

CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

1.1 Introduction

Malaria, a historic disease, continues to be a modern health threat. In 2011, the World Health Organization (WHO) estimated that 3.3 billion people worldwide were at risk of being infected (WHO, 2012). Favourable malaria prevention and control statistics were short lived, as the emergence of drug resistant strains increased human mortality and morbidity. The prevalence and distribution of malaria is concentrated in the tropical and sub-tropical areas of sub-Saharan Africa, the Middle East, Latin America, the Indian sub-continent, South-East Asia and Oceania (WHO, 2012).

Antimalarial chemotherapy focal dependence is from seven drug classes, namely 4-aminoquinolines, arylaminoalcohols, 8-aminoquinolines, artemisinin, antifolates, inhibitors of the respiratory chain and antibiotics (Schlitzer, 2008; Rudrapal, 2011).

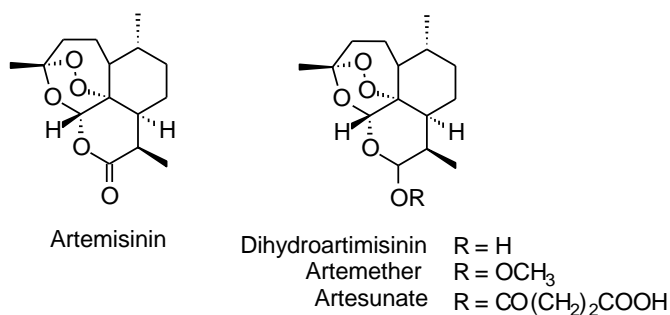


Figure 1.1: Structures of artemisinin and its derivatives.

The artemisinins are the most potent antimalarial drug class, due to widespread drug resistance towards traditional chemotherapy. The physicochemical limitations of artemisinin paved the way for synthetic and semi-synthetic derivatives, including artemether, arteether and artesunate. Artemether and arteether are oil soluble compounds with increased gastrointestinal absorption after oral administration (Kokwaro, *et al.*, 2007). Artesunate is water soluble and the most potent of the artemisinins, but is highly unstable and undergoes rapid elimination (Woodrow *et al.*, 2005). Activity against late ring and small ring stages are unique to the artemisinins.

Artemisinins are fast acting, potent blood schizontocides, with early gametocytic stage activity (White, 2002; Woodrow *et al.*, 2005). Poor physicochemical properties and short plasma half-lives, however, limit their therapeutic efficiency as chemotherapy (Ploypradith, 2004). Monotherapy of the artemisinins necessitates a prolonged course treatment of a minimal of seven days for successful pharmacotherapy (Price, 2013). The increased incidence of artemisinins tolerance led to adapted treatment policies. The WHO now recommends ACT's (artemisinin-based combination therapy) as first-line treatment for uncomplicated *Plasmodium falciparum* (*P. falciparum*) malaria (Amuasi *et al.*, 2012; Fink *et al.*, 2013).

Artemisinin based combination therapies (ACT's) combine fast acting, highly potent artemisinin derivatives with a longer acting, partner compound. The short acting, highly potent artemisinin derivative reduces the parasitemia density to very low levels. The artemisinin derivative also reduces gametocytogenesis by eight- to eighteenfold (Price *et al.*, 1996). Afterwards, the slower eliminated partner compound prevents parasite recrudescence (Price, 2013). Piperaquine-dihydroartemisinin, pyronaridine-artesunate and dapson-chlorproguanil-artesunate (LapDap⁺) are new generation ACT co-formulations in clinical trials (Price, 2013). The extensive use of traditional chemotherapy in sub-therapeutic doses and the escalation in drug resistance can affect the longevity of new generation ACT's (Davis *et al.*, 2005; Wongsrichanalai, 2013).

A strategy to impede drug resistance development is the synthesis of hybrid molecules (Burgess *et al.*, 2006). Hybrids are two or more chemical entities with differing structural domains and biological activities, covalently linked together (Hulsman *et al.*, 2007; Meunier, 2008). The hybridisation of compounds have shown to impart favourable physicochemical properties of one entity to another (Walsh *et al.*, 2007; Meunier, 2008). The selection of an appropriate linker with the desired metabolic stability or liability can help to either overcome resistance, or achieve independent receptor binding (Walsh & Bell, 2009). The manufacturing cost, instability of proposed linkers and inferior antimalarial activity to the parent compounds can be a disadvantage though.

Acridine base compounds were the foundation of antimalarial drug research (Pérez *et al.*, 2013). The acridine pharmacophore was the first blood schizontocide and was used as first line treatment of malaria during World War II (Elueze *et al.*, 1996). The emergence of parasite resistance to chloroquine has renewed interest in the acridine pharmacophore antimalarial activity. Synthetic acridines, i.e. pyronaridine and quinacrine, exhibited mean 50% inhibitory concentration (IC₅₀) values of 15 and 30.4 nM, respectively, compared to chloroquine's mean IC₅₀ value of 148 nM for various strains, as tested by Elueze *et al.* (1996). Winter *et al.* (2006) had synthesised a 2-methoxy-6-chloro-9-aminoacridine moiety with IC₅₀ values of 18 and 42 nM against D6 and Dd2 strains of *P. falciparum*, respectively (Winter *et al.*, 2006).

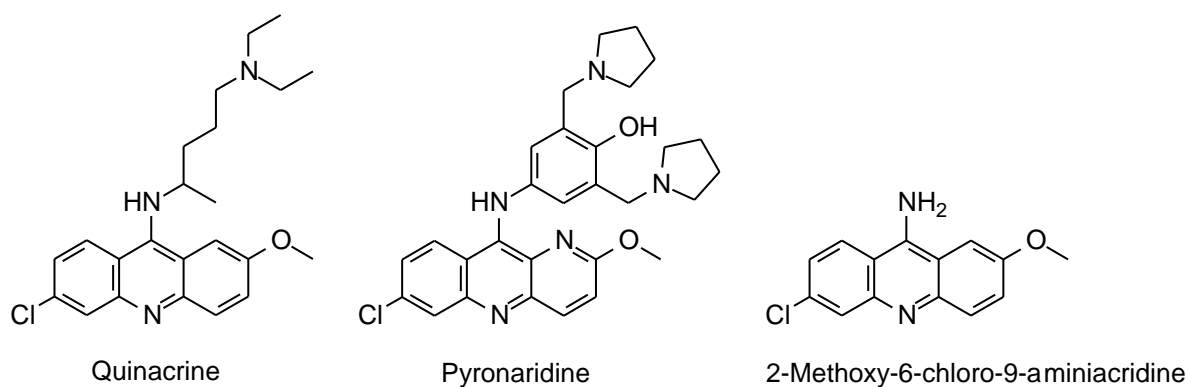


Figure 1.2: Structures of acridine derivatives.

Previous studies of artemisinin-acridine hybrids are best elucidated by Jones *et al.* (2009). A comparison of the most potent hybrid with dihydroartemisinin (DHA) generated IC_{50} values of 5.9 nM (hybrid) and 2.3 nM (DHA) against 3D7 of the *P. falciparum* strain, indicating that DHA displayed superior antimalarial activity. The observed loss of activity of the hybrids could have been due to the competitive, intramolecular aromatic stacking interaction between acridine and the trioxane moiety (Jones *et al.*, 2009). Although these hybrids displayed antitumor and antimalarial activity, the fluorescent characteristics of acridine gave the ability to determine the cellular location of the individual compound *via* confocal microscopy.

1.2 Aim and objectives of the study

In light of the aforementioned considerations, the aim of this study was to synthesise a series of 9-aminoacridines and artemisinin-acridine hybrids, containing acridine and artemisinin pharmacophores, to evaluate their antimalarial activities in comparison with dihydroartemisinin, artesunate and chloroquine and to 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 infrared spectroscopy, nuclear magnetic resonance spectroscopy and high resolution mass spectroscopy.
- Evaluate the antimalarial activity *in vitro* of the synthesised compounds against a chloroquine sensitive (NF54) strain and 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, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay and FITC Annexin V/Dead Cell Apoptosis Kit with FITC annexin V and PI for flow cytometry assay.