Chapter 6: Summary and Conclusion

Malaria is a vector borne disease of parasites in the genus *Plasmodium* that were responsible for the deaths of 660 000 people in 2011, whilst an estimated 3.3 billion people are at risk of contracting this deadly disease (WHO, 2012). Malaria is commonly found in tropical and subtropical regions, such as Africa and Asia (Singh *et al.*, 2005) and is endemic in 106 countries, worldwide.

Current treatment regimes include the highly effective artemisinin class of compounds, with the WHO recommending either artesunate, or quinine-artemether as the artemisinin combined therapy (ACT) of choice for severe *P. falciparum* malaria. Other ACTs form the preferred first line treatments against uncomplicated malaria (WHO, 2010). The artemisinin class is the last line of defence against malaria, since widespread multi-drug resistance has rendered other chemotherapeutical antiplasmodial drugs ineffective. Unfortunately, artemisinins suffer severe drawbacks, such as very short pharmacological half-lives, paired with low water or oil solubility, despite a quick onset of action (Li and Zhou, 2010). The progress made against malaria eradication hangs in the balance, with reports of clinical tolerance having developed towards artemisinin in South-Asia and along the Cambodia-Thailand border (Kumar *et al.*, 2010; WHO, 2012), which could have disastrous consequences to the fight against this disease. There hence exists a dire need for identifying and developing new and effective antiplasmodial agents.

Genetic advances have identified several new unique targets of the *Plasmodium*, which can be exploited through chemotherapy, including the parasite induced permeation pathways and malarial cysteine proteases. The main function of malarial cysteine protease is the hydrolysis of haemoglobin in the food vacuole (Rosenthal, 2004), while it may also be involved in the rupture of the erythrocyte membranes (Aly and Matuschewski, 2005). Numerous authors have already reported on the antiplasmodial activity of the naturally based compounds, chalcones, which are further believed to be cysteine proteases inhibitors (Reddy *et al.*, 2008; Zheng *et al.*, 2011; Powers *et al.*, 1998; Larsen *et al.*, 2005; Go *et al.*, 2004). Despite the fact that these chalcones overall exhibit relatively low potencies towards *Plasmodium* parasites, the combination of these moieties with other pharmacophores in hybrid fashion may prove beneficial to the search for new and effective antimalarials.

Hybridization is one method through which resistance can be subjugated. Hybrid molecules combine two drugs in a single molecule with the aim of creating a chemical entity with two or more structural domains, having different biological functions and dual activity that are medicinally/therapeutically more effective than its individual components (Meunier, 2008 33; Walsh and Bell, 2009).

In this study, a hybrid approach was adopted, in which chalcone based compounds were combined with quinoline, ferrocene and dihydroartemisinin in three separate groups of compounds. Series 1 and 2 (Chapters 3 and 4) were motivated by the observation by Chibale *et al.* (2000) that the length of the methylene spacer between two nitrogens in the side chain of 4-aminoquinolines is a major determinant of activity against CQ resistant strains of *P. falciparum*. The first series of compounds involved novel 4-aminoquinolinyl-chalcone amides, whilst the second comprised novel aminoferrocenyl-chalcone amides. In both series, the two pharmacophores were separated by means of methylene spacers. Investigation of the third series was motivated by the fact that Singh *et al.* (2008) had shown that ester derivatives of DHA had shown better oral activity than artemether and artesunate, together with the demonstration by Cloete *et al*. (2013) that 10-alkyl/aryl esters derivatives of DHA had shown superior antimalarial activity, compared to 10-aminoethylethers of artemisinin (Cloete *et al.*, 2013).

Chapter 3: "Synthesis, *in vitro* antimalarial activity and cytotoxicity of novel 4-aminoquinolinyl-chalcone amides", was the first article integrated into this thesis. This article describes the synthesis and biological activity of a series of nine novel 4-aminoquinolinyl-chalcone amides, **11 – 19**, which were synthesized synthesised in a three-step process involving amino-functionalizing functionalising quinolines through aromatic nucleophilic substitution (SN_{Ar}). The synthesis of the carboxylic acid-functionalized functionalised chalcone was attained by Claisen-Schmidt condensation of 4-formylbenzoic acid and 2-acetyl-5-methylfuran in basic methanolic water. The last step involved the amidation of the chalcone and the amino-functionalizing functionalising quinolines with 1,1'-carbonyldiimidazole (CDI) as coupling reagent. Routinely used techniques, such NMR, HRMS and IR were performed to confirm their structures.

DSC and TGA analyses were used to reveal their amorphous structures and thermal stability, respectively, while ADMET served to predict their drug-like properties. The targeted hybrid compounds were screened alongside CQ and proved to be active with IC_{50} values ranging between 0.05 - 0.53 µM and 0.07 - 1.8 µM against the 3D7 and W2 strains of *P. falciparum*, respectively. The amides displayed an overall loss of activity against the CQR

strain, as compared to the CQS, which resulted in resistance index values over the unit. No resistance to CQ was therefore overcome in the study. These compounds also showed moderate to high selective toxicity towards the mammalian cells in the presence of the parasitic cells. In this study, the activity was found to increase as the lipophilicity and the linker chain length increased.

The tertiary amides were the least active, which emphasised the influence of hydrogen bonding on the activity of this compound type. Amide **15**, featuring 1,6-diaminohexne linker was found the most active of all, being as potent as and two-fold more potent than CQ against the 3D7 and W2 strains, respectively, despite its predicted unfavourable high lipophilicity, low solubility and poor absorption properties.

An interesting addition to the generated results would have been the inclusion of the intermediate amino-functionalized quinolines, as well as the aminoquinolines in a 1:1 molar ratio with the chalcone. These results would have provided a more definitive conclusion about the role of the chalcone. Additionally the *in vivo* evaluation of amide **15** must also be investigated.

Chapter 4: “Synthesis, antimalarial and cytotoxic activity of novel aminoferrocenyl-chalcone amides”, was the second article incorporated into this thesis. In this article a series of nine novel aminoferrocenyl-chalcone amides, **11** - **19** were synthesized in a similar manner as the compounds in Chapter 3. Firstly, an amino functionalized ferrocenyl intermediate was prepared through reductive amination of ferrocene carboxaldehyde with the appropriate amine. The same carboxylic acid-functionalized chalcone was used with 1,1'-carbonyldiimidazole (CDI) as coupling reagent to prepare the novel aminoferrocenyl-chalcone amides. Routinely used techniques, such as NMR, HRMS and IR were performed to confirm their structures.

Despite favourable drug-like properties, as determined by preliminary ADMET software calculations, the observed antimalarial activities of these compounds were relatively low. During screen tests done alongside CQ, all of these amides proved to be active with IC_{50} values ranging between 0.5 - 4.5 µM and 2.1 - 6.6 µM against the 3D7 and FCR3 strains of *P. falciparum*, respectively. The amides overall displayed retention of activity against the FCR3 strain, as compared to the CQS, except amides **12** and **18**. They also showed low selective toxicity towards the parasitic cells in the presence of the mammalian ones. In this study, no clear SAR could be drawn, since no trend could be observed in terms of activity with an increase in the chain length. The observed activities were not intrinsic, but rather

cytotoxicity, most likely due to the formation of a ferricenium cation. The reduction potentials of all targeted hybrid compounds, together with ferrocene and ferrocene carboxaldehyde as standards, were determined as 0.55 – 0.6V.

Due to the increased observed activity for the 1:1 molar combination of ferrocene carboxaldehyde and the chalcone against the CQR strain (FCR3), the results being obtained may have been strengthened by the inclusion of the intermediate amino-functionalized ferrocenyl compounds, as well as the ferrocenyl intermediates in a 1:1 molar ratio with the chalcone. These results would once again have given a more conclusive outcome regarding the role of the chalcone in combination with ferrocene.

Chapter 5: "Synthesis and biological evaluation of dihydroartemisinyl-chalcone esters", comprised the third article included into this thesis. It entailed the synthesis and *in vitro* antiplasmodial activity of a series of novel dihydroartemisinyl-chalcone esters, **7** – **12**, cytotoxicity and antitumor activities. The intermediate chalcones **1** - **6** were prepared through condensation of 4-formylbenzoic acid with the appropriate aryl ketone in basic methanolic water. Esterification was performed under mild conditions, involving the carboxylic acid-functionalized chalcones and DHA, using either 1,1'-carbonyldiimidazole (CDI) as coupling agent, or oxalyl chloride as activation reagent. Routinely used techniques, such NMR, HRMS and IR served to confirm their structures. For some of the targeted hybrid compounds (**7**, **9** and **12**), HRMS proved to be too harsh, therefore only fragmentation was observed without a nominal mass. For this reason elemental analysis were conducted on all targeted compounds.

DSC and TGA were used to reveal the amorphous structures of the targeted hybrid compounds **7** - **12**, with DHA and AS as references. The hybrids demonstrated superior thermal stability compared to DHA and similar thermal stability than AS. In screen tests performed alongside CQ, DHA and AS, all of these esters proved active with IC_{50} values ranging between 1.9 – 10.7 nM and 1.6 – 10.6 nM against 3D7 and W2 strains of *P. falciparum*, respectively, despite possessing predicted unfavourable drug-like properties. The carboxylic acid functionalized chalcones **1** - **6**, on the contrary, were found less active with IC_{50} values ranging between 10.8 – 41.0 µM and 10.8 – 62.2 µM against 3D7 and W2, respectively, regardless of predicted favourable drug-like properties.

The dihydroartemisinyl-chalcone esters **7**, **10** and **11**, featuring oxygenated aryls as ring B in the chalcone, were found to be equipotent to DHA, three to four times more potent than artesunate (AS) against both the 3D7 and W2 strains, with more than forty-fold higher

activity than CQ against the W2 strain. These esters displayed an overall gain of activity against the CQR strain, as compared to the CQS, which resulted in resistance index values under the unit. In this study, activity was found to increase with the presence of oxygenated aryl groups (electron donating ring B), independent of their position on the aryl ring. Compounds with electron withdrawing groups seemed to be less potent. The targeted hybrid compounds also showed excellent selective toxicity towards the mammalian cells in the presence of the parasitic cells.

Additionally, regardless of lower or equipotent activities observed for the targeted hybrids, compared to DHA, the observation that chalcones with higher IC_{50} values (**1**, **4** and **5**) resulted in hybrid compounds with increased activity and thermal stability, may be encouraging. The incorporation of a chalcone with increased activity could therefore result in a hybrid with improved activity. The 1:1 combinations of the other chalcones (**2**, **3** and **6**) should also be investigated. Several of the targeted hybrid compounds displayed potent antitumor activity, with compounds **7**, being overall the most active.

In summary, all of the targeted hybrid compounds synthesized during this study showed activity against both the CQS and CQR strains. The compounds in series 3 were found the most active, due to the incorporation of the highly effective artemisinin pharmacophore. Owing to the ease and low cost of the synthesis of chalcones, this pharmacophore has a vast scope for future development. The most active compounds of the three series could serve as potential leads in the future development of more effective antimalarial drugs. During this study, hybrid **7** performed admirable compared to DHA and AS against both the CQS and CQR strains of *P*. *falciparum*, with a good RI value (0.7), excellent SI value (36464), overall potent antitumor activities (~3 µM) against all three cancer cells lines, as well as increased thermal stability.