Chapter 1: Introduction and Problem Statement

1.1. Background

With over three billion people at risk and around 660 000 deaths annually (WHO, 2012), malaria poses a major public health threat, globally (Nayyar *et al.*, 2012; Ploypradith, 2004; Totino *et al.*, 2009). Of these reported deaths, 91% were in the African region, with 86% being children under the age of five (WHO, 2012). Malaria is a vector borne infectious disease, caused by protozoan parasites of the genus *Plasmodium* and are transmitted by an infected female *Anopheles* mosquito. There are five species of *Plasmodium* that can infect humans *viz*. *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, of which the former is responsible for the most severe cases (Carrico *et al.*, 2004). The fifth species, *P. knowlesi* causes malaria in macaques, but is also capable of infecting humans (van Hellemond *et al.*, 2009). Malaria is commonly found in tropical and subtropical regions, such as Africa and Asia (Singh *et al.*, 2005) and is considered endemic in 106 countries worldwide (**Fig. 1.1**).

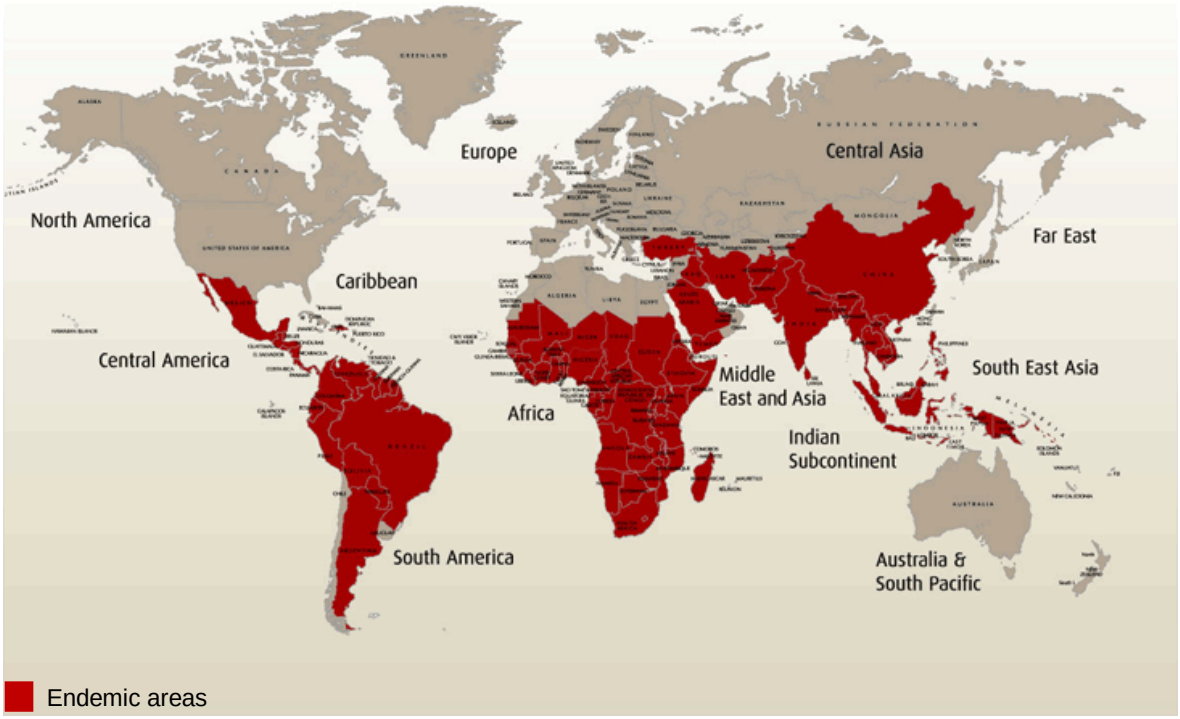
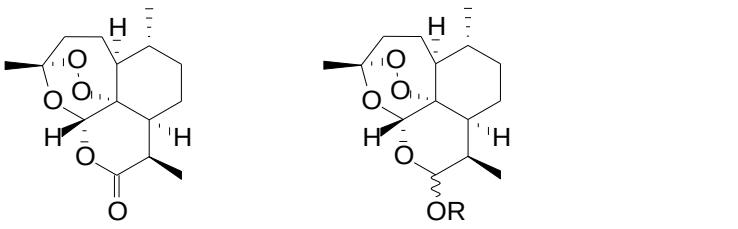


Figure 1.1: Global prevalence of malaria in 2010 (ANON, 2010).

Malaria symptoms present as fever and as a flu-like illness, shaking chills, headaches, muscle aches and tiredness, while symptoms like nausea, vomiting and diarrhoea may also occur (Beare *et al.*, 2006). A conventional method of vector control was with the use of insecticides, such as DDT, but this has been abandoned due to its associated carcinogenicity (van den Berg, 2009). Widespread drug resistant parasite strains have reduced the number of clinically effective drugs available, signifying an imperative need for more efficient antimalarial therapeutics (Carrico *et al.*, 2004).

The World Health Organization (WHO) recommends either artesunate, or quinine-artemether as the drug/drug combination of choice for severe *P. falciparum* malaria (WHO, 2010), while artemisinin based combined therapy (ACT) is preferred as the first line treatment against uncomplicated malaria. Regrettably, artemisinin and its derivatives (**Fig. 1.2**) suffers from severe drawbacks, such as very short pharmacological half-lives, paired with low water or lipid solubility (Barradell *et al.*, 2005), which require frequent dosing. While these compounds show high efficacy when administered through systemic routes, they are fairly less effective when given by oral route (Singh *et al.*, 2008). Since most symptomatic malaria is treated in minor health centres, or remote villages where facilities are limited, the use of artemisinin based compounds is quite challenging. Additionally, alarming reports of tolerance towards artemisinin in South-Asia and along the Cambodia-Thailand border (Kumar *et al.*, 2010; WHO, 2012) jeopardise the use of ACT, the last line of defence against malaria. This signifies the urgent need for identifying and developing new combination therapies that can act on unique targets.



Artemisinin **Dihydroartimisinin** **R = H**
Artemether **R = OCH_3**
Arteether **R = OCH_2CH_3**
Artesunate **R = $CO(CH_2)_2COOH$**

Figure 1.2: Artemisinin and its derivatives.

Recent advents in genetic sequencing of the *Plasmodium* genome have identified several new unique targets, such as the parasite induced permeation pathways and malarial cysteine proteases, which can be specifically targeted with minimal toxicity to the host.

Cysteine protease mediates protein hydrolysis *via* nucleophilic attack on a carbonyl of a susceptible peptide bond. The main function of malarial cysteine protease is the hydrolysis of haemoglobin in the food vacuole (Rosenthal, 2004). This enzyme is also presumed to be involved in the rupture of the erythrocyte membrane (Aly and Matuschewski, 2005).

A known cysteine protease inhibitor is E64 (**Fig. 1.3**), a natural modified peptide, containing an active epoxide functional group. Other inhibitors include naturally based compounds, such as chalcones (1,3-diaryl propenone) and isatins. The first reported chalcone with antimalarial activity was Licochalcone A (**Fig. 1.3**), a natural product isolated from Chinese liquorice roots, with an IC_{50} of 6.5 µM against 3D7 clones (Larsen *et al.*, 2005; Go *et al.*, 2004). Ever since, interest in these compounds has ignited. It has been shown that chalcones with electron deficient groups on ring A displayed strong antimalarial activity (Kaur *et al.*, 2010), while chalcones with one of the rings replaced by a heterocyclic ring, showed better antimicrobial activity, especially the ones containing furan rings (Zheng *et al.*, 2011). Numerous authors have reported chalcones containing basic nitrogen, or sulphur groups to possess both antimalarial and cytotoxic activity (Kumar *et al.*, 2010; Reddy *et al.*, 2008).

Besides their antimalarial activity, chalcones have a vast array of biological functions, such as antibacterial, antifungal, antiviral, anti-inflammatory and antitumour activities (Mishra *et al.*, 2008; Kumar *et al.*, 2010). For these reasons, the chalcone moiety could prove beneficial when coupled to other antimalarial pharmacophores with known activity, with an electron withdrawing ring A, in an attempt to procure both antimalarially active target compounds that trounce resistance.

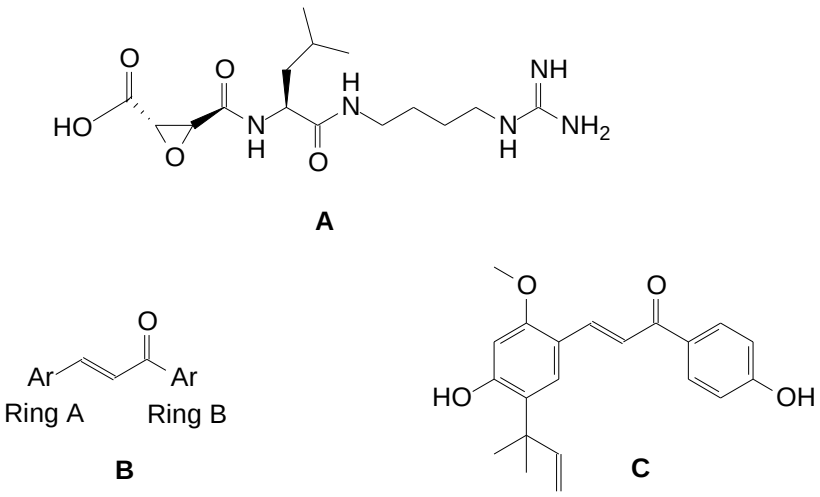
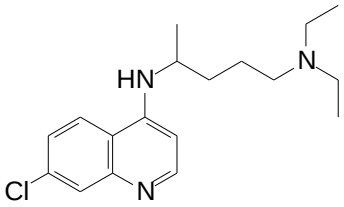


Figure 1.3: Cysteine protease inhibitor E64 (**A**), general structure of a chalcone (**B**) and Licochalcone A (**C**).

Despite major resistance currently being associated with chloroquine (**Fig. 1.4**) within the majority of endemic areas, the quinoline pharmacophore still remains an important class of antimalarials, due to its low cost of synthesis and the diverse application of this group (O'Neill *et al.*, 2012b; Yadav *et al.*, 2012). An alternative method being proposed for overcoming the development of antiplasmodial resistance is through the incorporation of a second pharmacophore *via* a chemical bond, forming a hybrid drug molecule. Hybrid molecules combine two drugs in a single molecule with the aim of creating a chemical entity, having two or more structural domains with different biological functions and dual activity that are medically/therpeutically more effective than its individual components (Meunier, 2008).

Due to an increasing emphasis on fixed-dose combinations in antimalarial therapy, combining these drugs into a hybrid molecule may offer several advantages over combination therapy, such as that the two components may act synergistically, restore the antimalarial activity of the individual compound and lower toxicity, as well as being potentially cheaper to manufacture (Walsh and Bell, 2009). For these reasons, the hybrid drug approach was considered during this study.



CQ

Figure 1.4: Clinically used chloroquine (CQ).

A series of substituted quinolinyl-chalcones (**Fig. 1.5**) synthesized by Sharma *et al.* (2009), showed disappointing activity against the NF-54 strain of *P. falciparum* (Sharma *et al.*, 2009). However, when designing 4-aminoquinoline based compounds, the length of the methylene spacer between two nitrogens in the side chain of CQ analogues is a major determinant of activity against CQ resistant *P. falciparum* (Chibale *et al.*, 2000). For this reason, the chalcone moiety was coupled to a 4-aminoquinoline based compound, using methylene spacers to obtain various hybrids during this study (Chapter 3).

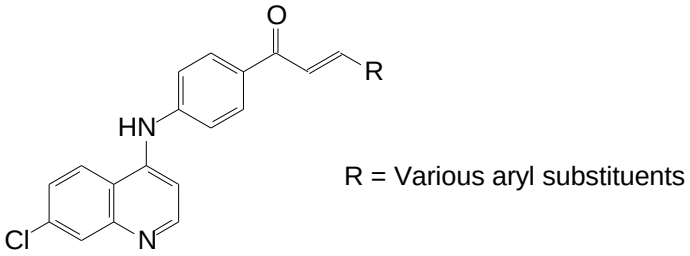


Figure 1.5: General structure of quinolinyl-chalcones (Sharma *et al.*, 2009).

Recent reports on ferroquine, a ferrocene derivative of CQ with increased efficacy towards CQ resistant strains of *P. falciparum*, have attracted much attention and a focus towards organometallic compounds (Mathiyalagan *et al.*, 2012; Gimeno *et al.*, 2011). The ferrocene moiety has also been proven as a successful addition to compounds, such as penicillin, cephalosporine and tamoxifen (Gimeno *et al.*, 2011). Numerous studies have been conducted on ferrocene containing chalcones, where one of the aryl groups had been replaced with ferrocene, in order to evaluate their biological activity, as well as the function of ferrocene (Wu *et al.*, 2006), although with limited success.

The Fe(II) centre may undergo redox reactions, which may influence the redox cycling of the parasite. The ease of oxidation of Fe(II) in the ferrocenyl moiety may be influenced by nearby chemical groups, in the vicinity which in turn impact on the electrochemical potential of the compound. Recently, it has been reported that chalcones also possess radical-scavenging properties, resulting in oxidative stress (Nabi and Liu, 2011; Jayasinghe *et al.*, 2004). Therefore, combining the chalcone entity with ferrocene, separated by methylene spacers, might improve the efficacy of these compounds compared to traditional ferrocenyl-chalcones, in which the ferrocene replaces one aryl ring of the chalcone.

The recommendation by the World Health Organization (WHO) to replace the mono-therapeutic use of artemisinins with ACTs is an attempt to slow the spread of tolerance and to avoid artemisinins' suffering widespread resistance (WHO, 2012). However, the development of multi-drug resistant strains is, nevertheless, inevitable (Bhattacharya *et al.*, 2009). In the search for alternative combinations, Bhattacharya *et al.* (2009) studied the *in vitro* pharmacodynamics of chalcone derivatives in combination with artemisinin against *P. falciparum* and found that the combinations being evaluated showed synergistic or additive interactions. Additionally, Cloete *et al*. (2013) synthesised a series of 10-alkyl/aryl esters and 10-aminoethylethers of artemisinin, of which the ester derivatives showed superior activity (Cloete *et al.*, 2013). Consequently, the combination of chalcones with dihydroartemisinin with a hydrolysable ester linker may prove worthy. If the ester linker is kept intact, the newly

formed hybrids would act as a new entity. When this group is hydrolysed, however, a synergistic or additive interaction is expected. Furthermore, Singh *et al.* (2008) showed that ester derivates of DHA showed better oral activity than artemether and artesunic acid, which may be beneficial to the administration of these antimalarial drugs in rural areas.

1.2 Aim

In light of the above considerations, the aim of this study was the synthesis, characterisation, *in vitro* antimalarial acitvity and cytotoxicity of three series of novel chalcone based compounds.

1.3 Objectives

In order to achieve the aim of this study, the following objectives were set:

- Synthesis of three chalcone based series of hybrid compounds, including 4-aminoquinolinyl-chalcone amides (Chapter 3), aminoferrocenyl-chalcone amides (Chapter 4) and dihyroartemisinyl-chalcone esters (Chapter 5).
- Characterisation of all intermediate and hybrid compounds by means of NMR, HRMS and IR.
- Determination of physicochemical properties by means of Discovery Studio version 3.1 computer software.
- Evaluation of the thermal stability and physical states of 4-aminoquinolinyl-chalcone amides and dihyroartemisinyl-chalcone esters using TGA and DSC analyses.
- Determination of the electrochemical potential of aminoferrocenyl-chalcone amides.
- Determination of the *in vitro* antiplasmodial activity of all targeted hybrid compounds together with some of the precursors.
- Determination of the *in vitro* cytotoxicity of synthesized hybrid compounds.