CHAPTER 4 SUMMARY AND CONCLUSION

Malaria instils an immense burden on global public health, with 3.3 billion people at risk and 660 000 malaria related deaths reported in 2011. Malaria is predominantly found in tropical and subtropical regions of sub-Saharan Africa, the Middle East, Latin America, the Indian sub-continent, South-East Asia and the Oceania (WHO, 2012).

The resurgence and distribution of this very treatable disease are due to the occurrence of widespread drug resistance. The continuous fight against this disease has become increasingly difficult, as the malaria parasite has an intrinsic ability to develop drug resistance through various mechanisms (Gregson & Plowe, 2005). The escalation in drug resistance, especially multi-drug resistance, has rendered traditionally used drugs, such as chloroquine, therapeutically ineffective. Prescribing measures to safeguard the artemisinins against drug resistance development had led the WHO to recommend artemisinin based combination therapies (ACT's) as first line treatment for uncomplicated *P. falciparum* malaria (Amuasi *et al*., 2012; Fink *et al*., 2013).

Unfortunately, artemisinins possess poor solubility in oil and water, have short half-lives and irregular absorption patterns (Rogers *et al*., 2009). Reports from Western Thailand, China, India, Sierra Leone, Nigeria and Madagascar exhibit slow parasitemia clearance times and elevated recrudescence rates (Rogers *et al*., 2009), indicative of reduced susceptibility in parasites to the artemisinins (Sahr *et al*., 2001; Yang *et al*., 2003). The implosion of artesunate-mefloquine therapy, partly due to widespread mefloquine resistance (Rogers *et al*., 2009), had been a devastating setback to the fight against malaria and has necessitated the introduction of novel antimalarial drugs as chemotherapy. While no artemisinin resistant strain of *Plasmodium* parasites has been isolated from patients, the slow parasitemia clearance rates and the occurrence of recrudescence are indicative of the development of clinical tolerance, but not yet resistance. This detection is alarming, since the artemisinins are the last bullet in the arsenal against malaria.

The current ACT's used as chemotherapy against malaria are physical and not chemical co-formulations. By chemically linking two distinct pharmacophores with a covalent bond, the hybrid might offer several advantages to the co-formulation. Hybridisation of two active pharmacophores can lead to the inhibition of drug resistance development (Walsh *et al*., 2007; Walsh & Bell, 2009). The choice of linker can either allow the intact hybrid to dissociate into its individual components, resulting in synergistic activity, or to be metabolically resistant in view of overcoming resistance (Walsh & Bell, 2009).

The aim of this study was to synthesise a series of 9-aminoacridines and artemisinin-acridine hybrids containing the acridine and artemisinin pharmacophores, evaluate their antimalarial activities in comparison with dihydroartemisinin (DHA), artesunate (AS) and chloroquine (CQ) and assess their cytotoxicities against various mammalian cell lines.

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

- Synthesise 9-aminoacridines and artemisinin-acridine hybrids and confirm their chemical structures by means of IR, NMR and HRMS.

- Evaluate the antimalarial activity *in vitro* of the synthesised compounds against a chloroquine sensitive (NF54) strain and a chloroquine resistant (Dd2) strain of *P. falciparum*.

- Test compounds *in vitro* for cytotoxicity against various mammalian cell lines, including Chinese hamster ovarian- (CHO), cervical cancer- (HeLa), human hepatocellular- (HepG2) and human neuroblastoma (SH-SY5Y) cells.

The novel 9-aminoacridines (**2** – **6**) and artemisinin-acridine hybrids (**7** - **11**) were synthesised in one- and three-step synthetic routes, respectively. The target hybrids were synthesised, using a microwave assisted radiation method, by covalently linking artemisinin and acridine pharmacophores by means of a liable aminoethyl ether linker. Special precautions with regards to reaction conditions, such as low temperatures and limited exposure to acidic conditions were taken to ensure that the integrity of the endoperoxide bridge of the artemisinin moiety was not compromised in the resultant hybrids. All structures were confirmed by means of IR, NMR and HRMS.

Overall, hybrids **7** - **11** showed minimum weight losses, with predictable stabilities in extreme conditions (240°C), as determined by TGA. DSC data revealed that compounds **7**, **10** and **11** had undergone endothermic phase transitions, characteristic of a glass transition and had possessed amorphous structures. These hybrids therefore would be able to withstand the typically harsh storage conditions, prevailing in the malaria endemic countries.

The calculated logP values of the 9-aminoacridines were in the targeted range (**1 - 5**), i.e. low aqueous solubility, good absorption and high to medium blood brain penetration. They therefore showed favourable drug-likeness properties, conducive to better biological activity.

Contrary, the hybrids, displayed high logP values above the limits of both the targeted and ideal ranges, thus extreme to very low solubility levels and very low-to-low absorption levels. All of the hybrids thus possessed unfavourable ADMET predicted drug-likeness properties in comparison with the 9-aminoacridine intermediates.

The IC_{50} values of compounds **2** - **11** were determined, with DHA, AS and CQ as references. All compounds were found to be active against both NF54 and Dd2 *Plasmodium* strains. None of the compounds had the ability to overcome resistance, whilst all of the compounds exhibiting lower activities than the CQ sensitive and CQ resistant strains. Generally, none of the compounds were found to be highly cytotoxic, expressing significantly less toxicity than the control drug, emetine.

Overall, hybrid **7** (IC_{50} values of 2.6 nM and 35.3 nM against NF54 and Dd2, respectively), featuring an ethylenediamine moiety in the linker, was the most active compound. This was in support of previous findings about short chain hybrids exhibiting enhanced antimalarial activities (Jones *et al*., 2009).

Compound **10**, with a 2-methylpiperazine linker, was the most promising compound, with good antimalarial and selective cytotoxicities, irrespective of the *P. falciparum* strain, nor of the origin of the cell lines. Hybrid **10** also possessed anticancer activity comparable to DHA and AS against HeLa cell line. Melphalan and CQ exhibited twelve- and five-fold inferior antineoplastic activity against the HeLa cell line, compared to compound **10**. This compound may thus stand as drug candidate for further investigation in the search for new anticancer drugs against cervical cancer, rather than as antimalarial.

A shortcoming of this study was the non-inclusion of a 1:1 molar ratio of the individual parent compounds in screen tests, to determine whether the hybrids would show advantages over a 1:1 combination of the two individual pharmacophores. In light of the results from this study, the screening of 9-aminoacridine and DHA in 1:1 molar ratio combinations seemed necessary in order to ascertain the existence of advantages, if any, that the hybrids may have over the physical combinations.

The hybridisation of acridine and artemisinin pharmacophores did not lead to a reduction in the constituent drugs' toxicities, nor was resistance of the malaria parasites to CQ overcome. From the screen tests outcomes, no synergistic activity was demonstrated by combining both pharmacophores. The hybrid strategy therefore resulted in limited benefits.

Since all of the compounds were tested *in vitro* without having undergone biotransformation, it would be valuable to determine whether the reported activities and cytotoxicities would be carried over *in vivo*, and attest whether these results had solely emanated from either the hybrid entities, the individual pharmacophores, or both.