alkylate 2.2a (200 mg, 0.75 mmol) with (3-bromopropyl)benzene (149 mg, 0.75 mmol) using potassium carbonate (207 mg, 1.5 mmol) in DMF (5 mL). It was purified by flash chromatography (Hexanes:EtOAc = 1:1;) to afford 2.2f (159 mg, 55%) as yellow solid. R_f = 0.24 in EtOAc. 1H NMR (400 MHz, CDCl₃) δ 8.38 – 8.31 (m, 1H), 7.85 (d, J = 8.7 Hz, 1H), 7.82 – 7.76 (m, 1H), 7.68 – 7.58 (m, 1H), 7.59 – 7.49 (m, 1H), 7.45 – 7.34 (m, 1H), 7.33 – 7.24 (m, 2H), 7.24 – 7.14 (m, 3H), 2.81 (s, 2H), 2.78 – 2.70 (m, 2H), 2.70 – 2.64 (m, 2H), 2.60 – 2.40 (m, 4H), 2.25 – 2.13 (m, 2H), 1.94 – 1.78 (m, 4H). 13C NMR (101 MHz, CDCl₃) δ 191.73, 157.37, 142.20, 137.87, 129.61, 128.50, 128.44, 128.06, 126.32, 125.90, 125.55, 123.50, 121.62, 120.65, 115.21, 79.23, 58.16, 49.30, 47.78, 34.64, 33.88, 28.97.

1'-(2-(3,4-difluorophenyl)-2-oxoethyl)spiro[benzo[h]chromene-2,4'-piperidin]-4(3H)-one  (2.2g): To a solution of 2.2a (200 mg, 0.74 mmol) and 2-bromo-1-(3,4-difluorophenyl)ethanone (176 mg, 0.74 mmol) in acetonitrile (6 mL) was added sodium bicarbonate (126 mg, 1.49 mmol) at 0°C. The reaction mixture was allowed to stir to room temperature and held at room temperature for 4 hours. The acetonitrile was removed under reduced pressure, the residue was diluted with water and extracted with EtOAc (10 mL X 3). The combined organic layer was dried with sodium sulfate, the solvent was evaporated and the residue was purified by flash chromatography (DCM: MeOH = 75: 1) to afford 2.2g (180 mg, 57%) as yellow solid. R_f (DCM:MeOH = 10:1) = 0.71. 1H NMR (399 MHz, CDCl₃) δ 8.40 – 8.28 (m, 1H), 7.94 – 7.85 (m, 1H), 7.85 – 7.72 (m, 3H), 7.66 – 7.58 (m, 1H), 7.58 – 7.50 (m, 1H), 7.37 (d, J = 8.6 Hz, 1H), 7.26 – 7.17 (m, 1H), 3.81 (s, 2H), 2.91 – 2.77 (m, 4H), 2.76 – 2.64 (m, 2H), 2.27 – 2.11 (m, 2H), 1.99 – 1.79 (m, 2H). 13C NMR (100 MHz, CDCl₃) δ 194.24, 191.50, 157.20, 153.64 (dd, J = 25.8, 14.5 Hz), 150.52 (dd, J = 252.5, 14.5 Hz), 137.90, 133.06 (dd, J = 7.5, 3.2 Hz), 129.70, 128.14, 126.43, 125.50 (dd, J = 7.6, 3.7 Hz), 56.
123.40, 121.62, 120.84, 117.86 (d, J = 18.3 Hz), 117.63 (d, J = 17.8 Hz), 115.22, 78.56, 64.90, 49.56, 47.73, 34.44.

**2-(3,4-difluorophenyl)oxirane:** General procedure C was used with 3,4-difluorobenzaldehyde (500 mg, 3.5 mmol) and trimethylsulfonium iodide (Me$_3$SI) (1.1 g, 5.4 mmol) in acetonitrile (8 mL) and water (20 µL) and potassium hydroxide pellets 3.92 mg, 7.0 mmol) to afford 4 (489 mg, 89% as a clear oil. R$_f$ (hexanes:EtOAc = 4:1) = 0.55.

**1’-(2-(3,4-difluorophenyl)-2-hydroxyethyl)spiro[benzo[h]chromene-2,4’-piperidin]-4(3H)-one (2.2h):** General procedure D was used with 2.2a (500 mg, 1.8 mmol) and epoxide (292 mg, 1.8 mmol) in ethanol (30 mL). It was purified by flash chromatography (hexanes:EtOAc = 2:1) to afford 2.2h (498 mg, 63%) as a yellow solid. R$_f$ = 0.69 in EtOAc.

**1’-(2-(3,4-dichlorophenyl)-2-hydroxyethyl)spiro[benzo[h]chromene-2,4’-piperidin]-4(3H)-one (2.2i):** General procedure D was used with 2.2a (500 mg, 1.8 mmol) and epoxide (354 mg, 1.8 mmol) in ethanol (30 mL). It was purified by flash chromatography (hexanes:EtOAc = 2:1) to afford 2.2i (628 mg, 74%) as a yellow solid. R$_f$ = 0.31 in EtOAc. $^1$H NMR (399 MHz, CDCl$_3$) $\delta$ 8.40 – 8.26 (m, 1H), 7.85 (d, $J$ = 8.7 Hz, 1H), 7.80 (d, $J$ = 8.1 Hz, 1H), 7.69 – 7.59 (m, 1H), 7.58 – 7.52 (m, 1H), 7.50 (d, $J$ = 2.0 Hz, 1H), 7.45 – 7.37 (m, 2H), 7.25 – 7.16 (m, 1H), 4.71 (dd, $J$ = 10.6, 3.4 Hz, 1H), 3.06 – 2.94 (m, 1H), 2.94 – 2.73 (m, 3H), 2.73 – 2.55 (m, 3H), 2.54 – 2.41 (m, 1H), 2.36 – 2.18 (m, 2H), 1.99 – 1.77 (m, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 191.43, 157.14, 142.54, 137.89, 132.65, 131.41, 130.47, 129.73, 128.17, 127.93, 126.45, 125.43, 125.24, 123.31, 121.61, 120.91, 115.21, 78.74, 67.93, 66.02, 50.62, 47.77, 47.70, 34.73, 34.55.

**1’-(3,4-difluorophenethyl)spiro[benzo[h]chromene-2,4’-piperidin]-4(3H)-one (2.2j):** General procedure F was used to alkylate 2.2a (110 mg, 0.4 mmol) with 4-(2-bromoethyl)-1,2-
difluorobenzene (91 mg, 0.4 mmol) using potassium carbonate (110 mg, 0.8 mmol) in DMF (4 mL). It was purified by flash chromatography (Hexanes:EtOAc = 2:1) to afford **2.2j** (117 mg, 72%) as yellow solid. Rf = 0.26 in EtOAc. 1H NMR (600 MHz, CDCl3) δ 8.53 – 8.26 (m, 1H), 7.84 (d, J = 8.6 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.70 – 7.58 (m, 1H), 7.60 – 7.49 (m, 1H), 7.38 (d, J = 8.6 Hz, 1H), 7.18 – 6.97 (m, 2H), 6.96 – 6.80 (m, 1H), 2.82 (s, 2H), 2.81 – 2.74 (m, 4H), 2.69 – 2.63 (m, 2H), 2.63 – 2.54 (m, 2H), 2.32 – 2.14 (m, 2H), 1.95 – 1.77 (m, 2H).

1′-(3,4-dichlorophenethyl)spiro[benzo[h]chromene-2,4′-piperidin]-4(3H)-one (2.2k):

General procedure F was used to alkylate **2.2a** (260 mg, 0.97 mmol) with 4-(2-bromoethyl)-1,2-dichlorobenzene (247 mg, 0.97 mmol) using potassium carbonate (270 mg, 1.9 mmol) in DMF (8 mL). It was purified by flash chromatography (Hexanes:EtOAc = 2:1) to afford **2.2k** (316 mg mg, 74%) as yellow solid. Rf = 0.26 in EtOAc. 1H NMR (399 MHz, CDCl3) δ 8.35 (d, J = 8.2 Hz, 1H), 7.84 (d, J = 8.7 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.62 (t, J = 7.3 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.43 – 7.27 (m, 3H), 7.03 (dd, J = 8.2, 2.0 Hz, 1H), 2.81 (s, 2H), 2.80 – 2.72 (m, 4H), 2.71 – 2.53 (m, 4H), 2.31 – 2.13 (m, 2H), 1.94 – 1.75 (m, 2H). 13C NMR (126 MHz, CDCl3) δ 191.61, 157.25, 140.65, 137.86, 132.30, 130.71, 130.38, 130.12, 129.65, 128.28, 128.09, 126.37, 125.48, 123.39, 121.60, 120.74, 115.19, 79.00, 59.97, 49.25, 47.76, 34.56, 33.04.

1'-((2-(3,4-difluorophenyl)acetyl)spiro[benzo[h]chromene-2,4′-piperidin]-4(3H)-one (2.2l):

General procedure H was used in the amidation reaction of **2.2a** (600 mg, 2.24 mmol) and 2-(3,4-difluorophenyl)acetic acid (386 mg, 2.24 mmol) with EDCI (860 mg, 4.5 mmol) and DMAP (55 mg, 0.45 mmol) in DCM (15 mL). It was purified by flash chromatography (Hexanes:EtOAc = 2:1) to afford **2.2l** (724 mg, 77%) as yellow solid. Rf = 0.60 in EtOAc. 1H NMR (600 MHz, CDCl3) δ 8.29 (d, J = 8.2 Hz, 1H), 7.85 – 7.78 (m, 2H), 7.66 – 7.60 (m, 1H), 7.57 – 7.52 (m, 1H), 7.41 (d, J = 8.6 Hz, 1H), 7.12 – 7.05 (m, 2H), 6.98 – 6.93 (m, 1H), 4.53 (d, J = 13.4 Hz, 1H), 3.75 – 3.64
(m, 3H), 3.56 (t, J = 11.9 Hz, 1H), 3.26 – 3.18 (m, 1H), 2.80 (q, J = 16.5 Hz, 2H), 2.28 – 2.17 (m, 2H), 1.73 – 1.64 (m, 1H), 1.54 – 1.46 (m, 1H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 190.72, 168.69, 156.75, 150.47 (dd, J = 246.1, 11.9 Hz), 149.59 (dd, J = 246.1, 11.9 Hz), 137.92, 131.92 (dd, J = 5.8, 3.9 Hz), 129.87, 128.24, 126.65, 125.23, 124.88 (dd, J = 5.9, 3.4 Hz), 123.01, 121.52, 121.26, 117.93 (d, J = 17.4 Hz), 117.54 (d, J = 17.4 Hz), 115.18, 78.85, 47.63, 42.04, 39.92, 37.81, 34.50, 34.33.

3,4-dihydrospiro[benzo[h]chromene-2,4’-piperidin]-4-ol (2.3a): General procedure B was used to deprotect 2.3 (1.48 g, 4.0 mmol) in DCM (40 mL) and TFA (13 mL) to afford 2.3a (1 g, 97%) as a brown solid. R$_f$ (DCM:MeOH = 10:1) = 0.20 $^1$H NMR (600 MHz, CDCl$_3$) δ 8.30 – 8.23 (m, 1H), 7.78 – 7.73 (m, 1H), 7.53 – 7.49 (m, 1H), 7.49 – 7.44 (m, 2H), 7.40 (d, J = 8.3 Hz, 1H), 4.96 – 4.88 (m, 1H), 3.22 – 3.13 (m, 1H), 3.13 – 3.03 (m, 1H), 2.94 – 2.56 (m, 4H), 2.22 – 2.13 (m, 1H), 2.08 – 2.01 (m, 1H), 1.97 – 1.82 (m, 2H), 1.75 – 1.53 (m, 2H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 147.55, 134.34, 127.63, 126.58, 125.56, 125.54, 125.49, 122.24, 120.09, 120.05, 74.71, 63.12, 42.61, 42.17, 42.04, 36.74, 34.76.

1'-ethyl-3,4-dihydrospiro[benzo[h]chromene-2,4’-piperidin]-4-ol (2.3b): General procedure E was used to reduce 2.2a (150mg, 0.5mmol) in absolute ethanol (4 ml) using sodium borohydride (58mg, 1.5mmol). It was purified by flash chromatography (DCM:MeOH = 20:1;) to afford 2.3b (134 mg, 89%) as white solid. R$_f$(DCM:MeOH = 10:1) = 0.22 $^1$H NMR (600 MHz, CDCl$_3$) δ 8.29 – 8.14 (m, 1H), 7.89 – 7.65 (m, 1H), 7.56 – 7.41 (m, 3H), 7.43 – 7.30 (m, 1H), 4.99 – 4.75 (m, 1H), 2.82 – 2.63 (m, 2H), 2.57 – 2.43 (m, 4H), 2.44 – 2.35 (m, 1H), 2.23 – 2.02 (m, 2H), 1.97 – 1.86 (m, 2H), 1.86 – 1.77 (m, 1H), 1.77 – 1.66 (m, 1H), 1.21 – 1.00 (m, 3H).
1'-benzyl-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidin]-4-ol (2.3c): General procedure E was used to reduce 2.2c (250 mg, 0.7 mmol) in absolute ethanol (5 mL) using sodium borohydride (79 mg, 2.1 mmol). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.3c (198 mg, 79%) as white solid. Rf (DCM:MeOH = 10:1) = 0.44 1H NMR (400 MHz, CDCl3) δ 8.26 – 8.16 (m, 1H), 7.81 – 7.69 (m, 1H), 7.54 – 7.43 (m, 3H), 7.40 (d, J = 8.5 Hz, 1H), 7.38 – 7.31 (m, 4H), 7.31 – 7.23 (m, 1H), 5.02 – 4.85 (m, 1H), 3.62 (s, 2H), 2.80 – 2.72 (m, 1H), 2.72 – 2.64 (m, 1H), 2.64 – 2.58 (m, 1H), 2.58 – 2.48 (m, 1H), 2.20 (dd, J = 13.7, 6.1 Hz, 1H), 2.14 – 2.04 (m, 1H), 2.02 – 1.93 (m, 1H), 1.93 – 1.83 (m, 2H), 1.82 – 1.72 (m, 1H). 13C NMR (101 MHz, CDCl3) δ 147.63, 137.98, 134.37, 129.51, 128.36, 127.62, 127.27, 126.57, 125.55, 125.44, 122.29, 120.08, 118.07, 74.27, 63.46, 63.33, 49.32, 49.14, 42.18, 35.95, 34.11.

1'-phenethyl-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidin]-4-ol (2.3d): General procedure E was used to reduce 2.2d (220 mg, 0.59 mmol) in absolute ethanol (5 mL) using sodium borohydride (67 mg, 1.78 mmol). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.3d (169 mg, 76%) as white solid. Rf (DCM:MeOH = 10:1) = 0.52 1H NMR (400 MHz, CDCl3) δ 8.34 – 8.15 (m, 1H), 7.84 – 7.72 (m, 1H), 7.59 – 7.46 (m, 3H), 7.42 (d, J = 8.3 Hz, 1H), 7.31 (t, J = 7.3 Hz, 2H), 7.23 (d, J = 7.6 Hz, 3H), 4.97 (t, J = 6.7 Hz, 1H), 3.01 – 2.77 (m, 4H), 2.78 – 2.65 (m, 3H), 2.64 – 2.49 (m, 2H), 2.33 – 2.12 (m, 2H), 2.09 – 1.88 (m, 2H), 1.88 – 1.74 (m, 1H), 1.32 – 1.14 (m, 1H). 13C NMR (101 MHz, CDCl3) δ 147.58, 140.30, 134.38, 128.82, 128.55, 127.66, 126.59, 126.23, 125.57, 125.52, 122.22, 120.14, 118.12, 74.16, 63.38, 60.83, 49.48, 49.35, 42.23, 35.88, 34.15, 33.73.

1'-(2-cyclohexylethyl)-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidin]-4-ol (2.3e): General procedure E was used to reduce 2.2e (200 mg, 0.53 mmol) in absolute ethanol (5 mL) using sodium borohydride (60 mg, 1.59 mmol). It was purified by flash chromatography...
(DCM:MeOH = 30:1;) to afford 2.3e (143 mg, 71%) as white solid. Rf (DCM:MeOH = 10:1) = 0.60 1H NMR (400 MHz, CDCl3) δ 8.33 – 8.18 (m, 1H), 7.84 – 7.72 (m, 1H), 7.56 – 7.45 (m, 3H), 7.40 (d, J = 8.5 Hz, 1H), 5.07 – 4.84 (m, 1H), 2.90 – 2.68 (m, 2H), 2.60 – 2.35 (m, 5H), 2.29 – 2.16 (m, 1H), 2.17 – 2.05 (m, 1H), 2.07 – 1.82 (m, 2H), 1.82 – 1.75 (m, 1H), 1.76 – 1.58 (m, 5H), 1.55 – 1.38 (m, 2H), 1.34 – 1.06 (m, 5H), 1.04 – 0.78 (m, 2H). 13C NMR (101 MHz, CDCl3) δ 147.65, 134.38, 127.64, 126.55, 125.58, 125.52, 125.48, 122.28, 120.06, 118.15, 74.33, 63.40, 57.03, 49.62, 49.49, 42.30, 36.61, 36.00, 34.69, 34.12, 33.62, 26.73, 26.45.

1'-(3-phenylpropyl)-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidin]-4-ol (2.3f): General procedure E was used to reduce 2.2f (200 mg, 0.57 mmol) in absolute ethanol (5 mL) using sodium borohydride (65 mg, 1.71 mmol). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.3f (161 mg, 73%) as white solid. Rf (DCM:MeOH = 10:1) = 0.53 1H NMR (400 MHz, CDCl3) δ 8.35 – 8.17 (m, 1H), 7.82 – 7.70 (m, 1H), 7.58 – 7.46 (m, 3H), 7.40 (d, J = 8.5 Hz, 1H), 7.36 – 7.27 (m, 2H), 7.25 – 7.15 (m, 3H), 5.03 – 4.69 (m, 1H), 2.89 – 2.62 (m, 5H), 2.62 – 2.51 (m, 1H), 2.51 – 2.39 (m, 3H), 2.24 – 2.12 (m, 1H), 2.13 – 2.03 (m, 1H), 2.02 – 1.80 (m, 5H), 1.80 – 1.67 (m, 1H). 13C NMR (101 MHz, CDCl3) δ 147.60, 142.17, 134.33, 128.48, 128.43, 127.61, 126.52, 125.89, 125.54, 125.49, 122.26, 120.02, 118.15, 74.29, 63.27, 58.39, 49.46, 49.34, 42.24, 36.01, 34.01, 33.96, 28.78.

1-(3,4-difluorophenyl)-2-(4-hydroxy-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidin]-1'-yl)ethanone (2.3g): To a solution of 2.3a (500 mg, 1.85 mmol) and 2-bromo-1-(3,4-difluorophenyl)ethanone (436 mg, 1.85 mmol) in acetonitrile (15 mL) was added sodium bicarbonate (311 mg, 3.70 mmol) at 0 °C. The reaction mixture was allowed to stir to room temperature and held at room temperature for 4 hours. The acetonitrile was removed under reduced pressure, the residue was diluted with water and extracted with EtOAc (20 mL X 3). The
combined organic layer was dried with sodium sulfate, the solvent was evaporated and the residue was purified by flash chromatography (DCM: MeOH = 50:1) to afford 2.3g (400 mg, 51%) as yellow solid. Rf (DCM:MeOH = 10:1) = 0.45

1'-((3,4-difluorophenyl)-2-hydroxyethyl)-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidin]-4-ol (2.3h): General procedure E was used to reduce 2h (100 mg, 0.23 mmol) in absolute ethanol (2 mL) using sodium borohydride (27 mg, 0.7 mmol). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.3h (88 mg, 88%) as white solid. Rf (DCM:MeOH = 10:1) = 0.37

1'-((3,4-dichlorophenyl)-2-hydroxyethyl)-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidin]-4-ol (2.3i): General procedure E was used to reduce 2.2i (160 mg, 0.35 mmol) in absolute ethanol (3 mL) using sodium borohydride (240 mg, 1.0 mmol). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.3i (140 mg, 87%) as white solid. Rf (DCM:MeOH = 10:1) = 0.52

1H NMR (500 MHz, CDCl3) δ 8.36 – 8.05 (m, 1H), 7.89 – 7.72 (m, 1H), 7.60 – 7.45 (m, 4H), 7.47 – 7.33 (m, 2H), 7.24 – 7.12 (m, 1H), 4.97 (t, J = 6.6 Hz, 1H), 4.81 – 4.59 (m, 1H), 3.11 – 2.70 (m, 2H), 2.73 – 2.48 (m, 3H), 2.42 (ddd, J = 12.5, 10.7, 8.4 Hz, 1H), 2.31 – 2.07 (m, 2H), 2.08 – 1.91 (m, 2H), 1.92 – 1.67 (m, 2H).

13C NMR (100 MHz, CDCl3) δ 147.49, 142.72, 134.39, 132.61, 131.32, 130.43, 127.94, 127.71, 126.70, 125.66, 125.50, 125.44, 125.25, 122.14, 120.30, 117.90, 73.91, 67.85, 66.12, 66.10, 63.47, 63.46, 50.94, 50.79, 47.87, 47.72, 42.08, 36.12, 35.86, 34.63, 34.63.

1'-((3,4-difluorophenethyl)-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidin]-4-ol (2.3j): General procedure E was used to reduce 2.2j (100 mg, 0.24 mmol) in absolute ethanol (3 mL) using sodium borohydride (28 mg, 0.73 mmol). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.3j (89 mg, 91%) as white solid. Rf (DCM:MeOH = 20:1) = 0.42
$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.47 – 8.05 (m, 1H), 7.89 – 7.65 (m, 1H), 7.57 – 7.45 (m, 3H), 7.40 (d, $J$ = 8.4 Hz, 1H), 7.24 – 6.96 (m, 2H), 6.97 – 6.80 (m, 1H), 5.09 – 4.76 (m, 1H), 3.03 – 2.69 (m, 4H), 2.68 – 2.58 (m, 3H), 2.59 – 2.48 (m, 2H), 2.29 – 2.15 (m, 1H), 2.15 – 2.03 (m, 1H), 2.01 – 1.90 (m, 2H), 1.90 – 1.81 (m, 1H), 1.83 – 1.66 (m, 1H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 150.14 (dd, $J$ = 246.4, 12.8 Hz), 148.89 (dd, $J$ = 246.4, 12.8 Hz), 147.41, 137.23 (dd, $J$ = 4.3, 2.6 Hz), 134.24, 127.53, 126.47, 125.44, 125.40, 125.34, 124.46 (dd, $J$ = 5.8, 3.3 Hz), 122.05, 120.03, 117.95, 117.37 (d, $J$ = 17.0 Hz), 116.97 (d, $J$ = 17.0), 73.98, 63.24, 60.19, 49.31, 49.20, 42.07, 35.76, 34.00, 32.76.

$1'$-(3,4-dichlorophenethyl)-3,4-dihydrospi[benzo[h]chromene-2,4'-piperidin]-4-ol (2.3k): General procedure E was used to reduce 2.2k (100 mg, 0.22 mmol) in absolute ethanol (3 mL) using sodium borohydride (28 mg, 0.68 mmol). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.3k (83 mg, 83%) as white solid. $R_f$ (DCM:MeOH = 10:1) = 0.34 $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.33 – 8.15 (m, 1H), 7.85 – 7.66 (m, 1H), 7.59 – 7.45 (m, 3H), 7.40 (d, $J$ = 8.4 Hz, 1H), 7.38 – 7.29 (m, 2H), 7.11 – 6.92 (m, 1H), 5.06 – 4.83 (m, 1H), 2.85 – 2.69 (m, 4H), 2.68 – 2.55 (m, 3H), 2.57 – 2.46 (m, 1H), 2.28 – 2.15 (m, 1H), 2.15 – 2.06 (m, 1H), 2.02 – 1.89 (m, 2H), 1.90 – 1.80 (m, 1H), 1.79 – 1.64 (m, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 147.52, 140.66, 134.33, 132.27, 130.71, 130.36, 130.07, 128.28, 127.65, 126.60, 125.59, 125.50, 125.46, 122.18, 120.14, 118.08, 74.11, 63.31, 60.14, 49.39, 49.28, 42.21, 35.92, 34.00, 32.82.

2-(3,4-difluorophenyl)-1-(4-hydroxy-3,4-dihydrospi[benzo[h]chromene-2,4'-piperidin]-1'-yl)ethanone (2.3l): General procedure E was used to reduce 2.2l (340 mg, 0.81 mmol) in absolute ethanol (10 mL) using sodium borohydride (92 mg, 2.4 mmol). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.3l (250 mg, 73%) as white solid. $R_f$ (DCM:MeOH = 20:1) = 0.69 $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.31 – 8.04 (m, 1H), 7.84 – 7.68 (m,
1H, 7.56 – 7.44 (m, 3H), 7.44 – 7.37 (m, 1H), 7.08 (t, J = 9.1 Hz, 2H), 6.99 – 6.85 (m, 1H), 5.02 – 4.79 (m, 1H), 4.55 – 4.31 (m, 1H), 3.65 (s, 3H), 3.58 – 3.42 (m, 1H), 3.30 – 3.07 (m, 1H), 2.65 (s, 1H), 2.22 – 2.05 (m, 2H), 2.03 – 1.93 (m, 1H), 1.93 – 1.79 (m, 1H), 1.72 – 1.53 (m, 1H), 1.53 – 1.33 (m, 1H).

tert-butyl 3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidine]-1'-carboxylate (2.4): General procedure E followed by general procedure G was used to convert 2 to 4 over 2 steps in 47% yield.

3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidine] (2.5a): General procedure B was used to deprotect 4 (1.4 g, 4.0 mmol) in DCM (40 mL) and TFA (13 mL) to afford 2.5a (982 mg, 97%) as a brown solid. Rf (DCM:MeOH = 10:1) = 0.25.

1'-ethyl-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidine] (2.5b): General procedure F was used to alkylate 2.5a (60 mg, 0.23 mmol) with bromoethane (25 mg, 0.23 mmol) using potassium carbonate (64 mg, 0.46 mmol) in DMF (2.5 mL). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.5b (45 mg, 68%) as brown oil. Rf(DCM:MeOH = 10:1) = 0.28

1'H NMR (400 MHz, CDCl3) δ 8.35 – 8.14 (m, 1H), 7.96 – 7.64 (m, 1H), 7.58 – 7.39 (m, 2H), 7.33 (d, J = 8.3 Hz, 1H), 7.16 (d, J = 8.3 Hz, 1H), 2.89 (t, J = 6.8 Hz, 2H), 2.86 – 2.70 (m, 2H), 2.62 – 2.43 (m, 4H), 2.02 – 1.95 (m, 2H), 1.91 (t, J = 6.8 Hz, 2H), 1.82 – 1.72 (m, 2H), 1.15 (t, J = 7.2 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 147.94, 133.45, 127.84, 127.84, 127.55, 125.92, 125.65, 125.20, 121.55, 119.29, 115.07, 73.06, 52.69, 49.17, 34.81, 32.33, 22.07, 12.37.

1'-benzyl-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidine] (2.5c): General procedure F was used to alkylate 2.5a (50mg, 0.19mmol) with (bromomethyl)benzene (33mg, 0.19mmol) using potassium carbonate (55mg, 0.39mmol) in DMF (2 mL) for 1 h. It was purified by flash
chromatography (Hexanes:EtOAc = 10:1) to afford 2.5c (55 mg, 81%) as white solid. Rf = 0.54 in EtOAc. 1H NMR (500 MHz, CDCl3) δ 8.24 (dd, J = 8.0, 1.7 Hz, 1H), 7.76 (dd, J = 7.4, 1.8 Hz, 1H), 7.56 – 7.42 (m, 2H), 7.41 – 7.31 (m, 5H), 7.31 – 7.27 (m, 1H), 7.16 (d, J = 8.3 Hz, 1H), 3.62 (s, 2H), 2.89 (t, J = 6.8 Hz, 2H), 2.83 – 2.68 (m, 2H), 2.67 – 2.51 (m, 2H), 2.09 – 1.83 (m, 4H), 1.84 – 1.71 (m, 2H). 13C NMR (101 MHz, CDCl3) δ 147.97, 138.60, 133.44, 129.40, 128.31, 127.82, 127.53, 127.09, 125.93, 125.64, 125.20, 121.61, 119.25, 115.04, 73.04, 63.49, 49.46, 34.83, 32.25, 22.06.

1'-phenethyl-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidine] (2.5d): General procedure F was used to alkylate 2.5a (50 mg, 0.19 mmol) with (2-bromoethyl)benzene (37 mg, 0.19 mmol) using potassium carbonate (55 mg, 0.39 mmol) in DMF (2 mL). It was purified by flash chromatography (Hexanes:EtOAc = 8:1) to afford 2.5d (57 mg, 81%) as white solid. Rf = 0.51 in EtOAc. 1H NMR (500 MHz, CDCl3) δ 8.32 – 8.19 (m, 1H), 7.81 – 7.72 (m, 1H), 7.55 – 7.42 (m, 2H), 7.39 – 7.29 (m, 3H), 7.29 – 7.21 (m, 3H), 7.18 (d, J = 8.3 Hz, 1H), 2.99 – 2.82 (m, 6H), 2.80 – 2.70 (m, 2H), 2.70 – 2.58 (m, 2H), 2.09 – 1.97 (m, 2H), 1.94 (t, J = 6.8 Hz, 2H), 1.87 – 1.72 (m, 2H). 13C NMR (126 MHz, CDCl3) δ 147.93, 140.62, 133.45, 128.86, 128.53, 127.84, 127.57, 126.16, 125.92, 125.69, 125.25, 121.56, 119.33, 115.07, 72.99, 61.04, 49.58, 34.87, 34.06, 32.35, 22.08.

1'-(2-cyclohexylethyl)-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidine] (2.5e): General procedure F was used to alkylate 2.5a (50 mg, 0.19 mmol) with (2-bromoethyl)cyclohexane (38 mg, 0.19 mmol) using potassium carbonate (55 mg, 0.39 mmol) in DMF (2 mL). It was purified by flash chromatography (Hexanes:EtOAc = 5:1;) to afford 2.5e (54 mg, 75%) as yellow solid. Rf = 0.49 in EtOAc. 1H NMR (500 MHz, CDCl3) δ 8.31 – 8.18 (m, 1H), 7.75 (dd, J = 7.7, 1.5 Hz, 1H), 7.51 – 7.39 (m, 2H), 7.33 (d, J = 8.3 Hz, 1H), 7.16 (d, J = 8.3 Hz, 1H), 2.88 (t, J = 6.8 Hz,
2H), 2.84 – 2.69 (m, 2H), 2.60 – 2.49 (m, 2H), 2.48 – 2.33 (m, 2H), 2.07 – 1.93 (m, 2H), 1.90 (t, \(J = 6.8\) Hz, 2H), 1.84 – 1.58 (m, 7H), 1.53 – 1.37 (m, 2H), 1.35 – 1.10 (m, 5H), 1.05 – 0.78 (m, 2H).

1’-(3-phenylpropyl)-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidine] (2.5f): General procedure F was used to alkylate 2.5a (50 mg, 0.19 mmol) with (3-bromopropyl)benzene (39 mg, 0.19 mmol) using potassium carbonate (55 mg, 0.39 mmol) in DMF (2 mL). It was purified by flash chromatography (Hexanes:EtOAc = 5:1) to afford 2.5f (50 mg, 69%) as yellow solid. \(R_f = 0.48\) in EtOAc. \(\text{\(1^H\) NMR (500 MHz, CDCl}_3\)} \(\delta 8.31 – 8.18\) (m, 1H), 7.85 – 7.68 (m, 1H), 7.54 – 7.40 (m, 2H), 7.38 – 7.29 (m, 3H), 7.25 – 7.19 (m, 3H), 7.17 (d, \(J = 8.3\) Hz, 1H), 2.90 (t, \(J = 6.8\) Hz, 2H), 2.83 – 2.73 (m, 2H), 2.69 (t, \(J = 7.8\) Hz, 2H), 2.61 – 2.43 (m, 4H), 2.02 – 1.95 (m, 2H), 1.95 – 1.86 (m, 4H), 1.83 – 1.71 (m, 2H). \(\text{\(1^C\) NMR (126 MHz, CDCl}_3\)} \(\delta 147.95, 142.33, 133.42, 128.51, 128.43, 127.82, 127.53, 125.91, 125.86, 125.64, 125.19, 121.58, 119.25, 115.03, 73.06, 58.50, 49.60, 34.87, 34.03, 30.44, 29.09, 22.05.

1’-(3,4-difluorophenethyl)-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidine] (2.5h): General procedure F was used to alkylate 2.5a (130 mg, 0.51 mmol) with 4-(2-bromoethyl)-1,2-difluorobenzene (113 mg, 0.51 mmol) using potassium carbonate (142 mg, 1.0 mmol) in DMF (4 mL). It was purified by flash chromatography (DCM: MeOH = 30:1) to afford 2.5h (134 mg, 67%) as yellow solid. \(R_f (\text{DCM:MeOH} = 20:1) = 0.46\)

1-(3,4-difluorophenyl)-2-(3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidin]-1'-yl)ethanol (2.5i): General procedure D was used with 2.5a (200 mg, 0.79 mmol) and epoxide (123 mg, 0.79 mmol) in ethanol (12 mL). It was purified by flash chromatography (DCM: MeOH = 100:1) to afford 2.5i (194 mg, 60%) as white solid. \(R_f (\text{DCM:MeOH} = 10:1) = 0.52\)
2-(3,4-difluorophenyl)-1-(3,4-dihydrospiro[benzo[h]chromene-2,4′-piperidin]-1′-yl)ethanone (2.5j): General procedure H was used in the amidation reaction of 2.5a (100 mg, 0.39 mmol) and 2-(3,4-difluorophenyl)acetic acid (68 mg, 0.39 mmol) with EDCI (151 mg, 0.79 mmol) and DMAP (10 mg, 0.078 mmol) in DCM (15 mL). It was purified by flash chromatography (Hexanes:EtOAc = 2:1) to afford 2.5j (126 mg, 79%) as yellow solid. $R_f = 0.69$ in EtOAc.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.26 – 8.08 (m, 1H), 7.82 – 7.64 (m, 1H), 7.54 – 7.40 (m, 2H), 7.41 – 7.32 (m, 1H), 7.22 – 7.05 (m, 3H), 7.04 – 6.90 (m, 1H), 4.91 – 4.15 (m, 1H), 3.70 (s, 3H), 3.67 – 3.53 (m, 1H), 3.29 – 3.11 (m, 1H), 2.92 – 2.76 (m, 2H), 2.11 – 1.93 (m, 2H), 1.94 – 1.80 (m, 2H), 1.66 – 1.51 (m, 1H), 1.49 – 1.32 (m, 1H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.63, 150.31 (dd, $J = 246.2, 12.4$ Hz), 149.4 (dd, $J = 247.2, 13.1$ Hz), 147.41, 133.47, 132.15 (d $J = 6.2, 4.2$ Hz), 127.71, 127.69, 125.87, 125.69, 125.48, 124.78 (dd, $J = 6.1, 3.6$ Hz), 121.13, 119.87, 117.85 (d, $J = 17.2$ Hz) 117.30 (d, $J = 17.5$ Hz), 114.87, 72.97, 42.35, 39.97 (d, $J = 1-3$ Hz), 38.14, 35.00, 34.45, 32.19, 21.77.

tert-butyl 3,4,7,8,9,10-hexahydrospiro[benzo[h]chromene-2,4′-piperidine]-1′-carboxylate (2.17): General procedure E followed by general procedure G was used to convert 2.2 to 2.17 over 2 steps in 17% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 6.83 (d, $J = 7.8$ Hz, 1H), 6.61 (d, $J = 7.7$ Hz, 1H), 3.95 (s, 2H), 3.20 (s, 2H), 2.81 – 2.70 (m, 4H), 2.68 (t, $J = 6.3$ Hz, 2H), 1.88 – 1.69 (m, 8H), 1.54 (dd, $J = 13.0, 5.0$ Hz, 2H), 1.49 (s, 9H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 154.98, 150.51, 136.42, 126.18, 125.73, 120.61, 117.34, 79.46, 72.38, 39.54, 34.90, 32.35, 29.61, 28.58, 23.27, 23.04, 22.96, 21.40.

3,4,7,8,9,10-hexahydrospiro[benzo[h]chromene-2,4′-piperidine] (2.5m): General procedure B was used to deprotect 2.17 (200 mg, 0.56 mmol) in DCM (8 mL) and TFA (2.5 mL) to afford 2.5m (117 mg, 81%) as a yellow solid. $R_f$ (DCM:MeOH = 10:1) = 0.20. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 67.
6.82 (d, J = 7.8 Hz, 1H), 6.61 (d, J = 7.8 Hz, 1H), 4.20 (s, 2H), 3.12 (t, J = 12.2 Hz, 2H), 2.98 (d, J = 11.8 Hz, 2H), 2.87 – 2.58 (m, 6H), 2.10 – 1.70 (m, 7H), 1.70 – 1.55 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 150.50, 136.33, 126.13, 125.67, 120.50, 117.34, 72.25, 41.85, 35.00, 32.52, 29.58, 23.28, 23.01, 22.93, 21.24.

1'-ethyl-3,4,7,8,9,10-hexahydropyrobenzo[h]chromene-2,4'-piperidine] (2.5n): General procedure F was used to alkylate 2.5m (30 mg, 0.11 mmol) with bromoethane (12 mg, 0.11 mmol) using potassium carbonate (32 mg, 0.233 mmol) in DMF (1 mL). It was purified by flash chromatography (Hexanes:EtOAc = 2:1) to afford 2.5n (19 mg, 57%) as white solid. Rf = 0.44 in EtOAc.

¹H NMR (500 MHz, CDCl₃) δ 6.81 (d, J = 7.7 Hz, 1H), 6.59 (d, J = 7.7 Hz, 1H), 3.35 (s, 1H), 2.90 – 2.77 (m, 2H), 2.77 – 2.68 (m, 4H), 2.66 (t, J = 6.3 Hz, 2H), 2.53 (q, J = 7.2 Hz, 2H), 2.43 (t, J = 11.8 Hz, 2H), 1.97 – 1.82 (m, 2H), 1.82 – 1.64 (m, 7H), 1.15 (t, J = 7.2 Hz, 3H).

1'-(3,4-difluorophenethyl)-3,4,7,8,9,10-hexahydropyrobenzo[h]chromene-2,4'-piperidine] (2.5o): General procedure F was used to alkylate 2.5m (50 mg, 0.19 mmol) with 4-(2-bromoethyl)-1,2-difluorobenzene (43 mg, 0.19 mmol) using potassium carbonate (54 mg, 0.38 mmol) in DMF (2 mL). It was purified by flash chromatography (Hexanes:EtOAc = 10:1) to afford 2.5o (39 mg, 51%) as white solid. Rf = 0.44 in EtOAc.

¹H NMR (500 MHz, CDCl₃) δ 7.17 – 6.99 (m, 2H), 6.99 – 6.89 (m, 1H), 6.83 (d, J = 7.7 Hz, 1H), 6.60 (d, J = 7.7 Hz, 1H), 2.86 – 2.70 (m, 8H), 2.68 (t, J = 6.3 Hz, 2H), 2.66 – 2.60 (m, 2H), 2.54 – 2.40 (m, 2H), 1.95 – 1.83 (m, 2H), 1.83 – 1.72 (m, 6H), 1.72 – 1.63 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 150.72, 150.28 (dd, J = 246.4, 114 Hz), 149.00 (dd, J = 246.4, 114 Hz), 137.64 (dd, J = 5.7, 3.9 Hz), 136.34, 126.19, 125.75, 124.62 (dd, J = 6.0, 3.4 Hz), 120.43, 117.54 (d, J = 16.6 Hz), 117.53, 117.07 (d, J = 17.0 Hz), 72.13, 60.54, 49.52, 34.93, 33.18, 32.38, 29.67, 23.44, 23.10, 23.06, 21.62.
tert-butyl spiro[benzo[h]chromene-2,4'-piperidine]-1'-carboxylatecarboxylate (2.18): General procedure E was used to reduce 2.2 (1.4 g, 3.8 mmol) in absolute ethanol (30 mL) using sodium borohydride (432 mg, 11.4 mmol). To the above product was added PTSA.H₂O (36 mg, 5 mol %) and refluxed for overnight. The reaction was cooled down, quenched with sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with brine, dried with sodium sulfate and the solvent was removed under reduced pressure. It was purified by flash chromatography (H:EA = 45:1;) to afford 2.18 (800 mg, 61%) as white solid.

spiro[benzo[h]chromene-2,4'-piperidine] (2.5p): General procedure B was used to deprotect 2.18 (1.4 g, 4.0 mmol) in DCM (40 mL) and TFA (13 mL) to afford 2.5 (982 mg, 97%) as a brown solid. Rᵣ(DCM:MeOH = 10:1) = 0.25.

1'-ethylspiro[benzo[h]chromene-2,4'-piperidine] (2.5q): General procedure F was used to alkylate 2.5a (60 mg, 0.23 mmol) with bromoethane (25 mg, 0.23 mmol) using potassium carbonate (64 mg, 0.46 mmol) in DMF (2.5 mL) . It was purified by flash chromatography (DCM:MeOH = 30:1;) to afford 2.5q (45 mg, 68%) as brown oil. Rᵣ(DCM:MeOH = 10:1) = 0.28.

1'-{(3,4-difluorophenethyl)spiro[benzo[h]chromene-2,4'-piperidine] (2.5r): General procedure F was used to alkylate 2.5a (200 mg, 0.79 mmol) with 4-(2-bromoethyl)-1,2-difluorobenzene (176 mg, 0.79 mmol) using potassium carbonate (220 mg, 1.6 mmol) in DMF (5 mL). It was purified by flash chromatography (DCM: MeOH = 250:1) to afford 2.5r (157 mg, 51%) as yellow solid. Rᵣ(DCM:MeOH = 20:1) = 0.46

tert-butyl 4-oxospiro[chromane-2,4'-piperidine]-1'-carboxylate (2.16a): General procedure A was used in the reaction of 1-(2-hydroxyphenyl)ethan-1-one (1 g, 7.3 mmol), tert-butyl 4-oxopiperidine-1-carboxylate (1.5 g, 7.3 mmol) and pyrrolidine (1.42 g, 719.8 mmol) in absolute
methanol (10 mL). It was purified by flash chromatography (hexanes:EtOAc = 7:1) to afford \textbf{2.16a} (2.18 g, 97%) as a yellow solid. R_f (hexanes:EtOAc = 5:1) = 0.37. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.96 – 7.74 (m, 1H), 7.58 – 7.40 (m, 1H), 7.08 – 6.77 (m, 2H), 4.02 – 3.69 (m, 2H), 3.35 – 3.03 (m, 2H), 2.70 (s, 2H), 2.11 – 1.79 (m, 2H), 1.69 – 1.52 (m, 2H), 1.44 (s, 9H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 191.66, 159.06, 154.80, 136.46, 126.71, 121.31, 120.84, 118.37, 79.86, 77.90, 48.14, 39.21, 34.07, 28.51.

\textit{tert}-butyl 4-oxo-3,4-dihydrospiro[benzo[g]chromene-2,4'-piperidine]-1'-carboxylate (\textbf{2.16b}) was synthesized according to general procedure A in 96% yield. $^1$H NMR (399 MHz, CDCl$_3$) $\delta$ 8.48 (s, 1H), 7.87 (dd, $J$ = 8.3, 1.1 Hz, 1H), 7.70 (dd, $J$ = 8.4, 1.0 Hz, 1H), 7.64 – 7.47 (m, 1H), 7.45 – 7.29 (m, 2H), 3.89 (s, 2H), 3.26 (t, $J$ = 12.7 Hz, 2H), 2.81 (s, 2H), 2.02 (d, $J$ = 13.9 Hz, 2H), 1.83 – 1.54 (m, 2H), 1.46 (s, 9H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 192.46, 154.86, 154.23, 138.24, 130.18, 129.36, 128.87, 128.50, 126.65, 124.90, 121.71, 113.40, 79.88, 77.43, 49.05, 39.23, 34.42, 28.54.

\textit{tert}-butyl 1-oxo-1,2-dihydrospiro[benzo[f]chromene-3,4'-piperidine]-1'-carboxylate (\textbf{2.16c}) was synthesized according to general procedure A in 91% yield. $^1$H NMR (399 MHz, CDCl$_3$) $\delta$ 9.68 – 9.13 (m, 1H), 7.93 (d, $J$ = 9.0 Hz, 1H), 7.74 (dd, $J$ = 8.0, 1.4 Hz, 1H), 7.72 – 7.55 (m, 1H), 7.57 – 7.34 (m, 1H), 7.11 (d, $J$ = 9.0 Hz, 1H), 3.88 (s, 2H), 3.26 (t, $J$ = 12.7 Hz, 2H), 2.83 (s, 2H), 2.11 (d, $J$ = 13.8 Hz, 2H), 1.90 – 1.52 (m, 2H), 1.47 (s, 9H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 192.74, 161.14, 154.83, 137.79, 131.34, 129.82, 129.13, 128.45, 125.67, 124.90, 119.17, 112.20, 79.92, 78.16, 49.25, 39.51, 33.84, 28.54.

\textit{spiro}[chroman-2,4'-piperidin]-4-one (\textbf{2.2aa}): General procedure B was used to deprotect \textbf{2.16a} (2 g, 6.3 mmol) in DCM (40 mL) and TFA (13 mL) to afford \textbf{2.2aa} (1.32 g, 97%) as a brown solid.
$R_f$ (DCM:MeOH = 10:1) = 0.21 $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 – 7.72 (m, 1H), 7.50 – 7.37 (m, 1H), 7.03 – 6.87 (m, 2H), 3.37 – 2.90 (m, 2H), 2.92 – 2.74 (m, 2H), 2.68 (s, 3H), 2.17 – 1.88 (m, 3H), 1.80 (s, 1H), 1.72 – 1.41 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 192.10, 159.23, 136.25, 126.53, 120.95, 118.40, 78.40, 48.39, 41.80, 35.28.

$1'$-ethylspiro[chroman-2,4'-piperidin]-4-one (2.2ab): General procedure F was used to alkylate 2.2aa (500 mg, 2.3 mmol) with bromoethane (250 mg, 2.3 mmol) using potassium carbonate (636 mg, 4.60 mmol) in DMF (10 mL). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.2ab (468 mg, 83%) as yellow solid. $R_f$ (DCM:MeOH = 10:1) = 0.45 $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.99 – 7.70 (m, 1H), 7.62 – 7.34 (m, 1H), 7.11 – 6.37 (m, 2H), 2.69 (s, 4H), 2.55 – 2.29 (m, 4H), 2.21 – 1.95 (m, 2H), 1.89 – 1.64 (m, 2H), 1.07 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 192.14, 159.29, 136.25, 126.62, 120.99, 118.47, 77.93, 52.34, 48.44, 48.11, 34.37, 12.29.

$1'$-(3,4-difluorophenethyl)spiro[chroman-2,4'-piperidin]-4-one (2.2ac): General procedure F was used to alkylate 2.2aa (300 mg, 1.38 mmol) with 4-(2-bromoethyl)-1,2-difluorobenzene (305 mg, 1.38 mmol) using potassium carbonate (382 mg, 2.76 mmol) in DMF (8 mL). It was purified by flash chromatography (DCM:MeOH = 30:1) to afford 2.2ac (360 mg, 73%) as yellow solid. $R_f$ (DCM:MeOH = 20:1) = 0.33 $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.90 – 7.78 (m, 1H), 7.52 – 7.38 (m, 1H), 7.13 – 6.92 (m, 4H), 6.92 – 6.80 (m, 1H), 2.79 – 2.72 (m, 2H), 2.73 – 2.66 (m, 4H), 2.64 – 2.56 (m, 2H), 2.55 – 2.44 (m, 2H), 2.12 – 1.96 (m, 2H), 1.82 – 1.67 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 192.09, 159.27, 150.30 (dd, $J$ = 246.6, 12.7 Hz), 149.04 (dd, $J$ = 246.2, 12.7 Hz), 137.41 (dd, $J$ = 9.8, 5.2 Hz), 136.33, 126.69, 124.59 (dd, $J$, 5.8, 3.4 Hz), 121.10, 120.96, 118.48, 117.48 (d, $J$ = 16.8 Hz), 117.10 (d, $J$ = 16.9 Hz), 77.82, 60.02, 48.85, 48.12, 34.43, 33.09.
spiro[benzo[g]chromene-2,4'-piperidin]-4(3H)-one (2.2ba) was synthesized according to general procedure B in 96% yield.

1'-ethylspiro[benzo[g]chromene-2,4'-piperidin]-4(3H)-one (2.2bb) was synthesized according to general procedure F in 67% yield. $^{13}$C NMR (100 MHz, CDCl$_3$) δ 192.96, 154.43, 138.20, 130.13, 129.18, 128.70, 128.33, 126.56, 124.69, 121.84, 113.36, 77.42, 52.37, 49.01, 48.44, 34.66, 12.31.

1'-(3,4-difluorophenethyl)spiro[benzo[g]chromene-2,4'-piperidin]-4(3H)-one (2.2bc) was synthesized according to general procedure F in 71% yield. $^1$H NMR (399 MHz, CDCl$_3$) δ 8.47 (s, 1H), 7.87 (d, $J = 8.2$ Hz, 1H), 7.70 (d, $J = 8.3$ Hz, 1H), 7.50 (t, $J = 7.6$ Hz, 1H), 7.41 – 7.30 (m, 2H), 7.13 – 6.96 (m, 2H), 6.94 – 6.81 (m, 1H), 2.81 (s, 2H), 2.80 – 2.67 (m, 4H), 2.67 – 2.59 (m, 2H), 2.59 – 2.50 (m, 2H), 2.14 – 2.01 (m, 2H), 1.88 – 1.74 (m, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 192.87, 154.40, 150.28 (dd, $J = 247.8$, 13.2 Hz), 149.01 (dd, $J = 245.2$, 13.2 Hz) 138.23, 137.41 (dd, $J = 5.2$, 3.8 Hz), 130.16, 129.24, 128.76, 128.39, 126.59, 124.75, 124.58 (dd, $J = 6.0$, 3.5 Hz), 121.86, 117.48 (d, $J = 16.9$ Hz), 117.09 (d, $J = 16.9$ Hz), 113.39, 77.31, 60.06, 49.00, 48.83, 34.72, 33.08.

1'-((2-(3,4-difluorophenyl)-2-hydroxyethyl)spiro[benzo[g]chromene-2,4'-piperidin]-4(3H)-one (2.2bd) was synthesized according to general procedure D in 69% yield.

tert-butyl 1-oxo-1,2-dihydropir[benzo[f]chromene-3,4'-piperidine]-1'-carboxylate (2.16c) was synthesized according to general procedure A in 94% yield. $^1$H NMR (399 MHz, CDCl$_3$) δ 9.68 – 9.13 (m, 1H), 7.93 (d, $J = 9.0$ Hz, 1H), 7.74 (dd, $J = 8.0$, 1.4 Hz, 1H), 7.72 – 7.55 (m, 1H), 7.57 – 7.34 (m, 1H), 7.11 (d, $J = 9.0$ Hz, 1H), 3.88 (s, 2H), 3.26 (t, $J = 12.7$ Hz, 2H), 2.83 (s, 2H), 2.11 (d, $J = 13.8$ Hz, 2H), 1.90 – 1.52 (m, 2H), 1.47 (s, 9H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 192.74,
spiro[benzo[f]chromene-3,4'-piperidin]-1(2H)-one (2.2ca) was synthesized according to general procedure B in 97% yield.

1'-ethylspiro[benzo[f]chromene-3,4'-piperidin]-1(2H)-one (2.2cb) was synthesized according to general procedure F in 61% yield.

1'-(3,4-difluorophenethyl)spiro[benzo[f]chromene-3,4'-piperidin]-1(2H)-one (2.2cc) was synthesized according to general procedure F in 69% yield. $^{13}$C NMR (100 MHz, CDCl$_3$) δ 150.65, 150.32 (dd, $J = 247.6$, 12.4 Hz), 149.08 (dd, $J = 246.53$, 12.4 Hz), 137.28 (dd, $J = 4.8$, 2.9 Hz), 132.84, 130.55, 129.43, 128.74, 127.20, 124.65 (dd, $J = 6.2$, 3.6 Hz), 123.65, 122.70, 119.66, 117.55 (d, $J = 16.9$ Hz), 117.15 (d, $J = 16.9$ Hz), 114.81, 72.65, 61.16, 60.18, 49.31, 49.16, 41.37, 36.41, 33.50, 32.85.

1'-(2-(3,4-difluorophenyl)-2-hydroxyethyl)spiro[benzo[f]chromene-3,4'-piperidin]-1(2H)-one (2.2cd) was synthesized according to the general procedure D in 60% yield.

1'-(3,4-difluorophenethyl)spiro[chroman-2,4'-piperidin]-4-ol (2.3ac): General procedure E was used to reduce 2.2ac (160 mg, 0.44 mmol) in absolute ethanol (4 mL) using sodium borohydride (51 mg, 1.34 mmol). It was purified by flash chromatography (DCM:MeOH = 40:1) to afford 2.3ac (148 mg, 92%) as white solid. $R_f$ (DCM:MeOH = 10:1) = 0.6 $^1$H NMR (500 MHz, CDCl$_3$) δ 7.43 (d, $J = 7.6$ Hz, 1H), 7.18 (t, $J = 7.9$ Hz, 1H), 7.12 – 6.96 (m, 2H), 6.96 – 6.86 (m, 2H), 6.84 (d, $J = 8.2$ Hz, 1H), 4.98 – 4.71 (m, 1H), 2.84 – 2.62 (m, 5H), 2.63 – 2.49 (m, 3H), 2.49 – 2.33 (m, 1H), 2.17 – 2.05 (m, 1H), 2.03 – 1.92 (m, 1H), 1.90 – 1.73 (m, 3H), 1.73 – 1.57 (m, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 152.59, 151.16 (dd, $J = 247.8$, 13.4 Hz), 148.96 (dd, $J = 247.8$, 13.4 Hz), 148.96 (dd, $J = 247.8$, 13.4 Hz), 148.96 (dd, $J = 247.8$, 13.4 Hz), 148.96 (dd, $J = 247.8$, 13.4 Hz), 148.96 (dd, $J = 247.8$, 13.4 Hz).
247.83, 13.46 Hz), 137.42 (dd, J = 5.6, 3.8 Hz), 129.31, 128.03, 125.05, 124.55 (dd, J = 6.0, 3.7 Hz), 120.62, 117.46 (d, J = 16.7 Hz), 117.36, 117.05 (d, J = 17.0 Hz), 73.61, 62.94, 60.19, 48.96, 48.94, 42.13, 36.28, 33.66, 32.85.

1'-ethyl-3,4-dihydrospiro[benzo[g]chromene-2,4'-piperidin]-4-ol (2.3bb) was synthesized according to the general procedure E in 70% yield. \(^1\)H NMR (399 MHz, CDCl\(_3\)) \(\delta\) 7.94 (s, 1H), 7.72 (d, \(J = 8.2\) Hz, 1H), 7.65 (d, \(J = 8.2\) Hz, 1H), 7.48 – 7.32 (m, 1H), 7.33 – 7.24 (m, 1H), 7.21 (s, 1H), 5.14 – 4.74 (m, 1H), 2.85 – 2.58 (m, 2H), 2.58 – 2.42 (m, 3H), 2.43 – 2.27 (m, 1H), 2.20 – 2.02 (m, 1H), 2.00 – 1.87 (m, 1H), 1.88 – 1.74 (m, 3H), 1.73 – 1.58 (m, 1H), 1.11 (t, \(J = 7.2\) Hz, 3H). \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 151.06, 134.46, 128.79, 127.80, 127.73, 127.39, 126.35, 126.24, 123.65, 111.92, 74.00, 63.10, 52.44, 48.49, 42.37, 36.61, 33.48, 29.80, 12.01.

1'-(3,4-difluorophenethyl)-3,4-dihydrospiro[benzo[g]chromene-2,4'-piperidin]-4-ol (2.3bc) was synthesized according to general procedure E in 73% yield. \(^1\)H NMR (399 MHz, CDCl\(_3\)) \(\delta\) 7.97 (s, 1H), 7.75 (d, \(J = 8.2\) Hz, 1H), 7.67 (d, \(J = 8.2\) Hz, 1H), 7.50 – 7.35 (m, 1H), 7.30 (t, \(J = 7.5\) Hz, 1H), 7.26 (d, \(J = 3.5\) Hz, 1H), 7.15 – 6.98 (m, 2H), 6.96 – 6.84 (m, 1H), 5.05 (dd, \(J = 8.9, 6.1\) Hz, 1H), 2.87 – 2.68 (m, 4H), 2.69 – 2.58 (m, 3H), 2.57 – 2.44 (m, 1H), 2.28 – 2.16 (m, 1H), 2.07 – 1.97 (m, 1H), 1.97 – 1.82 (m, 3H), 1.82 – 1.70 (m, 1H). \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 150.96, 150.32 (dd, \(J = 247.1, 13.5\) Hz), 149.08 (dd, \(J = 245.5, 13.5\) Hz), 137.28 (dd, \(J = 4.8, 2.9\) Hz), 134.58, 128.88, 127.85, 127.48, 127.45, 126.55, 126.32, 124.63 (dd, \(J = 6.1, 3.2\) Hz), 123.83, 117.53 (d, \(J = 16.6\) Hz), 117.15 (d, \(J = 16.8\) Hz), 112.11, 73.90, 63.57, 60.18, 49.01, 42.36, 36.63, 33.91, 32.87.

1'-(2-(3,4-difluorophenyl)-2-hydroxyethyl)-3,4-dihydrospiro[benzo[g]chromene-2,4'-piperidin]-4-ol (2.3bd) was synthesized according to general procedure E in 69% yield. \(^1\)H NMR
(399 MHz, CDCl$_3$) $\delta$ 7.96 (s, 1H), 7.75 (d, $J = 8.3$ Hz, 1H), 7.67 (d, $J = 8.3$ Hz, 1H), 7.43 – 7.34 (m, 1H), 7.35 – 7.27 (m, 1H), 7.27 – 7.19 (m, 2H), 7.17 – 7.02 (m, 2H), 5.21 – 5.00 (m, 1H), 4.84 – 4.55 (m, 1H), 3.01 – 2.71 (m, 2H), 2.74 – 2.52 (m, 3H), 2.51 – 2.38 (m, 1H), 2.26 – 2.17 (m, 1H), 2.15 – 1.93 (m, 2H), 1.93 – 1.79 (m, 2H), 1.79 – 1.64 (m, 1H).

1'-ethyl-1,2-dihydrospiro[benzo[f]chromene-3,4'-piperidin]-1-ol (2.3cb) was synthesized according to general procedure E in 76% yield. $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 150.51, 132.94, 130.34, 129.38, 128.56, 127.01, 123.54, 123.02, 119.53, 114.94, 72.49, 60.76, 52.43, 48.75, 48.50, 41.47, 35.88, 33.04, 11.73.

1'-(3,4-difluorophenethyl)-1,2-dihydrospiro[benzo[f]chromene-3,4'-piperidin]-1-ol (2.3cc) was synthesized according to the general procedure E in 71% yield.

1'-(2-(3,4-difluorophenyl)-2-hydroxyethyl)-1,2-dihydrospiro[benzo[f]chromene-3,4'-piperidin]-1-ol (2.3cd) was synthesized according to the general procedure E in 73 % yield. $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 150.58, 150.54 (dd, $J = 247.9$, 12.7 Hz), 149.75 (dd, $J = 247.1$, 12.7 Hz), 139.48 (dd, $J = 4.8$, 2.8 Hz), 132.81, 132.79, 130.55, 129.41, 128.74, 127.20, 123.66, 122.65, 122.63, 121.77 (dd, $J = 6.6$, 3.5 Hz), 119.61, 117.14 (dd, $J = 17.6$ Hz), 114.92 (d, $J = 17.6$ Hz), 114.72, 72.52, 72.50, 67.90, 66.15, 66.06, 61.07, 61.06, 50.65, 50.42, 47.88, 47.69, 41.25, 36.81, 36.57, 33.79, 33.56.

(R)-spiro[benzo[h]chromene-2,3'-piperidin]-4(3H)-one (2.10a) was prepared with similar procedure to 2.10d. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.74 – 8.61 (m, 1H), 7.95 – 7.85 (m, 1H), 7.77 – 7.64 (m, 2H), 7.64 – 7.55 (m, 1H), 7.54 – 7.44 (m, 1H), 3.32 – 3.23 (m, 1H), 3.09 – 2.94 (m, 3H), 2.84 – 2.73 (m, 1H), 2.74 – 2.64 (m, 1H), 2.12 – 1.98 (m, 1H), 1.80 – 1.64 (m, 2H), 1.56
– 1.45 (m, 1H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 190.56, 156.52, 137.17, 129.82, 127.78, 126.50, 124.80, 124.05, 120.86, 120.61, 114.53, 78.28, 51.13, 44.24, 43.84, 30.38, 20.60.

**(R)-1'-ethylspiro[benzo[h]chromene-2,3'-piperidin]-4(3H)-one (2.10b):** General procedure F was used to alkylate 2.10a (135 mg, 0.51 mmol) with bromoethane (55 mg, 0.51 mmol) using potassium carbonate (140 mg, 1.0 mmol) in DMF (4 mL). It was purified by flash chromatography (DCM:MeOH = 100:1) to afford 2.10b (113 mg, 75%) as yellow solid. $R_f$ (DCM:MeOH = 10:1) = 0.6 $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.44 – 8.28 (m, 1H), 7.83 (d, $J$ = 8.7 Hz, 1H), 7.79 – 7.71 (m, 1H), 7.66 – 7.55 (m, 1H), 7.56 – 7.45 (m, 1H), 7.44 – 7.31 (m, 1H), 3.05 (d, $J$ = 16.8 Hz, 1H), 2.86 (d, $J$ = 16.7 Hz, 1H), 2.77 – 2.61 (m, 2H), 2.58 – 2.49 (m, 1H), 2.48 – 2.33 (m, 3H), 2.09 – 1.91 (m, 1H), 1.91 – 1.77 (m, 2H), 1.73 – 1.58 (m, 1H), 1.00 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 191.76, 157.85, 137.79, 129.53, 127.85, 126.12, 125.48, 123.88, 121.58, 120.45, 114.98, 80.04, 60.18, 53.17, 52.16, 44.94, 33.91, 22.12, 12.14.

**(R)-1'-(3,4-difluorophenethyl)spiro[benzo[h]chromene-2,3'-piperidin]-4(3H)-one (2.10c):** General procedure F was used to alkylate 2.10a (150 mg, 0.6 mmol) with 4-(2-bromoethyl)-1,2-difluorobenzene (124 mg, 0.6 mmol) using potassium carbonate (166 mg, 1.2 mmol) in DMF (5 mL). It was purified by flash chromatography (DCM:MeOH = 200:1) to afford 2.10c (169 mg, 69%) as yellow solid. $R_f$ (DCM:MeOH = 20:1) = 0.70 $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.44 – 8.22 (m, 1H), 7.83 (d, $J$ = 8.7 Hz, 1H), 7.80 – 7.71 (m, 1H), 7.67 – 7.58 (m, 1H), 7.57 – 7.50 (m, 1H), 7.42 – 7.33 (m, 1H), 7.07 – 6.94 (m, 1H), 6.95 – 6.86 (m, 1H), 6.84 – 6.72 (m, 1H), 3.00 – 2.93 (m, 1H), 2.83 (t, $J$ = 15.3 Hz, 2H), 2.71 – 2.53 (m, 6H), 2.51 – 2.33 (m, 1H), 2.14 – 1.99 (m, 1H), 2.00 – 1.85 (m, 1H), 1.83 – 1.67 (m, 1H), 1.71 – 1.51 (m, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 191.44, 157.64, 150.03 (dd, $J$ = 247.6, 12.6 Hz), 148.82 (dd, $J$ = 246.3, 14.1 Hz), 137.72, 137.3 (dd, $J$ = 8.5, 5.2 Hz), 129.58, 127.78, 126.17, 125.32, 124.52 (dd, $J$ = 6.1, 3.7 Hz), 123.73, 121.39,
120.50, 117.33 (dd, J = 17.2 Hz), 116.74 (dd, J = 17.2 Hz), 114.79, 79.63, 60.01, 59.14, 53.52,
45.11, 33.59, 32.37, 21.80.

(S)-spiro[benzo[h]chromene-2,3'-piperidin]-4(3H)-one (2.10d): A solution of 2.9 (300 mg, 0.6
mmol) in a 1:1 mixture of ethanol and hydrochloric acid (10 mL) was refluxed overnight. The
organic solvent was removed under reduced pressure, neutralized with 10% NaOH and then
extracted 3X with DCM. The organic layer was combined, dried with sodium sulfate and
concentrated under reduced pressure. It was purified by flash chromatography (DCM:MeOH=
30:1) to afford 2.10d (160 mg, 89%) as a yellow solid. Rf(DCM:MeOH = 10:1) = 0.6

13C NMR (126 MHz, DMSO- d6) δ 190.13, 156.25, 137.18, 129.89, 127.74, 126.50, 124.72, 124.35, 120.87,
120.80, 114.54, 77.92, 49.87, 44.09, 42.98, 29.17, 19.29.

(S)-1'-ethylspiro[benzo[h]chromene-2,3'-piperidin]-4(3H)-one (2.10e): General procedure F
was used to alkylate 2.10d (180 mg, 0.67 mmol) with bromoethane (73 mg, 0.67 mmol) using
potassium carbonate (186 mg, 1.3 mmol) in DMF (4 mL). It was purified by flash chromatography
(DCM:MeOH = 100:1) to afford 2.10e (146 mg, 73%) as yellow solid. Rf(DCM:MeOH = 10:1) =
0.6

1H NMR (400 MHz CDCl3) δ 8.39 – 8.28 (m, 1H), 7.83 (d, J = 8.7 Hz, 1H), 7.78 – 7.71 (m,
1H), 7.65 – 7.56 (m, 1H), 7.55 – 7.46 (m, 1H), 7.39 – 7.28 (m, 1H), 3.05 (d, J = 16.8 Hz, 1H), 2.86
(d, J = 16.7 Hz, 1H), 2.76 – 2.61 (m, 2H), 2.57 – 2.46 (m, 1H), 2.46 – 2.27 (m, 3H), 2.02 – 1.91
(m, 1H), 1.90 – 1.76 (m, 2H), 1.74 – 1.56 (m, 1H), 0.99 (t, J = 7.1 Hz, 3H).

13C NMR (101 MHz,
CDCl3) δ 191.74, 157.84, 137.84, 129.52, 127.84, 126.12, 125.47, 123.87, 121.57, 120.44, 114.97,
80.03, 60.17, 53.16, 52.15, 44.93, 33.91, 22.12, 12.14.

(S)-1'-(3,4-difluorophenethyl)spiro[benzo[h]chromene-2,3'-piperidin]-4(3H)-one (2.10f):
General procedure F was used to alkylate 2.10d (150 mg, 0.6 mmol) with 4-(2-bromoethyl)-1,2-
difluorobenzene (124 mg, 0.6 mmol) using potassium carbonate (166 mg, 1.2 mmol) in DMF (5 mL). It was purified by flash chromatography (DCM: MeOH = 200: 1) to afford $2.10f$ (169 mg, 69%) as yellow solid. $R_{f}$ (DCM:MeOH = 20:1) = 0.70 $^1H$ NMR (500 MHz, CDCl$_3$) $\delta$ 8.32 (s, 1H), 7.83 (d, $J = 8.6$ Hz, 1H), 7.78 (d, $J = 8.1$ Hz, 1H), 7.68 – 7.59 (m, 1H), 7.57 – 7.48 (m, 1H), 7.37 (d, $J = 8.6$ Hz, 1H), 7.02 – 6.95 (m, 1H), 6.95 – 6.86 (m, 1H), 6.84 – 6.71 (m, 1H), 3.02 – 2.93 (m, 1H), 2.92 – 2.76 (m, 2H), 2.69 – 2.53 (m, 6H), 2.52 – 2.39 (m, 1H), 2.17 – 1.99 (m, 1H), 2.00 – 1.88 (m, 1H), 1.83 – 1.72 (m, 1H), 1.71 – 1.58 (m, 1H). $^{13}C$ NMR (126 MHz, CDCl$_3$) $\delta$ 191.59, 157.76, 150.02 (dd, $J = 246.8, 13.3$ Hz), 148.76 (dd, $J = 246.8, 13.3$ Hz), 137.81, 137.38 (dd, $J = 5.7, 3.8$ Hz), 129.66, 127.88, 126.23, 125.43, 124.51 (dd, $J = 6.1, 3.1$ Hz), 123.81, 121.50, 120.56, 117.33 d, $J = 16.9$Hz), 116.72 (d, $J = 16.81$ Hz), 114.89, 79.76, 60.18, 59.25, 53.64, 45.21, 33.73, 32.52, 21.97.

$(2R)$-1'-ethyl-3,4-dihydrospiro[benzo[h]chromene-2,3'-piperidin]-4-ol ($2.11a$): General procedure E was used to reduce $2.10b$ (90 mg, 0.31 mmol) in absolute ethanol (3 mL) using sodium borohydride (35 mg, 0.91 mmol). It was purified by flash chromatography (DCM:MeOH = 20:1) to afford $2.11a$ (63 mg, 69%) as white solid. $R_{f}$ (DCM:MeOH = 10:1) = 0.39 $^1H$ NMR (400 MHz, CDCl$_3$) $\delta$ 8.36 – 8.17 (m, 1H), 7.90 – 7.66 (m, 1H), 7.55 – 7.49 (m, 1H), 7.50 – 7.43 (m, 2H), 7.43 – 7.38 (m, 1H), 5.41 – 4.63 (m, 1H), 2.73 (d, $J = 11.3$ Hz, 1H), 2.70 – 2.57 (m, 1H), 2.58 – 2.17 (m, 5H), 2.03 – 1.66 (m, 4H), 1.26 (s, 3H), 1.17 – 0.96 (m, 3H). $^{13}C$ NMR (101 MHz, CDCl$_3$) $\delta$ 148.16, 148.02, 134.32, 134.25, 127.86, 127.47, 127.46, 126.46, 126.41, 125.93, 125.43, 125.34, 125.32, 125.25, 122.49, 119.95, 119.83, 118.13, 117.82, 114.02, 75.96, 75.31, 63.27, 62.97, 60.87, 59.69, 53.88, 53.60, 52.45, 52.35, 38.35, 35.69, 34.26, 29.81, 22.41, 21.97, 12.03, 11.72.

$(2R)$-1'-(3,4-difluorophenethyl)-3,4-dihydrospiro[benzo[h]chromene-2,3'-piperidin]-4-ol ($2.11b$): General procedure E was used to reduce $2.10c$ (98 mg, 0.24 mmol) in absolute ethanol (3
mL) using sodium borohydride (27 mg, 0.72 mmol). It was purified by flash chromatography (DCM:MeOH = 75:1) to afford 2.11b (69 mg, 70%) as white solid. Rf (DCM:MeOH = 10:1) = 0.58

(2S)-1'-ethyl-3,4-dihydropino[benzo[h]chromene-2,3'-piperidin]-4-ol (2.11c): General procedure E was used to reduce 2.10e (90 mg, 0.31 mmol) in absolute ethanol (3 mL) using sodium borohydride (35 mg, 0.91 mmol). It was purified by flash chromatography (DCM:MeOH = 20:1) to afford 2.11c (63 mg, 69%) as white solid. Rf (DCM:MeOH = 10:1) = 0.39

(2S)-1'-(3,4-difluorophenethyl)-3,4-dihydropino[benzo[h]chromene-2,3'-piperidin]-4-ol (2.11d): General procedure E was used to reduce 2.10f (98 mg, 0.24 mmol) in absolute ethanol (3 mL) using sodium borohydride (27 mg, 0.72 mmol). It was purified by flash chromatography (DCM:MeOH = 75:1) to afford 2.11d (69 mg, 70%) as white solid. Rf (DCM:MeOH = 10:1) = 0.58

1H NMR (500 MHz, CDCl3) δ 8.38 – 8.15 (m, 1H), 7.87 – 7.68 (m, 1H), 7.59 – 7.44 (m, 3H), 7.40 (d, J = 8.6 Hz, 1H), 7.12 – 6.87 (m, 2H), 6.88 – 6.71 (m, 1H), 5.03 – 4.67 (m, 1H), 2.84 – 2.61 (m, 3H), 2.62 – 2.36 (m, 6H), 2.31 – 2.09 (m, 1H), 2.00 – 1.86 (m, 2H), 1.87 – 1.74 (m, 1H), 1.74 – 1.49 (m, 1H). 13C NMR (126 MHz, CDCl3) δ 150.115 (ddd, J = 247.3, 13.7, 1.78 Hz), 148.84 (dd, J = 246.2, 13.0 Hz), 148.08, 148.06, 137.73 (dd, J = 5.4, 3.8 Hz), 137.56 (dd, J = 5.7, 3.9 Hz), 134.33, 134.31, 127.48, 126.60, 125.65, 125.47, 125.45, 125.40, 125.26, 124.68 (dd, J = 6.2, 3.5 Hz), 124.62 (dd, J = 6.1, 3.5 Hz), 122.54, 122.52, 119.97, 117.67 (dd, J = 22.1 Hz), 117.54 (d, J = 16.5 Hz), 117.43 (d, J = 16.7 Hz), 116.88 (d, J = 1.6 Hz), 116.75 (d, J = 1.6 Hz), 75.63, 75.29, 63.40, 63.25, 61.44, 60.27, 59.59, 59.56, 53.96, 53.90, 38.95, 38.87, 35.17, 33.94, 32.58, 32.34, 22.23, 22.01.
1'-<(3,4-difluorophenethyl)spiro[chroman-2,4'-piperidine] (2.22b): General procedure F was used to alkylate 2.22a (170 mg, 0.83 mmol) with 4-(2-bromoethyl)-1,2-difluorobenzene (184 mg, 0.836 mmol) using potassium carbonate (231 mg, 1.67 mmol) in DMF (5 mL). It was purified by flash chromatography (Hexanes:EtOAc = 3:1;) to afford 2.22b (221 mg, 77%) as white solid. \( R_f = 0.32 \) in EtOAc. \(^1\text{H NMR} (500 \text{ MHz, CDCl}_3) \delta 7.18 – 6.98 (m, 4H), 6.98 – 6.89 (m, 1H), 6.88 – 6.80 (m, 2H), 2.84 – 2.76 (m, 4H), 2.76 – 2.68 (m, 2H), 2.68 – 2.59 (m, 2H), 2.58 – 2.45 (m, 2H), 1.94 – 1.85 (m, 2H), 1.82 (t, \( J = 6.9 \text{ Hz, 2H} \)), 1.74 – 1.62 (m, 2H). \(^{13}\text{C NMR} (126 \text{ MHz, CDCl}_3) \delta 153.39, 150.23 (dd, \( J = 247.7, 13.3 \text{ Hz} \)), 148.95 (dd, \( J = 245.6, 13.3 \text{ Hz} \)), 137.62 (dd, \( J = 5.5, 3.8 \text{ Hz} \)), 129.60, 127.33, 124.57 (dd, \( J = 6.1, 3.8 \text{ Hz} \)), 121.50, 119.95, 117.48 (d, \( J = 16.5 \text{ Hz} \)), 117.42, 117.05 (d, \( J = 17.1 \text{ Hz} \)), 72.35, 60.30, 49.18, 34.74, 33.13, 32.10, 21.65.

spiro[chroman-2,4'-piperidine] (2.22a): General procedure B was used to deprotect 2.21a (500 mg, 1.65 mmol) in DCM (10 mL) and TFA (3 mL) to afford 2.22a (325 mg, 97%) as a yellow solid. \( R_f (\text{DCM.MeOH}=10:1) = 0.21 \).

(S)-2-bromo-1-(3,4-difluorophenyl)ethan-1-ol (2.13) A solution of 12 (1 g, 4.2 mmol) in anhydrous tetrahydrofuran (15 mL) was added to a solution of (-)-DIP-chloride\(^\text{TM} \) (1.5 g, 4.7 mmol) in anhydrous tetrahydrofuran (9 mL) at -25 °C under nitrogen. The resulting solution was stirred at -25 °C for 60 h, and then warmed to 0 °C, and diethanolamine (894 mg g, 8.5 mmol) was added dropwise. The mixture was warmed to room temperature and stirred for further 2 h, whereupon the boranes precipitated as a complex which was filtered and washed with pentane. The combined solvents were removed under pressure, and the residue was purified by flash chromatography (hexanes:EtOAc = 4 :1) to afford 2.13 (755 mg, 75%) as a colorless oil. \(^1\text{H NMR} (399 \text{ MHz, CDCl}_3) \delta 7.30 – 7.20 (m, 1H), 7.20 – 7.12 (m, 1H), 7.12 – 7.06 (m, 1H), 5.24 – 4.61 (m, 1H), 3.89 – 3.52 (m, 1H), 3.55 – 3.29 (m, 1H), 2.85 – 2.45 (m, 1H). \(^{13}\text{C NMR} (100 \text{ MHz,}
CDCl₃ δ 150.59 (dddd, J = 249.1, 39.3, 26.7, 13.1 Hz), 150.21 (dddd, J = 149.1, 39.3, 26.7, 13.1 Hz), 137.38 (dd, J = 4.9, 3.9 Hz), 122.18 (dd, J = 10.5, 3.8 Hz), 117.58 (dd, J = 17.8 Hz), 115.26 (dd, J = 18.3 Hz), 72.69, 39.84.

(S)-2-(3,4-difluorophenyl)oxirane (2.14) A 15% solution of NaOH in water (12 mL) was added to a solution of 13 (500 mg, 2.1 mmol) in ethanol (12 mL). The mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated, treated with water, and extracted with ethyl acetate. The organic layer was washed with brine and water, and dried. The filtrate was evaporated under reduced pressure and the residue was purified by flash chromatography (hexanes:EtOAc = 10:1) to afford 2.14 (160 mg, 49 %) as a colorless oil.

(R)-1’-(2-(3,4-difluorophenyl)-2-hydroxyethyl)spiro[benzo[h]chromene-2,4’-piperidin]-4(3H)-one (2.2m) was synthesized according to the general procedure D in 63 % yield. ¹H NMR (399 MHz, CDCl₃) δ 8.34 (d, J = 8.2 Hz, 1H), 7.85 (d, J = 8.7 Hz, 1H), 7.80 (d, J = 8.1 Hz, 1H), 7.69 – 7.59 (m, 1H), 7.59 – 7.44 (m, 1H), 7.40 (d, J = 8.7 Hz, 1H), 7.29 – 7.18 (m, 1H), 7.17 – 6.94 (m, 2H), 4.75 – 4.58 (m, 1H), 3.06 – 2.93 (m, 1H), 2.94 – 2.79 (m, 3H), 2.73 – 2.57 (m, 3H), 2.53 – 2.39 (m, 1H), 2.34 – 2.17 (m, 2H), 1.98 – 1.76 (m, 2H), ¹³C NMR (100 MHz, CDCl₃) δ 191.38, 157.12, 150.54 (dd, J = 248.2, 13.0 Hz), 149.76 (dd, J = 247.9, 13.0 Hz), 139.31 (dd, J = 4.7, 3.9 Hz), 137.87, 129.69, 128.13, 126.40, 125.42, 123.28, 121.75 (dd, J = 6.3, 3.8 Hz), 121.57, 120.86, 117.16 (d, J = 19.1 Hz), 115.19, 114.90 (d, J = 19.1 Hz), 78.74, 68.03, 66.14, 50.57, 47.80, 47.65, 34.70, 34.51.

(R)-1’-((R)-2-(3,4-difluorophenyl)-2-hydroxyethyl)-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidin]-4-ol (2.3ma) To a stirred solution of 2.2ma in trimethylamine (120mg, 1.1mmol) at 0°C was added formic acid (54 mg, 1.1mmol) slowly under argon. After the white smoke
subsided, RuCl[(R,R)-TsDPEN](mesitylene) (0.4mg, 0.6µmol) was added. The resulting mixture was stirred at room temperature for 24 h. The reaction was quenched by adding water and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography (DCM:MeOH = 20:1) to afford **2.3ma** (33mg, 66 %) as a white solid. $^1$H NMR (399 MHz, CDCl$_3$) δ 8.27 – 8.19 (m, 1H), 7.86 – 7.67 (m, 1H), 7.60 – 7.45 (m, 3H), 7.42 (d, $J$ = 8.5 Hz, 1H), 7.30 – 7.18 (m, 1H), 7.17 – 7.08 (m, 1H), 7.08 – 7.01 (m, 1H), 4.96 (t, $J$ = 6.6 Hz, 1H), 4.82 – 4.56 (m, 1H), 3.16 – 2.77 (m, 2H), 2.71 – 2.58 (m, 2H), 2.58 – 2.52 (m, 1H), 2.49 – 2.37 (m, 1H), 2.28 – 2.16 (m, 1H), 2.16 – 2.07 (m, 1H), 2.07 – 1.92 (m, 2H), 1.92 – 1.80 (m, 1H), 1.78 – 1.66 (m, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 150.55 (dd, $J$ = 247.7, 13.4 Hz), 149.75 (247.1, 13.4 Hz), 147.50, 139.45 (dd, $J$ = 5.3, 3.5 Hz), 134.38, 127.70, 126.69, 125.64, 125.50, 125.44, 122.14, 121.76 dd, $J$ = 6.0, 3.4 Hz), 120.27, 117.93, 117.15 (dd, $J$ = 17.3 Hz), 114.91 (d, $J$ = 17.8 Hz), 73.95, 67.94, 66.26, 63.46, 50.94, 47.71, 42.06, 36.17, 34.32.

**(S)-1'-(R)-2-(3,4-difluorophenyl)-2-hydroxyethyl)-3,4-dihydrospiro[benzo[h]chromene-2,4'-piperidin]-4-ol (2.3mc)** $^1$H NMR (399 MHz, CDCl$_3$) δ 8.48 – 8.10 (m, 1H), 7.99 – 7.67 (m, 1H), 7.56 – 7.45 (m, 3H), 7.43 (d, $J$ = 8.5 Hz, 1H), 7.30 – 7.19 (m, 1H), 7.18 – 7.02 (m, 2H), 4.99 (t, $J$ = 6.5 Hz, 1H), 4.85 – 4.58 (m, 1H), 2.98 (d, $J$ = 11.0 Hz, 1H), 2.92 – 2.78 (m, 1H), 2.74 – 2.56 (m, 3H), 2.50 – 2.32 (m, 1H), 2.32 – 2.13 (m, 2H), 2.08 – 1.90 (m, 2H), 1.91 – 1.76 (m, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 150.58 (dd, $J$ = 248.5, 13.9 Hz), 149.78 (dd, $J$ = 247.7, 12.9 Hz), 147.51, 139.45 (dd, $J$ = 5.2, 3.9 Hz), 134.42, 127.72, 126.70, 125.66, 125.51, 125.45, 122.14, 121.78 (dd, $J$ = 6.3, 3.5 Hz), 120.33, 117.92, 117.18 (d, $J$ = 17.4 Hz), 114.94 (d, $J$ = 18.2 Hz), 73.92, 67.96, 66.30, 63.50, 50.83, 47.94, 42.10, 35.85, 34.66.
(S)-1’-((S)-2-(3,4-difluorophenyl)-2-hydroxyethyl)-3,4-dihydrospiro[benzo[h]chromene-2,4’-piperidin]-4-ol (3md) \( ^1H \) NMR (399 MHz, CDCl\(_3\)) \( \delta \) 8.34 – 8.12 (m, 1H), 7.85 – 7.63 (m, 1H), 7.55 – 7.45 (m, 3H), 7.43 (d, \( J = 8.5 \) Hz, 1H), 7.28 – 7.19 (m, 1H), 7.17 – 7.10 (m, 1H), 7.09 – 7.03 (m, 1H), 4.99 (t, \( J = 6.6 \) Hz, 1H), 4.81 – 4.59 (m, 1H), 3.01 – 2.83 (m, 2H), 2.75 – 2.52 (m, 3H), 2.51 – 2.38 (m, 1H), 2.33 – 2.19 (m, 1H), 2.20 – 2.11 (m, 1H), 2.08 – 1.95 (m, 2H), 1.95 – 1.84 (m, 1H), 1.82 – 1.69 (m, 1H). \( ^13C \) NMR (100 MHz, CDCl\(_3\)) \( \delta \) 150.57 (dd, \( J = 247.4, 12.7 \) Hz), 149.27 (dd, \( J = 247.4, 12.7 \) Hz), 147.52, 139.46 (dd, \( J = 5.1, 3.7 \) Hz), 134.41, 127.71, 126.70, 125.66, 125.52, 125.43, 122.15, 121.77 (dd, \( J = 6.4, 3.7 \) Hz), 120.30, 117.92, 117.16 (d, \( J = 17.4 \) Hz), 114.92 (d, \( J = 17.8 \) Hz), 73.96, 67.95, 66.27, 63.51, 50.96, 47.74, 42.09, 36.17, 34.40.

2.10 References


Chapter 3. Asymmetric dihydropyridines as antimalarials

3.1 Introduction

The efforts to discover and develop new antimalarial drugs have been limited by inaccessibility to antimalarial chemotypes with novel modes of action.\(^1\) With an aim of identifying antimalarial having new mechanism of action, the laboratory of Kiplin Guy at St. Jude Children’s Research Hospital using a chemical genetic approach, screened 309,474 compounds against the 3D7 strain of Plasmodium falciparum. Interestingly, a class of compounds comprised of asymmetric dihydropyridines (DHPs) was discovered, which was synergizing with mefloquine.\(^2,3\) The promising effects of the DHPs led to a structure-activity relationship (SAR) study of this class of compounds. Extensive SAR on the 2-, 3-, 4- and 7-positions on the DHP was conducted by Van Horn et al. leading to the development of frontrunner DHP 3.0 (figure 3.1).

Figure 3.1: Frontrunner DHP 3.0

![Frontrunner DHP 3.0](image)

Van Horn et al synthesized asymmetric 1,4-dihydropyridines by reacting equal proportions of an acetoacetate derivative, a benzaldehyde, a 1,3-cyclohexanedione or dihydro-2,4-pyrrandione, and ammonium acetate in acetonitrile at 120°C (scheme 3.1).
Scheme 3.1: Synthesis of asymmetric 1,4-dihydropyridines

Conditions: for X = CH₂, (a): (i) acetone, NaOH (aq); (ii) diethyl malonate, NaOEt, ethanol; (iii) NaOH (aq), reflux; and (iv) H₂SO₄ (aq), reflux. For X = O, (b): (i) NaH, ethyl acetoacetate, 0°C then nBuLi; (ii) addition of benzaldehyde; and (iii) NaOH (aq).

The need to synthesize and identify the potent stereoisomer required synthetic approaches using a chiral auxiliary or the use of an enantiopure lactone intermediate in the Hantzsch reaction to generate a mixture of diastereoisomers, which was separated into their single stereoisomers via achiral chromatography. The approach utilizing a chiral auxiliary took advantage of l-threonine, which was attached to the DHP scaffold enabling the separation of four DHP stereoisomers. The esterification with thionyl chloride in methanol followed by an amidation with m-nitrobenzoyl chloride in water and ethyl acetate using potassium carbonate resulted in alcohol 3.1 which was treated with 2,2,6-trimethyl-1,3-dioxin-4-one in toluene at 150°C in a sealed tube providing the chiral acetoacetate 3.2 in 65% yield over three steps (scheme 3.2).

Scheme 3.2: Synthesis of chiral auxiliaries
Asymmetric Hantzsch cyclization of the chiral acetoacetate 3.2, \( o \)-chlorobenzaldehyde, 5-(4-chloro-3-methoxyphenyl)cyclohexane-1,3-dione and ammonium acetate in acetonitrile at 120\(^\circ\)C in a sealed tube resulted in 42% of a mixture of DHP diastereomers 3.3 (scheme 3.3).

**Scheme 3.3: Synthesis of chiral 1,4-dihydropyridines**

Diastereomeric separation using preparative HPLC gave 3 fractions: 2 diastereomerically pure fractions and 1 fraction consisting of an inseparable mixture of two diastereomers. A \( \beta \)-elimination using DBU in methanol afforded the free acid which was esterified using ethyl iodide in methanol to give ethyl ester 3.4. Interestingly, the inseparable mixture of two diastereomers became separable after the removal of the chiral auxiliary and transformation to the ethyl ester, thereby allowing access to all four DHP stereoisomers. However, all attempts to crystalize enantiopure samples of 3.3 or 3.4 so as to determine the absolute stereochemical configuration at the C4- and C7-positions failed. With the use of a second chiral auxiliary, \( R \)-4-benzyloxazolidin-2-one in the asymmetric Hantzsch synthesis, crystallization of enantiomerically pure DHPs was achieved using vapor diffusion with dimethyl sulfoxide (DMSO) as the solution solvent and an ethanol/water mixture as the diffusion solvents.
The X-ray structure of the one crystallized isomer showed that it was cis with a (4S,7S) configuration. All crystallization attempts of the other DHP isomers failed.

There was a large difference in antiplasmodial activity of the four isomers revealing the importance of the stereochemistry in the activity of the DHP. The trans configuration was more active in vitro compared to the cis configuration (table 3.1).

Table 3.1: Comparison of activities of stereoisomers of 3.0

<table>
<thead>
<tr>
<th>Stereochemistry</th>
<th>3D7 EC₅₀ nM</th>
<th>K1 EC₅₀ nM</th>
<th>W2 EC₅₀ nM</th>
<th>TM90-C2B EC₅₀ nM</th>
<th>J774A.1 EC₅₀ µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>4S,7S</td>
<td>&gt;6000</td>
<td>&gt;6000</td>
<td>2130</td>
<td>2110</td>
<td>&gt;20</td>
</tr>
<tr>
<td>4R,7R</td>
<td>17</td>
<td>10.5</td>
<td>200</td>
<td>514</td>
<td>&gt;20</td>
</tr>
<tr>
<td>4R,7S</td>
<td>2.6</td>
<td>3.2</td>
<td>18.1</td>
<td>49.1</td>
<td>&gt;20</td>
</tr>
<tr>
<td>4S,7R</td>
<td>&gt;6000</td>
<td>&gt;6000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

Chloroquine (EC₅₀K₁ = 1360 ± 960 nM, EC₅₀3D7 = 48 ± 25 nM, EC₅₀W₂ = 302 ± 176 nM, EC₅₀TM90-C₂B = 174 ± 87 nM), mefloquine (EC₅₀K₁ = 31 ± 18 nM, EC₅₀3D7 = 62 ± 31 nM), atovaquone (EC₅₀W₂ = 1.56 ± 0.83 nM, EC₅₀TM90-C₂B = 20.6 ± 12.7 µM) and dihydroartemisinin (EC₅₀W₂ = 4.96 ± 1.02 nM, EC₅₀TM90-C₂B = 6.19 ± 0.42 nM) are the internal controls for the in vitro anti-malarial activity assays. Podophyllotoxin is the internal control for the in vitro cytotoxicity assay with EC₅₀ = 250 ± 10 nM against J774A.1.
In an attempt to improve oral bioavailability and in vivo efficacy, an aminoethoxy-substituent was incorporated into the C2-position of the DHP 3.0.1 (figure 3.2). However, a major step in the synthesis, which involved bromination at the C2-position has to be optimized as it was problematic and low yielding due to tendency of excessive bromination and decarboxylation of the C3-position.

**Figure 3.2: Aminoketo DHP**

A metabolic stability study in human liver microsomes focusing on the identification and characterization of potential metabolites identified two major metabolic transformations including a demethylation of one of the methoxy groups and an oxygenation at the phenyltetrahydroquinone region. The demethylation of a methoxy group made up over 85% of the metabolite product while an oxidation of the phenyltetrahydroquinone region accounted for less than 15% of the metabolites (figure 3.3).
The microsomal stability of the compound was improved by replacing the sites of major metabolism. The methoxy group at the para-position of the C7 was replaced by a chloro substituent, which not only improved the stability but also the antimalarial activity.

3.2. Optimization of the bromination step

In order to synthesize large amounts of the para-chloro on C7 analogue with improved microsomal stability and antimalarial activity, the synthesis had to be improved, especially the bromination step on the C2-position. Attempts to use N-bromosuccinimide (NBS) as the brominating agent was not promising. While using pyridinium tribromide as the brominating agent in a halogenated solvent with pyridine as the base, different equivalents of the base, different halogenated solvents and varied reaction temperatures were screened.\(^7\) Optimized conditions required DCM, with slightly less equivalence of pyridine at a temperature of -41 °C. Additional improvements were achieved by avoiding acidic workup by direct column chromatography at the completion of the reaction affording the mono-brominated product in excellent yields. Alkylating the DHP with tert-butyl (2-hydroxyethyl)carbamate or 2-azidoethanol to Boc-protected DHP 3.12 or azido DHP 3.13 respectively followed by the treatment with benzenesulfonic acid or a reduction of the azido group before treating with benzenesulfonic acid respectively afforded the besylate salt 3.14 (scheme 3.4).
Scheme 3.4: Bromination and alkylation of DHP

Reactions and conditions: (a) For $R = \text{NHBoc}$: benzene sulfonic acid, Tol, MeOH (b) For $R = \text{N}_3$: $\text{H}_2$, Pd/C; then benzene sulfonic acid.

For the synthesis of lactone DHPs, a lactone with the desired $S$ stereochemistry on the $C7$ was used in the Hantzsch reaction. The reasoning behind the use of the lactone was setting of one stereocenter prior to formation of the 1,4-dihydropyridine core. If the stereochemistry of the 7-position could be set, isolation of the pure stereoisomers would depend solely on separation of two diastereomers. It also allows for manipulation of the 2-position by incorporating the aminoethoxy substituent into the acetoacetate derivative, thereby avoiding the bromination step entirely.

The synthesis of the lactone started by an alkylation of 4-chloro-3-methoxybenzaldehyde using (+)-$B$-allyldiisopinocampheylborane in diethyl ether at -100 °C to afford the allylic alcohol 3.7 with good enantiomeric excess (ee). This was followed by an oxidative cleavage using modified Lemieux-Johnson oxidation with osmium tetroxide and sodium periodate to afford the $\beta$-hydroxyaldehyde. C-H insertion using ethyl diazoacetate and tin (II) chloride to afford 3.8 which was cyclized in sodium hydroxide to the $\beta$-ketolactone 3.9 (scheme 3.5).
Scheme 3.5: Synthesis of chiral lactone and its use in synthesis of enantiopure DHPs

Manipulation of the 2-position of the DHP by incorporating the aminoethoxy substituent started by a substitution reaction of bromo alcohol with azide in water to azido alcohol 3.15a which was used in alkylating ethyl 4-chloro-3-oxobutanoate to the azido acetoacetate derivative 3.15b. Direct alkylation of ethyl 4-chloro-3-oxobutanoate with tert-butyl (2-hydroxyethyl)carbamate afforded the tert-butyl carbamate derivative of acetoacetate 3.15c (scheme 6).

Scheme 3.6: Synthesis of aminoethoxy acetoacetate derivative

The Hantzsch reaction using the chiral lactone 3.9 with the aminoethoxy acetoacetate derivative 3.15b or 3.15c afforded the enantiopure DHP 3.16 and 3.17 respectively after column chromatography. Treatment of the Boc-protected DHP 3.17 with benzenesulfonic acid directly afforded the besylate salt 3.18. In another attempt, azido DHP 3.16 was initially reduced to the corresponding amino DHP before it was transformed into the besylate salt 3.18 by treatment with benzenesulfonic acid (scheme 7).
Scheme 3.7: Synthesis of lactone DHP

Reactions and conditions: (a) For R = NHBoc: benzenesulfonic acid, Tol, MeOH (b) For R = N\textsubscript{3}: H\textsubscript{2}, Pd/C; then benzenesulfonic acid.

3.3 Discussion

The besylate salt formation of amino lactone-DHP 3.18 was more challenging than for amino keto-DHP 3.14. There was oxidation of the amino lactone-DHP to the to the pyridine analogue. The oxidation byproduct was observed when either the azido DHP 3.16 or the tert-butyl carbamate DHP 3.17 was transformed to the besylate salt 3.18. Furthermore, the oxidation continued, albeit slowly but gradually even after the formation of the besylate salt. This suggests that the oxidation has to do with not only the reaction conditions used for the salt formation but also on the inherent unstable nature of the molecule itself. A possible alternative will be to employ a different salt formation/formulation technique with milder synthetic conditions for the lactone-DHP.
The *in vitro* activity testing showed that the amino keto-DHP 3.14 was more active than the amino lactone-DHP 3.18. Due to the instability of the amino lactone-DHP, it was not possible to get an extensive pharmacokinetic study on 3.18. The *in vitro* studies to determine parasite viability after treatment showed that the amino keto-DHP showed a moderate killing profile similar to pyrimethamine. The pharmacokinetic study on 3.14 showed that it exhibits a moderate apparent half-life of 3.7 hours following IV administration, high plasma volume of distribution and low plasma clearance. Following oral administration, the half-life was similar to that of IV administration. The maximum plasma concentration was observed 4 hour post-dose suggesting a relatively slow absorption and the apparent oral bioavailability was approximately 30%. The oral therapeutic efficacy studies showed that 3.14 do induce elimination of *Plasmodium falciparum* from peripheral blood of mice upon once a day oral administration. Interestingly, there was no observed adverse reactions or compound-related side effects associated with 3.14 indicating that it is safe.

### 3.4 Conclusion

The asymmetric dihydropyridines (DHPs) are novel and interesting compounds with antimalarial activity. Using the result from the metabolic stability studies, an amino keto-DHP 3.14 with improved microsomal stability was identified. The problem with its synthesis was fixed by optimization of a crucial bromination step, which afforded a route to access large amounts for pharmacokinetic studies. The amino keto-DHP 3.14 is orally bioavailable and noncytotoxic. It has shown to be slow acting with high plasma volume of distribution and low plasma clearance, which makes it a good candidate for a combination therapy. Though the synthesis of amino lactone-DHP 3.18 was also improved by incorporation of the aminoethoxy substituent on the C2 position before Hantzsch cyclization, its instability made it impossible to be evaluated extensively.
3.4 Experimental

**General.** All reagents and solvents were obtained from Aldrich Chemical Co. and used without further purification. NMR spectra were recorded at ambient temperature on a 400 MHz or 500 MHz Varian NMR spectrometer in the solvent indicated. All $^1$H NMR experiments are reported in $\delta$ units, parts per million (ppm) downfield of TMS, and were measured relative to the signals of chloroform (7.26 ppm) and dimethylsulfoxide (2.50 ppm) with $^1$H decoupled observation. Data for $^1$H NMR are reported as follows: chemicals shift ($\delta$ ppm), multiplicity ($s$ = singlet, $d$ = doublet, $t$ = triplet, $q$ = quartet, $p$ = pentet, sext = sextet, sept = septet, oct = octet, $m$ = multiplet), integration and coupling constant (Hz) whereas $^{13}$C NMR analyses were reported in terms of chemical shift. NMR data was analyzed by using MestReNova Software version 6.0.2-5475. The purity of the final compounds was determined to be $\geq$95% by high-performance liquid chromatography (HPLC) using an Agilent 1100 LC/MSD-VL with electrospray ionization. Low-resolution mass spectra were performed on an Agilent 1100 LC/MSD-VL with electrospray ionization. High-resolution mass spectra (HRMS) were performed on an Agilent LC/MSD TOF system G3250AA. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 precoated plates (0.25 mm) from EMD Chemical Inc., and components were visualized by ultraviolet light (254 nm). EMD silica gel 230-400 (particle size 40-63 $\mu$m) mesh was used for all flash column chromatography.

$$(2S,3R)$-methyl 2-(3-nitrobenzamido)-3-((3-oxobutanoil)oxy)butanoate ($2S,3R$) (3.2): 3 equivalents of thionyl chloride were added to 1 equivalent of 2 M D-threonine in MeOH. After twenty hours the solvent was removed by evaporation. The residue was taken up in a 2:1 ratio of ethyl acetate to water for a 1.3 M total concentration. To this solution in an ice bath was added 1.5 equivalents of potassium carbonate followed by 1 equivalent of 3-nitrobenzoyl chloride. After five
hours the organic layer was separated and washed with an equal volume of brine three times to
give 1. After concentration, 1 was mixed with 1 equivalent of 2,2,6-trimethyl-1,3-dioxin-4-one
and heated to 150 °C in a sealed tube for fifteen hours. Column chromatography using 3:1 hexanes
to ethyl acetate resulted in a 65% overall yield of the title compound. Rf = 0.22 (hexanes to ethyl
acetate 1:1) 1H NMR (400 MHz, CDCl3) δ 8.60 (t, J = 1.9 Hz, 1H), 8.26 – 8.22 (m, 1H), 8.12 (dd,
J = 7.8, 1.0 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.45 (d, J = 8.9 Hz, 1H), 4.74 (dd, J = 8.9, 2.5 Hz,
1H), 4.41 (qd, J = 6.4, 2.5 Hz, 1H), 3.68 (s, 3H), 3.30 (s, 1H), 1.20 (d, J = 6.4 Hz, 3H). 13C NMR
(101 MHz, CDCl3) δ 171.41, 165.91, 148.15, 135.20, 133.49, 129.86, 126.41, 122.34, 68.00,
58.22, 52.82, 20.15.

(4R,7S)-(2S,3R)-4-methoxy-3-(3-nitrobenzamido)-4-oxobutan-2-yl 7-(4-chloro-3-
methoxyphenyl)-4-(2-chlorophenyl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-
carboxylate (3.3): 1.80 g (4.91 mmol) of 3.2, 0.55 mL (4.91 mmol) of 2-chlorobenzaldehyde, 1.22
g (4.91 mmol) of 5-(4-chloro-3-methoxyphenyl)cyclohexane-1,3-dione and 0.45 g (5.8 mmol) of
ammonium acetate were mixed in 5 mL of acetonitrile and heated to 120°C in a sealed tube for
three hours. Flash chromatography using 1:1 hexanes to ethyl acetate resulted in 1.49 g (2.07
mmol) of the diastereomers in 42% yield. Prep-HPLC using 1:1 acetonitrile to water with 0.05%
TFA resulted in the separation of the enantiomers to the stereoisomer of interest. 1H NMR (399
MHz, CDCl3) δ 8.67 (t, J = 2.0 Hz, 1H), 8.43 – 8.32 (m, 1H), 8.24 – 8.07 (m, 1H), 7.70 (t, J = 8.0
Hz, 1H), 7.36 (dd, J = 7.7, 1.8 Hz, 1H), 7.33 – 7.22 (m, 3H), 7.20 (dd, J = 7.8, 1.5 Hz, 1H), 7.16
– 7.06 (m, 1H), 7.06 – 6.97 (m, 1H), 6.76 – 6.63 (m, 2H), 6.48 (s, 1H), 5.51 (dd, J = 6.6, 3.7 Hz,
1H), 5.37 (s, 1H), 4.87 (dd, J = 8.5, 3.6 Hz, 1H), 3.85 (s, 3H), 3.73 (s, 3H), 3.23 – 3.00 (m, 1H),
2.89 – 2.62 (m, 1H), 2.62 – 2.44 (m, 3H), 2.35 (s, 3H), 1.13 (d, J = 6.6 Hz, 3H). 13C NMR (100
MHz, CDCl3) δ 194.31, 170.36, 166.43, 165.70, 155.24, 148.90, 148.43, 145.93, 144.05, 142.60,
(4R,7S)-ethyl 7-(4-chloro-3-methoxyphenyl)-4-(2-chlorophenyl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (3.4): 0.26 g (0.36 mmol) of 3.3 and 0.16 mL (1.1 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene were mixed in 10 mL of MeOH for two hours. After evaporation of solvent the residue was diluted with 25 mL of water and washed with 15 mL of diethyl ether three times. The aqueous layer was acidified to pH 2 using 5 M HCl (aq) and extracted with dichloromethane. Separation of the organic layer followed by drying on sodium sulfate and evaporation of the solvent resulted in the crude acid. The residue was taken up in 10 mL of DMF followed by the addition of 0.06 g (0.4 mmol) of potassium carbonate and 58 μL (0.72 mmol) of ethyl iodide. After fifteen hours the solution was diluted between water and dichloromethane and the organic layer dried and evaporated. Flash chromatography using 1:1 hexanes to ethyl acetate resulted in 0.16 g (0.35 mmol) of 3.4 in 98% yield. ¹H NMR (399 MHz, Chloroform-d) δ 7.41 (dd, J = 7.7, 1.7 Hz, 1H), 7.37 – 7.25 (m, 1H), 7.23 (dd, J = 7.9, 1.4 Hz, 1H), 7.18 – 7.08 (m, 1H), 7.09 – 6.97 (m, 1H), 6.81 – 6.58 (m, 3H), 5.43 (s, 1H), 4.07 – 3.95 (m, 2H), 3.85 (s, 3H), 3.32 – 3.11 (m, 1H), 2.84 – 2.58 (m, 1H), 2.60 – 2.35 (m, 3H), 2.30 (s, 3H), 1.17 (t, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 194.49, 167.48, 155.18, 149.81, 144.05, 143.70, 142.78, 133.32, 132.24, 130.48, 129.82, 127.56, 126.54, 121.15, 119.24, 112.05, 110.94, 105.62, 60.07, 56.22, 44.31, 38.90, 36.14, 34.78, 19.33, 14.32.

(S)-1-(4-chloro-3-methoxyphenyl)but-3-en-1-ol (3.7): Anhydrous ether (20 mL) was added to (-)-IPC₂Ball (3.26 g, 9.96 mmol), and the resulting solution was cooled to -100 °C. A solution of 4-chloro-3-methoxy benzaldehyde (1.7 g, 9.96 mmol) in ether (10 mL) was slowly added along the
side of the flask to the solution of (-)-IPC$_2$BAI at -100 °C. The reaction mixture was stirred for 1.5 h at -100 °C and methanol (2 mL) was added. The reaction mixture was then brought to room temperature (1 h) and treated with 3 N NaOH (6 mL) and 30% H$_2$O$_2$ (12 mL). The completion of oxidation was ensured by refluxing the reaction mixture for 3 h. The usual workup and distillation afforded (S)-homoallyl alcohol in 90% yield (1.9 g) as pale white solid. (92% ee)

$$^1$$H NMR (399 MHz, CDCl$_3$) δ 7.31 (d, $J = 8.1$ Hz, 1H), 6.97 (d, $J = 1.8$ Hz, 1H), 6.85 (dd, $J = 8.1$, 1.9 Hz, 1H), 5.99 – 5.65 (m, 1H), 5.21 – 5.16 (m, 2H), 5.15 (d, $J = 1.1$ Hz, 1H), 4.87 – 4.56 (m, 1H), 3.91 (s, 3H), 2.67 – 2.32 (m, 2H), 2.15 (dd, $J = 3.2$, 1.3 Hz, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 155.09, 144.16, 134.13, 130.07, 121.37, 118.99, 118.69, 109.58, 72.86, 56.23, 44.06.

(S)-ethyl 5-(4-chloro-3-methoxyphenyl)-5-hydroxy-3-oxopentanoate (3.8): To a solution of homoallyl alcohol 3.5 (2.0 g, 9.4 mmol) in dioxane-water (3:1, 106 mL) was added OsO$_4$ (900 µL, 4% in H$_2$O, 36 mg, 0.141 mmol) and was stirred for 5 minutes during which time the solution became dark brown (due to osmate ester formation). 2,6-lutidine (2.2 mL, 18.8 mmol) was then added followed by portion-wise addition of NaIO$_4$ (8.075 g, 37.6 mmol) over 30 minutes. The tan-colored slurry was then stirred for additional 1.5 hours. The reaction was diluted with water and extracted with DCM (50 mL X 3). The organic layer was washed with dilute HCl, brine and the dried with sodium sulfate. The solvent was removed under reduced pressure and the aldehyde was used without further purification.

To anhydrous tin (II) chloride (159 mg, 0.84 mmol) was added dry DCM (30 mL) followed by ethyl diazoacetate (900 µL, 8.4 mmol) with stirring at room temperature. To this suspension were slowly added a few drops of crude aldehyde (1.8 g, 8.4 mmol) in dry DCM. When N$_2$ evolution began, the remaining soln. of crude aldehyde was added drop wise within 10 min. After N$_2$
evolution had stopped (3 h), the mixture was transferred to a separatory funnel with brine soln. (30 mL) and extracted with DCM (3 X 30 mL). The combined org. layer was dried (Na$_2$SO$_4$), the solvent evaporated, and the residue purified by flash chromatography to yield title compound (1.3 g, 46%) as yellow liquid.

(S)-6-(4-chloro-3-methoxyphenyl)dihydro-2H-pyran-2,4(3H)-dione (3.9): The beta-keto ester 3.8 (1.3 g, 4.3 mmol) was taken in a round bottom flask and 60 mL of 0.2 M NaOH was added and the solution stirred for 30 min at room temperature. Then the reaction mixture was shaken two times with 30 mL of ether. The aqueous layer was acidified using 1 M HCl until the pH reached 4. The reaction mixture was extracted with EtOAc (3 x 30 mL). The combined organic layers was dried with sodium carbonate, the solvent evaporated, and the residue purified by flash chromatography to yield the lactone (646 mg, 59%) as a pale yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.40 (d, $J = 7.0$ Hz, 1H), 6.99 (s, 1H), 6.88 (d, $J = 7.3$ Hz, 1H), 5.68 (d, $J = 10.7$ Hz, 1H), 3.93 (s, 3H), 3.68 (d, $J = 19.0$ Hz, 1H), 3.50 (d, $J = 19.5$ Hz, 1H), 2.96 (d, $J = 18.1$ Hz, 1H), 2.84 (t, $J = 14.4$ Hz, 1H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 198.96, 166.76, 155.76, 136.62, 130.84, 123.68, 118.53, 109.55, 76.05, 56.44, 47.14, 45.39.

(4R,7S)-ethyl 2-((2-azidoethoxy)methyl)-7-(4-chloro-3-methoxyphenyl)-4-(2-chlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (3.12): To an ice-cooled solution of the alcohol in THF was added NaH and stirred at that temperature for 30 mins. The bromide 3.11 in dry THF was added the solution and then warmed to room temperature. The solvent evaporated, and the residue purified by flash chromatography. $^1$H NMR (399 MHz, CDCl$_3$) $\delta$ 7.43 (dd, $J = 7.7$, 1.7 Hz, 1H), 7.38 (s, 1H), 7.34 – 7.22 (m, 2H), 7.21 – 7.13 (m, 1H), 7.13 – 7.03 (m, 1H), 6.82 – 6.66 (m, 2H), 5.45 (s, 1H), 4.93 – 4.68 (m, 2H), 4.10 – 3.93 (m, 2H), 3.87 (s, 3H), 3.84 – 3.64 (m,
2H), 3.57 – 3.42 (m, 2H), 3.33 – 3.11 (m, 1H), 2.81 – 2.67 (m, 1H), 2.68 – 2.54 (m, 2H), 2.54 – 2.44 (m, 1H), 1.17 (t, J = 7.1 Hz, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 194.44, 167.05, 155.18, 148.96, 144.27, 143.71, 142.76, 133.49, 132.50, 130.51, 129.95, 127.68, 126.51, 121.14, 119.25, 112.39, 110.89, 103.12, 70.40, 68.24, 60.17, 56.21, 50.92, 44.31, 38.86, 36.17, 34.73, 14.28.

2-(((4S,7S)-7-(4-chloro-3-methoxyphenyl)-4-(2-chlorophenyl)-3-(ethoxycarbonyl)-5-oxo-1,5,7,8-tetrahydro-4H-pyrano[4,3-b]pyridin-2-yl)methoxy)ethan-1-aminium benzenesulfonate (3.18): $^1$H NMR (399 MHz, CDCl$_3$) δ 8.32 (s, 1H), 7.83 (s, 2H), 7.71 (d, J = 7.6 Hz, 2H), 7.36 (t, J = 7.1 Hz, 1H), 7.18 – 7.12 (m, 0H), 7.08 (d, J = 7.6 Hz, 0H), 6.83 (s, 0H), 6.64 (d, J = 8.1 Hz, 0H), 5.40 (s, 0H), 4.92 (d, J = 12.7 Hz, 0H), 4.69 – 4.34 (m, 1H), 4.11 – 3.91 (m, 0H), 3.79 (s, 1H), 3.51 (d, J = 6.7 Hz, 1H), 2.95 (s, 1H), 2.79 (d, J = 16.7 Hz, 0H), 2.59 – 2.38 (m, 0H), 1.14 (t, J = 7.1 Hz, 1H).

3.5 References


Chapter 4. Menoctone as antimalarial

4.1 Introduction

Menoctone is a hydroxynaphthoquinone that was developed as an antimalarial agent by Fieser and co-workers at Harvard University in collaboration with Sterling-Winthrop Research Institute in 1967.\textsuperscript{1-3} Historically, interest in hydroxynaphthoquinone as antimalarial agents commenced in early 1940s during the drive to find a replacement for quinine. However, the emergence of primaquine and chloroquine led to a decline in interest in the hydroxynaphthoquinones.\textsuperscript{4} Due to the development of drug resistance to established antimalarials in the early 1960s, drug discovery on a novel antiplasmodial agent again led to the identification of menoctone. It demonstrated suppressive effects against blood-induced rodent malarias and was tested against sporozoite-induced \textit{Plasmodium berghei} infections in a susceptible white mouse strain where it displayed some schizonticidal activity.\textsuperscript{5} It was selected for study in man, but disappointingly failed to live up to its early promise (WHO 1973) as it showed no gametocidal, sporontocidal or causal prophylactic activity against \textit{Plasmodium falciparum} in humans at an oral dose. Menoctone’s inactivity, when administered orally, has been attributed to its poor solubility, its poor absorption from the gastrointestinal tract, and its strong binding to plasma proteins.\textsuperscript{5-9}

Interestingly, through the repurposing of drugs, a little over a decade after its discovery, menoctone was found to be effective at low intravenous doses against \textit{Theileria parva}, a parasitic tick-borne protozoan that causes the cattle disease East Coast Fever (ECF) that kills about half a million cattle annually. However, despite the impressive efficacy of menoctone against \textit{T. parva}, the complexity of its synthesis and high manufacturing costs made it uneconomical to market it as a treatment for theileiosis.\textsuperscript{10-12}
The development of resistance against the current antimalarials has not only driven the urgent need for new antimalarial candidates but also an understanding on how the parasites develop resistance. For example, atovaquone induce rapid appearance of resistant malaria parasites in *Plasmodium falciparum* when used as a single agent.\(^{13}\) Menoctone has been considered to be a good candidate to study the induction or resistance because of its structural similarity to atovaquone and its unique profile of higher potency in the liver stage of malaria than in the blood stage.

**4.2 Synthesis of Menoctone**

Fieser and co-workers at Harvard University in collaboration with scientists at the Sterling-Winthrop Research Institute reported the first synthesis of menoctone.\(^{3-6}\) In 1969, Lorenz patented a synthetic route starting from 5-phenylvaleric acid.\(^{7}\) These synthesis had many shortcomings such as the need for specialized starting materials, harsh reaction conditions, costly raw materials, and very low yield due to byproduct formation. It was reported as tedious and uneconomical to make.\(^{11}\) Herein, a simple and facile synthesis of menoctone using modern and classical transformations with fewer, high-yielding steps is reported.

The isochroman-1,4-dione 4.1 was synthesized from 2-acetylbenzoic acid, which was first brominated, and then cyclized by hydrolysis.\(^{15}\) Chloroalkene 4.2 was synthesized from 1-bromo-7-chloroheptane and allylmagnesium bromide using copper mediated alkane synthesis. Substitution of the chloride with an iodide via Finkelstein reaction afforded the iodoalkene 4.3 which was then treated with cyclohexylmagnesium bromide in another copper mediated alkane synthesis to afford the allylcyclohexane 4.4.\(^{16}\) Lemieux-Johnson oxidation of the allyl 4.4 yielded the aldehyde 4.5 which upon condensation with isochroman-1,4-dione 4.1 afforded 3-(2-
cyclohexylethylidene)isochroman-1,4-dione 4.6 which was rearranged to menoctone using sodium methoxide in methanol (Fig. 4.1).

Scheme 4.1: Synthesis of menoctone

4.3 Antimalarial Activity Testing and Resistant Parasite Generation

The antimalarial activity testing and resistant parasite generation with menoctone was carried out by Kyle’s laboratory at the University of South Florida. The potency of menoctone was determined against blood and liver stages of malaria in vitro. Using the *Plasmodium falciparum* W2 strain, menoctone was determined to have an IC$_{50}$ value of 0.75 nM against the blood stage parasites. Against the liver stage of the parasite, *Plasmodium berghei* lucanka ANKA in vitro analysis was determined to be 0.41 nM. Menoctone was found to be about 3-fold more active than atovaquone in these assays. In order to generate menoctone resistance in *P. berghei* (MEN), a similar method used by Peters and co-workers was used to generate a new menoctone resistant parasite from *P.*
Infected mice were treated with menoctone, recrudescent parasites were inoculated into new mice, and subsequently treatment with higher doses of drug led to resistance at a dose of 300 mg/kg. Initial assessment for the potential for cross-resistance between menoctone and atovaquone in the MEN line revealed incomplete resistance. Pulse exposures of atovaquone to mice infected with MEN result in rapid selection of atovaquone resistance. Under pressure from menoctone, *P. berghei lucanka* ANKA developed a mutation of methionine 133 into an isoleucine (M133I), identical to previous reports of *P. berghei* under atovaquone pressure. The mutation identified by Sanger sequencing genomic alignment, was located directly outside the quinol oxidation site (Qo), the known binding site for atovaquone.

4.4 Conclusion

A facile synthesis of menoctone has been designed and optimized. This novel synthesis involving modern and classical transformations has fewer steps and higher yields than previously reported methods. The overall yield for the shortest linear (over 10) steps reported synthetic scheme was 2.3% while herein, a convergent synthetic route with 7 steps and an overall yield of 11.6% yield was presented. Hydroxynaphthoquinones have been identified as class of compounds with antiplasmodial activity. Menoctone showed better potency than atovaquone against the W2 strain of *P. falciparum* asexual stage parasites (IC$_{50} = 0.75$ nM) and liver stages of *P. berghei lucanka* ANKA in vitro (IC$_{50} = 0.41$ nM). A new *P. berghei lucanka* ANKA menoctone resistance parasite was developed by successive dosing of infected mice with menoctone. On sequencing the resistant parasite, it was found that it demonstrates cytochrome b mutation M133I located outside the Qo site, the same resistance mutation for atovaquone. Subsequently, the resistance parasite also
demonstrated incomplete resistance to atovaquone. The study to access the efficacy of menoctone against liver stages and gametocytes of menoctone resistant *P. berghei* is currently ongoing.

4.5 Experimental

**General.** All reagents and solvents were obtained from Aldrich Chemical Co. and used without further purification. NMR spectra were recorded at ambient temperature on a 400 MHz or 500 MHz Varian NMR spectrometer in the solvent indicated. All $^1$H NMR experiments are reported in δ units, parts per million (ppm) downfield of TMS, and were measured relative to the signals of chloroform (7.26 ppm) and dimethylsulfoxide (2.50 ppm) with $^1$H decoupled observation. Data for $^1$H NMR are reported as follows: chemicals shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, sept = septet, oct = octet, m = multiplet), integration and coupling constant (Hz) whereas $^{13}$C NMR analyses were reported in terms of chemical shift. NMR data was analyzed by using MestReNova Software version 6.0.2-5475. The purity of the final compounds was determined to be $\geq 95\%$ by high-performance liquid chromatography (HPLC) using an Agilent 1100 LC/MSD-VL with electrospray ionization. Low-resolution mass spectra were performed on an Agilent 1100 LC/MSD-VL with electrospray ionization. High-resolution mass spectra (HRMS) were performed on an Agilent LC/MSD TOF system G3250AA. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 precoated plates (0.25 mm) from EMD Chemical Inc., and components were visualized by ultraviolet light (254 nm). EMD silica gel 230-400 (particle size 40-63 μm) mesh was used for all flash column chromatography.

**General Procedure A: Copper Mediated Alkane synthesis.** To a suspension of Copper (II) chloride (0.05 equiv.) in Et$_2$O (1.0 M relative to the alkyl halide) at 78°C was added a 2.0 M solution of a Grignard reagent (2 equiv.) in THF followed by the addition of an alkyl halide (Br or
I) (1.0 equiv.). The mixture was allowed to warm to room temperature and held at that temperature until the starting alkene was judged consumed by TLC analysis (4 h unless otherwise stated). The mixture was then cooled to 0° C and quenched with 1 N HCl (50 X the volume of Et₂O) and extracted into EtOAc (50 X the volume of Et₂O). The organic layer was then dried with Na₂SO₄, evaporated to dark green oil, and immediately purified by flash chromatography.

**10-chlorodec-1-ene (4.2):** General procedure A was used to couple 1-bromo-7-chloroheptane (3.7 g, 17.3 mmol) and allylmagnesium bromide (5.0 g, 34.7 mmol). It was purified by flash chromatography (hexanes) to afford 4.2 (2.3 g, 75%) as a clear and colorless oil. Rₚ (hexanes; KMnO₄) = 0.88. ¹H NMR (399 MHz, CDCl₃) δ: 6.19 – 5.50 (m, 1H), 5.24 – 4.60 (m, 2H), 3.53 (t, J = 6.8 Hz, 2H), 2.17 – 1.93 (m, 2H), 1.93 – 1.64 (m, 2H), 1.50 – 1.34 (m, 4H), 1.34 – 1.20 (m, 6H). ¹³C NMR (100 MHz, CDCl₃) δ: 139.26, 114.32, 45.30, 33.92, 32.79, 29.45, 29.15, 29.02, 28.99, 27.02.

**10-iododec-1-ene (4.3):** Sodium iodide (5.4 g, 35 mmol) was added to a solution of 4.2 (1.2 g, 7 mmol) in acetone 10ml and heated to reflux for 12 h. The reaction was evaporated to dryness and purified by flash chromatography (hexanes) to afford 4.3 (1.6 g, 87%) as a clear and colorless oil. Rₚ (hexanes; KMnO₄) = 0.85. ¹H NMR (399 MHz, CDCl₃) δ: 6.23 – 5.53 (m, 1H), 5.22 – 4.57 (m, 2H), 3.18 (t, J = 7.1 Hz, 2H), 2.25 – 1.89 (m, 2H), 1.89 – 1.72 (m, 2H), 1.47 – 1.32 (m, 4H), 1.34 – 1.06 (m, 6H) ppm. ¹³C NMR (100 MHz, CDCl₃) δ: 139.27, 114.33, 33.91, 33.70, 30.63, 29.40, 29.15, 29.01, 28.64, 7.46.

**dec-9-en-1-ylcyclohexane (.44):** General procedure A was used to couple 10-iododec-1-ene (700 mg, 2.6 mmol) and cyclohexylmagnesium bromide (986 mg, 5.2 mmol). It was purified by flash
chromatography (hexanes) to afford **4.4** (474 mg, 81%) as a clear and colorless oil. R$_f$ (hexanes; KMnO$_4$) = 0.88

**9-cyclohexylnonanal (4.5):** To a solution of **4.4** (700 mg, 3.1 mmol) in dioxane (30 mL) and water (15 mL) was added OsO$_4$ (12 mg, 0.047 mmol) and was stirred for 5 minutes during which time the solution became dark brown (due to osmate ester formation). 2,6-lutidine (674 mg, 6.2 mmol) was then added followed by portion-wise addition of NaIO$_4$ (2.7 g, 12.5 mmol) over 30 minutes. The tan-colored slurry was then stirred for additional 1.5 hours. The reaction was diluted with water and extracted with DCM (50 mL X 3). The organic layer was washed with dilute HCl, brine and the dried with sodium sulfate. The DCM was removed under reduced pressure and the residue was purified by flash chromatography (hexanes: EtOAc = 50:1) to afford **4.5** (606 mg, 86%) as a clear oil. R$_f$ (hexanes : EtOAc = 5:1) = 0.68. $^1$H NMR (399 MHz, CDCl$_3$) δ: 9.76 (s, 1H), 2.60 – 2.10 (m, 2H), 1.80 – 1.48 (m, 7H), 1.42 – 1.20 (m, 11H), 1.20 – 1.01 (m, 5H), 0.99 – 0.65 (m, 2H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$) δ: 203.09, 44.07, 37.82, 37.67, 33.60, 30.04, 29.60, 29.50, 29.32, 26.97, 26.92, 26.61, 22.24 ppm.

**3-(9-cyclohexylnonylidene)isochroman-1,4-dione (4.6):** Isochromandione **4.1** (347 mg, 2.14 mmol) and isobutylamine (157 mg, 2.14 mmol) was added to a stirred solution of the aldehyde **4.5** in acetic acid. The reaction was heated to 40 °C for 4 hours and allowed to cool to room temperature. The acetic acid was removed under reduced pressure and the residue was purified by flash chromatography (hexanes : EtOAc = 100:1) to afford **4.6** (347 mg, 44%) as a white solid. R$_f$ (hexanes : EtOAc = 5:1) = 0.58. $^1$H NMR (399 MHz, CDCl$_3$) δ: 8.31 (dd, J = 5.7, 3.4 Hz, 1H), 8.23 (dd, J = 5.7, 3.3 Hz, 1H), 7.85 (dd, J = 5.8, 3.3 Hz, 2H), 6.53 (t, J = 7.9 Hz, 1H), 2.61 – 2.13 (m, 2H), 1.79 – 1.55 (m, 5H), 1.57 – 1.42 (m, 2H), 1.42 – 1.05 (m, 16H), 0.97 – 0.62 (m, 2H) ppm.
$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 176.62, 158.83, 146.73, 135.25, 135.10, 133.60, 130.85, 127.64, 127.22, 126.99, 37.89, 37.75, 33.68, 30.15, 29.79, 29.66, 29.61, 28.58, 27.06, 27.00, 26.69, 25.75.

2-(8-cyclohexyloctyl)-3-hydroxynaphthalene-1,4-dione (Menoctone): A 30 wt. % solution of sodium methoxide in methanol (39 mg, 0.7 mmol) was added to a suspension of 4.6 in methanol (215 mg, 0.58 mmol). The solids rapidly dissolved and the resulting red solution was stirred at room temperature for 24 hours. The reaction was quenched with aqueous solution of acetic acid and extracted with DCM (50 mL X 3). The organic layer was washed with brine and the dried with sodium sulfate. The DCM was removed under reduced pressure and the residue was purified by flash chromatography (hexanes : EtOAc = 200:1) to afford menoctone (123 mg, 58%) as a yellow solid. R$_f$ (hexanes : EtOAc = 5:1) = 0.63. $^1$H NMR (399 MHz, CDCl$_3$) $\delta$: 8.11 (dd, $J = 7.7$, 1.3 Hz, 1H), 8.07 (dd, $J = 7.6$, 1.4 Hz, 1H), 7.79 – 7.71 (m, 1H), 7.71 – 7.62 (m, 1H), 7.30 (s, 1H), 2.90 – 2.28 (m, 2H), 1.73 – 1.58 (m, 5H), 1.57 – 1.46 (m, 2H), 1.41 – 1.19 (m, 11H), 1.19 – 1.05 (m, 5H), 0.91 – 0.71 (m, 2H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 184.85, 181.62, 153.13, 134.97, 133.11, 132.98, 129.60, 126.93, 126.19, 125.01, 37.84, 37.71, 33.62, 30.14, 29.97, 29.77, 29.64, 28.48, 27.02, 26.95, 26.63, 23.56.

4.6 References


