SYNTHESIS, CHARACTERISATION AND EVALUATION OF BENXOXABOROLE-BASED HYBRIDS AS ANTIPLASMODIAL AGENTS

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT THE DEGREE

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ABSTRACT

Malaria is a mosquito-borne disease, which continues to pose a threat to the entire humanity. About 40% of the world population is estimated to be at risk of infections by malaria. Despite efforts undertaken by scientific community, government entities and international organizations, malaria is still rampant. The major problem is drug resistance, where the *Plasmodium* spp have over the past decades developed drug resistance against available drugs. In order to counter this problem, novel antimalarial drugs that are efficacious and with novel mode of action are of great necessity.

Benzoxaborole derivatives have been shown to exhibit promising antimalarial activity against *Plasmodium falciparum* strains. Previous studies reported on the compounds such as 6-(2-(alkoxycarbonyl)pyrazinyl-5-oxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaboroles, which showed good antimalarial activity against both W7 and 3D7 strains without significant toxicity. On the other hand, chloroquine (CQ) and cinnamic acids have a wide variety of biological activity including antimalarial activity.

Herein, a hybridisation strategy was employed to synthesise new CQ-benzoxaborole and cinnamoyl-benzoxaborole hybrids. CQ-Benzoxaborole **2.12a-c** and cinnamoyl-benzoxaborole **2.11a-g** hydrid molecules were synthesised in low to good yields. Their structural identities were confirmed using conventional spectroscopic techniques (^{1}H and ^{13}C NMR, and mass spectrometry). CQ-benzoxaborole compounds, however, showed instability, and only **2.12b** was used for *in vitro* biological assay and showed activity comparable to CQ.

Furthermore, *in vitro* biological assay revealed that compounds **2.11a-g** poorly inhibited the growth of *P. falciparum* parasites. Interestingly, these compounds, however, exhibited satisfactory activity against *Trypanosoma brucei* with $IC_{50} = 0.052$ µM for compound **2.11g**. The cell cytotoxicity assay of all final compounds confirmed that all CQ-benzoxaborole **2.12b** and cinnamoyl-benzoxaborole **2.11a-g** hybrids were non-toxic against HeLa cell lines.

However, efforts to further expand the structure-activity relationship (SAR) of CQ-benzoxaborole by increasing the length of the linker with one extra carbon (**Scheme 2.10**) were not possible as an important precursor 6-formylbenzoxaborole **2.29** could not be synthesized in sufficient yields.

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δ	Chemical shift
QN	Quinine
MQ	Mefloquine
ART	Artemisinin
DDT	Dichlorodiphenyltrichloroethane
SP	Sulfadoxine-Primethamine
RBM	Roll Back Malaria
MMV	Medicines for Malaria Venture
P*f*ATP4	P-type sodium transporter ATPase
FQ	Ferroquine
GSK	GlaxoSmithKline
MVI	Malaria vaccine initiative
HAT	Human African Trypanomiasis
PK	Pharmacokinetics
PD	Pharmacodynamics
CYP	Cytochrome P40
ADME	Absorption Distribution Metabolism Excretion
DV	Digestive Vacuole
P*f*CRT	*P. falciparum* CQ resistance transpoter
CAD	Cinnamic acids derivatives
CA	Cinnamic acids
DEPT135	Distortionless Enhancement by Polarization Transfer
HRMS	High resolution mass spectroscopy
MSC	Methanesulfonyl chloride
HBr	Hydrogen bromide
DMAP	Dimethylamine pyridine
DMF	Dimethylformamide
ESI	Electron spray ionozation
PTLC	Preparative thin layer chromatography

ppm	Parts per million
µM	Micromolar
HPLC	High performance liquid chromatography
pLDH	Parasite lactate dehydrogenase
^{13}C NMR	Carbon Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
ACT	Artemisinin combination therapies
$CDCl_3$	Deuterochloroform
CQ	Chloroquine
d	Doublet (in ^{1}H NMR)
DCM	Dichloromethane
dd	Doublet of doublet (in ^{1}H NMR)
DMSO-*d6*	Deuterodimethylsulfoxide
EC_{50}	50% Effective concentration
eq	Equivalent(s)
EtOAc	Ethyl acetate
EtOH	Ethanol
Fe[III]PPIX	Ferriprotoporphyrin IX
h	Hour(s)
H_2O	Water
Hz	Hertz
IC_{50}	50% Inhibitory concentration
K_2CO_3	Potassium carbonate
Leu	Leucine
LeuRS	Leucine transfer ribonucleic acid synthase
m	Multiplet (in ^{1}H NMR)
m.p.	Melting point
MeOH	Methanol
$MgSO_4$	Magnesium sulfate
min	Minute(s)
ml	Millilitre
mM	Millimolar

mmol	Millimole
q	Quartet (in ^{1}H NMR)
R_f	Retention factor
RNA	Ribonucleic acid
s	Singlet (in ^{1}H NMR)
SAR	Structure-activity relationship
t	Triplet (in ^{1}H NMR)
td	Triple of doublets (in ^{1}H NMR)
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin layer chromatography
tRNA	Transfer ribonucleic acid
WHO	World Health Organization

DEDICATION

TO MY MUM
Ester Gumbo

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CHAPTER ONE

1 INTRODUCTION AND LITERATURE REVIEW

1.1 MALARIA EPIDEMIOLOGY AND IMPACT

Malaria is a protozoan disease prominent in regions around the equator and in the sub-Saharan Africa.[1] Approximately more than half of the world population is affected by this deadly disease.[1,2] It is responsible for highest adults and children death cases in developing countries especially in the sub-Saharan Africa, with the distribution shown on **Fig. 1.1**.[2] An estimated 85 % of these deaths occur in immuno-compromised individuals like children of ages 0-5 years, pregnant women and foetuses.[3,4] This disease consumes up to 40 % of public health expenditure in developing countries with intensive malaria transmission.[5]

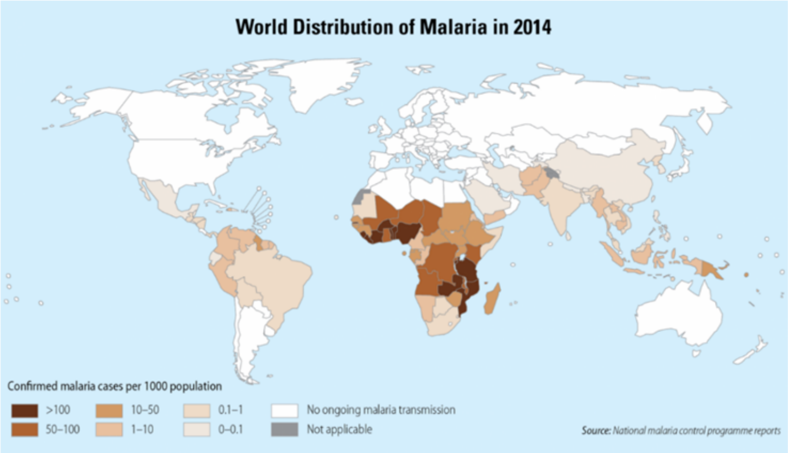


Figure 1.1: World distribution of malaria.[6]

1.1.1 Economic impact of malaria

Economically, less developed countries are faced with two main challenges in combating the disease, and these include the high incidences of drug resistance and escalating antimalarial drugs costs.[7] This leads to reduction of annual economic growth by approximately 1.3 % and the long term impact is a reduction of the gross domestic products (GDP) by more than half.[8] While the malaria burden is still enormous, there has been a noteworthy decline in malaria cases and mortality rates due to malaria for the past 15 years.[9] The cases and deaths due to malaria have declined by 18 % and 48 %, respectively. About 33 countries are moving toward total eradication of malaria and 16 of these have reached a zero indigenous malaria cases.[6]

1.2 *PLASMODIUM,* MALARIA CAUSATIVE AGENT

Malaria is caused by a single-celled protozoan organisms of the genus *Plasmodium*. The *Plasmodium* genus has five species that infect humans and animals, namely *P. falciparum, P. knowlesi, P. vivax, P. malariae* and *P. ovale*. *P. knowlesi* is common in South East Asia, and was formerly reported to affect only monkeys.[10] However, there are reports that this species is also wide spread in humans and is life threatening due to its violent symptoms.[11] *P. vivax* is widely distributed in Asia and it is responsible for a significant number of malaria cases, although rarely fatal. *P. ovale* has the lowest prevalence, but most common in West and Central Africa, its symptoms are generally very mild coupled with low infection cases.[12] The *P. vivax* and *P. ovale* species are able to stay inactive in the liver (hypnozoites) for several years and later cause clinical manifestations at regular intervals.[13] On the other hand, *P. malariae* is the least studied *Plasmodium* species attributably to its low prevalence. In worst cases, its symptoms are associated with severe anaemia and sometimes death.[14] The most important of these species is *P. falciparum,* as it is ascribed to the majority of malaria manifestations and deaths. Up to 90 % of deaths due to malaria are caused by this species and it is widely spread in the continent of Africa.[15]

1.1.2 The *Plasmodium falciparum* life cycle

The *P. falciparum* parasite undergoes a series of changes both in the vector (mosquito) and partly in the host (human).[16] Anopheles (female) mosquitoes are the vectors responsible for the spread of malaria. **Fig. 1.2** illustrates the events occurring during the *P. falciparum* life cycle. Briefly, the infested *Anopheles* vectors, during the blood meal, inoculate sporozoites into the human host.[16] The sporozoites migrate to the liver where they invade the hepatocytes, hiding from the human immune system. Within the hepatocytes, the sporozoites differentiate and undergo asexual reproduction, resulting in the formation of millions of merozoites. The merozoites burst from the hepatocytes into the blood circulation where they invade the erythrocytes and undergo further multiplication to produce more merozoites per schizont. The rupture of infected erythrocytes leads to the release of merozoites into the blood stream. This results in the clinical manifestation of malaria, with fever and chills as symptoms.[17] *P. falciparum* also secretes special proteins to the cell membrane of the infected erythrocyte that make the erythrocytes adhere to the walls of pre-venous capillaries, and this becomes an impediment to the flow of blood in the vessels. In cerebral malaria, sequestration of infected erythrocytes in blood vessels of the brain leads to unconsciousness, and is fatal if not immediately cured.[13]

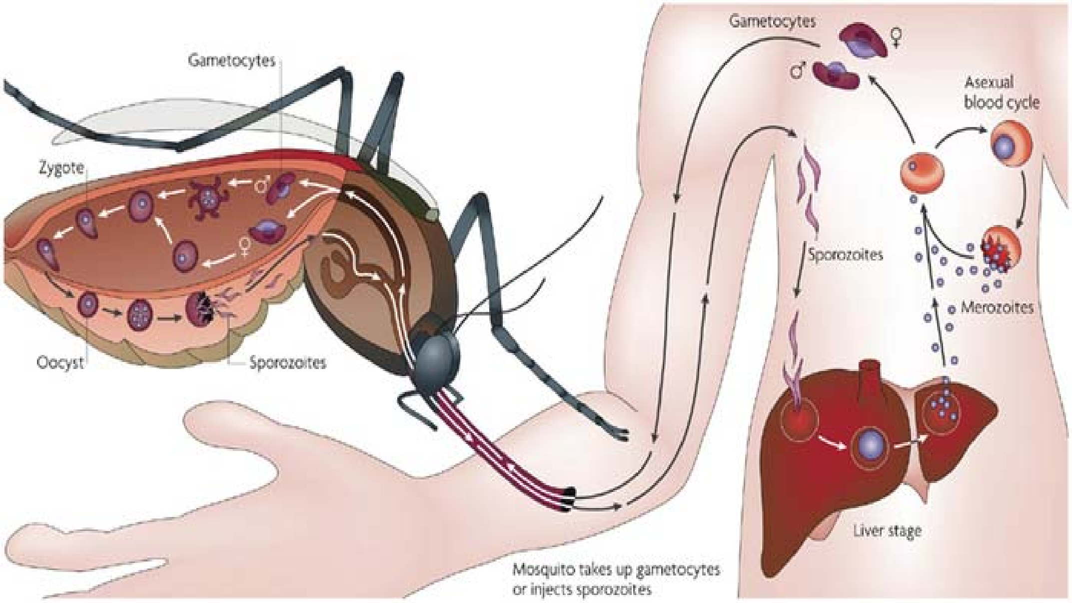


Figure 1.2: *P. falciparum* life cycle.[15]

Not all the merozoites reproduce in the infected erythrocytes to form more merozoites. Some differentiate and transform into sexual cells called gametocytes. Some gametocytes are then taken in by the *Anopheles* vector during the blood meal. Within the vector, the gametocytes undergo further transformation and later fuse together to form zygotes. These develop into oocysts in the gut, which rupture to release sporozoites, and these migrate into the salivary gland. During a blood meal the sporozoites will be discharged in the human blood circulation system, thus completing the life cycle.[15]

1.3 HEMOGLOBIN DEGRADATION

Within the erythrocytes the parasite matures in the cytoplasm and undertakes processes to degrade hemoglobin.[18] It is believed that the parasite breaks down the hemoglobin (major cytosolic protein of the host erythrocytes) as a source of amino acid for metabolism, growth and maturity since the *P. falciparum* has limited capacity to synthesise its own amino acids (**Fig. 1.3**).[19] The parasite makes use of a structure called a cytostome to transport hemoglobin from host erythrocyte cytosol to the digestive food vacuole in intracellular malaria parasites. The cytostome is a tubular structure formed from the vacuolar membrane that surrounds the intra-erythrocytic parasite and the parasite plasma membrane.[20] Hemoglobin-containing vesicles are transported from the cytostome and travel to the digestive vacuole where the hemoglobin hydrolysis occurs.

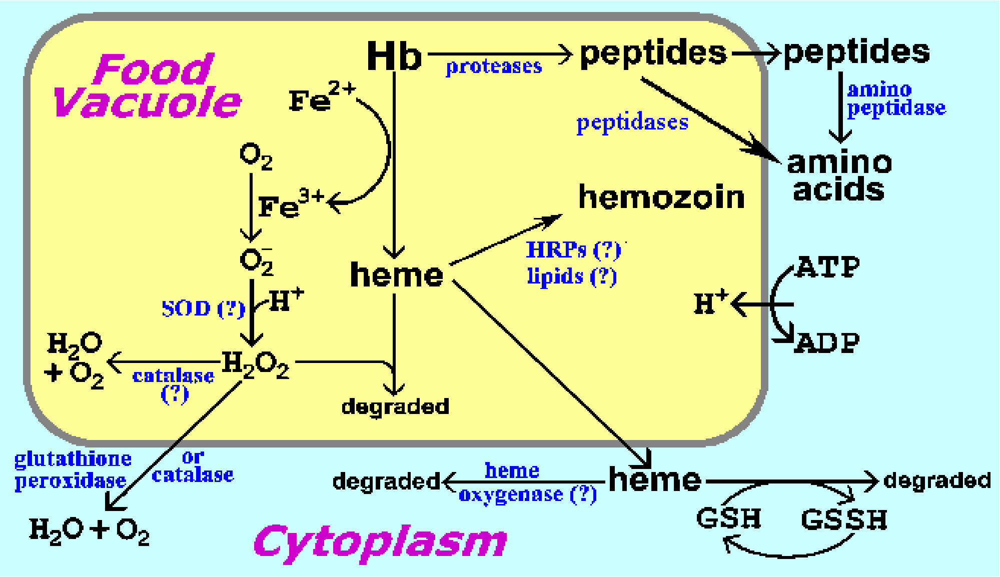
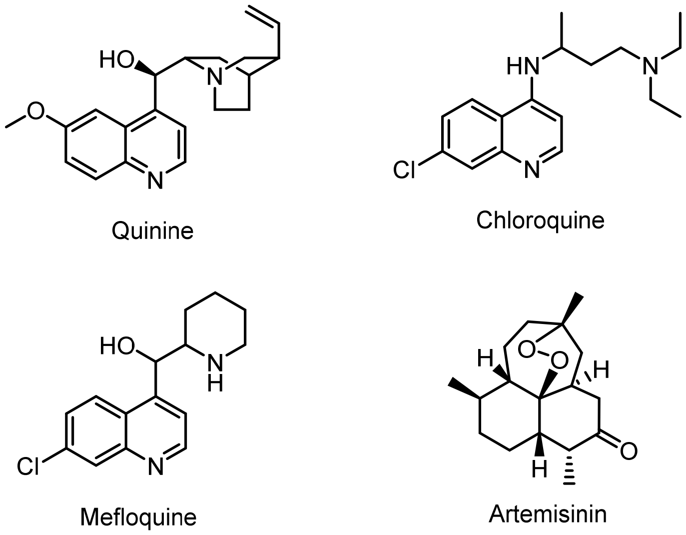


Figure 1.3: Hemoglobin degradation.[20]

The hydrolysis of hemoglobin is mediated by various proteases, which are capable of breaking down hemoglobin (**Fig. 1.3**). The aspartic proteases, which are very specific for hemoglobin degradation, separates the heme from the globin.[21] The heme moiety is composed of a porphyrin ring, which is complexed with iron (Fe) ion. Within the parasitic food vacuole, Fe(II)-protoporphyrin IX (FP) oxidizes into a toxic ferric form, Fe(III)-protoporphyrin IX (FP), that can damage the parasitic biological membranes and interfere with various enzymes including proteases. It has also been observed that during the oxidation process free radicals and hydrogen peroxides are produced resulting in the food vacuole being more acidic than the parasite can endure.[22] To overcome the unfavourable conditions, the parasite develops survival tactics to continue with degradation of hemoglobin with minimal drawbacks. One of its mechanisms is detoxification of the heme Fe(III)-protoporphyrin IX (FP) complex. The malaria parasite has evolved a biochemical solution to bio-crystallise heme to form hemozoin, which is non-toxic (**Fig. 1.3**). The hemozoin is deposited in the digestive food vacuole as an insoluble crystalline pigment,[23] often referred to as the malaria pigment.

1.4 CONTROL EFFORTS OF ERADICATING MALARIA

Historically, malaria was recorded as early as the 4th century as fever.[24] In those ancient times, traditional herbs were used to treat malaria. In China they used *Artemisia annua* to treat fever related illnesses.[25] *Kalanchoe pinnata* was also used in Africa, Asia and Latin America. Whilst, *Cinchona* plants were commonly used in Europe and Asia.[26] The use of these traditional herbs led to the development of modern drugs, like quinine (QN), chloroquine (CQ), mefloquine (MQ) and artemisinin (ART) (**Fig. 1.4**).[27] Despite efforts made over the years to come up with new antimalarial drugs and control of the vectors, to date malaria remains a burden in many countries, killing millions of people annually.



Quinine

Chloroquine

Mefloquine

Artemisinin

Figure 1.4: Chemical structure of early antimalarial drugs.

In the early 1900s, there were elevated cases of malaria in Asia and the rest of the world.[1] Introduction of QN resulted in a massive decrease of the mortality rate. This coincided with a period where dichlorodiphenyltrichloroethane (DDT) was heavily used as an insecticide to control vectors, which resulted in the decrease in deaths due to malaria (**Fig. 1.5**).[28]

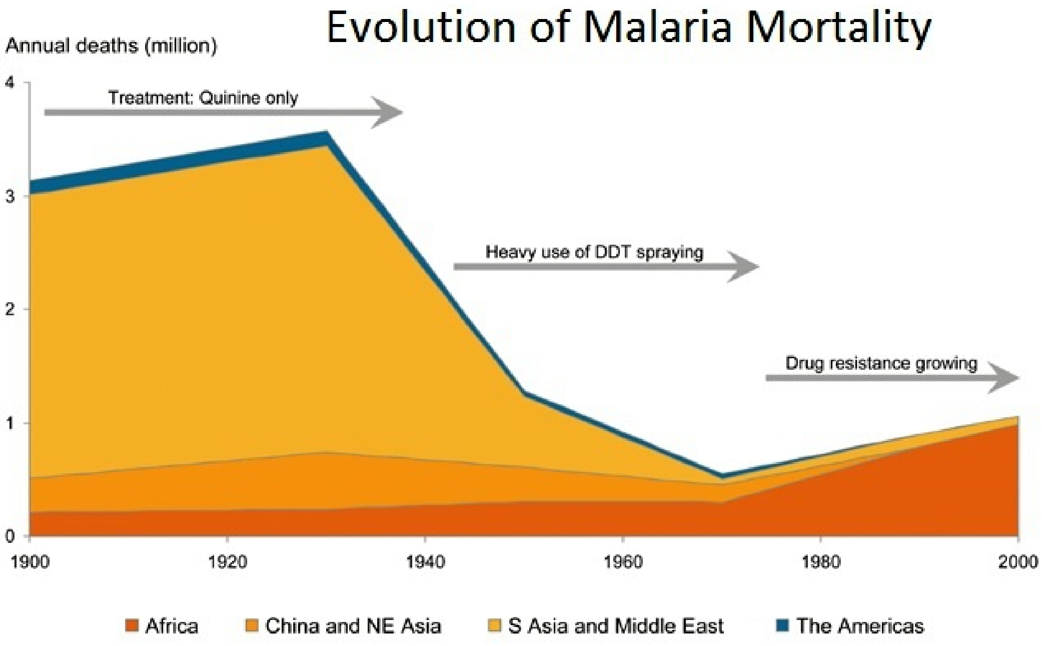


Figure 1.5: Malaria prevalence over the years.[29]

DDT is also a mosquito repellent and an irritant to the vectors. This makes vectors feed and rest outdoors, thereby reducing vector and host contact, ultimately contributing to effective disease-transmission control. There are safety concerns about the use of DDT including other insecticides of possible long-term toxicity. Over the years, many malaria-endemic countries switched to alternative insecticides, mostly pyrethroids and this compromised the vector-control programmes, leaving mosquitoes rampant and spreading the disease.[30,31]

1.1.3 The rise and fall of chloroquine

In the 1940s, the introduction of CQ as the first choice drug for treatment of malaria resulted in a significant decrease in mortality rate.[32] A decade later, the parasite developed drug resistance against the CQ.[33] This coupled with resistance against DDT in some parts of the world, resulted in the increase of death rate.[31] Chloroquine combination therapy became an option as the drug resistance had become wide spread. This combination chemotherapy generally offered a faster rate of clinical recovery, minimal adverse effects, cheap and effective (in earlier years) in a single dose.[34] High levels of resistance to one or both components (especially chloroquinolines) meant that chemotherapy was ineffective in many geographical locations.[35] Below are some non-artemisinin combination therapies that were used to combat drug resistance;

- Quinine (QN) + doxycycline

- Sulfadoxine-pyrimethamine (SP)
- SP + chloroquine
- SP + amodiaquine
- SP + mefloquine.[36]

The problem with the quinine doxycycline system was the comparatively complicated drug schedule, where quinine must be taken at 8 hour intervals for 7 days. There were also substantial undesirable side effects with the use of QN and tetracyclines, especially in children and pregnant women.[37] In the 2000s, these combination chemotherapies were only used to treat uncomplicated malaria (malaria sensitive to chloroquine based medication).[36] With the wide spread of resistance against CQ based chemotherapy, a switch to artemisinin based chemotherapy was recommended.[38]

1.1.4 Emergence of drug resistance

Following emergence of drug resistance against CQ, the hope for cure of malaria was brought about by the discovery of artemisinin and its derivatives. As wide spread resistance to CQ based treatment rose, artemisinin combination therapies (ACT) were introduced as the first line treatment for malaria. Generally, this combination therapy is very effective, even to CQ-resistant and complicated *P. falciparum* malaria.[39] Despite great efficacy, there are challenges associated with the use of ACTs, such as short plasma half-life, poor solubility in oil and water and a limited yield from nature resulting in elevation of the prices for malaria treatment.[40] Below are the combination therapies currently used;

- artesunate + sulfadoxine–pyrimethamine
- artesunate + mefloquine
- dihydroartemisinin + piperaquine
- artemether-lumefantrine
- artesunate + amodiaquine
- artesunate + pyronaridine tetraphosphate.[41]

In 2007, the Bill and Melinda Gates Foundation unveiled funds to help eradicate malaria.[42] Several other institutes such as the Roll Back Malaria (RBM) partnership of the World Health Organization (WHO) and other non-profit partnerships like Medicines for Malaria Venture (MMV) also partner in this vision. These partnerships have boosted antimalarial research, as evident by a numbers of potential antimalarial drug candidates in clinical trials and also an encouraging global antimalarial portfolio.[7]

1.1.5 Artemisinin-based chemotherapy and its limitations

Despite various efforts, drug resistance remains a major concern when it comes to malaria treatment. To the ACTs, which form first line drugs for treatment of malaria, *P. falciparum* has developed drug resistance as well. The cases of drug resistant strains were first recorded in areas around Cambodia and East Thailand in 2012, and it is likely to spread to Africa and the rest of the world, because the same pattern was observed with CQ resistance.[12,43] There are also serious pharmacokinetics and pharmacodynamics challenges faced by artemisinin.[44] Therefore, there is an urgent need to work on new antimalarial medications with novel chemical structures to counter-resistance, which are safe, cheap and easy to use. This will help increase the number of antimalarial drug candidates entering clinical trials, and eventually a long lasting cure and total eradication of this deadly disease.

1.5 PROMISING ANTIMALARIAL DRUGS IN CLINICAL TRIALS

There has been tremendous research efforts in the past decade from researchers worldwide regarding malaria treatment. This is evident in a number of antimalarials in clinical trials and and also a vaccine in developmental stage.[42] For example, **OZ349** (a second generation endoperoxide) is in phase II clinical trials for treatment of malaria. More importantly, it has pharmacokinetic properties superior to that of artemisinins and demonstrates clinical efficacy as a single entity.[45,46] **P218**, **DSM265**, **GN156** and **NITD609** are some of the drugs in clinical trials (**Fig. 1.6)**. **P218** was developed from pyrimethamine through structure-based design and it exhibits high affinity for both parent and mutant dihydrofolate reductase (DHFR) enzymes. This drug has a good pharmacokinetic profile and is highly efficacious with an effective concentration of 0.5 nM (EC_{50}) (**Fig. 1.6**) and has a good safety margins.[47] **DSM265** is a triazolopyrimidine-based compound with a novel mode of action, and it acts by inhibiting the *P. falciparum* enzyme dihydrooratate dehydrogenase (*Pf*DHODH). Like other drugs shown in **Fig. 1.6**, it has good potency and an acceptable safety profile.[48]

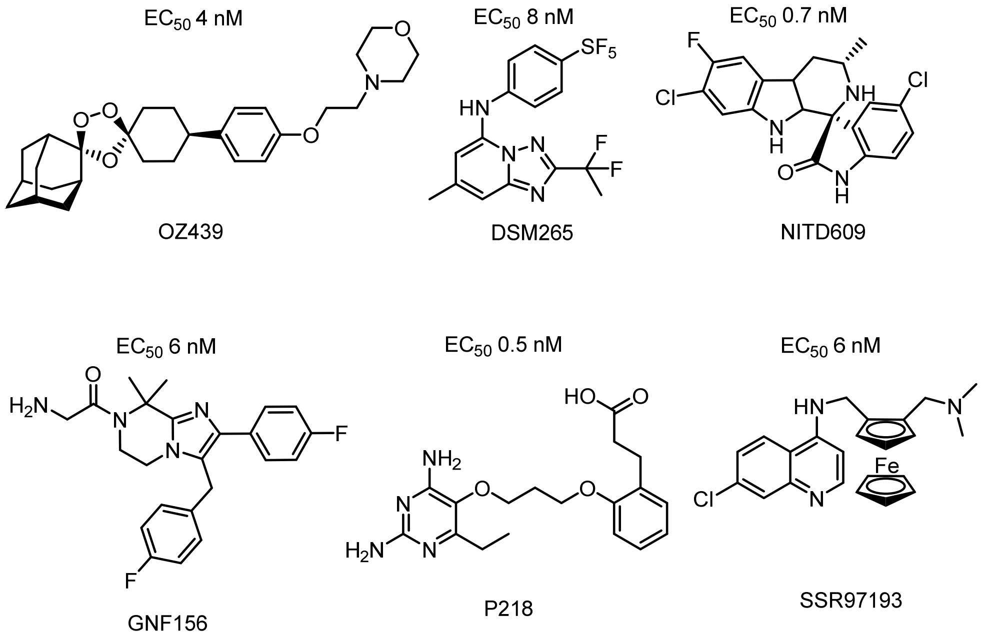


Figure 1.6: Some antimalarial drugs in clinical trials.

GNF156 is an imidazolopiperazine compound, with high activity against blood and liver stages of *P. falciparum in vitro* and *in vivo.* Interestingly, it has also been shown to kill gametocytes suggesting that this can potential block malaria transmission.[49] **NITD609** is a synthetic spiroindolone discovered by screening a library of natural products and ‘natural product-like’ compounds.[50] It has great efficacy and it is attractive in the sense that it clears both *P. vivax* and *P. falciparum* in patients infected with these parasites. Studies show that this compound acts by inhibiting the P-type sodium transporter ATPase (*Pf*ATP4) of the parasite, resulting in an increase of sodium ion concentrations in parasitic cells, and this leads to death by toxicity.[51] Ferroquine (**FQ, SSR97193**), a cutting-edge organometallic drug composed of a quinolone ring fused to a ferrocene moiety, is also an appealing drug currently in clinical trials. This drug is in advanced stages of phase II clinical trials as a treatment for uncomplicated malaria, and it is efficacious against chloroquine-sensitive and chloroquine-resistant strains of *P. vivax* and *P. falciparum* parasites.[52]

1.6 LATEST DEVELOPMENTS ON A MALARIA VACCINE

Another innovative development by GlaxoSmithKline Biologicals (GSK) and the Malaria Vaccine Initiative (MVI), with support from the Bill & Melinda Gates Foundation, is the development of a malaria vaccine (**RTS,S**). The vaccine is currently in phase III clinical trials and has shown 39% efficacy in infants.[53] However, there are limitations associated with this vaccine and these include its short lived efficacy, additional risk of febrile seizures within the first week of administration and its unsuitability for all age groups.[53] Despite the tremendous progress, the vaccine is not yet prequalified by the WHO, so chemotherapy remains paramount in the treatment of malaria.[42]

1.7 OTHER PROTOZOAN PARASITIC DISEASES

Our primary aim for this project being development of compounds for treatment of malaria, it was interesting to explore our target compounds for potential anti-trypanosomal activity against *Trypanosoma brucei*, a causative agent human African trypanosomiasis (HAT) or African sleeping sickness.

1.7.1 African sleeping sickness

Trypanosoma brucei (T. brucei) parasites are the causative agents of African sleeping sickness (HAT) in humans and Nagana (animal African trypanosomiasis) in cattle.[54] The parasite is transmitted to the host through a painful tsetse fly bite. This disease is endemic in sub-Saharan Africa, with tens of thousands affected annually and million others at risk of contracting the disease.[55] This disease is extremely dangerous as the parasite can travel through the blood-brain barrier and resides in the brain cells leading to neurological death with symptoms of hallucinations, sleep disorder, coma and eventually death.[54] The sub species responsible for Nagana (*T. brucei brucei*) is not infective to humans and is commonly used for drug screening. There are a wide range of benzoxaborole compounds that exhibit antitrypanosomal activity.[55-57]

1.8 HYBRIDIZATION STRATEGY

In the quest for new effective drugs to address challenges faced by drugs currently in the market, scientists employ various strategies to identify new effective drugs and these approaches include:

- Inventive chemical manipulation of old drugs;
- Improving efficiency of existing drugs e.g. quinolines and artemisinin derivatives;
- Affirmation of novel molecular targets using information of parasite physiology and biology.[43]

Scientists can make use of all these three strategies at once by using hybridisation as a tool of drug discovery, in a hope to produce safe and efficient new drugs to cure malaria.[58] Hybridisation is a very appealing approach in drug discovery and it involves combining two or more pharmacophores with multiple pharmacological targets offering diverse modes of action, improved efficacy, cheaper synthetic routes and counter-resistance.[59] **Fig. 1.7** shows various ways of chemically combining two pharmacophores to form various hybrid molecules.

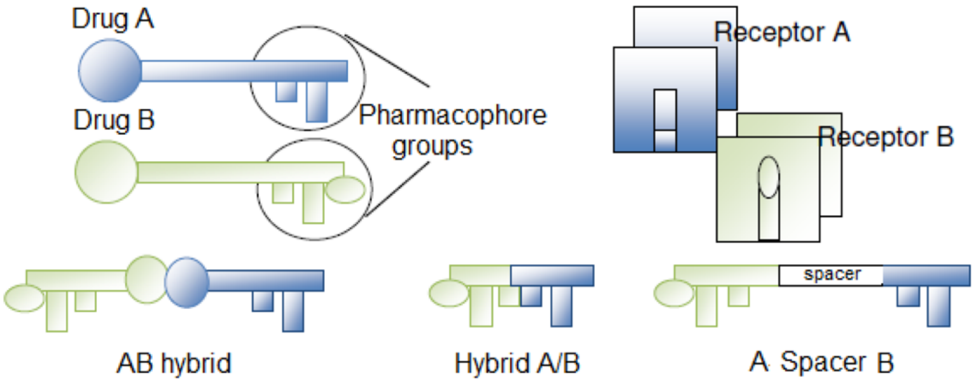


Figure 1.7: Molecular hybridisation strategy.[60]

Classification of hybridized compounds can be described as:

- **Conjugates:** Pharmacophoric centres for each target are joined by a linking (spacer) group that is absent in either of the individual drugs like **A spacer B** (**Fig. 1.7**). It has been observed that most conjugates contain a linker that is metabolically stable.[61,62]
- **Cleavage conjugates**: Pharmacophoric units are joined together by a linker that is susceptible to metabolism to release the two drugs that interact independently with their respective complementary targets.
- **Fused hybrids:** Molecules contain a linker of reduced size such that the frameworks of the pharmacophoric units are essentially touching **AB Hybrid** (**Fig. 1.7**).[58]
- **Merged hybrids:** Combined pharmacophoric frameworks by taking advantage of common groups in the structures of the starting compounds, and this gives rise to smaller and simpler molecules like **Hybrid A/B** on (**Fig. 1.7**).[62,63]

The hybridization strategy has shown some successes in cancer therapy.[64] Many researchers are using this strategy to develop novel drugs to combat drug resistance. Hybridisation offers a possible solution in addressing the drug resistant complications of the *P. falciparum* parasite and the drugs may be accessed through cheaper synthetic routes and are more effective compared to combination therapy.[65] In modern clinical setups the common practice is poly-pharmacy, whereby patients take a number of drugs at the same time to treat multiple co-ailments or for increased efficacy. There is often a need to increase dosage for reduction of drug resistance risks; this is common in the treatment of infectious diseases.[64] However, this does not fully address the issue of drug resistance since it remains a major concern globally.

1.8.1 Hybridization, combination therapies and prodrugs

In current malaria treatment, artemisinin-based chemotherapies (ACTs) serve as the first line treatment option recommended by the WHO in endemic countries.[44,60] The strategy of combination therapies takes advantage of possible synergistic or additive pharmacokinetics and pharmacodynamics activities. The rational co-administration of two or more bioactive compounds is likely to elicit an overall biological response that is superior to that achieved when either of the compounds is administered individually.[44,66] In this approach, the synergistic effect demonstrated by co-administering of two drugs results in achievement of more rapid therapeutic outcomes by using effective lower doses and minimizing the risk of concurrent development of resistance to the co-administered drugs by the targeted microorganism.[65]

Hybridization is quite different from prodrug strategy which is particularly useful when addressing the pharmacokinetics and pharmacodynamics profiles of a promising drug candidate with poor bioavailability.[67] The attractiveness of hybridization strategy is based on its potential to contributing to the discovery of highly efficacious novel chemical entities often with dual modes of action. Hybridization is considered a success when it leads to effects that are more pronounced than those of either parent compound. Other advantages that could be offered by hybridization include low dosage (implying reduced toxicity), minimum risk of drug-drug interactions and cheaper pre-clinical evaluations.[62] Hybridization at the same time results in delaying or evading the development of resistance. In choosing pharmacophores for hybridisation, one has to consider the activity of these pharmacophores against the target parasite and their individual modes of actions.

1.8.2 The downside of hybridization strategy

When hybridising pharmacophores into a single molecule, ideally the drug should have greater activity than its independent pharmacophores. Furthermore, it is also anticipated that some of the liabilities exhibited by the individual parent entities will be addressed in the hybrid drug, but recent studies prove otherwise.[68] In hybrid drugs, it is likely that various patients react differently to relative rates of metabolism of the hybrid drugs. This may be a major drawback as it can produce highly complex pharmacokinetic (PK) and pharmacodynamics (PD) relationships; this might result in prolonged and expensive clinical studies. Prescribing combinations therapies that offer greater dose flexibility may be preferred because of lower cost treatment, especially in generic drugs.[61]

The hybridised drugs may show undesirable effects like high toxicity and pharmacokinetic liabilities, which may have not been present in the individual pharmacophores.[68,69] For instance, Thelingwani and colleagues reported artemisinin-chloroquine hybrids with excellent efficacy (similar to artemisinin), potential to inhibit hemozoin formation, and moderately selective to *P. falciparum* compared to mammalian cells (selectivity was less than that for CQ). On the contrary, these hybrids had a shorter half-life (rapid metabolic clearance) compared to that of CQ, and inhibited major cytochrome P450 (CYP) enzymes, which are responsible for their metabolism.[68,69] All the 5 of 6 major CYP isoforms were inhibited and this raised concerns about the risk of drug-drug interactions with the substrates requiring these enzymes for metabolism.[70] This study led to the conclusion that hybrid drugs are highly advantageous over combination therapy; however, they can also lose some favourable absorption, distribution, metabolism and excretion (ADME) properties and give rise to new liabilities that would need to be addressed.[69]

1.9 CHLOROQUINE HISTORY AND PROPERTIES

Chloroquine (CQ) was initially synthesised in 1934 by Torsell and rose to be the preferred antimalarial drug.[32, 71] Due to drug resistance, WHO recommends ACTs as first line treatment for malaria, but world-wide deployment of ACT is limited by factors such as imbalance in demand and supply, safety in pregnancy and higher cost of treatment as mentioned previously.[44] Although the resistance to CQ and associated 4-aminoquinoline antimalarial drugs has arisen, the designing of innovative antimalarials containing the quinoline pharmacophore has discrete benefits due to exceptional pharmacological effect of 4-aminoquinoline remedies.[72]

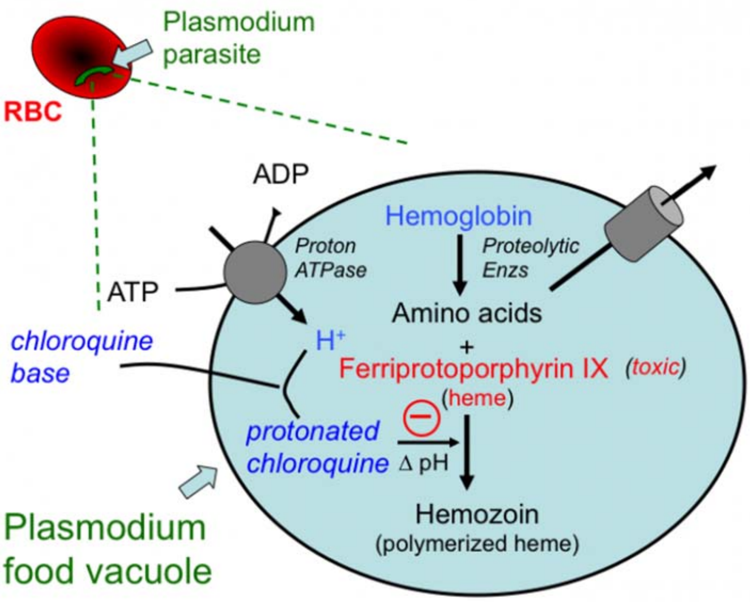


Figure 1.8: Proposed CQ inhibition process in parasite digestive (food) vacuole.[73]

Since CQ is a diprotic weak base (pK_{a1} = 8.1, pK_{a2} = 10.2) in its unprotonated form, it diffuses through the membranes of the parasite infected erythrocyte and accumulates in the acidic digestive vacuole (DV) (pH 5 – 5.2) where it becomes protonated to form CQ^{2+}, **Fig. 1.8**. Inside the DV, CQ can therefore not leave by diffusion because, in its protonated ionized form, it cannot diffuse across the lipid membrane of the DV; this phenomenon is called pH trapping, whereby, CQ accumulates in the proton-rich vacuole.[74] Within the DV it binds to heme (FP Fe (II)) and/or the hydroxo- or aqua complex of heme (ferriprotoporphyrin IX, Fe (III) FP) through π-π stacking (**Fig. 1.9**) and therefore prevents the detoxication of heme to form insoluble dimeric/polymeric hemozoin.[75]

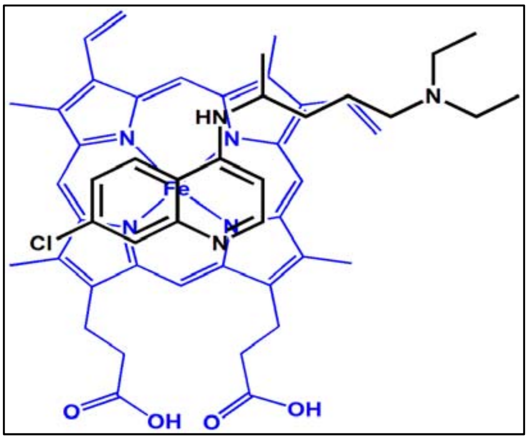


Figure 1.9: Proposed pi-pi stacking between CQ and heme.

Accumulation of the heme will eventually lead to the death of the parasite.[72] However, antimalarial drugs that are quinoline-based have been greatly compromised over the years due to the development and spread of parasite resistance.

1.9.1 Mechanism of chloroquine resistance

The *P. falciparum* parasite has developed a mechanism of removing CQ from its digestive vacuole by efflux.[76] CQ resistance is due to mutations in the *Pf*CRT (*P. falciparum* chloroquine resistance transporter) gene that encodes for the protein and is thought to facilitate efflux of the drug from the food vacuole (DV) of the parasite resulting in the reduction of drug concentrations (**Fig. 1.10**).[77] The resistant *P. falciparum* parasite discharges chloroquine 40 to 50 fold more swiftly than the vulnerable parasite. The variance in the rates of chloroquine discharge from digestive vacuole is dependable to the differences in the steady-state escalations of chloroquine and in their chloroquine therapeutic doses.[78] Studies of CQ uptake kinetics have shown that CQ-resistant *P. falciparum* parasites amass substantially less CQ than CQ-sensitive strains.[74] Other studies disclose that *Pf*CRT variants can also distress parasite fitness, guard immature gametocytes against chloroquine action, and alter *P. falciparum* vulnerability to present-day first-line therapies.[33]

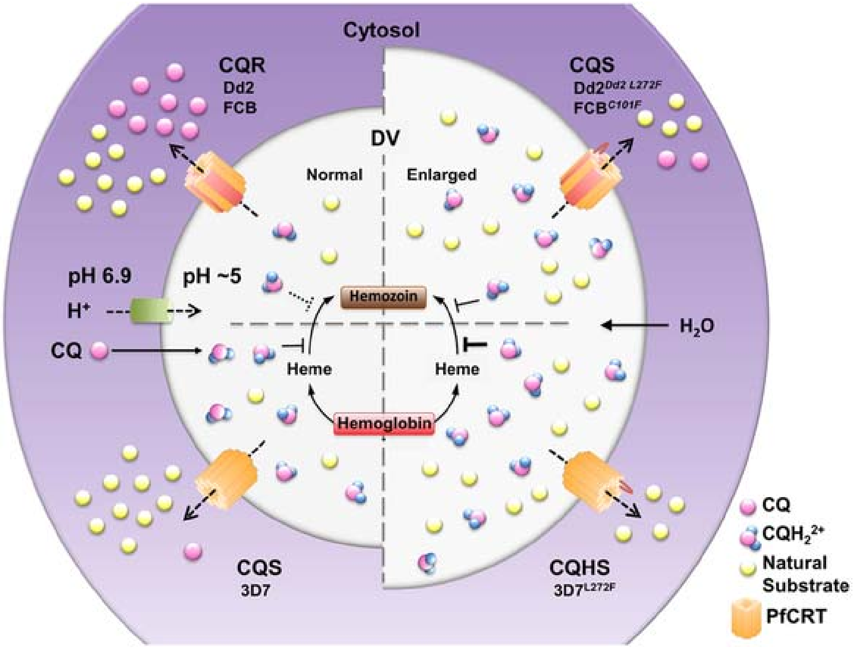


Figure 1.10: Mechanism of CQ efflux by *P. falciparum*.[79]

Modifications of most CQ based drugs are often made on the alkyl side chain of CQ since it is the site for parasite resistant recognition.[80] Availability of a secondary or tertiary amine on the side chain modification should be taken into consideration as the amino group will enhance weak base properties of CQ resulting in pH trapping of CQ in the digestive vacuole.[72]

1.10 BENZOXABOROLE AND BORON COMPOUNDS IN DRUG DISCOVERY

Benzoxaboroles (**Fig. 1.11**) are a class of compounds made up of a benzene ring fused with an oxaborole ring. In 1957, benzoxaboroles were first synthesized and characterized.[55] They have a very stable oxaborole ring; the boron–carbon bond is highly hydrolytic-resistant and has better solubility in water compared to the corresponding boronic acids. In recent years, applications of benzoxaboroles have been receiving much attention in various fields such as organic chemistry, glycopeptides recognition[81], supramolecular chemistry[82] and medicinal chemistry.[83]

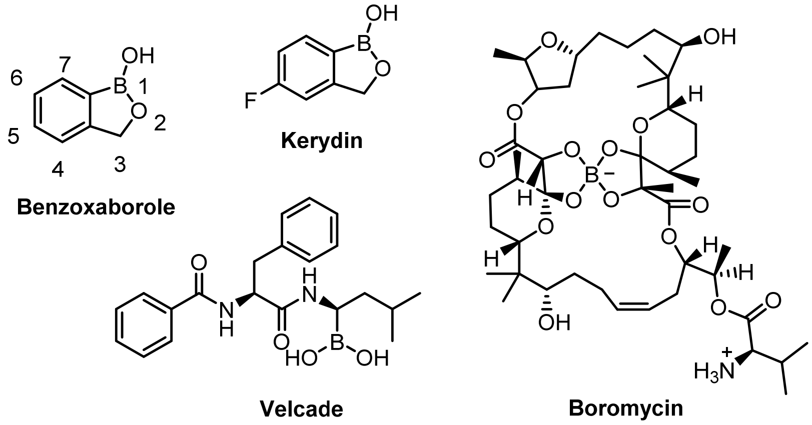


Figure 1.11: Chemical structure of benzoxaborole and boron-based drugs

In medicinal chemistry, applications were sparked by the discovery of Velcade in 1999 and Kerydin (5-flurobenzoxaborole (AN2690)) in 2006 (**Fig. 1.11**).[84] The latter was found to have antifungal activity while the former is used to treat relapsed mantle cell lymphoma. Subsequently, tremendous research efforts revealed benzoxaboroles with broad bioactivities including antibacterial,[57] antiviral,[85] anti-parasitic,[54,86] and anti-inflammatory.[87] Boron containing compounds exist mostly as boric acid in nature and it is a vital plant nutrient.[88] Humans get an average of 3 – 7 mg boron daily from fruits, vegetables and nuts. The first naturally occurring boron compound to be isolated was boromycin (**Fig. 1.11**), which is a polyether macrolide antibiotic that is active against Gram-positive bacteria.[89] Boromycin acts by targeting the cytoplasmic membrane of Gram-positive bacteria and effects a release of potassium ions.[89] In addition, boromycin has been demonstrated to have anti-HIV activity.[90] Currently, AN2690 (Kerydin) is already on the market for treatment of fungal infections, while AN5568 (SCYX-6759) is in clinical trials for the treatment of stage 2 HAT.[56] The benzoxaborole AN2728 is currently being investigated for activity against psoriasis and atopic dermatitis.[91]

1.10.1 Proposed mode of action of benzoxaboroles

The benzoxaboroles are attractive due to their high affinity for diols under physiological conditions (**Fig. 1.12**).[90] It is believed that AN2690 disrupts antifungal protein synthesis through the inhibition of leucyl tRNA synthetase (LeuRS).[57] This drug was recently approved as a topical agent for treatment of onychomycosis. In amino acids translation, the enzyme LeuRS leucincyl-transfer RNA synthetase ensures that the translation occurs accurately by proof-reading the 3'-aminoacylated end of *t*RNALeu from the synthetic active site.[92] Due to this high affinity for diols, benzoxaboroles bind to the *cis*-diols of the ribose sugar on the terminal ribonucleotide of *t*RNALeu to form a tetrahedral complex, thereby trapping the benzoxaborole in the 3' end of *t*RNALeu at the editing site,[83] and thus preventing the translocation the 3' end of *t*RNALeu to the synthetic active site (**Fig. 1.12**). This ultimately inhibits protein synthesis due to failure of *t*RNALeu aminoacylation.[92]

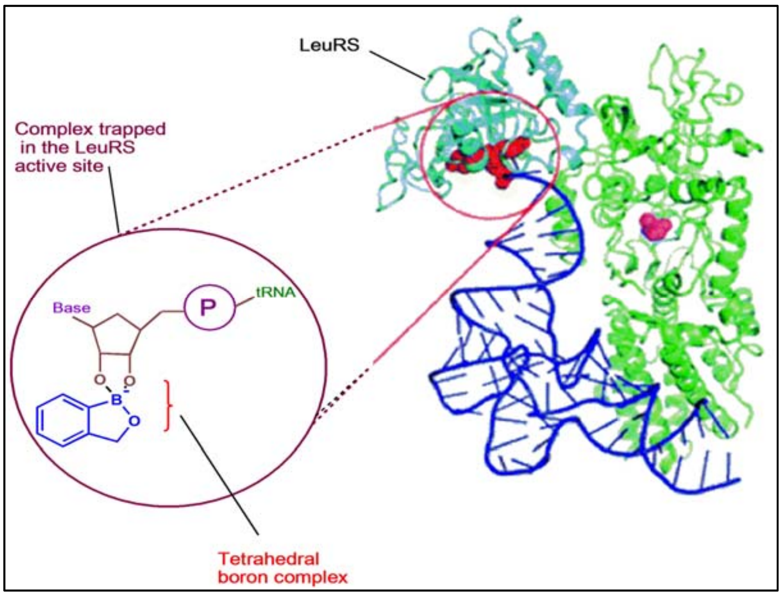


Figure 1.12: Inhibition of Leucine tRNA by benzoxaborole

1.11 CINNAMIC ACIDS AND THEIR PROPERTIES

Cinnamic acids are naturally occurring products, belonging to the class of auxin and found in fruits, vegetables, flowers, plants and are ingested by humans as dietary phenolic compounds.[94] This class of compounds are found throughout *Kingdom Plantae* and are constituents of most natural oils and resins; and they play various important roles in plant metabolism. Generally, they are found in all green plants in minute quantities, in the reproductive organs of flowering plants. These compounds exist in *trans* or *cis* structures. *Trans*-cinnamic acid has long been used by humans in flavourings and scents.[95] In the 19th century, *trans*-cinnamic acid, which is characteristically found in cinnamon (*Cinnamomum verum*) and storax (*Liquidambar orientalis*), was used for treatment of tuberculosis (TB).[96] Novel substituted cinnamic acid scaffolds have been employed for the development of selective anti-TB agents.[97] *O*-prenylation and *O*-geranylation of 4-hydroxycinnamic acids have been found to yield enhanced anti-TB efficacy in a series of azide, thioester and amide derivatives. [98] Deamination of phenylalanine in plants and bacteria and consecutive enzyme-catalyzed reductions starting from the corresponding cinnamyl CoA-esters, produce natural hydroxyl cinnamic acids (**Fig. 1.13**) like *p*-coumaric acid,[99] caffeic acid,[100] ferulic acid,[101] and sinapic acid.[98,102] The cinnamic acids are also present in diverse secondary metabolites of phenyl-propanoid biogenesis origin.[95] Cinnamic acids play many vital roles in plants, such as;

- Regulating cell growth and differentiation (as plant hormones),[93]
- Oxidative polymerization of the hydroxyl cinnamic acids in the biochemical process for the formation of lignin in plants,[103] which is the most abundant biopolymer.

Ferulic acid **Caffeic acid**

Coumaric acid **Sinapic acid**

Figure 1.13: Naturally occuring cinnamic acids

Derivatives of *trans*-cinnamic acid have drawn much attention due to their antitubercular,[104] antimalarial,[7,105] leishmanicidal and anti-trypanosomal properties.[106] Sharma Prateek (2011) reported that natural cinnamic acid derivatives show activity against a wide range of ailments and conditions, which include antidiabetic, cytotoxic, antioxidant, antimicrobial, hepatoprotective, CNS depressant, antiviral, anxiolytic, anticholesterolemic, antifungal and fungitoxic, antihyperglycemic, and anti-inflammatory.[104]

1.11.1 Antimalarial properties of cinnamic acids

In drug discovery, cinnamic acids are often utilized in the design of bioactive molecules due to the presence of a β-unsaturated system, which can be regarded as a pharmacophoric unit since it is a Michael acceptor.[107] Cinnamic acids play an essential role in the genesis of commercially key intermediate molecules which are imperative for the production of diverse pharmaceutical ingredients.[106]

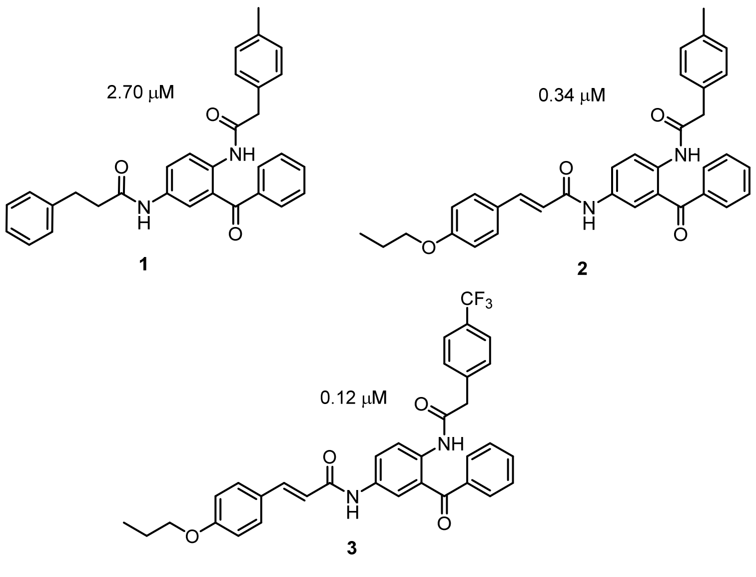


Figure 1.14: Cinnamic acid based antimalarial lead compounds.

From a high throughput screening (HTS) campaign, Wiesner *et al* identified compound **1** (**Fig. 1.14**) as a novel class of anti-malarial agents.[108] The structure-activity relationship studies (SARs) around this compound revealed that replacement of the phenylpropionyl group of the lead **1** by a 4-propoxycinnamic acid resulted in **2**, which showed an improved anti-malarial efficacy by 8-fold in comparison to compound **1**.[109] This further emphasises that the cinnamic acid phamacophore exhibits antimalarial properties. With further structural modifications on compound **2**, replacing the *para*- methyl group with a trifluoromethyl group (**3**) resulted in an improved antimalarial efficacy by a 3-fold measure.[110] A further modifications resulted in improved antimalarial activity even greater than that of CQ.[105] Cinnamic acid derivatives' (CADs) antimalarial modes of action are known to be due to:

- Inhibition of carrier-mediated lactate and pyruvate transport across cellular plus mitochondrial membranes.[111]
- Inhibiting the transport of glucose, glycine and lactate.[105]
- Inhibitors to all phases of parasite growth.

1.12 AIMS AND OBJECTIVES OF CURRENT STUDY

1.12.1 Objective

The primary objective is to synthesise novel benzoxaborole hybrid compounds derived from bioactive scaffolds with *in vitro* antiplasmodial and antitrypanosomal potencies.

1.12.2 Specific aims

- To synthesise and characterised benzoxaborole-based hybrids as bioactive small molecules.
- To pharmacologically evaluate synthesised hybrid compounds for their *in vitro* biological activity against *P. falciparum* and *T. brucei.*

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CHAPTER TWO

2 SYNTHESIS AND CHARACTERISATION OF BENZOXABROLE-BASED DERIVATIVES

2.1 INTRODUCTION

This chapter describes the synthesis and characterization of benzoxaborole based hybrid compounds as novel potential antiplasmodial and antitrypanosomal agents. The synthesis of these hybrids was conducted using conventional synthetic methods and resultant compounds were fully characterized using common analytical techniques such as NMR, MS, HPLC and melting point determination.

2.1.1 Rationale: Pharmacophore hybridization (chloroquinoline and cinnamoyl with benzoxaborole motif)

The antimalarial activity of CQ has long been established and the mode of action (i.e. inhibition of hemozoin formation) is well studied. CQ has received much attention from many researchers due to cheap synthesis, great efficacy and outstanding pharmacokinetics and pharmacodynamics.[1] However, CQ and its corresponding analogues have lost their appeal given the parasite developed resistance against aminoquinoline based antimalarial drugs.[2] One way to restore activity of old organic drugs facing resistance is to modify the structure or append another pharmacophore yet still maintaining key structural features required for activity. During structural modification, CQ-based compounds often retained key structural features which are important for activity. These include the 4-aminoquinoline nucleus, which is required for hematin association, the 7-chloro group necessary for optimal inhibition of hemozoin formation, and amino groups in the side-chain, which serve as basic sites to facilitate drug accumulation by pH trapping.[3] To date, a number of CQ based hybrid compounds have been reported,[4,5] and majority of these compounds showed great potency against *P. falciparum* sensitive (IC_{50} values are shown in **Fig. 2.1**) and resistant strains, though some showed undesirable side effects.[6]

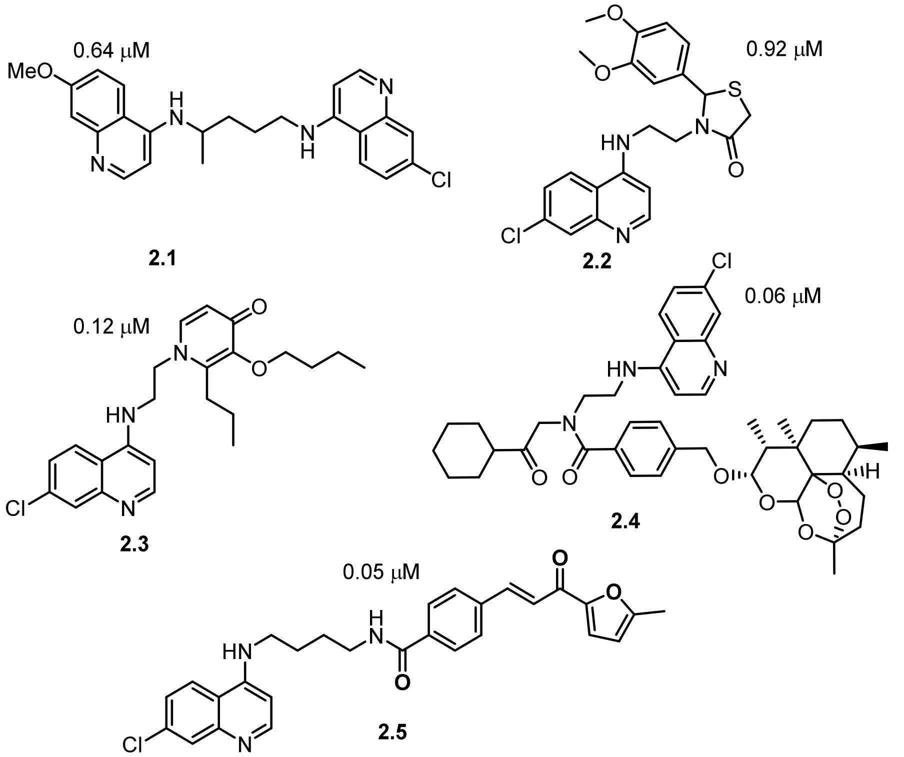
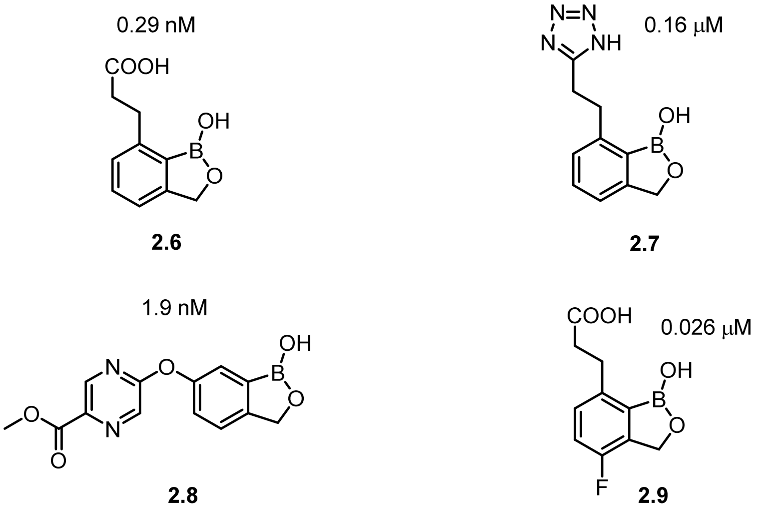


Figure 2.1: CQ-based hybrids as potent antimalarial drugs.

On the other hand, benzoxaboroles have received much attention due to their promising antiparasitic activities that recently surfaced in literature. Despite this, the chemistry of these compounds has been largely ignored, and the pioneering work of scientists from Anacor Phamaceutical in the development of benzoxaboroles as drugs for treatment of various ailments sparked interest to pursue this class of compounds as promising therapeutic agents.[7-10] Thus far, there are a number of benzoxaborole derivatives with outstanding activity against both drug sensitive and resistant strains of *P. falciparum* parasite that have been synthesized.

More importantly, benzoxaborole compounds such as **2.6 – 2.7** have been shown to exhibit promising antiplasmodial activity against *P. falciparum* strains. Some examples include among others 6-(2-(alkoxycarbonyl)pyrazinyl-5-oxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaboroles (**2.8**)[7] and fluoro-substituted 7-(2-carboxyethyl)-1,3-dihydro-1-hydroxy-2,1-benzoxaboroles (**2.9**) **Fig. 2.2.**[11] As alluded in the previous chapter, these compounds are being comprehensively studied for activity against the malaria parasite. The general mode of action of benzoxaboroles is perceived interesting, and it involves the prevention of protein synthesis by inhibiting the aminoacylation of the terminal ribose sugar of leucyl-tRNA synthetase.[12]

However, their mode of action against *P. falciparum* is yet to be fully studied and validated.[12] The structure activity relationship studies (SARs) of benzoxaborole based compounds suggests that the unsubstituted benzoxaborole structure is important for activity as it is responsible for inhibiting the protein synthesis.



2.6 **2.7** **2.8** **2.9**

Figure 2.2: IC_{50} values of benzoxaborole based antimalarial agents.

In most cases the structural modification of benzoxaborole based drugs is done by substitution at any position of the benzene ring of the benzoxaborole motif,[13] and positions 5, 6 and 7 on the benzene ring have been the most studied parts of this motif. Modifications can also be done on carbon (position 3) of the oxaborole ring. The significant biological importance of benzoxaborole against malaria and other infectious diseases, for example HAT, prompted us to design the CQ-benzoxaborole hybrid compounds and explore their biological activities against infections caused by the *P. falciparum* and trypanosomes parasites.

Our hypothesis is that the coupling of the CQ core with bioactive benzoxaborole unit would yield hybrid molecules with enhanced activity. In modification of CQ based drugs the obvious starting point is changing the alkyl side chain of CQ since it is the site for parasite resistance recognition (**Fig. 2.3**).[3] In this study, we proposed and limited structural changes of envisaged hybrid compounds along the alkyl side chain between the CQ and benzoxaborole pharmacophores. Herein, our focus is on hybridization of two pharmacophores into a single agent, with the length of the linker chain being varied (**Fig. 2.3**).

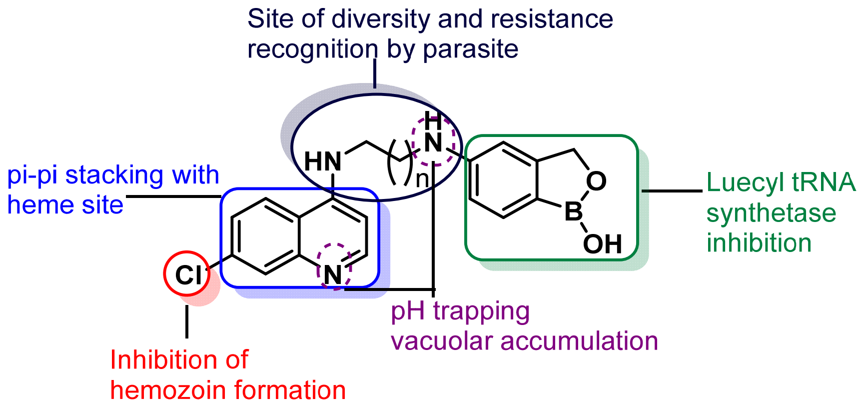
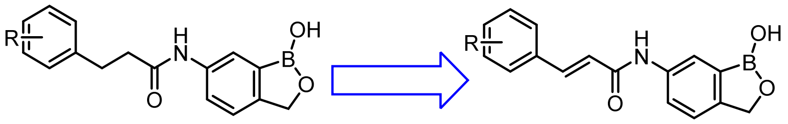


Figure 2.3: Hybridization of chloroquinoline and benzoxaborole.

Another class of compounds, which has attracted our attention, involves cinnamic acids (CAs). As stated in the previous chapter, CA compounds exhibit a broad range of biological properties including antiplasmodial activity.[14] Their mode of action against *P. falciparum* malaria is believed to be via the inhibition of carrier-mediated lactate and pyruvate transport across cellular and mitochondrial membranes.[15] In this study, we focused on the hybridization through an amide bond of various cinnamoyl derivatives with benzoxaboroles to form a new class of cinnamoyl-benzoxaborole hybrid compounds as shown in **Scheme 2.1**. The design of cinnamoyl-benzoxaborole hybrids was inspired by compound **2.10** (**Scheme 2.1**), which showed great activity (IC_{50} = 0.4 μg/mL) against *Trypanosoma brucei* strain S427. In designing these hybrids, we thought of replacing the phenyl and alkyl chain with cinnamic acid moiety and investigate the activity of resulting compounds against *P. falciparum* and *T. brucei*. In cinnamic acids, the α,β-carbonyl system commonly referred to as Michael acceptor, is considered an important pharmacophoric unit. However, others have described the α,β-carbonyl system as a toxicophore and often the toxicophores are excluded to limit the risk during drug discovery and development.[16]


IC50 (T. brucei S427) = 0.4 mg/mL

2.10 **2.11**

Scheme 2.1: Proposed cinnamoyl-benzoxaborole conjugates.

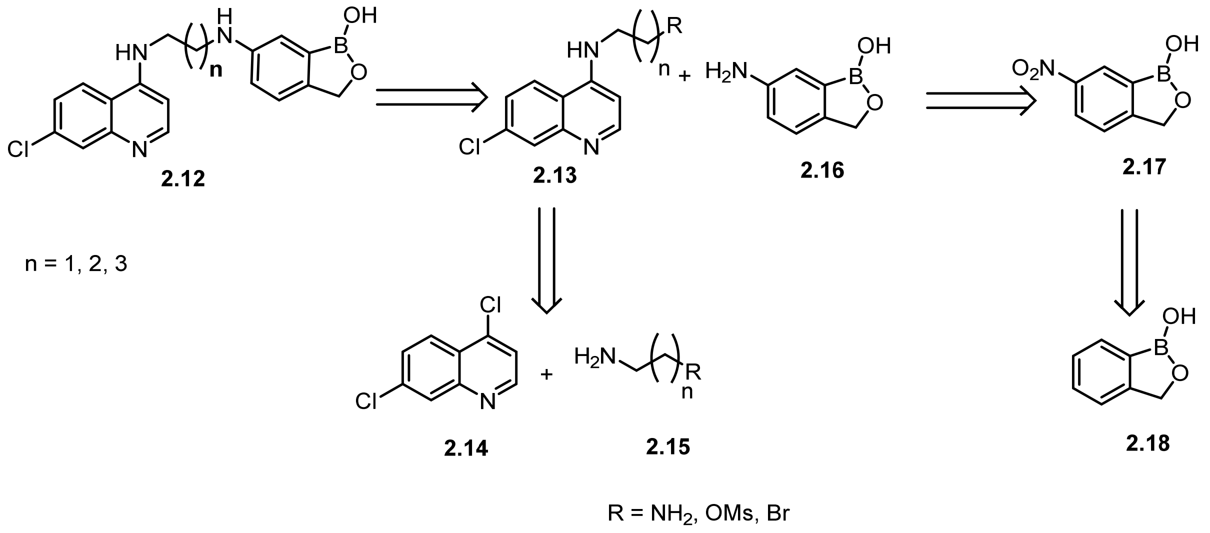
In cinnamoyl-benzoxaborole hybrids, we focus on hybridization of two pharmacophores into a single agent through the amide bond as a linker, with variations limited to the phenyl ring of the cinnamoyl portion of the proposed structures. We aim to assess the antiplasmodial and antitrypanosomal activities of the hybrids in comparison to their parent drugs. These hybrids are quite novel and they can hold potential to serve as a monotherapy or in combination with other clinically established drugs, and at the same time interacting with multiple targets.

2.2 RESULTS AND DISCUSSION

2.2.1 SYNTHESIS OF BENZOXABOROLE-BASED CHLOROQUINOLINE DERIVATIVES

2.2.1.1 Retrosynthesis analysis

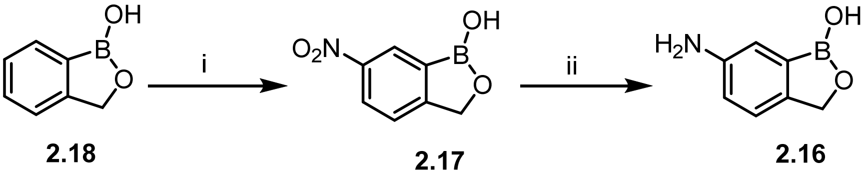
The synthesis of desired benzoxaborole-based chloroquinolines (**2.12**) was envisaged as achievable from commercially available 4,7-dichloroquinoline (**2.14**), aminoalcohols (**2.15**) and the key starting benzoxaborole (**2.18**).



Scheme 2.2: Retrosynthetic analysis of benzoxaborole-based aminoquinoline compounds.

2.2.1.2 Synthesis of key starting benzoxaborole

The synthesis of target compounds began with nitration of compound **2.18**. The nitration of aromatic rings is typically carried out using a mixture of nitric acid and sulfuric acid, with sulfuric acid acting as a catalyst, and reacting with the nitric acid to generate a strong electrophile in situ, the nitronium ion (NO_2^+).[17,18] In literature, nitric acid and sulfuric acid have been tried to nitrate the benzoxaboroxole, however, this resulted in low yields (34 %) and often long reaction times were required. Thus, 16 M HNO_3 is commonly used for nitration of benzoxaborole.[19] Thus, the commercially accessible benzoxaborole (**2.18)** was treated with 16 M fumic nitric acid (99.9 %) at a temperature ranging between -30 and -40 °C (**Scheme 2.3**). The mixture was allowed to stir at this temperature (-30 and -40 °C) for 3 h. The TLC showed disappearance of the starting material and a new spot could be observed. Nitration using 16 M fuming nitric acid (99.9 % HNO_3) at -40 °C was successful and the work-up was simply filtration and recrystallization in EtOAc to afford compound **2.17** as a pale yellow solid with 89 % yield.[20]



Scheme 2.3: Reagents and conditions i) -40 °C, fumic HNO_3, 3 h; ii) EtOH, 10% Pd/C, H_2, 2 h.

The formation of a nitrated compound **2.17** was confirmed by the ^1H NMR, and the spectrum showed a singlet at δ 9.60 ppm which is consistent with the B-OH. The protons corresponding to aromatic region appeared downfield compared to the unsubstituted benzoboroxole aromatic protons, which showed chemical signals between δ 7.76 – 8.57 ppm (**Fig. 2.4**). At δ 8.31 ppm, the signal peak due to H5 appeared as a double of doublets and the two other aromatic peaks due to H4 and H7 could be observed as doublets, and thus confirming the position of $-NO_2$ substituent. The $-CH_2$ group integrating for 2H could be observed as a singlet peak appearing at δ 5.11 ppm.

Theoretically, the benzoxaborole is expected to show seven signals on ^{13}C NMR, but practically only six signals were observed, which were agreeing with the aromatic unit and the presence of $-CH_2$ carbon in **2.17**. The quaternary carbon adjacent to boron atom does not show up on ^{13}C NMR[21]. This phenomenon has been noted by various researchers who observed a similar behaviour by benzoxaboroles.[10,21,22] Previous studies also suggested that the ^{13}C signal of the carbon adjacent to boron atom is significantly broadened, and therefore making it hardly visible on the ^{13}C solution NMR spectra, but can be observed on solid state ^{13}C NMR[21].

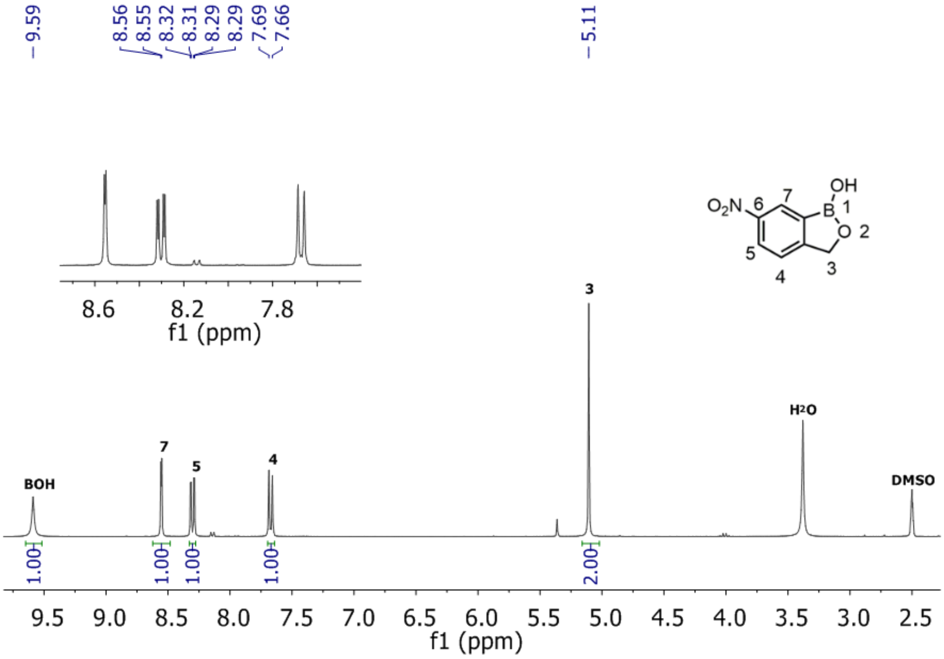
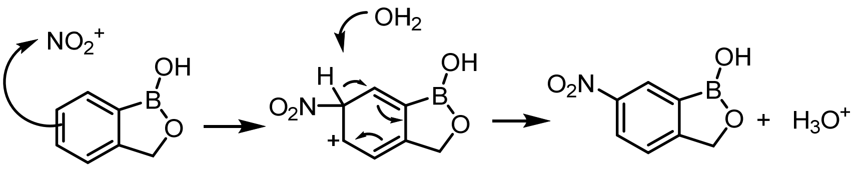


Figure 2.4: ^{1}H NMR spectrum (DMSO-d_6) of 6-nitro benzoxaborole.

Nitric acid has both acidic and basic properties,[23] and at temperatures close to its freezing point (-42 °C), it is able to undergo an autoprotolysis reaction or self-dehydration and form its own nitronium ions together with nitrate ion and water (NO_2^+, NO_3^- and H_2O).[23] As shown in **Scheme 2.4**, the π electrons of the benzene ring attacking the nitronium ion, have its aromaticity disrupted and give a resulting carbocation on the para position. The intermediate sigma complex then loses a proton to form 6-nitrobenzoxaborole as reported in the literature.[10] The boron, with an empty orbital, is a strong electrophile, substitution in the para position is therefore less favored, as it causes the positive (+) charge to build up at a carbon bearing the boron substituent. This problem does not exist for the resonance structures from meta substitution, hence the substitution occurring at position 6.[10,23]



Scheme 2.4: Reaction mechanism for the formation of 6-nitrobenzoxaborole.

The second step (**Scheme 2.3**) involves the conversion of 6-nitro benzoxaborole to form the desired aminobenzoxaborole (**2.16**). Therefore, the treatment of 6-nitrobenzoxabole (**2.17**) with 10 % Palladium on carbon under the hydrogen atmosphere resulted in the formation of

2.16 in 91 % yield. Aromatic nitro groups are easily reduced to primary amino groups by metal catalyzed (Zn, Pd, Sn) hydrogenation and various hydrogen sources include HCl, H_2, ammonium formate.[24] The TLC confirmed the reduction of NO_2 to NH_2 showing disappearance of the starting material spot, which was accompanied by a new fluorescent spot that could be clearly seen. IR also confirmed the disappearance of NO_2. Fluorescent boron compound synthesis is a growing field of study in technology and medicine,[25] and molecules like the 6-aminobenzoxaborole and its derivatives have much potential for various applications.[10]

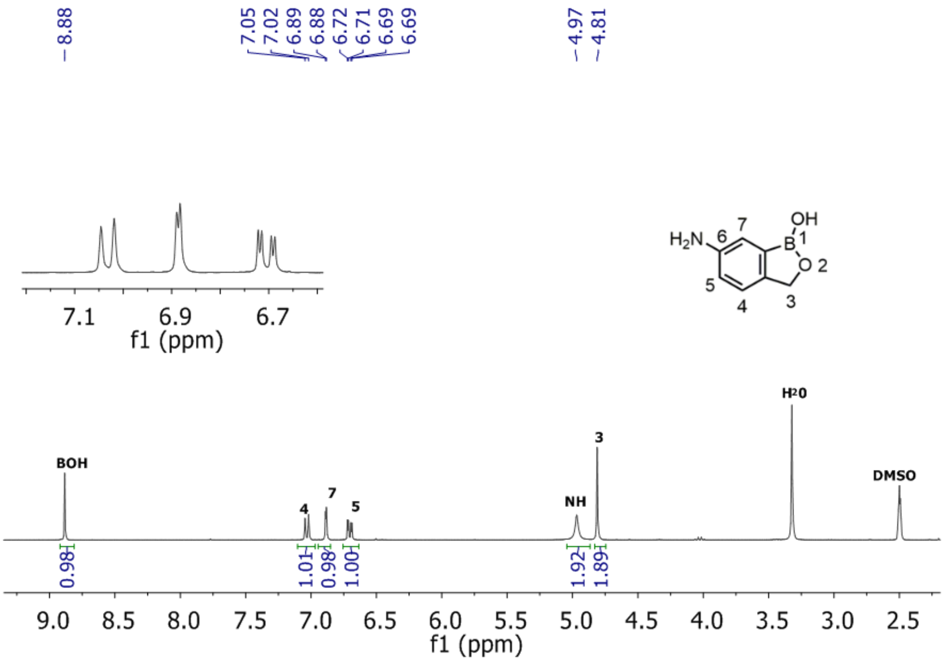


Figure 2.5: ^1H NMR spectrum (DMSO-d_6) of 6-amino benzoxaborole.

To confirm the structure of 6-aminobenzoxaborole (**2.16**), the ^1H NMR was performed and 6 characteristic peaks were evident. The B-OH singlet chemical resonance at δ 8.89 ppm and the aromatic signals could be seen appearing more up-field than the 6-nitrobenzoxaborole (**Fig. 2.5**). The NH_2 singlet peak was observed at δ 4.81 ppm and the CH_2 peak appeared at δ 4.70 ppm, respectively. The ^{13}C NMR (**Fig. 2.6**) showed 6 signals instead of 7 and this is due to the invisibility of the carbon adjacent to the boron atom, and this trend has been observed by other researchers in all benzoxaborole containing compounds.[21] The quaternary carbon next to C-3 was the most deshielded with the signal observed at δ 147.8 ppm, and at δ 141.8 ppm appeared the signal corresponding to C-6 and the remaining carbon signals were observed between δ 121.8 ppm and δ 114.9 ppm, respectively.

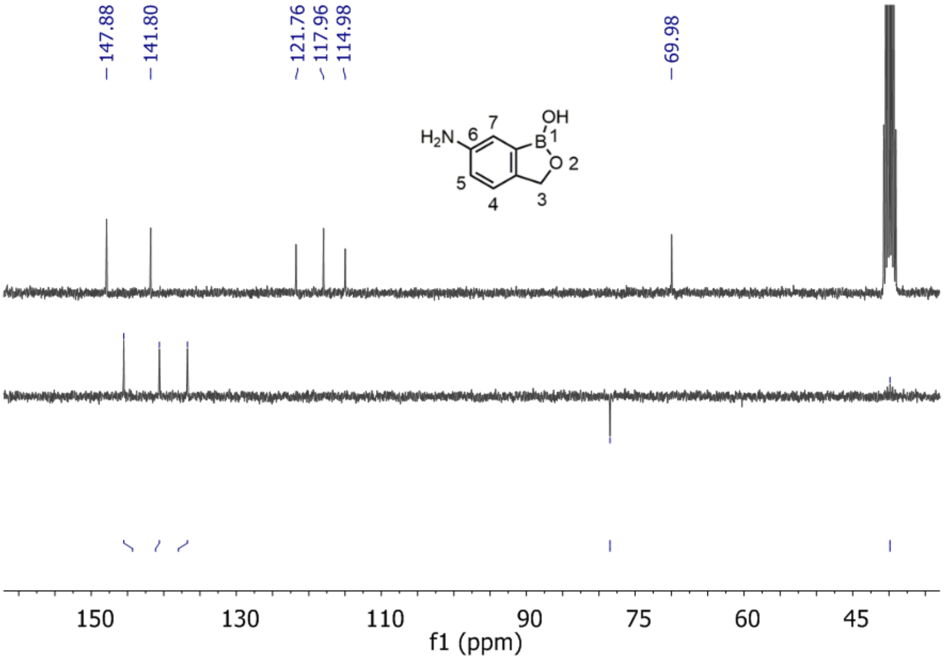


Figure 2.6: ^{13}C NMR (top) and DEPT135 NMR (bottom) spectra (DMSO-d_6) of 6-amino benzoxaborole.

The DEPT 135 NMR experiment confirmed the presence of 3 × CH signals, which can be observed in the aromatic region and a negative signal corresponding to the CH_2 at position 3 (**Fig. 2.6**) further confirming the skeletal structure of compound **2.16**. Additionally, the high resolution mass spectrophotometry (HRMS) also confirmed the successful synthesis of this key precursor with the molecular ion peak appeared at *m/z* 149.0243 (**Fig. 2.7**).

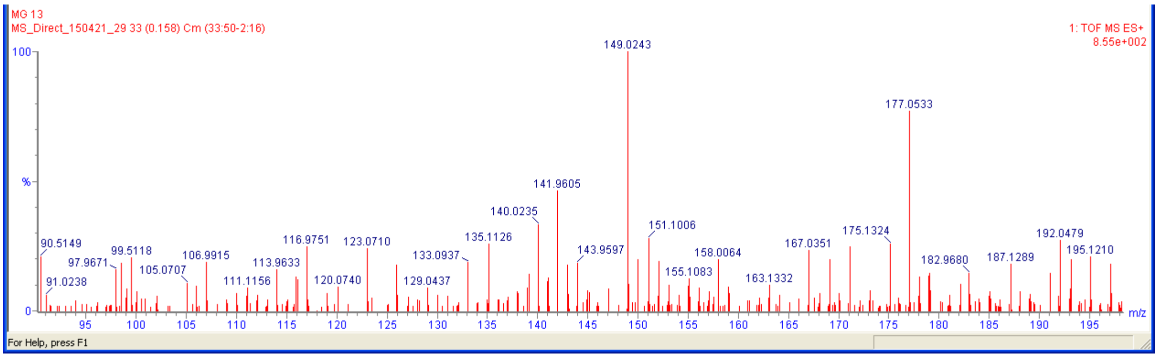
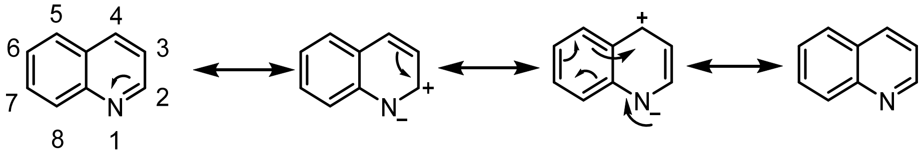


Figure 2.7: HRMS spectrum for compound 2.16.

2.2.1.3 Synthesis of 3-(7-chloroquinolin-4-ylamino) alcohols

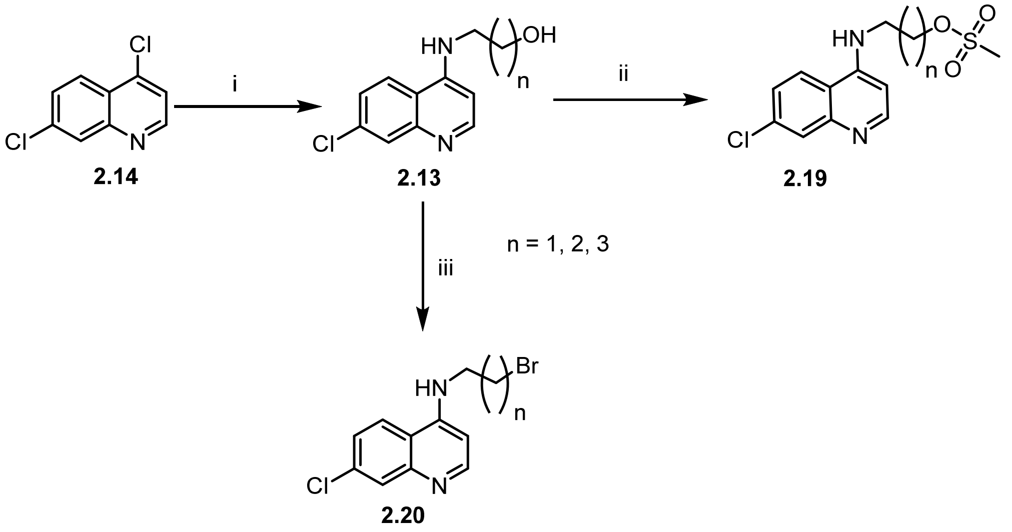
Following the successful synthesis of the required starting boron compound **2.16**, the next task was to prepare targeted key quinoline intermediates for final coupling with **2.16** to generate the required hybrids. A quinoline is a heterocyclic molecule that consists of a benzene ring fused with a pyridine ring. This ultimately means that we can view its reactivity in a similar perspective as its parental rings. For instance, we can predict that electrophilic substitution reactions are more likely to occur on the benzene ring while nucleophilic susceptibility spots are more dominant on the pyridine ring. The nitrogen atom being more electronegative causes more π-electron deficiency through mesomeric and inductive effects to the quinoline (**Scheme 2.5**).[26]



Scheme 2.5: Quinoline resonance forms.

As can be seen from the resonance stabilized structures, the positive charge is more localized on positions 2 and 4, which makes these positions more prone to nucleophilic attack.[27] It has been shown that reactivity at these positions can be further enhanced if there are substituents, such as halogens which are electron-withdrawing, on the ring.[28] The pyridine ring in quinoline is electron deficient. Therefore, nucleophilic substitution takes place at the 2- and 4-positions. The electron densities have been calculated for quinoline with the help of molecular orbital method and revealed electron deficiency at these two positions.[29]

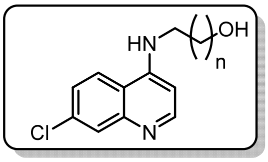
Thus, excess of amino-alcohols (2C, 3C and 4C) (**2.15**) that are available commercially, were individually reacted with 4,7-dichloroquinoline (**2.14**) under solvent free conditions, in the presence of catalytic amounts of triethylamine and K_2CO_3 (2.5 eq) to yield the corresponding 3-(7-chloroquinolin-4-ylamino) alcohols (**2.13**, **Scheme 2.6**) with yields in the range 64 – 86 % (**Table 2.1**). The role of trimethylamine is to remove the hydrogen chloride by-product from the reaction mixture through the formation of a triethylamine hydrochloride, this is necessary for the reaction to go to completion.[30]



Scheme 2.6: *Reagents and conditions* i) K_2CO_3, aminoalcohol, Et_3N, 140 °C, 4 h; ii) THF, MSC, Py, 0 °C, 5 h iii) 48 % HBr/H_2SO_4, 65 °C, 4 h.

The TLC showed total consumption of the starting material and also revealed a new spot with a smaller R_f value than the starting material. The general reactivity of the amino alcohol with 4,7-dichloroquinoline is that, the shorter the carbon chain of the amino alcohol the higher the reactive. The 2C (carbon chain) was more reactivity than 3C, and 4C was the least reactive. This trend has also been observed in literature.[31]

Table 2.1: Isolated yields of 3-(7-chloroquinolin-4-ylamino) alcohols.



Compound No.	n	Yield (%)
2.13a	1	86
2.13b	2	82
2.13c	3	64

The ^1H NMR spectrum (**Fig. 2.8**) of target compounds revealed key characteristic signals, which were consistent with the aromatic protons and 5 signals (for example, **2.13b**) suggesting the attachment of required alkyl chains. The doublet peak assigned to H-2 could be seen at δ 8.38 ppm, due to the deshielding effect of the neighbouring nitrogen atoms. A doublet of doublets, which was observed at δ 7.42 ppm was attributted to H-5, due to its coupling with H-4 and H-7 protons. NH appeared as a broad unresolved triplet signal at δ

7.29 ppm, and it was more down-field than expected due to the inductive effect of the quinoline ring. The starting material for this reaction was 4,7-dichloroquinoline, which only has 5 aromatic proton signals, therefore the appearance of the -NH, -OH and aliphatic proton signals further confirmed the formation of the desired product. The broad signal at δ 4.61 ppm corresponded to the -OH and the quintet at δ 1.81 ppm corresponded to H-9 due to its interaction with protons on C-8 and C-10.

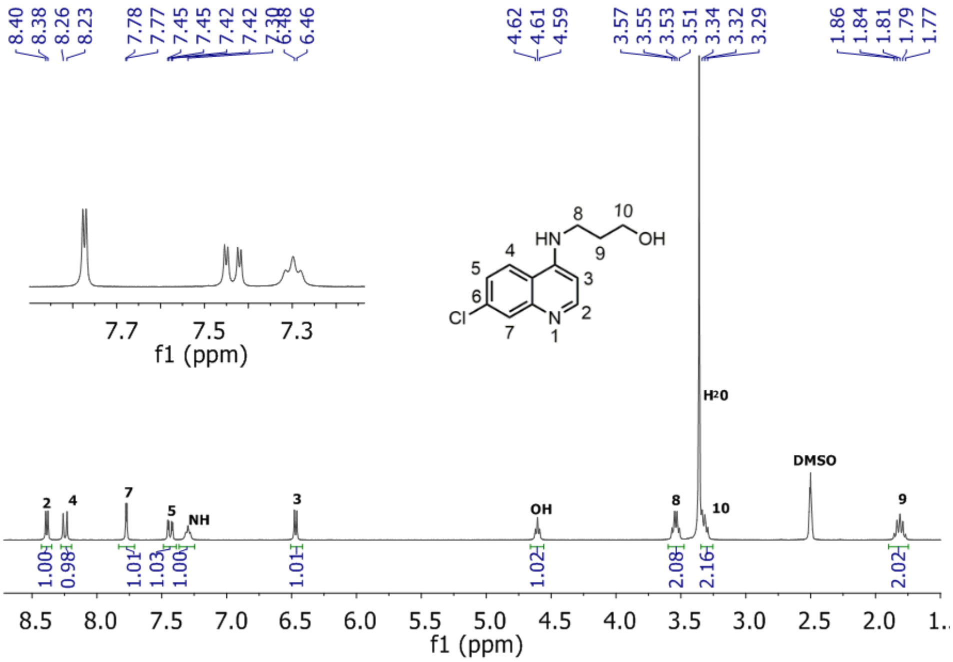


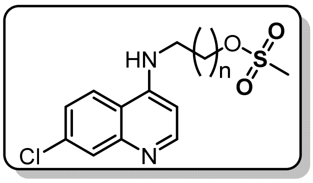
Figure 2.8: ^{1}H NMR (DMSO-d_6) of compound 2.13b.

^{13}C NMR showed 12 signals corresponding to the total number of carbons. At δ 152.3 ppm appeared the quaternary carbon adjacent to -NH group, this signal was the most deshielded, followed by C-2 appearing at δ 150.6 ppm. The second quaternary carbon adjacent to C-7 can be observed at δ 149.41 ppm with remaining aromatic signals appearing between δ 133.74 and δ 98.9 ppm. In the aliphatic region the corresponding number of carbons could also be observed. DEPT 135 NMR further confirmed the formation of the product as evident in the corresponding negative signals of the CH_2 groups and the 5 aromatic carbons signals. For compound **2.13b**, three negative signals also appeared as expected suggesting that the aminoalcohol was successfully attached to the 7-chloroquinoline. Similarly, the NMR spectra of other compounds **2.13a** and **2.13c** were consistent with expected structures.

2.2.1.4 Synthesis of methane sulfonyl derivatives

The activation of the OH in each of starting compounds **2.13a** – **c** was done through the attachment of methanesulfonyl leaving group. The prepared 3-(7-chloroquinolin-4-ylamino) alcohols **2.13a – c** were treated with methanesulfonyl chloride in anhydrous THF under inert nitrogen atmosphere in the presence of catalytic amounts of pyridine at 0 – 5 °C for 5 h. The TLC showed a new spot which was accompanied by the disappearance of starting alcohols. The target mesylated compounds **2.19a – b** were isolated in yields of 64 – 68 % (**Table 2.2**).

Table 2.2: Isolated yields of chloroquine methane sulfonyl esters.



Compound No.	n	Yield (%)
2.19a	1	64
2.19b	2	68
2.19c	3	-

Despite several attempts, the isolation of **2.19c** from reaction mixture was unsuccessful. Upon removal of water, the ^{1}H NMR experiment of the remaining crude product was inconsistent with the desired product. To confirm the structure of other methane sulfonyl derivatives, the ^{1}H NMR was performed and 5 characteristic signals were evident in the aromatic region and corresponding signals in the aliphatic region. As an example to illustrate the formation of these desired mesylated compounds, the appearance of a characteristic methyl signal at δ 3.20 ppm (**2.19b**, **Fig. 2.9**) served as evidence for the formation of mesylated products. All the ^{1}H NMR signals shifted downfield compared to the alcohols derivatives. The NH signal can be seen at δ 9.57 ppm more downfield than the alcohols. This was consistent with disappearance of the broad hydroxyl signal showing that indeed the substitution reaction had occured.

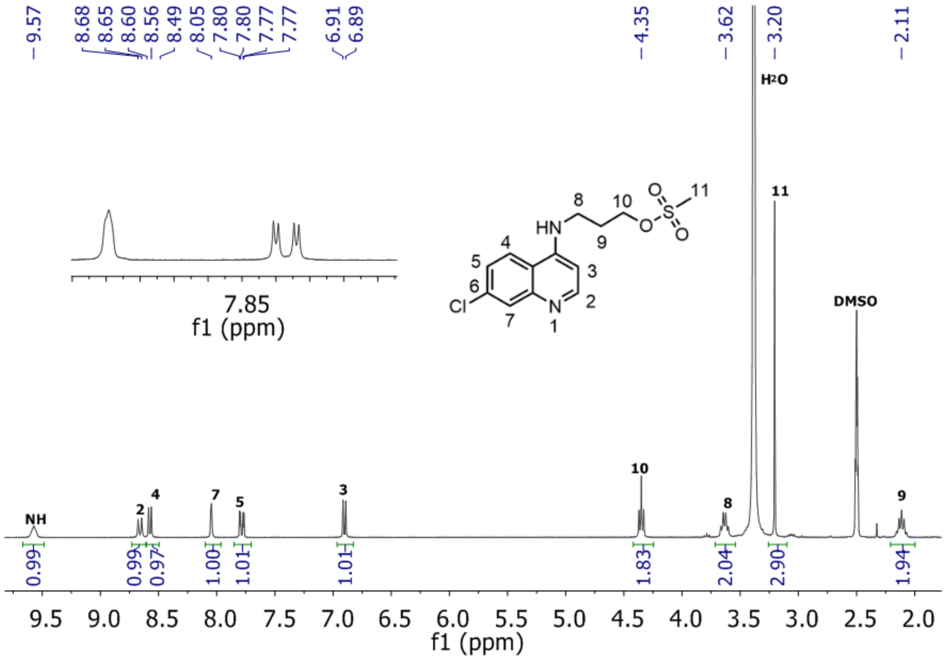


Figure 2.9: ^{1}H NMR (DMSO-d_6) spectrum of compound 2.19b.

To further confirm the successful synthesis of methane sulfonyl derivative compounds **2.19a** - **b**, the ^{13}C NMR spectra (**Fig. 2.10**) showed a required number of carbons, with a new methyl carbon of the methane sulfonyl moiety appearing at δ 37.1 ppm. The C-9 and C-8 were observed at δ 27.5 ppm and δ 40.1 ppm. The DEPT 135 NMR also indicated a positive signal in the aliphatic region again confirming that the mesylation of the alcohol was successful.

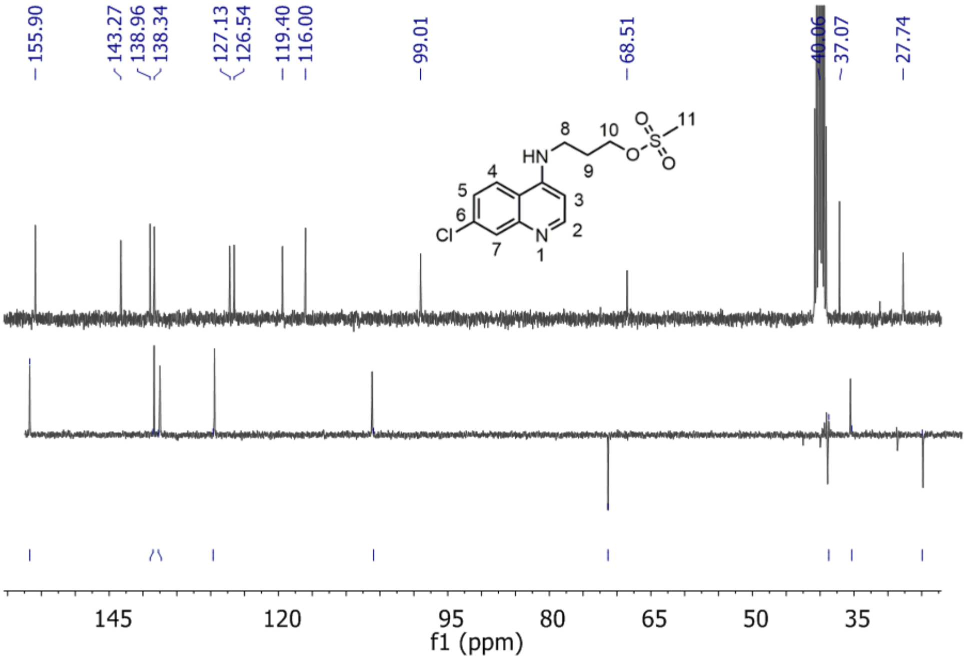
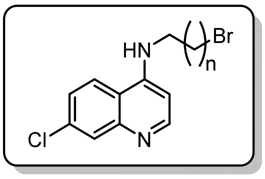


Figure 2.10: ^{13}C NMR (top) and DEPT135 (bottom) spectra (DMSO-d$_6$) of compound **2.19b.**

2.2.1.5 Synthesis of (7-chloroquinolin-4-yl amino) bromides

Halogens also serve as good leaving groups. Hydrogen halides can be used to produce halogenoalkanes. Hence, (7-chloroquinolin-4-yl amino) alcohols **2.13** were heated at 65 °C in 48 % HBr in the presence of catalytic amounts of H_2SO_4. The reaction mixture was allowed to stir for 4 h at the same temperature. The TLC showed a total consumption of alcohols, and new spots could be observed. Upon complete consumption of the alcohols (TLC) the reaction was quenched with $NaHCO_3$ and extracted with ethyl acetate followed by washing with saturated $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and solvent removed under reduced pressure to yield the crude products. Compound **2.20a** was, however, recovered by neutralizing the reaction mixture with $NaHCO_3$ and dried in the freeze dryer. All the compounds **2.20a - c** were purified using a silica gel column chromatography to yield desired brominated products in good yields (**Table 2.3**). Compounds **2.20a–c** are well known intermediates, and their melting points was not measured thereof.

Table 2.3: Isolated yields of (7-chloroquinolin-4-yl amino) bromides.



Compound No.	n	Yield (%)
2.20a	1	88
2.20b	2	84
2.20c	3	73

The ^{1}H NMR spectrum of each compound revealed the appearance of both aromatic and aliphatic signals downfield upon bromination. The correct number of protons could be accounted for each compound with -NH being more downfield appearing as a broad unresolved triplet signal at δ 9.40 ppm. At δ 8.60 ppm, a triplet as a result of overlapping doublets (triplet of doublets) integrating for 2H was observed, and this was assgined to (H-4) and (H-2) (**Fig. 2.11**). H-5 is observed as a doublet of a doublet appearing at δ 6.93 ppm, other aromatic protons can be observed (**Fig. 2.11**). Analysis of the ^{13}C NMR spectra indicated the expected number of carbons, and the significant difference between the alcohols and the brominated compounds is that the brominated compounds have all their carbon signals deshielded.

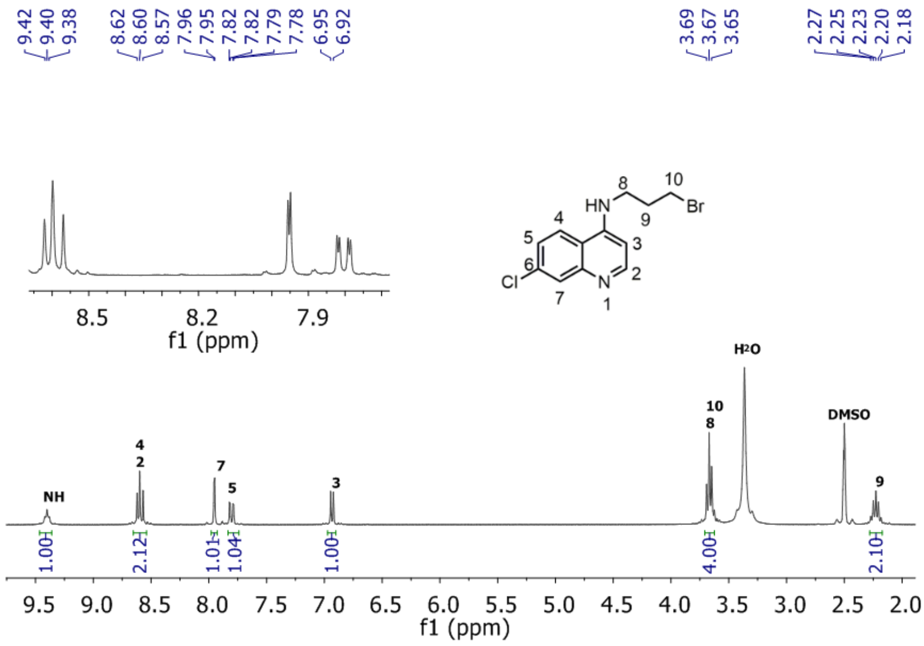
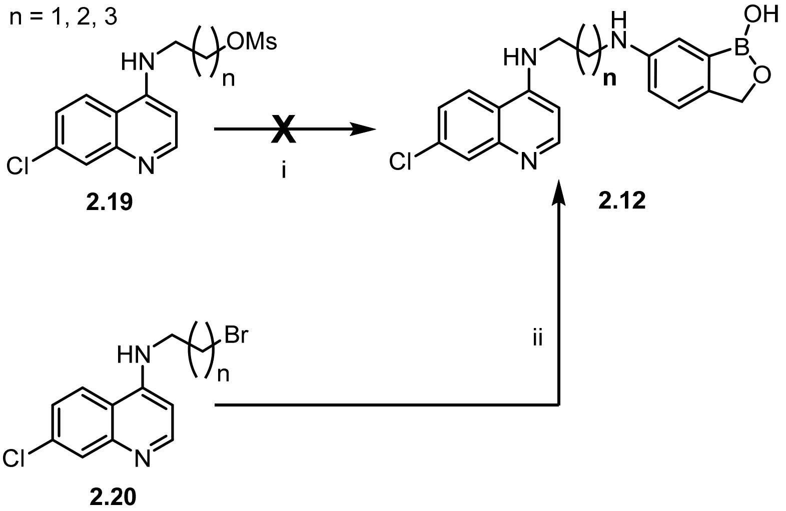


Figure 2.11: ^{1}H NMR (DMSO-d$_6$) of a brominated quinoline compound **2.20b**.

2.2.1.6 Synthesis of benzoxaborole-based chloroquinoline derivatives

Initially, the mesylated compounds **2.19a – b** were treated with NaH and **2.16** in DMF at 60 °C for 24 h; and K_2CO_3 in acetonitrile, but on several trials and prolonged reaction times, the products did not form. Following the failure to access the target compounds **2.12** via the displacement of the mesylate leaving groups (Step i), we resorted to access these compounds via the displacement of bromine (Step ii, **Scheme 2.7**). Amination reaction between amines and alkyl halides is a common reaction, which has led to the synthesis of various N-alkylated compounds. Thus, appropriate bromides (1.1 eq), and 6-aminobenzoxaborole (**2.16**) (1 eq) were initially reacted with various bases such as KOH, NaH, K_2CO_3, Et_3N and NaOH at room temperature for 24 h in DMF. NaOH and K_2CO_3 did not yield the desired product. On the other hand, NaH yielded the desired crude product as judged by the ^{1}H NMR experiment. However, the quantities isolated were very small.

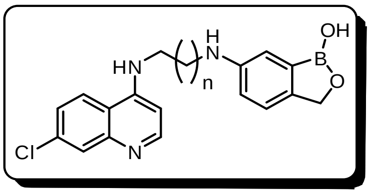


Scheme 2.7: *Reagents and conditions.* i) DMF, KOH, 120 °C, 1 h. ii) DMF, Et_3N, DMAP. r.t, 24 h

Reaction conditions were then altered with the coupling done in the presence of catalytic amounts of DMAP with Et_3N as a base. The progress of the reaction was monitored by TLC, which showed disappearance of the quinoline spot. Thus, the liquid-liquid extraction using EtOAc followed by washing with 5 % HCl, saturated $NaHCO_3$ and water yielded crude products **2.12a-c** solid residues. These crude products however proved difficult to purify. Subjecting the residues to silica gel column chromatography eluting with DCM/MeOH (9:1) resulted in the products cleaving of the new amine bond. All compounds **2.12a - c** were isolated as crude products (though with very little impurities) following poor separation.

Mass spectroscopy data of compounds **2.12a-c** could not be obtained due the instability of these compounds. Yields are shown on **Table 2.4**.

Table 2.4: Isolated yields of (7-chloroquinolin-4-yl amino) bromides.



Compound No.	n	Yield(%)[a]
2.12a	1	60
2.12b	2	55
2.12c	3	75

[a] Compounds isolated as crude products

The formation of desired hybrids (**2.12a – c**) was confirmed by NMR spectroscopy and mass spectrometry. Selecting compound **2.12b** as an example, the ^{1}H NMR spectrum (**Fig. 2.12**) revealed the appearance of new characteristic doublet signals at δ 7.65, 8.42 and δ 9.59 ppm, which corresponded to the aromatic protons of benzoxaborole after coupling with starting quinoline compound **2.20b**.

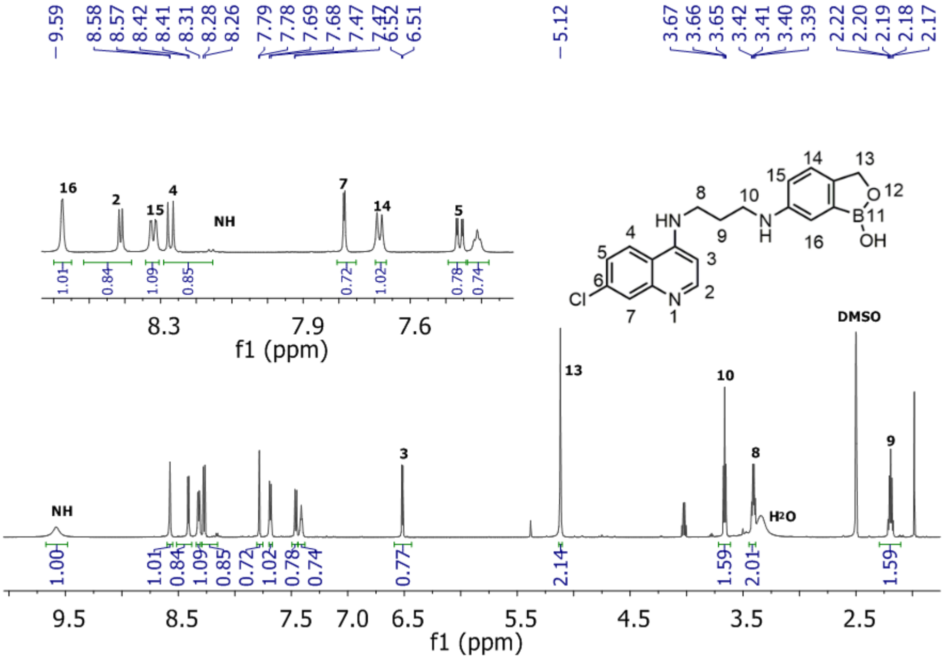
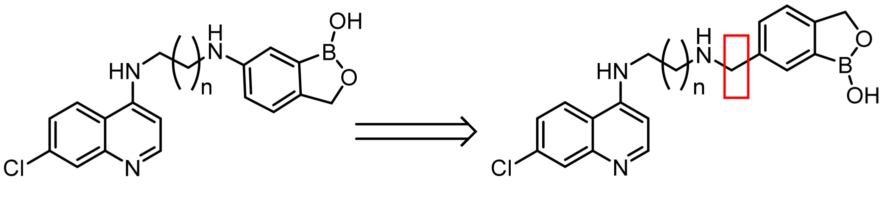


Figure 2.12: ^{1}H NMR (DMSO-d_6) of chloroquine benzoxaborole hybrid compound **2.12b.**

Additionally, the chemical shift signal at δ 5.12 ppm, which integrated for 2H, was attributed to the CH_2 of the oxaborole ring and this further served as a confirmation that 6-aminobenzoxaborole (**2.16**) coupled with quinoline **2.20b** to form compound **2.12b**. The ^{1}H NMR results were supported by the ^{13}C NMR spectrum for compound **2.12b**, which showed the signals appeared to be consistent with a number of carbons of the proposed structure. The distinct signal due to ^{13}C NMR of oxaborole ring was also observed at δ 70.5 ppm.The compounds, **2.12a – c** appeared to be unstable in solution, as within 24 h in solution a precipitate was observed and the ^{1}H NMR of isolated precipitates showed signals corresponding to starting materials. During storage, **2.12c** transformed into an orange paste, while others **2.12a - b** just disintegrated.

2.2.2 SYNTHESIS OF CHLOROQUINE -LIKE BENZOXABOROLE COMPOUNDS

As part of structural variation, we considered quinoline-like benzoxaborole compounds **2.21** by introducing a -CH_2 between terminal amine of CQ alkyl chain and benzoxaborole motif (**Scheme 2.8**). Herein, the targeted compounds required access to quinoline diamines (**2.30**) and benzoxaborole aldehyde (**2.29**) as starting materials.

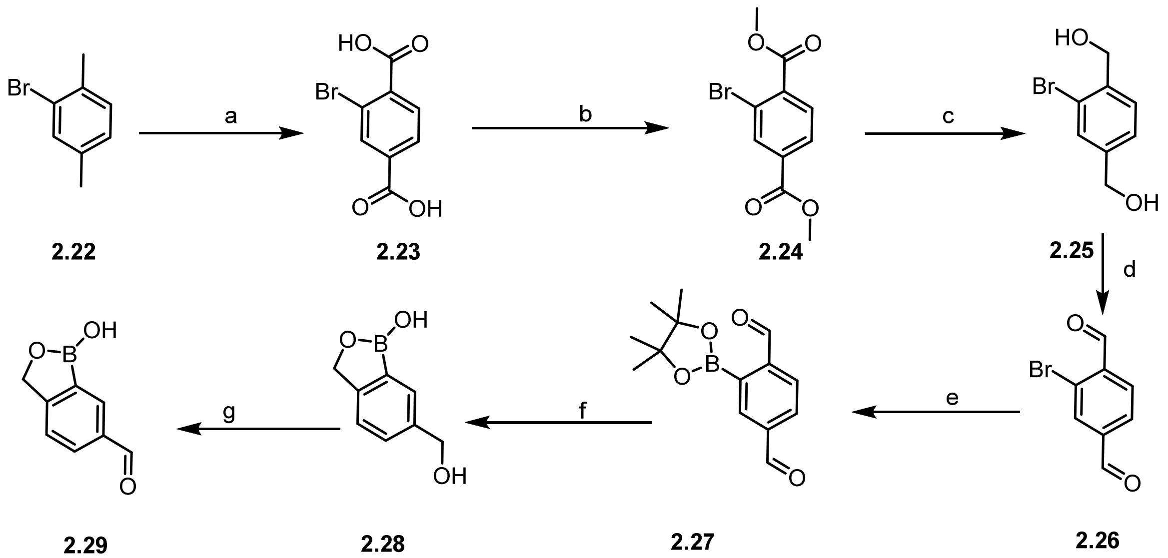


2.12 **2.21**

Scheme 2.8: Chloroquine-like benzoxaborole compounds.

2.2.2.1 Synthesis of precursor 6-formylbenzoxaborole

The synthesis of 6-formyl benzoxaborole is a seven step (**Scheme 2.9**) reaction starting from the commercially accessible compound **2.22**. Treatment of **2.22** with aqueous KOH at 70 °C for 0.5 h was followed by reacting the resulted mixture with $KMnO_4$ at 70 °C for 3 h to give the diacid **2.23**, which was isolated as a white solid upon workup step (filtration followed by acidification and filtration). The ^{1}H NMR spectrum of **2.22** showed aromatic proton signals, while those corresponding to the -COOH protons could not be observed. Unlike the ^{1}H NMR, the ^{13}C NMR was more informative in the sense that the spectrum revealed the characterestic COOH signals at δ 167.6 ppm and δ 165.9 ppm, which suggested the oxidation of **2.22** to form diacid **2.23**.



Scheme 2.9: *Reagents and conditions* a) i. KOH, H_2O, 70 °C, 0.5 h; ii. $KMnO_4$, 3 h. b) i. MeOH, $SOCl_2$, 0 °C; ii. Reflux, 100 °C, 1 h. c) $LiBH_4$, THF, r.t., 12 h. d) i. PCC/Celite, ii. DCM, 12 h. e) i. Pin_2B_2, KOAc, toluene; ii. $Pd(PPh_3)_2Cl_2$, PPh_3, 24 h. f) i. $NaBH_4$, EtOH; ii. 2 N HCl, 12 h. g) i. PCC/Celite; ii. DCM, 6 h.

Esterification of the diacid **2.23** was achieved using methanol in presence of $SOCl_2$. The ester **2.24** was isolated as a white solid with 95 % yield after recrystalization and drying the product under vacuum filtration. The ^1H and DEPT135 NMR spectra of compound **2.24** confirmed the esterification of the diacid as new signals due to CH_3 could clearly be observed in the aliphatic region at δ 3.95 and 3.94 ppm (1H NMR) and δ 52.8 and 52.7 ppm (DEPT135 NMR). Treatment of **2.24** with $NaBH_4$ partially reduced the ester to form an alcohol **2.25**, but the starting material was still visible (TLC). Thus, we opted to use a strong reducing agent, $LiBH_4$, which unlike $NaBH_4$ it is readily soluble in simple solvents such as ether.[32] It also possesses a much greater selectivity than $NaBH_4$ and it is more suitable for selective reduction reactions.[33] Thus, the ester **2.24** was treated with $LiBH_4$ in THF as a solvent to yield the alcohol **2.25** in 68 % yield. The formation of **2.25** was confirmed using the ^{13}C NMR, which showed the disappearance of the two carbonyl protons together with two methyl (CH_3) signals. The oxidation of the alcohol **2.25** using PCC (pyridinium chlorochromate) in DCM gave the desired aldehyde **2.26** in 45 % yield.[34,35] Evidence of the target aldehyde was confirmed by the ^1H NMR spectrum, which revealed the characteristic singlets corresponding to the aldehyde protons.

Having the desired aldehyde at hand, the next step was to prepare the key intermediate **2.27**. Thus, the Miyaura palladium cross-coupling of aldehyde **2.26** with bis(pinacolato)diboron (B_2pin_2) in basic medium yielded the borylated compound **2.27** as a brown oil in 91 % yield.[36] The identity of this compound was verified by the ^{1}H NMR, which showed the appearance of the methyl proton signals at δ 1.18 ppm that integrated for 12 protons. Without further purification, the borylated compound **2.27** was reacted with $NaBH_4$ and cyclized using 6 N HCl[37] to form, after purification by column chromatography, compound **2.28** in 51 % yield as a white solid.[10] The ^{1}H NMR spectrum of **2.28** showed the absence of proton signals due to aldehydes, which was accompanied by the appearance of new singlet signals at δ 4.85 ppm that corresponded to -CH_2 of the oxaborole ring and δ 4.54 ppm corresponded to methylene -CH_2 bonded to the alcohol group.[10] The ^{13}C NMR supported the ^{1}absence of carbonyl signals accompanied by appearance of new carbon signal due to the CH_2 of oxaborole ring. This confirmed that the cyclisation successfully occurred.

The oxidation of compound **2.28** with PCC gave the desired aldehyde, 6 formylbenzoxaborole (**2.29**) as a pale yellow solid albeit in very low yields (14 %). ^{1}H NMR (**Fig. 2.14**) revealed characteristic signals at δ 4.80 ppm corresponding to H-5, a formyl proton signals at δ 10.00 ppm and four characteristic aromatic signals including a BOH signal at δ 7.85 ppm. Aromatic protons can be observed at δ 7.63 – 7.45 ppm. Despite the poor yield, the desired compound could not be isolated in high purity (even after column chromatography) due to the difficulties with purification. Melting points of compounds **2.23** – **2.25** and **2.28** are well known intermediates, and could not measure their melting points.

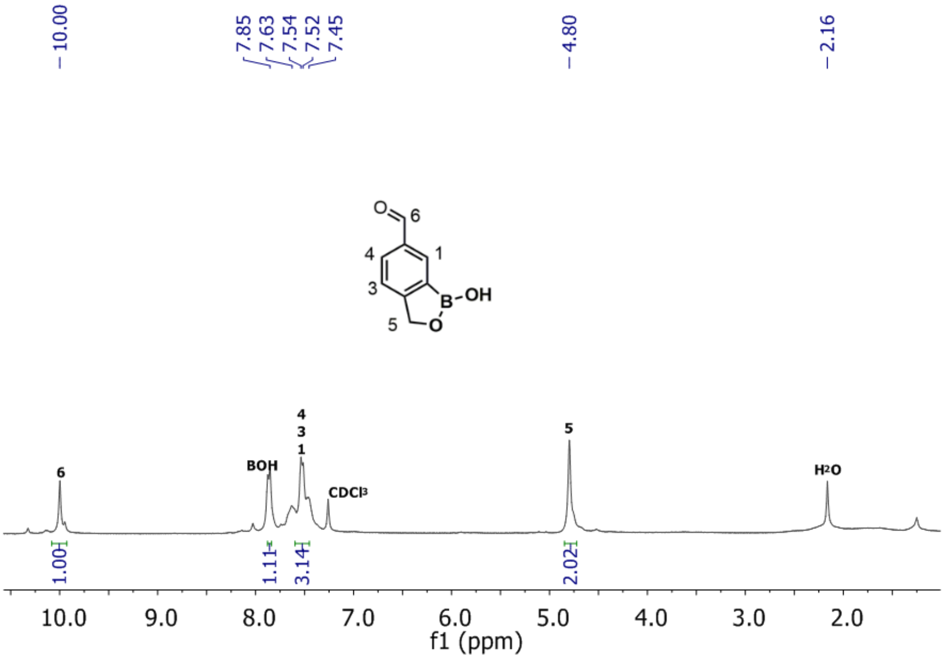
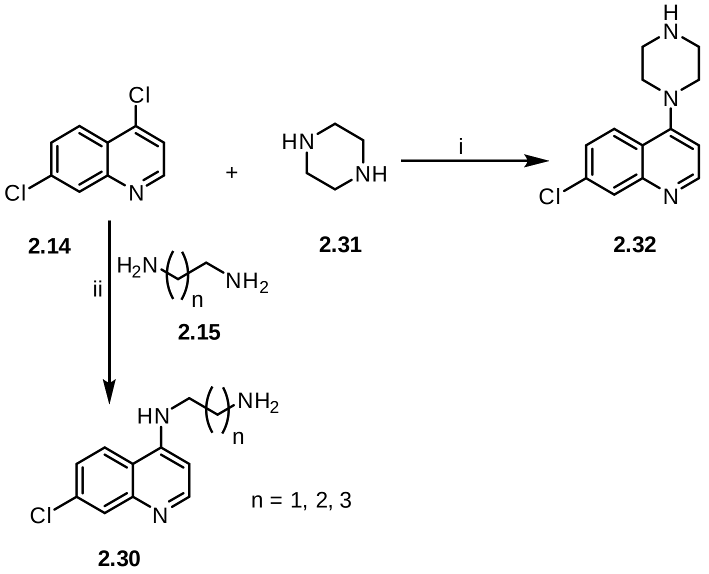


Figure 2.13: ^{1}H NMR ($CDCl_3$-*d*) of 6-formylbenzoxaborole (2.29).

2.2.2.2 Synthesis of quinoline diamines

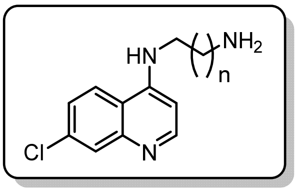
The other set of CQ intermediates was prepared by nucleophilic reaction involving 4,7-dichloroquinoline **(2.14)** and alkyl diamines (**2.15**) of 2C, 3C and 4C chains (**Scheme 2.9**) as reported in literature.[38]



Scheme 2.9: *Reagents and conditions* i) EtOH, 80 °C, 5 h ii) 80-110 °C, 5 h.

Excess of diamines (2C, 3C and 4C) that are available commercially, were individually reacted under reflux at 80 – 110 °C with 4,7-dichloroquinoline **2.14** under solvent free conditions (**Scheme 2.9**) to obtained the targeted intermediates with yields in the range 63 – 83 % (**Table 2.5**). The general reactivity of the diamines with 4,7 dichloroquinoline **2.14** showed that the shorter the carbon chain of the diamines the higher the reactivity. The same trend was also observed when the 4,7 dichloroquinoline (**2.14**) was treated with aminoalcohols.

Table 2.5: Isolated yields of (7-chloroquinolin-4-yl amino) amines.



Compound No.	**n**	**Yield (%)**
2.30a	1	83
2.30b	2	73
2.30c	3	63

The ^{1}H NMR spectra of target compounds **2.30a-c** revealed key characteristic signals, which were consistent with the aromatic proton signals suggesting the successful attachment of required diamines, and corresponding signals due to diamine CH_2 chains in the aliphatic region were also observed. ^{13}C and DEPT 135 NMR also showed signals that were consistent with the formation of these compounds.

As part of restricting the flexibility of aminoquinoline side chain, the 4,7-dichloroquinoline (**2.14**) was refluxed with excess of piperazine (**2.31**) in ethanol as a solvent (**Scheme 2.9**). Similarly, the TLC showed a total consumption of the starting materials. Upon the work-up step, compound **2.32** was recovered as pale yellow crystals in 71 % yield. The ^{1}H NMR (**Fig. 2.15**) showed all the five characteristic signals in the aromatic region between δ 8.72 - 6.82 ppm and a multiplet in the aliphatic region at δ 3.17 ppm, which integrated for 8 protons. The ^{13}C and DEPT 135 NMR also appeared consistent with the skeletal structure of compound **2.32**.

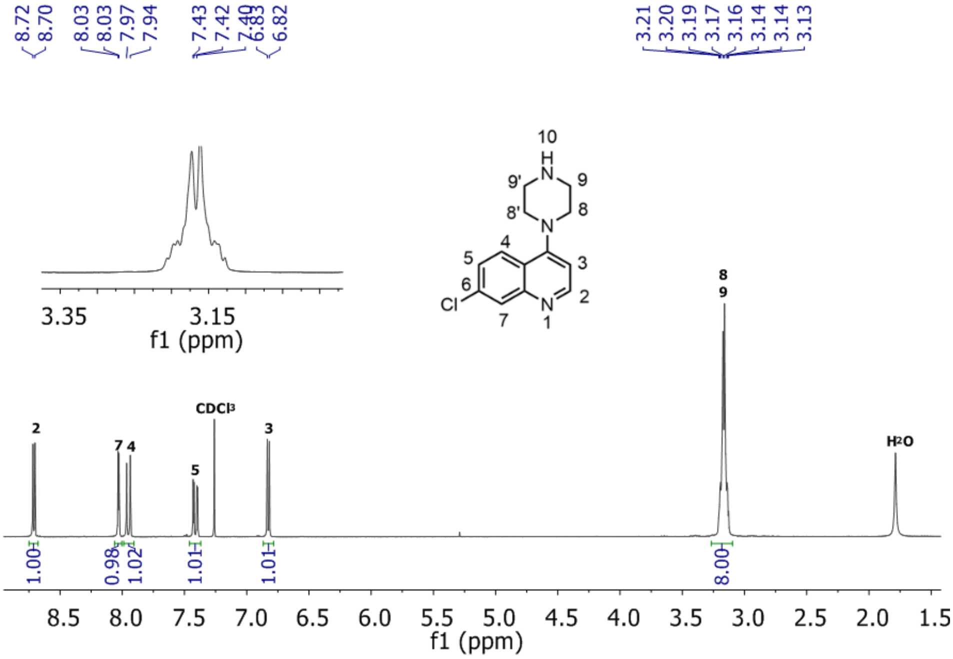
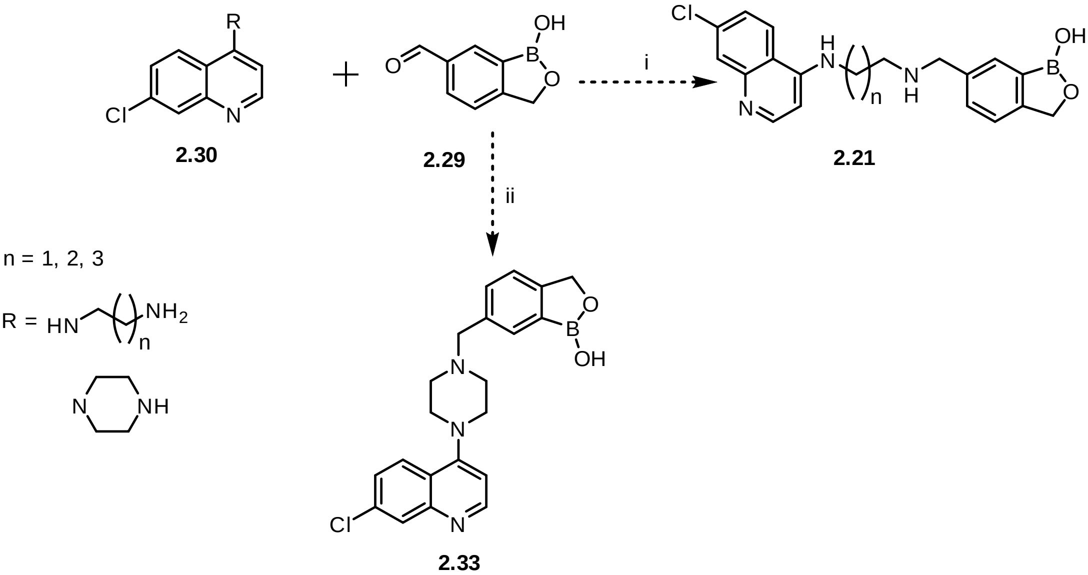


Figure 2.14: ^{1}H NMR ($CDCl_3$-*d*) spectrum of **2.32**.

2.2.2.3 Synthesis of chloroquine-like benzoxaborole compounds

As mentioned in **2.3.2** that the compounds in **Scheme 2.10** were designed in such a way that the chloroquine-benzoxaborole would be accessed via 6-formylbenzoxaborole (**2.29**), this was, however, not possible due to very low yields of 6-formylbenzoxaborole (**2.29**) and unsatistactory purity. Hence, we could not continue with this part of work.



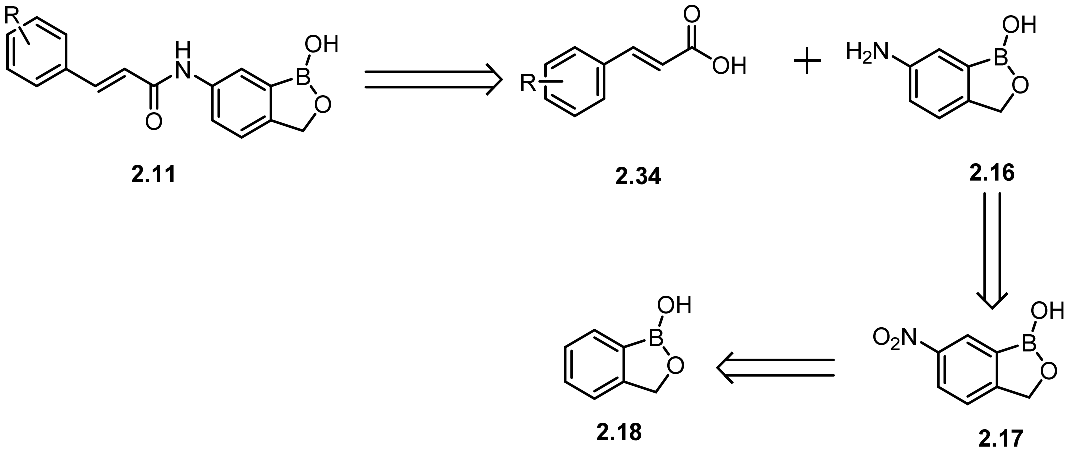
Scheme 2.10: Proposed synthesis of chloroquine-like benzoxaborole compounds.

2.2.3 SYNTHESIS OF CINNAMOYL-BASED BENZOXABOROLE DERIVATIVES

The second part of this project involved the investigation of cinnamic acids coupled with benzoxaborole scaffold as antiprotozoal agents. It has already been stated previously that this class of compounds which largely occur in nature exhibit a broad range of biological properties including antiprotozoal potencies.**Error! Reference source not found.**

2.2.3.1 Retrosynthetic analysis

The synthesis of desired cinnamoyl-benzoxaboroles was envisaged as achievable from commercially available cinnamic acids (**2.34**) with different substituents and 6-aminobenzoxaborole (**2.16**) and this is accessible from starting benzoxaborole (**2.18**) **Scheme 2.11**.

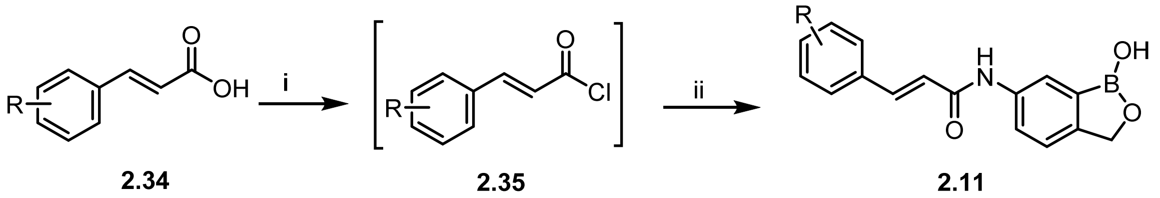


Scheme 2.11: Retrosynthetic analysis of cinnamoyl-based benzoxaborole compounds.

2.2.3.2 Synthesis of target cinnamoyl-benzoxaborole compounds

Initially, we treated cinnamic acids (**2.34a – g**) with 6-aminobenzoxaborole (**2.16**) using various coupling agents such as CDI, EDCI, HOBt, HOSu, but they all gave traces of desired products.[39] Another way of activating carboxylic acids, is by attaching a good leaving group to the acyl carbon of the acid to promote nucleophilic attack by the amino group.[40] The common synthetic approach of converting the acid to acyl chloride using $SOCl_2$ was attempted, but upon coupling with 6-aminobenzoxaborole (**2.16**) the reaction gave poor yields and in some cases the desired products could not be isolated.

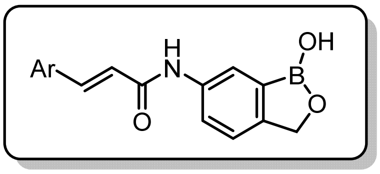
Then, we opted for the conversion of carboxylic acid to acyl chloride using $POCl_3$ in the presence of Et_3N. The presence of an organic base has been reported to be important for the acylation reaction as it maintains the basic pH conditions throughout the reaction.[41] Thus, the cinnamic acids (**2.34a – g**) were treated with triethylamine in anhydrous DCM under N_2 atmosphere.

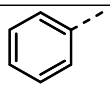
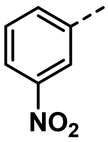
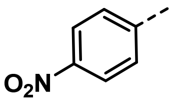
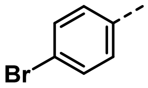
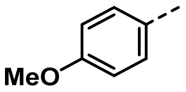
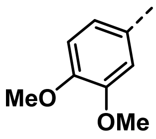
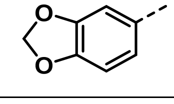


Scheme 2.12: Reagents and conditions i) DCM, Et_3N, N_2, $POCl_3$, 0.5 h, ii) DCM, DMAP, 6-aminobenzoxaborole, 12 h.

After letting the reaction to stir for 5 min, $POCl_3$ in anhydrous DCM was added to the reaction mixture and allowed to stir at room temperature for 0.5 h under N_2 atmosphere.[41] The reaction progression was monitored by TLC, which showed a complete conversion of starting materials. Without the isolation of acid chloride products (**2.35**), the resulting reaction mixture was reacted with the 6-aminobenzoxaborole (**2.16)** in presence of DMAP in DCM at 0 °C under N_2 atmosphere. The reaction was allowed to warm to ambient temperature where it was left stirring overnight. After elapsed of the reaction time, the reaction products were subjected to silica gel column chromatography to give pure compounds in yields between 30 and 50 % (**Table 2.6**).

Table 2.6: Isolated yields of cinnamoyl-benzoxaborole hybrids.



Compound No.	Ar	Yield (%)
2.11a		41
2.11b		48
2.11c		42
2.11d		29
2.11e		32
2.11f		46
2.11g		38

The synthesized cinnamoyl-benzoxaboroles (**2.11a – g**) were characterized using ^{1}H and ^{13}C NMR as well as MS spectrometry. Using compound **2.11a** as an example, the ^{1}H NMR spectrum of this compound showed the characteristic signal due to B-OH at δ 10.27 ppm and appearance of a signal due to NH of the amide at δ 9.24 ppm (**Fig. 2.16**). Furthermore, the spectrum revealed two doublets signals at δ 6.87 and 7.60 ppm with corresponding coupling constants (*J* values) of 15.0 and 15.6 Hz, and these were attributed to H-7 and H-8 protons. The coupling constants suggested that both H-7 and H-8 assumed a *trans* geometry. The key CH$_2$ (H-12) signal could also be observed at δ 4.96 ppm, which further confirmed the coupling of benzoxaborole and cinnamic acids.

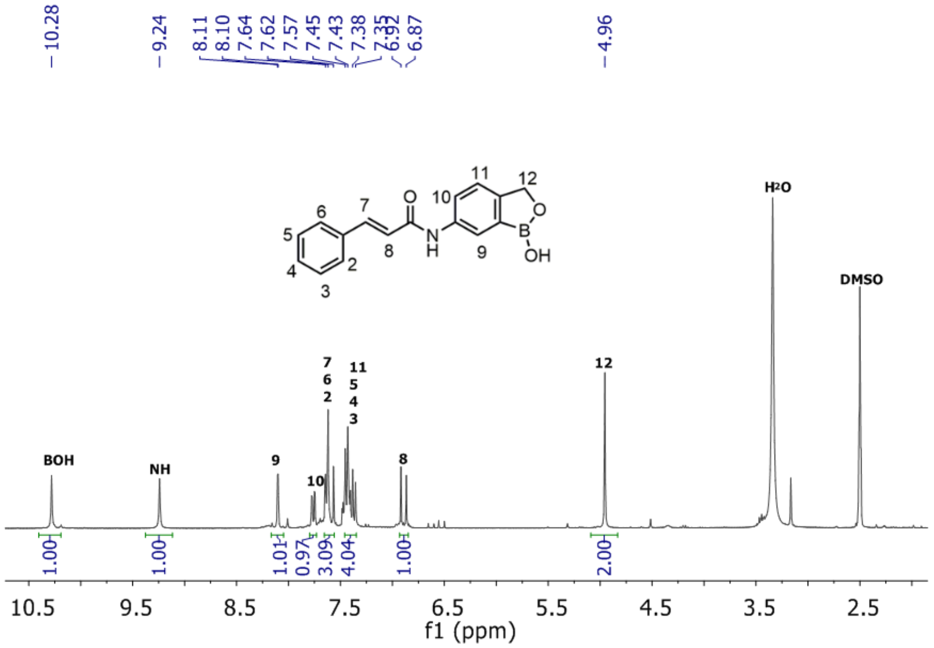


Figure 2.15: ^{1}H NMR (DMSO-d_6) spectrum of 2.11a.

The ^{13}C NMR spectrum (**Fig. 2.17**) of each compound confirmed the skeletal structures consistent with proposed compounds **2.11a – g**. The non-appearance of the carbon adjacent to the boron atom was noted, which is a common phenomenon in benzoxaborole containing compounds.[21]

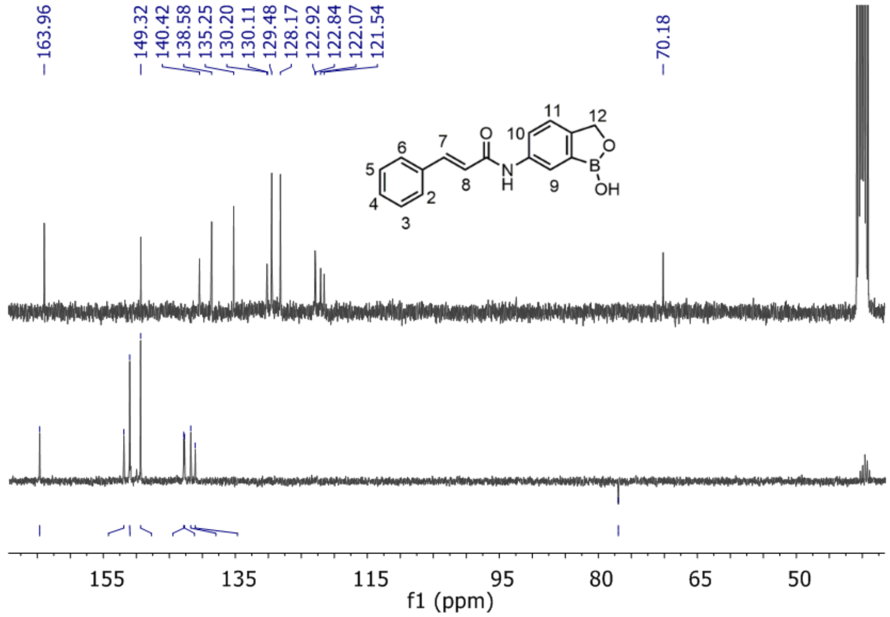


Figure 2.16: ^{13}C NMR (top) and DEPT135 NMR of (bottom) (DMSO-d_6) spectra of **2.11a**

Analysis of the high resolution mass spectrometry (HRMS) revealed molecular ion peaks agreeing with overall molecular structures of achieved compounds **2.11a-g**. The spectrum below showed a molecular ion peak consistent with structure of **2.11a** at *m/z* 280.1146 (**Fig. 2.18**).

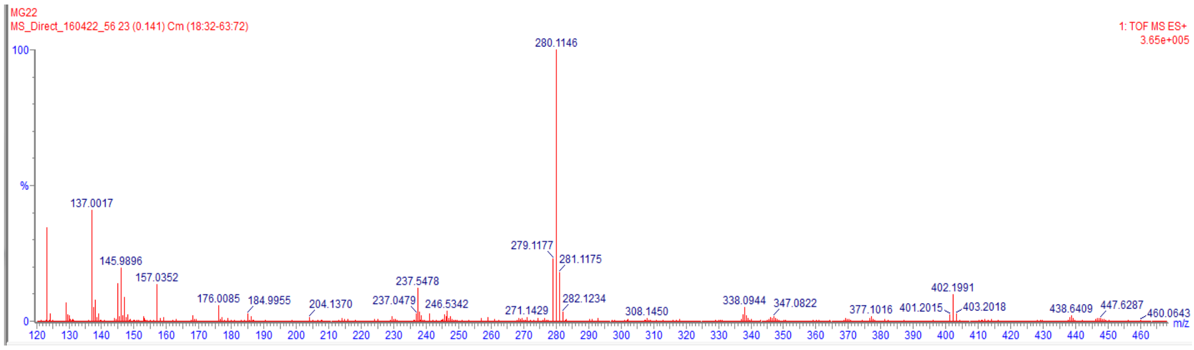


Figure 2.17: HRMS spectrum for **2.11a**

The purity of resulting compounds was verified using Qualitative HPLC (Reversed phase), which showed a single peak for the derivatives with relative purity as high as 99.3 % for **2.11a**. **Fig. 2.19** shows the chromatogram for **2.11a** with retention time of 1.01 minutes.

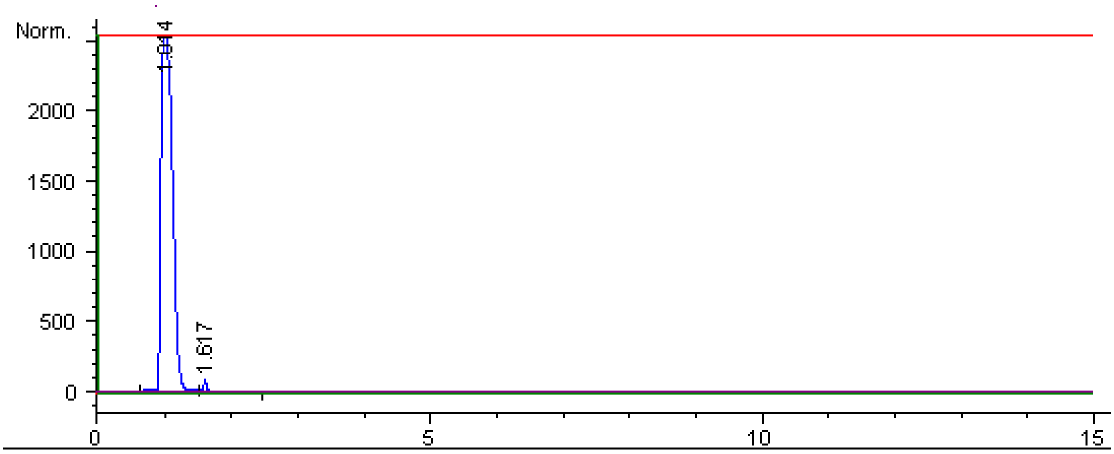


Figure 2.18: HPLC chromatogram for **2.11a**.

2.3 CONCLUSIONS

In conclusion, the synthesis of the key precursor, 6-aminobenzoxaborole (**2.16**) was achieved starting from the unsubstituted benzoxaborole (**2.18**), with the primary intention to couple it with the cinnamoyl and chloroquine pharmacophores to form the desired benzoxaborole-based hybrids. Lead compound **S427** prompted the synthesis of the cinnamoyl-benzoxaborole hybrids **2.11a-g**, which were successfully accessed through amidation. The first set of chloroquine-benzoxaborole hybrids **2.12a-c** were successfully synthesised and characterised, however, these compounds appeared to be unstable after standing at room temperature overnight. The second set of chloroquine-benzoxaborole hybrids **2.21a-c** and **2.33** could not be successfully accessed due to unbelievably low yields of vital precursor **2.29** and proved difficult to isolate. All precursors and target compounds were successfully characterised using various analytical and spectroscopic techniques such as ^{1}H, ^{13}C NMR and mass spectrometry. The purity of final compounds was measured using the HPLC.

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CHAPTER THREE

3 *IN VITRO* ANTIPLASMODIAL AND ANTITRYPANOSOMAL EVALUATION OF BENZOXABOROLE HYBRIDS

3.1 INTRODUCTION

The central objective in this research was to identify compounds based on the benzoxaborole scaffold with antiparasitic activities. The synthesised compounds were generated in order to assess their ability to inhibit plasmodial and trypanosomal growth by determining their IC_{50} values. The IC_{50} is defined as the drug concentration required to cause the measured parameter (parasite viability, in this case) to fall to 50 % of its original value. The lower the IC_{50} value of a compound, the better the efficacy.

Additionally, a good drug should exhibit good selectivity.[1] The more selective a drug for its target, the less chance that it will interact with different targets, which may lead to unwanted side effects. Thus, a compound displaying good efficacy (low IC_{50}) will not be a good drug if it exhibits poor selectivity between the host and the parasite. All experimental details relating to biological tests have been done in collaboration with the Centre for Chemico- and Biomedical Research at Rhodes University and detailed experimental procedures are provided in the experimental section.

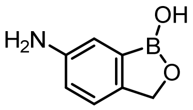
3.2 BIOLOGICAL RESULTS AND DISCUSSION

All the synthesised benzoxaborole compounds described in **Chapter 2** were evaluated in three *in vitro* assays. In the first instance, the compounds were evaluated *in vitro* against the *P. falciparum* chloroquine-sensitive (CQS) 3D7 strain with chloroquine (CQ) used as the control drug. The antitrypanasomal activities of the compounds against *T. brucei* parasite cultures were determined using the 427 strain of *T. brucei brucei.* Pentamidine, which is an existing drug for treatment of trypanosomiasis, was used as a positive control drug. The cytotoxicity evaluation of all compounds was assessed using HeLa (human cervix adenocarcinoma) cell line and emetine was used as a control drug. Assays were conducted in the Centre for Chemico and Biomedicinal Research (CCBR) bioassay facility at Rhodes

University, funded by the Medical Research Council SA University Flagship programme, in collaboration with the groups of Professors P.T. Kaye and H.C. Hoppe.

3.2.1 *In vitro* antiplasmodial activities of benzoxaborole-based compounds

Initially, the malaria parasite lactate dehydrogenase (pLDH) and cytotoxicity (HeLa cells) assays were performed using 20 μM solutions containing the prepared chloroquine-benzoxaborole hybrid **2.12b** in duplicate. The results are summarised in **Table 3.1**. At 20 μM, **2.12b** (**MG-15)** completely inhibited the viability of the malaria parasite while, by comparison, HeLa cell viability was reduced by <40 %. Interestingly, the non-hybridized 6-aminobenzoxaborole1 **2.16** (**MG-13**) showed no effect on the parasites with the percentage viability >90 % suggesting that the combination of CQ and benzoxaborole is important. Unfortunately, we were not able to test **2.12a** and **2.12c** as these appeared to be unstable as described in **Chapter 2**.


2.16

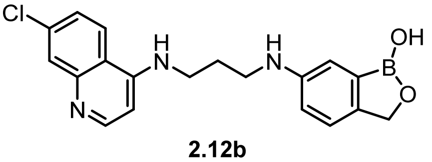

2.12b

Table 3.1: Residual % *P. falciparum* parasite viability and toxicity (HeLa cell viability) data at 20 μM.

Compound	**Percentage viability%**			
	P. falciparum	**SD***	**HeLa**	**SD**
2.12b	-2.68	16.9	61.2	7.39
2.14	95.1	4.65	85.2	1.73

*Standard deviation

Subsequently, the IC_{50} value was determined for compound **2.12b** as it completely inhibited the parasite at 20 μM. **Fig. 3.1** illustrates the percentage parasite viability plotted against the logarithm of concentration with IC_{50} value determined by non-linear regression as illustrated by the trend-line. Compound **2.12b** showed moderate activity against the 3D7 strain of *P. falciparum* parasite with an IC_{50} value of 3.43 μM. Under the same conditions, CQ was very active with an IC_{50} value of 0.0081 μM. This compound showed modest cytotoxicity towards the HeLa cell line with an IC_{50} value of 27.9 μM.

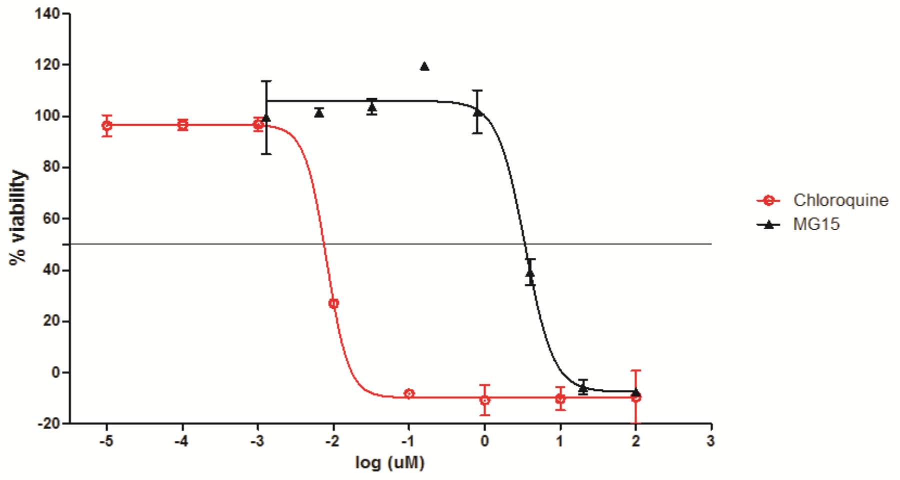


Figure 3.1: Inhibitory effect of **MG-15** (**2.12b**), showing percentage viability of *P. falciparum* at varying compound concentrations. The dose-response curve and IC_{50} value were generated by non-linear regression using GraphPad Prism.

The next set of compounds was cinnammoyl-benzoxaborole compounds **2.11a-g** (structures are displayed in **Table 3.2**), which are linked via an amide bond to form hybrid molecules. Similarly, the malaria pLDH assay and cytotoxicity (HeLa cells) screen were performed using 20 µM solutions containing prepared compounds in duplicate. CQ and emetine were included as positive controls. The results are summarised and displayed in **Fig. 3.2**.

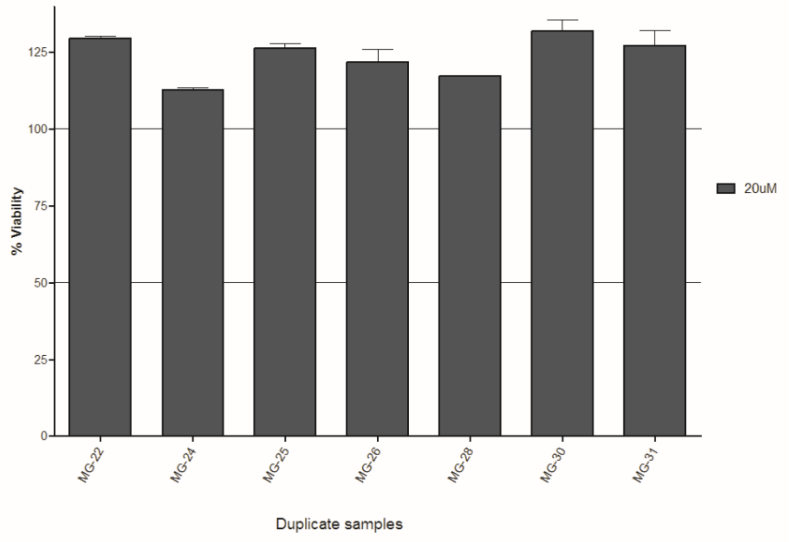


Figure 3.2: Antiplasmodial (*P. falciparum* pLDH) screening assay data for compounds **2.11a-g** at 20 µM. The graph shows percentage of living parasite (pLDH) after incubation with the compounds.

Unfortunately, none of these compounds significantly showed growth inhibition below 50 % and in most cases the malaria viability was >80 % at 20 µM. The growth of the HeLa cells was also not significantly affected indicating that these compounds are not generally cytotoxic.

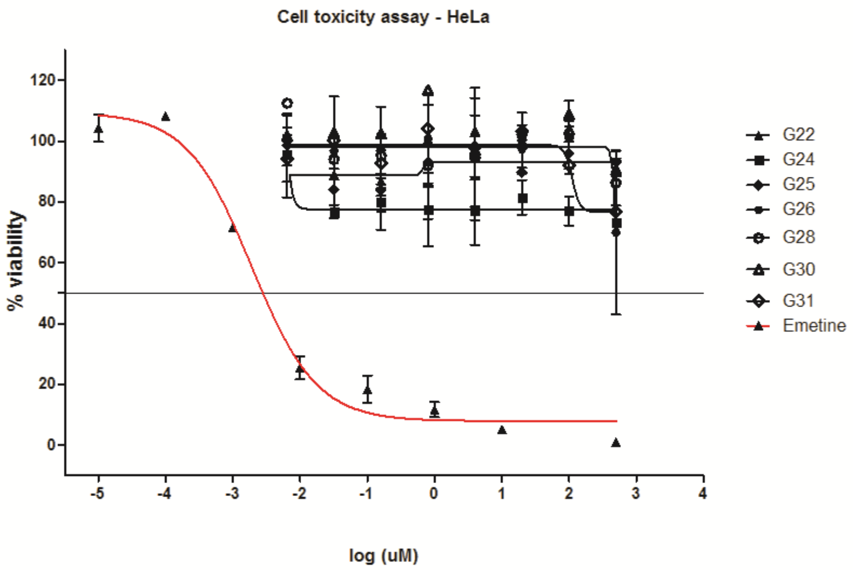


Figure 3.3: Cell cytotoxicity assay (HeLa cell viability) data at 20 µM.

Based on this data, these compounds were not recommended for further assay. The results obtained nullified our hypothesis that the cinnamoyl-benzoxaborole hybrid compounds could possess significant antiplasmodial activity.

3.2.2 *In vitro* antitrypanosomal activities of cinnamoyl-benzoxaborole hybrids

Inspired by hydrocinnamoyl-benzoxaborole compound **2.10** (**Scheme 2.1** in **Chapter 2**), a small series of cinnamoyl-benzoxaborole hybrid compounds **2.11a-g** (**G22 – 31**) linked through an amide bond were synthesised and evaluated for their potency against *T. brucei*. The cytotoxicity (HeLa cells) and *T. brucei* assays were also conducted using 20 µM solutions containing prepared compounds **2.11a-g** in duplicate. To our delight, compounds **2.11a-g** exhibited significant growth inhibition and often the percentage viability of trypanosomes for each compound was below 10 % at 20 µM (**Fig. 3.3**).

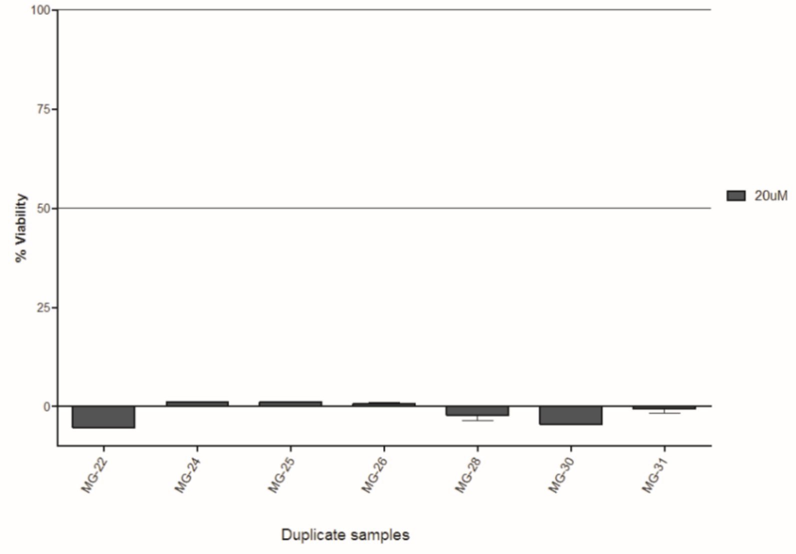
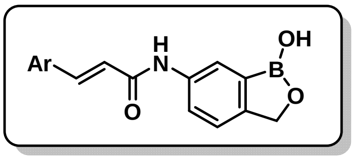


Figure 3.4: Antitrypanosomal screening assay data for compounds **2.11a-g** at 20 µM. The graph shows the residual parasite.

More importantly, at the same concentration these compounds showed no significant toxicity effects against the HeLa cells, and in most cases >80 % HeLa cell viability was observed. Based on the single concentration screening data (**Fig. 3.3**), the IC_{50} values were determined for compound **2.11a-g** (**G22-31**), and the results are summarised in **Table 3.2**.

Table 3.2: *In vitro* cytotoxicity and anti-trypanosomal activities of cinnamoyl-benzoxaborole hybrids, **2.11a-g**



Compound		**IC$_{50}$ (µM)**		**Selectivity index**
Number	**Code**	***T. brucei***	**%HeLa cells**	**HeLa IC$_{50}$/*T.b.* IC$_{50}$**
2.11a	**G22**	**0.13**	**108.5**	**834.6**
2.11b	**G24**	**0.13**	**87.0**	**669.2**
2.11c	**G25**	1.11	94.3	85.0
2.11d	**G26**	**0.46**	**94.1**	**2.3**
2.11e	**G28**	**0.66**	**102.5**	**155.3**
2.11f	**G30**	8.71	109.9	12.6
2.11g	**G31**	**0.052**	**131.5**	**2528.8**
Pentamidine		0.0012	-	
Ementine		-	3.19	

The hybrids all showed promising antitrypanosomal activity with IC$_{50}$ values in the low micromolar to sub-micromolar range. More importantly, half of the compounds (**2.11a**, **2.11b**, , **2.11d, 2.11e** and **2.11g**) showed good activity (highlighted in bold) with IC$_{50}$ values below 1 μM and compound **2.11g** emerged as the most active compound. However, compound **2.11g** was 40-fold less active than the standard drug pentamidine (**Table 3.2**)[3,4]. The more active compounds also displayed excellent selectivity for the trypanosmal parasites over human HeLa cells, with selectivity indexes ranging from 155 to >2500. Thus far, the preliminary structure-activity relationship suggested that mono-substituted aryl moieties are preferred for activity with electron donating groups being the most favoured by this class of compounds. More importantly, bicyclic aryl frameworks as observed for compound **2.11g** emerged as promising ring systems, which are worth for further exploration.

3.3 CONCLUSIONS

The most important precursor for this project 6-amino benzoxaborole **2.14** showed no antimalarial activity and toxicity on HeLa cells. However, one of the chloroquine-benzoxaborole hybrids showed promising antimalarial activity with an IC_{50} of 3.4 µM against *P. falciparum*, with only moderate toxicity (HeLa cell IC_{50} 28 µM). The cinnamoyl-benzoxaborole hybrids showed no antimalarial activity at 20 µM. On the contrary, the same compounds showed low to sub-micromolar IC_{50} values against *Trypanosoma brucei*. Compounds **2.11a-g** showed no cytotoxicity effects against HeLa cell line, producing selectivity indexes of 155-2500 for the best compounds. The lack of antimalarial and HeLa cell activity of the cinnamoyl-benzoxaborole hybrids suggests that they are not general eukaryotic toxins, but are specifically targeting a metabolic process that is vital to trypanosomes and may be promising scaffolds for the development of novel African sleeping sickness therapeutics.

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CHAPTER FOUR

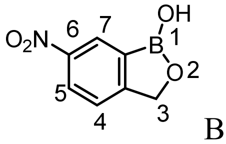
4 EXPERIMENTAL PROCEDURE

4.1 MATERIALS AND METHODS

Chemicals and reagents were purchased from Sigma-Aldrich and Merck, South Africa. Thin layer chromatography (TLC) was performed on pre-coated silica gel F254 plates (0.25mm, E. Merck). Purification of products was done by column chromatography using Silica 60 (0.063 – 0.2 mm) from Macherey-Nagel with pore size - 60 Å, pore volume - 0.75 mL/g, spec. surface BET- 500 m^2/g. Preparative thin layer chromatography (PTLC) was prepared and activated as prescribed by the supplier (Merck). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 300, 400 and 600 MHz spectrophotometers. The samples were analysed in deuterated solvents, DMSO-d_6 with chemical shifts 2.5 ppm in ^{1}H NMR and 39.5 ppm in ^{13}C NMR and $CDCl_3$-d with chemical shifts 7.26 ppm in ^{1}H NMR and 77.2 ppm in ^{13}C NMR. The chemical shifts were given in parts per million. Abbreviations d - doublet, dd - double of doublets, s - singlet, t - triplet, q - quartet and m - multiplet were used to describe NMR signals. Electron Spray Ionization mass spectrometer (ESI-MS) in the positive and negative ionization mode was used to record low resolution mass spectra. High resolution mass spectrometry data were recorded on Waters Snapt G2-TOF MS, University of Stellenbosch, and ion's mass-to-charge ratio was determined by time measurement. The mass spectrophotometer signals were recorded in *m/z* (mass to charge ratio). The melting points (M.p.) were determined on Reichert 281313 melting point apparatus. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR 400 instrument, absorption bands are reported as s = strong, m = medium, b = broad or w = weak.

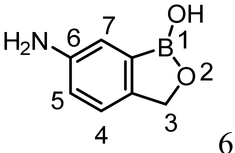
4.2 SYNTHESIS OF 6-AMINOBENZO[C][1,2]OXABOROLE-1(3H)-OL, 2.16

4.2.1 Synthesis of 6-aminobenzo[c][1,2]oxaborol-1(3H)-ol, 2.17



Benzoxaborole **2.18** (1.0 g, 4.9 mmol) was added portion-wise to 4.8 mL of fumic nitric acid (99.9 %) maintained at a temperature between -30 °C and -40 °C. The mixture was allowed to stir at temperature between -30 °C and -40 °C for 3 h. Ice and water (20 mL) were added to the mixture and a precipitate was observed, the mixture was then allowed to stir at room temperature for an additional 20 minutes. The solid product was filtered through the vacuum filtration, washed with water, dried overnight and recrystallized in EtOAc to give **2.17** as a pale yellow solid.[1] Yield = 89 %. M.p.: 195 – 200 °C (Lit. 200 °C).[1] ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 9.59 (1H, s, B-OH), 8.56 (1H, d, J = 1.2 Hz, Ar-H7), 8.31 (1H, dd, J = 2.3 and 6.2 Hz, Ar-H5), 7.69 (1H, d, J = 8.4 Hz, Ar-H4), 5.11 (2H, s, H3). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 155.1, 148.4, 143.4, 131.3, 121.7, 71.1.

4.2.2 Synthesis of 6-aminobenzo[c][1,2]oxaborol-1(3H)-ol, 2.16

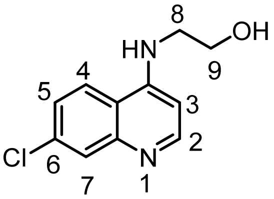


6-Nitrobenzo[c][1,2]oxaborol-1-(3H)-ol **2.17** (0.1 g, 0.56 mmol) was dissolved in ethanol (2 mL), followed by addition of 10 % palladium on carbon (0.04 g). The mixture was vacuumed three times to remove air and filled with hydrogen. The reaction mixture was allowed to stir at room temperature for 2 h. The reaction mixture was filtered through a celite pad and the filtrate was dried under reduced pressure to give **2.16** as a brick red solid.[1] Yield = 91 %. M.p.: 177 – 180 °C (Lit. 178-180 °C).[1] ν_{max} = 3244 (NH), 3028 (OH), 1738 (CH). ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 9.59 (1H, s, B-OH), 8.56 (1H, d, J = 1.2 Hz, Ar-H7), 8.31 (1H, dd, J = 2.3 and 6.2 Hz, Ar-H5), 7.69 (1H, d, J = 8.4 Hz, Ar-H4), 4.99 (2H, br, NH), 4.82 (2H, s, H3). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 147.9, 141.9, 121.7, 118.2, 115.1, 70.2. *m/z* (HRMS – ESI) found 146.0243 [M]$^+$; expected 149.0648.

4.3 GENERAL PROCEDURE FOR SYNTHESIS OF 2-((7-CHLOROQUINOLIN-4-YL)AMINO) ALCOHOLS

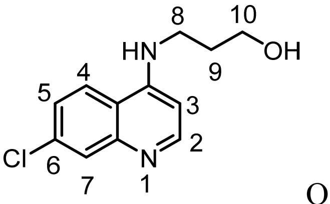
A mixture of 4,7-dichloroquinoline, **2.14** (2.5 mmol, 1 eq) and the corresponding amino-alcohols (50.0 mmol, 20 eq) was refluxed in the presence of K_2CO_3 (2.5 eq) and catalytic amounts of Et_3N at 140 °C for 4 h. The progress of the reaction was monitored by thin layer chromatography (TLC) which showed the consumption of starting material. The reaction mixture was then allowed to cool to room temperature, washed with brine and water; and recrystallized in methanol to yield corresponding (7-chloroquinolin-4yl amino) alcohols (**2.13a-c**) in good yields.[2]

4.3.1 2-((7-chloroquinolin-4-yl)amino)ethan-1-ol, 2.13a



Off white crystals. Yield = 66 %. M.p.: 213 – 215 °C (Lit. 214 °C).[2] ^{1}H NMR (600 MHz, DMSO- d_6) δ_H (ppm): 8.39 (1H, d, J = 9.1 Hz, Ar-H4), 8.38 (1H, d, J = 5.5 Hz, Ar-H2), 7.78 (1H, d, J = 2.2 Hz, Ar-H7), 7.41 (1H, br, NH), 7.42 (1H, dd, J = 2 .2 and 6.8 Hz, Ar-H5), 6.48 (1H, d, J = 5.5 Hz, Ar-H3), 5.07 (1H, br, OH), 3.68 (2H, t, J = 5.9 Hz, H8), 3.35 (2H, t, J = 5.6 Hz, H9). ^{13}C NMR (151 MHz, DMSO-d_6) δ_C (ppm): 152.3, 150.9, 149.5, 133.9, 127.8, 124.9, 124.4, 117.9, 99.1, 59.2, 45.8.

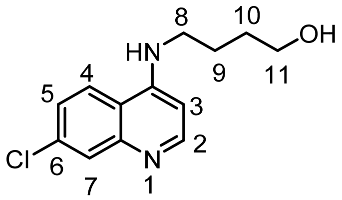
4.3.2 3-((7-chloroquinolin-4-yl)amino)propan-1-ol, 2.13b



Off white crystals. Yield = 62 %. M.p.: 141 – 144 °C (Lit.142 – 145 °C).[2] ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 8.39 (1H, d, J = 5.4 Hz, Ar-H2), 8.25 (1H, d, J = 9.0 Hz, Ar-H4), 7.77 (1H, d, J = 2.2 Hz, Ar-H7), 7.42 (1H, dd, J = 2.2 and 6.8 Hz, Ar-H5), 7.29 (1H, br, NH), 6.47 (1H, d, J = 5.49 Hz, Ar-H3), 4.6 (1H, br, OH), 3.53 (2H, t, J =

5.9 Hz, H8), 3.32 (2H, t, *J* = 6.8 Hz, H10), 1.86 – 1.77 (2H, m, H9). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 152.5, 150.5, 149.5, 133.9, 127.9, 124.4, 118.1, 99.1, 59.1, 40.0, 31.5.

4.3.3 4-((7-chloroquinolin-4-yl)amino)butan-1-ol, 2.13c

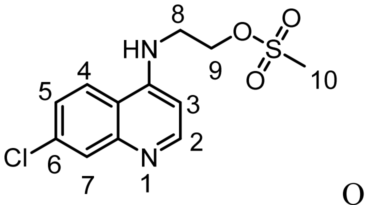


Off white crystals. Yield = 54 %. M.p.: 201 – 203 °C (Lit. 201 – 203 °C).[2] ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 8.37 (1H, d, *J* = 5.4 Hz, Ar-H2), 8.28 (1H, d, *J* = 9.0 Hz, Ar-H4), 7.76 (1H, d, *J* = 2.2 Hz, Ar-H7), 7.43 (1H, dd, *J* = 2.3 and 6.7 Hz, Ar-H5), 7.36 (1H, br, NH), 6.46 (1H, d, *J* = 5.5 Hz, Ar-H3), 4.51 (1H, br, OH), 3.44 (2H, t, *J* = 6.2 Hz, H8), 3.26 (2H, t, *J* = 6.8 Hz, H11), 1.71 – 1.63 (2H, m, H9), 1.57 – 1.48 (2H, m, H10). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 151.7, 150.2, 147.1, 134.8, 125.9, 125.1, 125.0, 117.5, 99.1, 60.9, 42.9, 30.4, 24.9.

4.4 GENERAL PROCEDURE FOR SYNTHESIS OF 2-((7-CHLOROQUINOLIN-4-YL)AMINO) ALKYL METHANESULFONATES

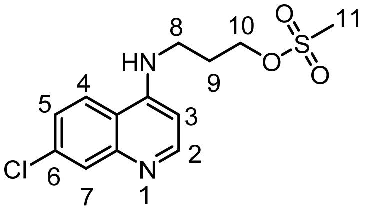
A suspension of 2-((7-chloroquinolin-4-yl)amino) alcohols (**2.13a-c**) in pyridine was allowed to stir at 0 °C for 30 minutes, followed by dropwise addition of methanesulfonylchloride. The mixture was allowed to stir between 0 - 5 °C for 5 h. The progress of the reaction was monitored by TLC which showed the consumption of starting material. Upon complete consumption of the alcohol 1 mL of 17 % ammonia solution was added and the desired product extracted three times using dichloromethane. The organic phase was dried over $MgSO_4$ and dried *in vacuo* followed by recrystalisation in methanol to yield corresponding mesylated compounds **2.19a** and **2.19b** in good yields.[3]

4.4.1 2-((7-chloroquinolin-4-yl)amino)ethyl methanesulfonate, 2.19a



Off white crystals. Yield = 64 %. M.p.: 137 – 140 °C (Lit. 138 – 140 °C).[2] ^{1}H NMR (600 MHz, DMSO-d_6) δ_H (ppm): 9.90 (1H, br, NH), 8.78 (1H, d, J = 9.2 Hz, Ar-H4), 8.60 (1H, d, J = 7.1 Hz, Ar-H2), 8.15 (1H, d, J = 2.0 Hz, Ar-H7), 7.76 (1H, dd, J = 2.0 and 7.08 Hz, Ar-H5), 6.96 (1H, d, J = 7.2 Hz, Ar-H3), 4.53 (2H, t, J = 5.1 Hz, H8), 3.93 (2H, t, J = 5.4 Hz H9), 3.20 (3H, s, H10). ^{13}C NMR (151 MHz, DMSO-d_6) δ_C (ppm): 156.3, 143.3, 139.0, 138.5, 127.4, 126.5, 119.5, 115.9, 99.4, 68.1, 42.7, 37.4, 31.2.

4.4.2 3-((7-chloroquinolin-4-yl)amino)propyl methanesulfonate, 2.19b

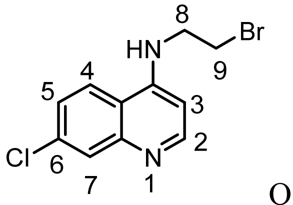


Off white crystals. M.p.: 128 – 130 °C (Lit. 130 – 135 °C).[2] Yield = 68 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 9.79 (1H, br, NH), 8.87 (1H, d, J = 9.2 Hz, Ar-H2), 8.62 (1H, d, J = 7.1 Hz, Ar-H4), 8.16 (1H, d, J = 2.0 Hz, Ar-H4), 7.81 (1H, dd, J = 2.0 and 7.1 Hz, Ar-H5), 6.96 (1H, d, J = 7.2 Hz, Ar-H3), 4.36 (2H, t, J = 6.1 Hz, H8), 3.63 (2H, t, J = 6.0 Hz, H10), 3.21 (3H, s, H11), 2.06 – 2.15 (2H, m, H9). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 155.9, 143.3, 139.9, 138.9, 138.3, 127.2, 126.5, 119.4, 116.0, 99.0, 68.5, 40.1, 37.1, 24.7.

4.5 GENERAL PROCEDURE FOR SYNTHESIS OF N-(2-BROMOALKYL)-7-CHLOROQUINOLIN-4-AMINES

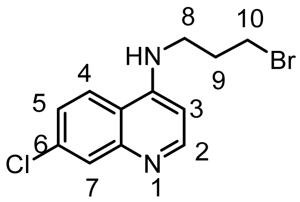
2-((7-chloroquinolin-4-yl)amino) alcohols, (**2.13a-c**) (2.12 mmol) were allowed to reflux at 65 °C in 48 % HBr and catalytic amounts of H_2SO_4. The reaction mixture was allowed to stir for 4 h. Upon complete consumption of the alcohol (TLC) the reaction was quenched with $NaHCO_3$ and product was extracted with ethyl acetate, washed with saturated $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and solvent removed under reduced pressure, followed by purification using column chromatography to yield **2.20a-c** in good yields.[2]

4.5.1 N-(2-bromoethyl)-7-chloroquinolin-4-amine, 2.20a



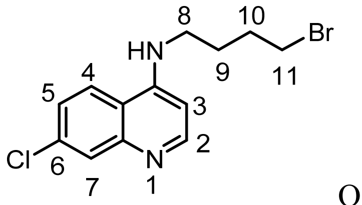
Off white crystals. Yield = 84 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 9.51 (1H, br, NH), 8.63 (1H, d, J = 7.1 Hz, Ar-H2), 8.59 (1H, d, J = 9.2 Hz, Ar-H4), 7.98 (1H, d, J = 2.1 Hz, Ar-H7), 7.81 (1H, dd, J = 2.1 and 6.9 Hz, Ar-H5), 7.04 (1H, d, J = 7.2 Hz, H3), 4.03 (1H, t, J = 5.9 Hz, Ar-H9), 3.78 (2H, t, J = 6.2 Hz, H8). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 156.0, 143.8, 138.9, 138.7, 127.7, 125.9, 119.7, 115.8, 99.5, 45.1, 31.4.

4.5.2 N-(3-bromopropyl)-7-chloroquinolin-4-amine, 2.20b



Off white solid. Yield = 88 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 9.41 (1H, br, NH), 8.62 (1H, d, J = 6.6 Hz, Ar-H2), 8.59 (1H, d, J = 8.8 Hz, Ar-H4), 7.93 (1H, d, J = 2.1 Hz, H7), 7.78 (1H, dd, J = 2.1 and 6.9 Hz, Ar-H5), 6.94 (1H, d, J = 7.2 Hz, H3), 3.71 – 3.59 (4H, m, H8/10), 2.12 – 2.21 (2H, m, H9). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 156.1, 143.5, 138.1, 138.9, 127.0, 125.5, 119.3, 115.8, 99.6, 45.10, 31.35, 28.4.

4.5.3 N-(4-bromobutyl)-7-chloroquinolin-4-amine, 2.20c

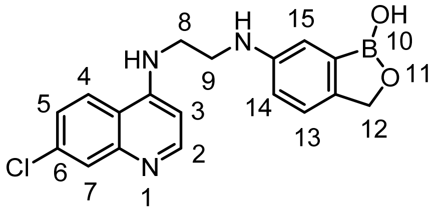


Off white solid. Yield = 73 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 9.48 (1H, br, NH), 8.63 (1H, d, J = 9.0 Hz, Ar-H2), 8.59 (1H, d, J = 9.0 Hz, Ar-H4), 7.96 (1H, d, J = 2.1 Hz, H7), 7.75 (1H, dd, J = 2.1 and 6.9 Hz, Ar-H5), 6.94 (1H, d, J = 7.2 Hz, H3), 3.56 – 3.65 (4H, m, H8, H11), 1.90 – 1.98 (2H, m, H10), 1.75 – 1.84 (2H, m, H9). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 155.8, 143.4, 138.8, 138.4, 127.3, 126.3, 119.4, 115.8, 99.1, 42.7, 35.3, 30.0, 26.7.

4.6 GENERAL PROCEDURE FOR SYNTHESIS OF CHLOROQUINE-BENZOXABOROLE HYBRIDES

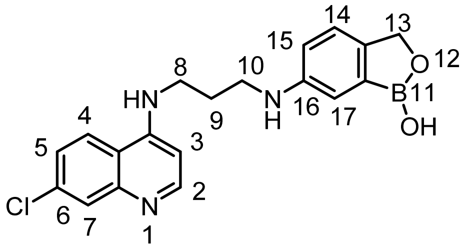
An appropriate N-(2-bromoalkyl)-7-chloroquinolin-4-amines, **2.20** (11 mmol), 6-aminobenzo[c][1,2]oxaborol-1(3H)-ol **2.16** (10 mmol) in DMF (5 mL) were added into a round bottom flask. To this reaction mixture Et$_3$N (10 mmol) and catalytic amounts of DMAP were added and the reaction mixture was left stirring at room temperature for 24 h. The progress of the reaction was monitored by TLC. After completion, the reaction product was extracted with ethyl acetate (3 × 15 mL) followed by washing with 5 % HCl, saturated NaHCO$_3$ and water. The organic layers were combined and dried over anhydrous MgSO$_4$ and concentrated under reduced pressure to give **2.12a-c** as crude products.[4]

4.6.1 6-((2-((7-chloroquinolin-4-yl)amino)ethyl)amino)benzo[c][1,2]oxaborol-1(3H)-ol, 2.12a



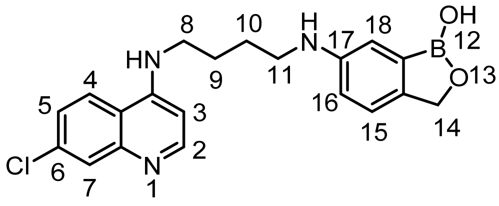
Yellow solid. Yield = 60 %. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ_H (ppm): 9.50 (1H, br, NH), 8.58 (1H, s, Ar-H15), 8.41 (1H, d, *J* = 5.4 Hz, Ar-H2), 8.31 (1H, d, *J* = 8.4 Hz, Ar-14), 8.24 (1H, d, *J* = 9.0 Hz, Ar-H4), 7.81 (1H, d, *J* = 1.9, Hz, Ar-H7), 7.69 (1H, d, *J* = 8.1 Hz, Ar-H13), 7.61 (1H, br, NH), 7.50 (1H, dd, *J* = 1.5 and 7.1 Hz, Ar-H5), 6.58 (1H, d, *J* = 5.4 Hz, Ar-H3), 5.12 (2H, s, H12), 3.70 – 3.78 (2H, t, *J* = 6.0 Hz, H8), 1.17 (1H, t, *J* = 7.1 Hz, H9). ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ_C (ppm): 161.0, 152.2, 150.0, 149.2, 147.9, 134.0, 127.8, 126.0, 125.9, 124.9, 124.4, 123.5, 117.7, 99.3, 70.4, 44.6, 31.6. *m/z* (ESI-MS) found 354.2000 [M+H]$^+$, expected 353.1102.

4.6.2 6-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)benzo[c][1,2]oxaborol-1(3H)-ol, 2.12b



Off white solid. Yield = 55 %. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H (ppm): 9.59 (1H, br, NH), 8.58 (1H, d, *J* = 1.4 Hz, Ar-H17), 8.42 (1H, d, *J* = 5.4 Hz, Ar-H2), 8.33 (1H, dd, *J* = 1.8 and 6.6 Hz, Ar-15), 8.25 (1H, d, *J* = 9.0 Hz, Ar-H4), 7.79 (1H, d, *J* = 2.2 Hz, Ar-H7), 7.69 (1H, d, *J* = 8.4 Hz, Ar-H14), 7.50 (1H, dd, *J* = 2.2 and 6.7 Hz, Ar-H5), 7.43 (1H, br, NH), 6.52 (1H, d, *J* = 5.5 Hz, Ar-H3), 5.12 (2H, s, H13), 3.67 (2H, t, *J* = 6.5 Hz, H10), 3.42 (2H, t, *J* = 6.6 Hz, H8), 2.22 – 2.17 (2H, m, H9). ^{13}C NMR (151 MHz, DMSO-d_6) δ_C (ppm): 161.1, 152.2, 150.7, 149.1, 147.7, 133.9, 127.7, 126.2, 125.9, 124.8, 124.5, 123.5, 117.9, 99.1, 70.5, 41.5, 33.0, 31.4. *m/z* (ESI-MS) found 368.1314 $[M+H]^+$, expected 367.1259.

4.6.3 6-((4-((7-chloroquinolin-4-yl)amino)butyl)amino)benzo[c][1,2]oxaborol-1(3H)-ol, 2.12c

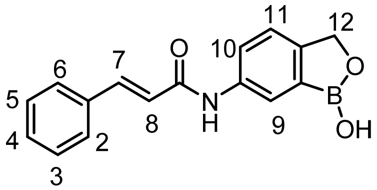


Yellow crystals. Yield = 75 % (crude). ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 8.42 (1H, d, *J* = 7.8 Hz, Ar-H4), 8.40 (1H, d, *J* = 5.40 Hz, Ar-H2), 7.95 (1H, br, B-OH), 7.90 (1H, d, *J* = 2.4 Hz, Ar-7), 7.49 (1H, dd, *J* = 2.4 and 6.9 Hz, Ar-H5), 7.00 (1H, d, *J* = 7.5, Hz, Ar-H15), 6.89 (1H, d, *J* = 1.8 Hz, Ar-H18), 6.70 (1H, dd, *J* = 2.1 and 6.0 Hz, Ar-H16), 6.58 (1H, d, *J* = 6.6 Hz, Ar-H3), 4.80 (2H, s, H14), 3.84 – 3.71 (2H, m, H10), 3.16 – 3.08 (4H, m, H8, H11), 2.03 – 1.90 (2H, m, H9). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 162.7, 153.9, 147.8, 143.9, 141.8, 139.8, 136.1, 129.3, 121.8, 118.00, 117.5, 115.0, 107.3, 103.0, 70.0, 53.1, 36.1, 31.3, 25.6. **MS data**: Could not be done due to instability of this compound.

4.7 SYNTHESIS OF N-(1-HYDROXY-1,3-DIHYDROBENZO[C][1,2]OXABOROL-6-YL)CINNAMAMIDES

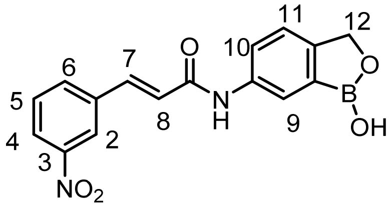
A solution of cinnamic acids **2.34a-g** (0.5 mmol) and Et_3N (1.2 mmol) in anhydrous CH_2Cl_2 (1.5 mL) under N_2 atmosphere was allowed to stir at 0 °C. After stirring for 5 min, $POCl_3$ (0.5 mmol) in anhydrous CH_2Cl_2 (1 mL) was added into the above reaction and the resulting reaction mixture allowed to stir at room temperature for 30 minutes. After complete consumption of the acids (TLC), a solution of DMAP (0.15 mmol) and 6-aminobenzo[c][1,2]oxaborol-1(3H)-ol (0.6 mmol) in anhydrous CH_2Cl_2 (0.5 mL) was added dropwise and the reaction mixture was allowed to stir overnight at room temperature. Upon completion of reaction (TLC), the reaction product was diluted with CH_2Cl_2 (30 mL). The organic phase was washed sequentially with ice-cold water (10 mL), 10 % aqueous HCl (10 mL), saturated aqueous $NaHCO_3$ solution (10 mL), and brine. The organic phase was dried over anhydrous $MgSO_4$, solvent removed under vacuum to give a solid residue, which was purified by flash column chromatography to afford the pure products,[5] **2.11a-g**.

4.7.1 (*E*)-N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)cinnamamide, 2.11a



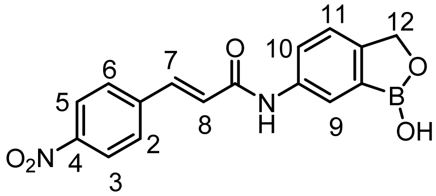
Off white solid. Yield = 41 %. M.p.: 182-184 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 10.28 (1H, s, B-OH), 9.25 (1H, s, NH), 8.11 (1H, d, *J* = 1.8 Hz, Ar-H9), 7.78 (1H, dd, *J* = 1.8 and 6.3 Hz, Ar-H10), 7.65 – 7.56 (3H, m, Ar-H7/H6/H2), 7.46 – 7.34 (4H, m, Ar-H11, Ar-H5, Ar-H4, Ar-H3), 6.90 (1H, d, *J* = 15.6 Hz, H8), 4.96 (2H, s, H12). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 163.6, 149.4, 140.4, 138.6, 135.3, 130.1, 129.6, 128.2, 122.9, 122.8, 122.1, 121.5, 70.2. *m/z* (ESI-MS) found 280.1146 $[M+H]^+$, expected 279.1067. HPLC Purity = 99.3 %, retention time = 1.01 minute.

4.7.2 (*E*)-N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-(3-nitrophenyl)acrylamide, 2.11b



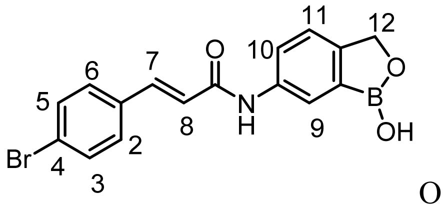
Yellow solid. Yield = 48 %. M.p.: 176-178 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 10.23 (1H, s, B-OH), 8.09 (1H, d, *J* = 3.0 Hz, Ar-H9), 8.77 (1H, dd, *J* = 3.0 and 6.0 Hz, Ar-H10), 7.52 (1H, d, *J* = 15.0 Hz, H7), 7.35 (1H, d, *J* = 6.0 Hz, Ar-11), 7.20 – 7.13 (2H, m, H4, H2),7.03 – 6.96 (1H, m, Ar-H6), 6.90 – 6.82 (1H, m, Ar-H5), 6.73 (1H, d, *J* = 15.0 Hz, H8), 4.94 (2H, s, H12). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 164.5, 149.4, 148.7, 140.4, 138.9, 129.9, 124.1, 122.9, 122.4, 121.7, 121.1, 109.4, 107.0, 102.2, 70.4. *m/z* (ESI-MS) found 324.1046 [M]$^+$, expected 324.0918. HPLC Purity = 92.5 %, retention time = 1.43 minutes.

4.7.3 (*E*)-N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-(4-nitrophenyl)acrylamide, 2.11c



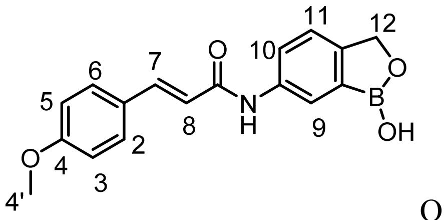
Yellow solid. Yield = 42 %. M.p.: 176-180 °C.^{1}H NMR (600 MHz, DMSO-d_6) δ_H (ppm): 10.42 (1H, s, B-OH), 8.29 (2H, *J* = 9.0 Hz, Ar-H3, Ar-H5), 8.10 (1H, d, *J* = 1.5 Hz, Ar-H9), 7.90 (2H, d, *J* = 9.0 Hz, Ar-H2, Ar-H6), 7.77 (1H, dd, *J* = 1.8 and 9.0 Hz, Ar-H10), 7.70 (1H, d, *J* = 15.6 Hz, H7), 7.39 (1H, d, *J* = 8.4 Hz, Ar-H11), 7.05 (1H, d, *J* = 15.0 Hz, H8), 4.96 (2H, s, H12). ^{13}C NMR (151 MHz, DMSO-d_6) δ_C (ppm): 163.3, 149.6, 148.1, 141.8, 138.4, 138.0, 129.2, 127.2, 124.6, 122.8, 122.2, 121.6. 70.3. *m/z* (ESI-MS) found 325.1005 [M+H]$^+$, expected 324.0918. HPLC Purity = 99.1 %, retention time = 1.33 minutes.

4.7.4 (*E*)-3-(4-bromophenyl)-N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)acrylamide, 2.11d



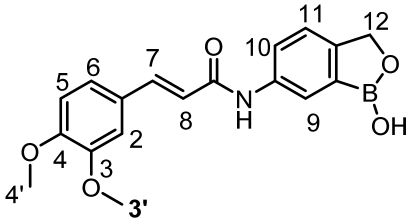
Off white solid. Yield = 29 %. M.p.: 206-210 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 10.33 (1H, s, B-OH), 8.06 (1H, d, J = 2.8 Hz, Ar-H9), 8.72 (1H, dd, J = 3.0 and 6.0 Hz, Ar-H10), 7.61 – 7.51 (4H, m, Ar-H2, H3, H5, H6), 7.53 (1H, d, J = 15.0 Hz, H7), 7.36 (1H, d, J = 9.0 Hz, Ar-11), 6.87 (1H, d, J = 15.0 Hz, H8), 4.94 (2H, s, H12). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 163.9, 149.6, 139.3, 139.3, 138.3, 134.4, 132.4, 130.2, 123.4, 123.0, 122.2, 121.6, 70.2. *m/z* (ESI-MS) found 358.0250 $[M+H]^+$, expected 357.0172. HPLC Purity = 98.4 %, retention time = 0.67 minutes.

4.7.5 (*E*)-N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-(4-methoxyphenyl)acrylamide, 2.11e



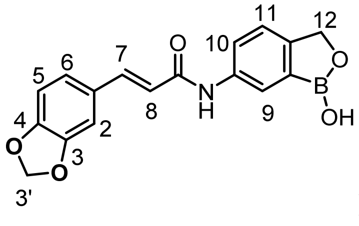
Off white solid. Yield = 32 %. M.p.: 190-192 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 10.19 (1H, s, B-OH), 9.25 (1H, s, NH), 8.09 (1H, d, J = 1.2 Hz, Ar-H9), 7.75 (1H, dd, J = 1.8 and 6.6 Hz, Ar-H10), 7.54 (1H, d, J = 15.0 Hz, H7), 7.36 (1H, d, J = 9.0 Hz, Ar-11), 7.01 (2H, d, J = 9.0 Hz, Ar-H2, H6), 6.97 (2H, d, J = 9.0 Hz, Ar-H5, H3), 6.74 (1H, d, J = 15.0, H8), 4.95 (2H, s, H12), 3.15 (3H, s, H4'). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 168.4, 164.4, 161.1, 149.3, 138.9, 130.5, 130.57, 130.0, 127.8, 127.7, 120.6, 115.1, 70.5, 56.1. *m/z* (ESI-MS) found 310.1252 $[M+H]^+$, expected 309.1172. HPLC Purity = 99.5 %, retention time = 0.953 minutes.

4.7.6 (*E*)-3-(3,4-dimethoxyphenyl)-N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)acrylamide, 2.11f



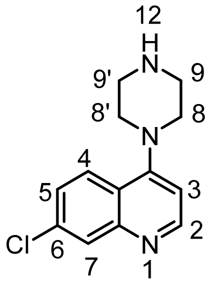
Pale yellow solid. Yield = 46 %. M.p.: 188 - 190 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 10.20 (1H, s, B-OH), 9.35 (1H, s, NH), 8.05 (1H, d, *J* = 2.6 Hz, Ar-H9), 7.71 (1H, dd, *J* = 3.0 and 6.0 Hz, Ar-H10), 7.51 (2H, d, *J* = 15.6 Hz, H7), 7.35 (1H, d, *J* = 9.0 Hz, Ar-11), 7.20 – 7.13 (2H, m, H5, H2), 6.98 (1H, d, *J* = 9.0 Hz, H6), 6.73 (1H, d, *J* = 15.0, H8), 4.93 (2H, s, H12), 3.79 (6H, s, H4', H3). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 168.9, 164.9, 151.2, 151.6, 149.7, 141.2, 138.8, 128.1, 127.7, 123.4, 122.7, 122.5, 122.0, 120.6, 110.9, 56.4, 56.3. *m/z* (ESI-MS) found 340.1357 [M+H]$^+$, expected 339.1278. HPLC Purity = 97.1 %, retention time = 1.276 minutes.

4.7.7 (*E*)-3-(benzo[d][1,3]^{6}dioxol-5-yl)-N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)acrylamide, 2.11g



Pale yellow solid. Yield = 38 %. M.p.: 145-148 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 10.64 (1H, s, B-OH), 9.30 (1H, s, NH), 8.47 (2H, s, Ar-H9), 8.24 (1H, d, *J* = 8.0 Hz, Ar-H10), 8.14 (1H, s, Ar-H2), 7.81 (1H, d, *J* = 6.3 Hz, Ar-11), 7.76 (1H, d, *J* = 7.6 Hz, Ar-6), 7.71 (1H, d, *J* = 16.0 Hz, H7), 7.37 (1H, d, *J* = 8.0 Hz, Ar-H5), 7.20 (1H, d, *J* = 16.0 Hz, H8), 4.98 (2H, s, H12), 1.98 (2H, s, H3'). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 170.7, 163.0, 149.4, 148.8, 138.4, 137.8, 134.4, 130.8, 125.9, 124.2, 122.1, 121.5, 70.1, 60.1. *m/z* (ESI-MS) found 325.0995 [M+H]$^+$, expected 323.0965. HPLC Purity = 100 %, retention time = 1.40 minutes.

4.8 SYNTHESIS OF 7-CHLORO-4-(PIPERAZIN-1-YL)QUINOLINE, 2.14

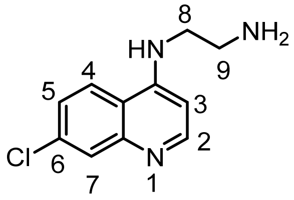


A mixture of 4,7 dichloroquinoline, **2.14** (0.5 g, 2.5 mmol) and piperazine, (0.4 g, 4.6 mmol) were allowed to reflux in 5mL ethanol at 78 °C for 12 h. The reaction mixture was cooled to room temperature and the precipitate filtered and rinsed with 10 mL cold methanol. The filtrate was evaporated to dryness and dissolved in 60 mL H_2O/DCM. The organic phase was washed with water three times and dried over $MgSO_4$ and the solvent was evaporated. The product was purified by recrystallization in 1:1 Hexane: EtOAc to give **2.32** as a pale yellow solid.[6] Yield = 71 %. M.p.: 112 – 114 °C (Lit. 113 – 114 °C).[6] ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ_H (ppm): 8.63 (1H, d, *J* = 5.04 Hz, Ar-H2), 7.96 (1H, d, *J* = 2.1 Hz, Ar-H7), 7.87 (1H, d, *J* = 9.0 Hz, Ar-H4), 7.34 (1H, dd, *J* = 2.2 and 9.0 Hz, Ar-H5), 3.04 - 3.41 (8H, m, H8/9/10/11).

4.9 GENERAL PROCEDUR FOR SYNTHESIS OF N^1-(7-CHLOROQUINOLIN-4-YL)ALKYL DIAMINE

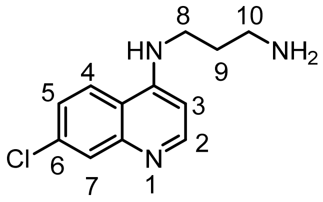
A mixture of 4,7-dichloroquinoline, **2.14** (2.5 mmol) and the corresponding diamines, **2.15a-c** (50.0 mmol) was heated at 80 °C for 1 h without stirring and then at 110 °C for 5 h with continued stirring to drive the reaction to completion. The reaction mixture was cooled to room temperature and extracted three times with 30 mL dichloromethane. The organic layer was dried in $MgSO_4$ and solvent was removed under reduced pressure to afford the desired products **2.30a-c** in good yields and in high purity.[7]

4.9.1 N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine, 2.30a



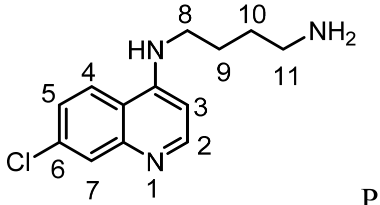
Pale yellow powder. Yield = 83 %. M.p.: 138-145 °C (Lit. 145-147 °C).[7] ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 8.38 (1H, d, *J* = 5.4 Hz, Ar-H2), 8.29 (1H, d, *J* = 9.0 Hz, Ar-H4), 7.78 (1H, d, *J* = 2.2 Hz, Ar-H7), 7.43 (1H, dd, *J* = 2.2 and 6.8 Hz, Ar-H5), 7.30 (1H, br, NH), 6.48 (1H, d, *J* = 5.5 Hz, Ar-H3), 3.25 (2H, t, *J* = 6.1 Hz, H9), 2.82 (2H, t, *J* = 6.5 Hz, H8).

4.9.2 N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine, 2.30b



Pale yellow powder. Yield = 75 %. M.p.: 94 – 97 °C (Lit. 96 – 98 °C).[7] ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 8.37 (1H, d, *J* = 5.4 Hz, Ar-H2), 8.21 (1H, d, *J* = 9.0 Hz, Ar-H4), 7.77 (1H, d, *J* = 2.2 Hz, Ar-H7), 7.52 (1H, br, NH), 7.42 (1H, dd, *J* = 2.1 and 6.7 Hz, Ar-H5), 6.44 (1H, d, *J* = 5.5 Hz, Ar-H3), 3.29 (2H, t, *J* = 6.8 Hz, H8), 2.66 (2H, t, *J* = 6.6 Hz, H10), 1.66 – 1.75 (2H, m, H9).

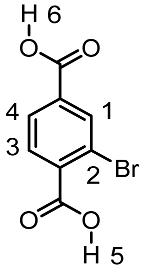
4.9.3 N^1-(7-chloroquinolin-4-yl)butane-1,4-diamine, 2.30c



Pale yellow powder. Yield = 63 %. M.p.: 121 – 125 °C (Lit. 122 – 124 °C).[7] ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm) 8.37 (1H, d, *J* = 5.4 Hz, Ar-H2), 8.27 (1H, d, *J* = 9.0 Hz, Ar-H4), 7.82 (1H, d, *J* = 2.2 Hz, Ar-H7), 7.42 (1H, dd, *J* = 2.2 and 6.8 Hz, Ar-H5), 6.45 (1H, d, *J* = 5.5 Hz, Ar-H3), 3.23 (2H, t, *J* = 6.9 Hz, H8), 2.58 (2H, t, *J* = 6.8 Hz, H11), 1.62 – 1.71 (2H, m, H10), 1.42 – 1.51 (2H, m, H9).

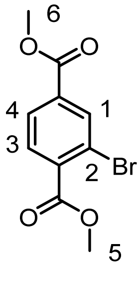
4.10 SYNTHESIS OF 1-HYDROXY-1,3-DIHYDROBENZO[C][1,2]OXABOROLE-6-CARBALDEHYDE

4.10.1 Synthesis of 2-bromoterephthalic acid, 2.23



A mixture of 2,5-dimethylbromobenzene (1.85 g, 10 mmol), KOH (0.60 g, 10.7 mmol), and H_2O (60 mL) was heated at 70 °C for 0.5 h. Thereafter, $KMnO_4$ (3.8 g, 24 mmol) was added in portions to the reaction mixture. The resultant reaction mixture was allowed to reflux for further 3 h until 2,5-dimethylbromobenzene was completely oxidized (TLC). After cooling to room temperature, a solution of $NaSO_3$ was added to oxidise residual $KMnO_4$. The mixture was filtered and residual manganese dioxide was washed with the solution of sodium hydroxide. The filtrate was acidified with concentrated hydrochloric acid to pH 3. The white solid precipitate was filtered, washed several times with water, and recrystallized in methanol to afford 2-bromoterephthalic acid (**2.23**) as a white powder. Yield = 70 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 8.18 (1H, d, J = 3.0 Hz, Ar-H1), 8.03 (1H, dd, J = 1.5 and 6.6 Hz, Ar-H4), 7.86 (1H, d, J = 8.0 Hz, Ar-H3). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 167.6, 165.9, 138.4, 134.6, 134.4, 131.0, 128.9, 120.1.

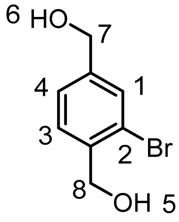
4.10.2 Synthesis of dimethyl 2-bromoterephthalate, 2.24



Thionyl chloride (1.3 mL, 18 mmol) was added drop wise to methanol (10 mL) at 0 °C. 2-Bromoterephthalic acid (2.72 g, 10 mmol) was added portion-wise and the reaction mixture allowed to stir for 10 minutes. The resultant reaction mixture was then heated at 100 °C. After 1 h TLC showed complete disappearance of the precursor. The reaction was cool to room temperature and concentrated *in vacuo* to give a white solid, which was recrystallized in 1:1 Hexane:EtOAc to yield dimethyl-2-bromoterephthalate (**2.24**) as a white solid.[8] Yield

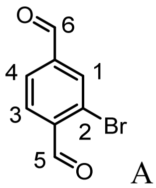
= 95 %. ^{1}H NMR (300 MHz, CDCl$_3$) δ_H (ppm): 8.18 (1H, d, *J* = 3.0 Hz, Ar-H1), 8.03 (1H, dd, *J* = 1.5 and 6.6 Hz, Ar-H4), 7.86 (1H, d, *J* = 8.0 Hz, H3), 3.88 (6H, s, H6, H5). ^{13}C NMR (75 MHz, CDCl$_3$) δ_C (ppm): 135.2, 131.0, 128.1, 52.8, 52.7.

4.10.3 Synthesis of (2-bromo-1,4-phenylene)dimethanol, 2.25



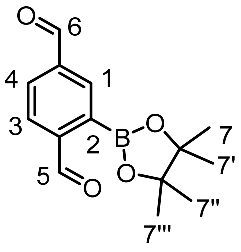
To a solution of 2-bromoterephthalate (1.5 g, 1 mmol) in THF (11.8 mL) was added LiBH$_4$ (0.267 g, 1.75 mmol) in THF (3.95 mL) slowly at 0 °C. Then 395 μL MeOH was added to the mixture. The reaction mixture was stirred at room temperature overnight, then neutralised with 1 M HCl to pH 7. Then the product was extracted with ethyl acetate, dried over MgSO$_4$, solvent evaporated to give a white solid, which was recrystallized in EtOAc to yield 2-bromo-1,4-phenylene)dimethanol (**2.25**) as a white solid.[9] Yield = 68 %. ^{1}H NMR (300 MHz, CDCl$_3$-*d*) δ_H (ppm): 8.22 (1H, d, *J* = 1.6 Hz, Ar-H1), 8.03 (1H, dd, *J* = 1.6 and 6.4 Hz, Ar-H4), 7.61 (1H, d, *J* = 7.9 Hz, Ar-H3), 4.81 (2H, s, H7), 4.79 (2H, s, H8), 3.9 (2H, br, OH). ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ_C (ppm): 143.9, 141.3, 139.5, 126.8, 126.0, 121.5, 63.3, 62.3.

4.10.4 Synthesis of 2-bromoterephthalaldehyde (2.26)



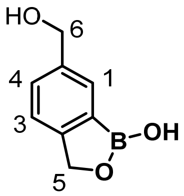
A mixture of 2-bromoterephthalaldehyde (0.120 g, 55.3 mmol), PCC (0.357 g, 165.9 mmol) and celite (0.536 g) in CH$_2$Cl$_2$ (5 mL) was stirred at room temperature overnight. The reaction mixture was filtered through celite and silica gel pad and the filtrate was evaporated *in vacuo*. Purification was done using flash column chromatography (1:1 Hexane:EtOAc) to obtained 2-bromoterephthalaldehyde (**2.26**) as a white solid.[10] Yield = 45 %. ^{1}H NMR (300 MHz, CDCl$_3$-*d*) δ_H (ppm): 10.38 (1H, s, H-6), 10.02 (1H, s, H-5), 8.10 (1H, s, Ar-H1), 8.00 (1H, d, *J* = 8.0 Hz, Ar-H3), 7.85 (1H, d, *J* = 8.8 Hz, Ar-H4). ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ_C (ppm): 192.4, 191.9, 141.1, 137.1, 135.5, 131.2, 130.8, 128.6, 126.3.

4.10.5 Synthesis of 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)terephthalaldehyde, 227



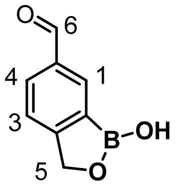
A solution of 2-bromoterephthalaldehyde (1.62 g, 5.0 mmol), bis(pinacolato)diborane (1.4 g, 5.5 mmol), $Pd(PPh_3)_2Cl_2$ (0.702 g, 0.1 mmol) and KOAc (2.45 g, 25 mmol) in dry toluene (50 mL) was heated at 80 °C under nitrogen atmosphere for 24 h. After cooling to room temperature, the reaction product was poured into water (200 mL) and the organic layer separated. The aqueous layer was extracted with dichloromethane (3 × 100 mL) and the combined layers were dried over anhydrous Na_2SO_4 and filtered. The organic solvent was evaporated under reduced pressure to give 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)terephthalaldehyde (**2.27**) as a brown oil.[11] Yield = 91 %. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ_H (ppm): 9.36 (1H, s, H-6), 9.13 (1H, s, H-5), 7.42 (1H, d, *J* = 8.0 Hz, Ar-H3), 7.35 (1H, dd, *J* = 1.2 and 6.8 Hz, Ar-H4), 7.81 (1H, d, *J* = 1.2 Hz, Ar-H3), 1.18 (12H, m, H-7).

4.10.6 Synthesis of 6-(hydroxymethyl)benzo[c][1,2]oxaborol-1(3H)-ol, 2.28



2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)terephthalaldehyde (0.23 g, 0.9 mmol) was dissolved in ethanol (10 mL) and cooled in an ice bath. Thereafter, $NaBH_4$ (0.23 g, 6.0 mmol) was added and the resultant reaction mixture was allowed to stir at room temperature for 2 h. Then 2 N HCl (30 mL) was added to the reaction mixture and left to stir at room temperature overnight. The resulting reaction product was extracted three times with isopropanol/chloroform (1:9). The combined extracts were dried over anhydrous $MgSO_4$, filtered, dried *in vacuo* and purified under column chromatography (10 % MeOH:EtOAc) to give a white solid.[12] Yield = 51 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 8.10 (1H, s, Ar-H1), 8.00 (1H, d, *J* = 8.0 Hz, Ar-H4), 7.85 (1H, d, *J* = 8.8 Hz, Ar-H3), 4.85 (2H, s, H5), 4.54 (2H, s, H6).

4.10.7 Synthesis 1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborole-6-carbaldehyde, 2.29



A mixture of 6-(hydroxymethyl)benzo[c][1,2]oxaborol-1(3H)-ol (0.120 g, 1 mmol), PCC (0.357 g, 3 mmol) and celite (0.536 g) in CH_2Cl_2 (5 mL) was stirred at room temperature for 6 h. After the reaction mixture was filtered through celite and silica gel pad and the filtrate was evaporated *in vacuo* to a crude product, which was purified using silica gel column chromatography (3:7 Hexane:EtOAc) to obtain 6-formylbenzoxaborole (**2.29**) as a yellow solid.[10] Yield = 14 %. Mp.: (125-128 °C, Lit 133-135 °C). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ_H (ppm): 10.00 (1H, s, 6-H), 7.85 (1H, s, B-OH), 7.63 (3H, m, Ar-H4/H3/H1), 4.85 (2H, s, H-5).

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CHAPTER FIVE

5 SUMMARY AND CONCLUSIONS

In conclusion, the synthesis and characterisation of the all the needed precursors was successfully done. The cinnamoyl-benzoxaborole hybrids **2.11a-g** were successfully accessed through amidation. The first set of chloroquine-benzoxaborole hybrids **2.12a-c** were successfully synthesised and characterised, however, the compounds appeared to be unstable room temperature. The second set of chloroquine-benzoxaborole hybrids **2.21a-c** and **2.33** could not be successfully accessed due to low yields of vital precursor **2.29** and difficulties during isolation. All precursors and target compounds were characterised using various analytical and spectroscopic techniques such as TLC, ^{1}H, ^{13}C NMR and stable final compounds were further characterised using mass spectrophotometry and purity was as measured using the HPLC.

Biological assays (antimalarial and antitrypanosomal) were performed and the main precursor for this project 6-amino benzoxaborole **2.14** showed no antimalarial activity and no toxicity on HeLa cells. However, one of the chloroquine-benzoxaborole hybrids showed promising antimalarial activity with an IC_{50} of 3.4 µM against *P. falciparum*, with only moderate toxicity (HeLa cell IC_{50} 28 µM). The cinnamoyl-benzoxaborole hybrids **2.11a-g** showed no antimalarial activity at 20 µM. On the contrary, the same compounds showed low to sub-micromolar IC_{50} values against *Trypanosoma brucei*. More importantly, these compounds **2.11a-g** showed no cytotoxicity effects against HeLa cell line, producing selectivity indexes of 155-2500 for the best compounds. The lack of antimalarial and HeLa cell activity of the cinnamoyl-benzoxaborole hybrids suggests that they are not general eukaryotic toxins, but are specifically targeting a metabolic process that is vital to trypanosomes and may be promising scaffolds for the development of novel African sleeping sickness therapeutics.

The aims and objectives of the current study were largely achieved, and future work which will be based on the research results is expected to include the following:

- Look into the reason behind instability of the chloroquinoline-benzoxaborole hybrids (**2.2.1.6**).
- Explore alternative synthetic routes and optimise yields for 6 formylbenzoxaborole (**2.2.2.1)**, so that coupling with the chloroquinolines can be possible.