

Chapter 1

Background and aim of the study

1.1 Background

Malaria is a major parasitic disease afflicting mankind, with an estimated 3.3 billion people at risk of being infected and an approximate 655 000 people that die annually. More than 80% of reported cases and 90% of all deaths are estimated to be in Africa, most of which are pregnant women and children under the age of 5 years (WHO 2011:1).

In regions with a high malaria incident, the disease may account for as much as 40% of public health expenditure, 30-50% of inpatient admissions and up to 50% of outpatient visits (WHO 2009:1).

Malaria is a protozoan infection of the red blood cells (RBCs) caused by the *Plasmodium* parasite. The five *Plasmodium* species capable of infecting humans are *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi* and *P. falciparum* with the latter accounting for the highest percentage of case fatalities (White 2008:1201, WHO 2011:1). The most deadly malaria species, *P. falciparum*, predominates in Africa and Asia (Fig. 1.1) (Snow *et al.*, 2005:2).

Antimalarial drugs fall into one of seven classes, *viz.* the 4-aminoquinolines, the aryl-amino alcohols, the 8-aminoquinolines, the antifolates, the hydroxynaphthoquinones, certain antibiotics and the artemisinin class of compounds (Schlitzer 2008:150). Regrettably the parasite has the ability to acquire resistance against most drugs used against it, making them obsolete in the majority of the areas afflicted by this disease (Petersen *et al.*, 2011:1553).

The artemisinin class of compounds is currently the basis of treatment preferred the by the World Health Organization (WHO) for uncomplicated *P. falciparum* (WHO 2010a:13). These drugs have been invaluable in the fight against malaria due to their potent antimalarial action, their lack of resistance/cross-resistance with other antimalarial drugs and their action against the gametocyte forms of *P. falciparum*. Despite these advantages this class suffers from poor water and oil solubility, a short elimination half-life and high parasite recrudescence after monotherapy treatment (Posner *et al.*, 2007:2516, Galal *et al.*, 2009:741). In an effort to overcome these shortcomings, artemether, arteether and sodium artesunate were synthesised as first generation analogs of artemisinin solving the solubility problems,

however, themselves struggling with relatively short elimination half-lives (Fig. 2.12) (Ploypradith 2004:330, Krishna *et al.*, 2004:234; Duthaler *et al.*, 2012:273).

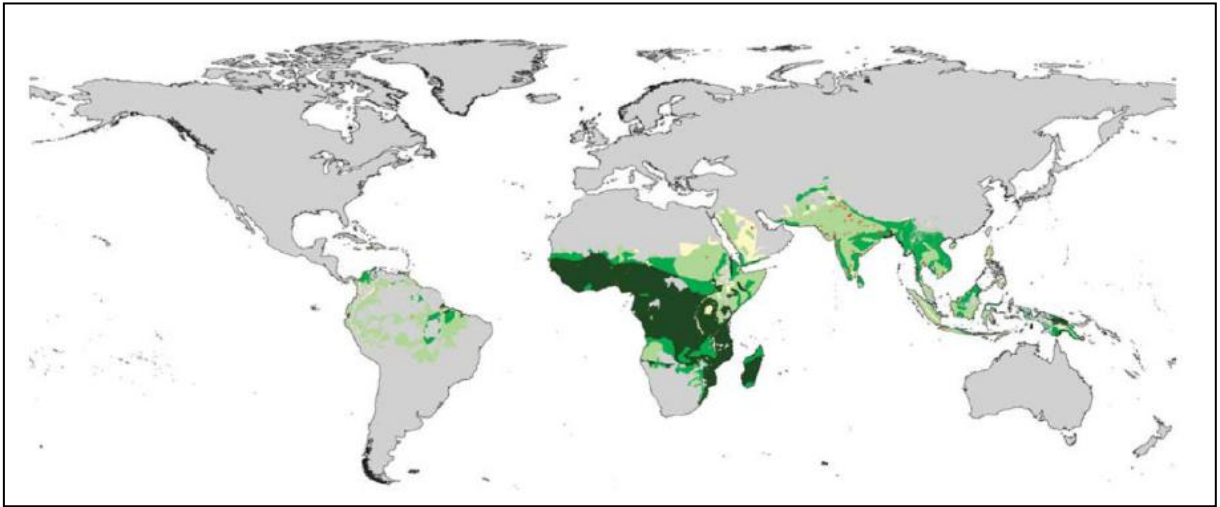


Figure 1.1: Prevalence of *P. falciparum* around the world (Snow *et al.*, 2005:7). Light green indicates hypoendemic areas, medium green indicates mesoendemic areas and dark green indicates hyperendemic and holoendemic areas.

In 1957 the WHO launched a campaign aimed at eradicating malaria, which showed initial success, however, failing in the end in part because of the parasite's ability to acquire drug resistance (Gardiner *et al.*, 2005:505). In 1998 the WHO began a new campaign called the "roll back malaria initiative" (RBM) with the aim of halving the burden of malaria by 2010, superseded by the RBM partnership's Global Malaria Action Plan after 2010 (Gardiner *et al.*, 2005:505). To prevent the parasite from acquiring resistance to the new and potent artemisinin class of drugs, the WHO recommended that the short acting highly efficacious class should not be used as monotherapy, but should rather always be combined with a longer acting drug of a distinct different class in the so called artemisinin-based combination therapies (ACTs) (WHO 2010a:14). The speed by which these ACTs reduce the parasite biomass has been reported to delay the onset of resistance against the artemisinin class (Na-Bangchang *et al.*, 2009:391). Regardless of these efforts, resistance against artesunate has already been reported at the Thai-Cambodian and Thai-Myanmar borders, where a significantly longer *in vivo* parasite clearance time has been observed (Dondorp *et al.*, 2009:466, Phyo *et al.*, 2012:6). This can prove to be problematic as the ACTs are considered the first and last line treatment in areas afflicted by multi drug resistant malaria (Pays 2010:224). The rapid spread in resistance to current malaria chemotherapy and the fact that no effective malaria vaccine are on the horizon can prove problematic, as losing the

artemisinin class would have dire consequences for current efforts to eradicate malaria (Bray *et al.*, 2005:3).

In addition to the ACTs, another method proposed to overcome the development of resistance is the incorporation of a second pharmacophore *via* a chemical bond, forming a hybrid drug molecule. A hybrid drug molecule is defined as a chemical entity with more than one structural domain, each having its own biological function. The advantage of hybrid drugs is not only their dual mechanism of action but also the fact that they tend to have more predictable pharmacokinetic properties and that one entity can be used to impart favourable qualities onto the other, e.g. increased solubility (Meunier 2008:69). The coupling of a second pharmacophore to artemisinin might thus not only decrease resistance against this class, but also increase the solubility of these compounds and reduce the incidence of recrudescence reported.

A different technique for increasing the activity of a compound and/or to overcome resistance is to couple a given pharmacophore to itself, forming a dimer, trimer, etc. It has been reported that these dimeric compounds showed an increase in activity and have been known to overcome resistance (Galal *et al.*, 2009:746, Chaturvedi *et al.*, 2010:20, Posner *et al.*, 2007:2516, Kaur *et al.*, 2010:3247). In previous studies, it was found that artemisinin dimers not only showed remarkable antimalarial but also anticancer activity (Posner *et al.*, 2008:1035, Posner *et al.*, 2007:2516, Grellepois *et al.*, 2005:5222, Chaturvedi *et al.*, 2010:20).

Targeted delivery is one more method of overcoming resistance, increasing activity or decreasing toxicity. This can be achieved by attachment of a specific moiety to the parent drug, increasing that drug's concentration at the site of action. Chadwick and co-workers attached spermidine to artemisinin to attain this goal (Chadwick *et al.*, 2010:2587). Polyamine compounds, e.g. spermidine, putrescine and spermine, have been found to have a large array of functions in the malaria parasite, mostly relating to cell differentiation and growth (Ramya *et al.*, 2006:579). The parasite is reliant on polyamine transport systems and other exogenous sources to provide it with the necessary quantities needed for growth (Chadwick *et al.*, 2010:2587). The natural occurring polyamine compounds exist as polycations *in vivo* and are taken up by the malaria parasite through a polyamine transport system that recognises specific point charges on these compounds, actively transporting them into the malarial cells (Bergeron *et al.*, 1997:1477, Chadwick *et al.*, 2010:2587). Polyamine compounds coupled to artemisinin have been shown in the past to increase the activity of the drug, both against malaria and cancer cells (Chadwick *et al.*, 2010:2589).

In addition to being actively transported into the parasite, polyamine compounds have been found to be active on their own, having antagonistic effects on crucial metabolic pathways inside the parasite (Ancelin *et al.*, 1998:1426). Calas and co-workers synthesised analogues of the natural occurring choline, ethanolamine, serine and other polar head compounds, and showed that they had substantial activity against malaria (Calas *et al.*, 1997:3560). These natural occurring polar head compounds are essential for phospholipid biosynthesis, a process necessary for membrane synthesis in the malaria parasite (Fig. 2.15). By competing with these naturally occurring polar head compounds, Calas and co-workers' compounds were able to block the synthesis of phospholipids, killing the parasite (Calas *et al.*, 2000:506). In their study, the length separating the two amino groups, the degree of substitution and the number of lipophilic moieties around the amines had a substantial influence on the activity (Calas *et al.*, 2000:509, Calas *et al.*, 2007:6307). Forming hybrid drug molecules using the described amine pharmacophore and artemisinin, might deliver compounds with increased activity and a more favourable solubility profile.

Besides the above mentioned applications of amines, ion trapping is another mechanism by which amines can be utilised in the elimination of malaria (Egan 2003:118). In the normal *in vivo* environment (pH 7.4) amino groups with low pK_a values are unprotonated and can easily cross biological membranes. When these neutral amino groups enter an acidic environment, e.g. the food vacuole of the malaria parasite (pH 4.5 - 5.5), they become protonated making it difficult for them to cross biological membranes, trapping the molecule inside the parasite (Fig. 2.14) (Egan 2003:118). Previous studies designed to increase the concentration of artemisinin by ion trapping succeeded with a modest increase in antimalarial activity (Hindley *et al.*, 2002:1055, O'Neil *et al.*, 1996:4513).

The type 1 quinoline containing 4-aminoquinoline compounds, chloroquine and amodiaquine (Fig. 2.5), are weak bases that have both aromatic and aliphatic amine moieties necessary for activity against malaria. These amines form part of the compound's pharmacophore and are also involved in ion trapping, which increases their concentration in the parasite allowing them to reach therapeutic concentrations (Kaschula *et al.*, 2002:3532, Egan *et al.*, 2000:288, Olliaro 2001:212). The type 2 quinoline containing aryl-amino alcohols, quinine, quinidine, halofantrine and mefloquine (Fig. 2.6), work by a different mechanism of action, however their amine moieties were also found to be paramount to the activity of these compounds (Dassonville-Klimpt *et al.*, 2011:27, Schlitzer 2008:150, Bhattacharjee *et al.*, 1996:4622).

The dihydrofolate reductase (DHFR) inhibitor class of compounds, sulfonamides, sulfones, pyrimethamine, cycloguanil, proguanil and trimethoprim (Fig. 2.9 & 2.11), also have amine moieties in their pharmacophores mimicking the pteridine ring of the natural substrate

dihydrofolate (DHF) and competing for the active site on the target enzyme. Cycloguanil and pyrimethamine contains the 1,3,5-triazine and phenyl pyrimidine moieties respectively, that mimic the pteridine ring. By inhibition of the folate pathway, a reduction in pyrimidine synthesis is observed, which leads to parasite death (Olliaro 2001:208).

Most of the drugs used against malaria have amine moieties that are of vital importance for their activity, either as a part of the compound's pharmacophore or by influencing the physicochemical properties of the compound to an extent necessary for activity.

Due to widespread resistance, the majority of drugs historically used are now ineffective in a number of malaria endemic areas, with even the more potent artemisinin class showing signs of reduced efficacy (Petersen *et al.*, 2011:1552, Wongsrichanalai *et al.*, 2002:209, Dondorp *et al.*, 2009:466, Phyo *et al.*, 2012:6). Vaccines to prevent people from contracting malaria are under investigation, but no success has yet been reported. The vaccine closest to public release, RTS,S/AS02A, created as far back as 1987, in trials only protected less than half the recipients from severe malaria and only reduced the risk of contracting clinical malaria by 35% (Alonso *et al.*, 2005:2017, Malaria Vaccine Initiative, 2008). As a result there is a great need for the development of new antimalarial drugs.

1.2 Aim of the study

The aim of this study was to synthesise three series of artemisinin-amine derivatives, to evaluate their antimalarial activity against both sensitive and resistant strains of *Plasmodium falciparum* and to determine their toxicity against mammalian cells. This may lead to new compounds with favourable properties to be used in the fight against malaria.

The compounds of the first series of derivatives (Fig. 1.2) consist of both the artemisinin moiety and an amino unit in accordance to that described by Calas and co-workers (Fig. 2.16) (Ancelin *et al.*, 1998:1427, Calas *et al.*, 2000:506). Calas and co-workers found that by increasing the alkyl chain length from 2 – 18 carbon atoms, there was an increase in activity of their mono amine compounds (Calas *et al.*, 1997:3560). They also found that by increasing either the lipophilicity of the substituents or the pK_a values of the amines, an increase in antimalarial activity was observed (Calas *et al.*, 1997:3560, Calas *et al.*, 2007:6312). The aminoether series was also screened alongside a series of eight ester compounds previously synthesised within our group to compare the antimalarial activity and investigate whether the same factors would influence the ester series.

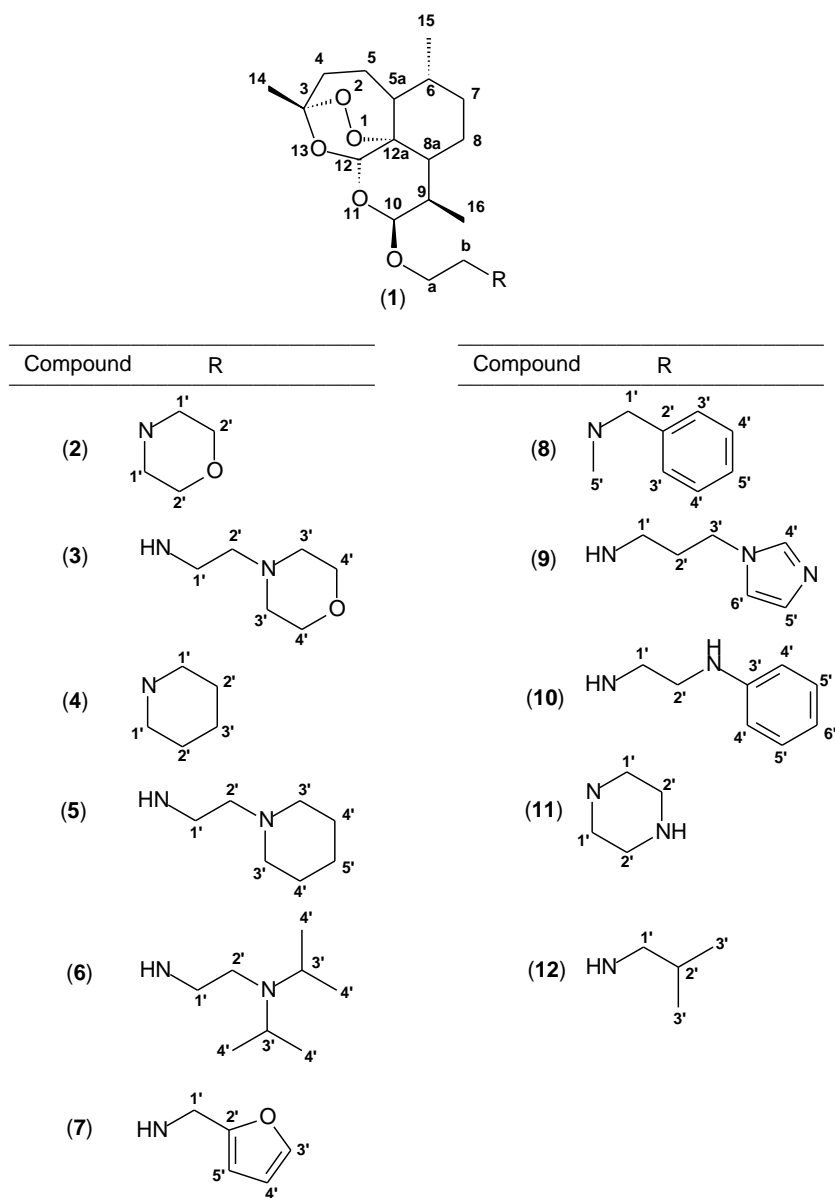


Figure 1.2: Target compounds of series 1 (see Chapter 3).

The second series of derivatives (Fig. 1.3) contain both the artemisinin and triazine pharmacophores with a variety of substituents on the triazine moiety. The 1,3,5-triazine substructure is a common moiety found in antifolate drugs (Fig. 2.8 & 2.9) and has good activity against malaria and other bacteria both on its own (Agarwal *et al.*, 2005:533, Zhou *et al.*, 2006:5452, Kinyanjui *et al.*, 1999:944) and when attached to other pharmacophores (Manohar *et al.*, 2010:324, Kumar *et al.*, 2009:6998). Both pK_a and $\log P$ values have important implications relevant to the antimalarial activity of triazine containing compounds (Agarwal *et al.*, 2005:532).

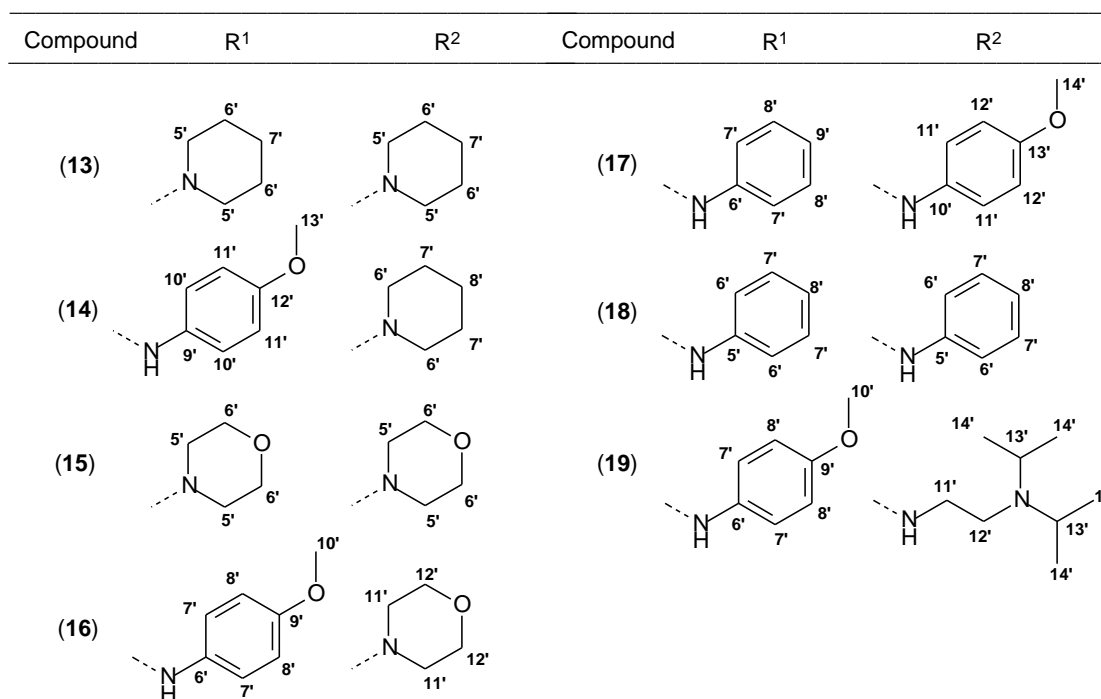
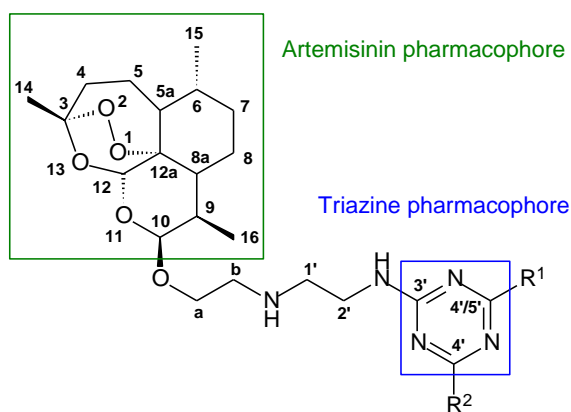


Figure 1.3: Target compounds of series 2 (see Chapter 5).

The third series of derivatives (Fig. 1.4) entailed the attachment of an additional artemisinin pharmacophore to the artemisinin-triazine derivatives, forming dimer compounds. Dimer compounds have been found to exhibit increased activity compared to their monomer counterparts (Galal *et al.*, 2009:746, Chaturvedi *et al.*, 2010:20, Posner *et al.*, 2007:2516).

Artemisinin dimers have been reported to have remarkable antimalarial and anticancer activity compared to artemisinin and related compounds (Posner *et al.*, 2008:1035, Posner *et al.*, 2007:2516, Grellepois *et al.*, 2005:5222, Chaturvedi *et al.*, 2010:20). With this series we intended to investigate whether the addition of a second artemisinin pharmacophores would lead to increased activity.

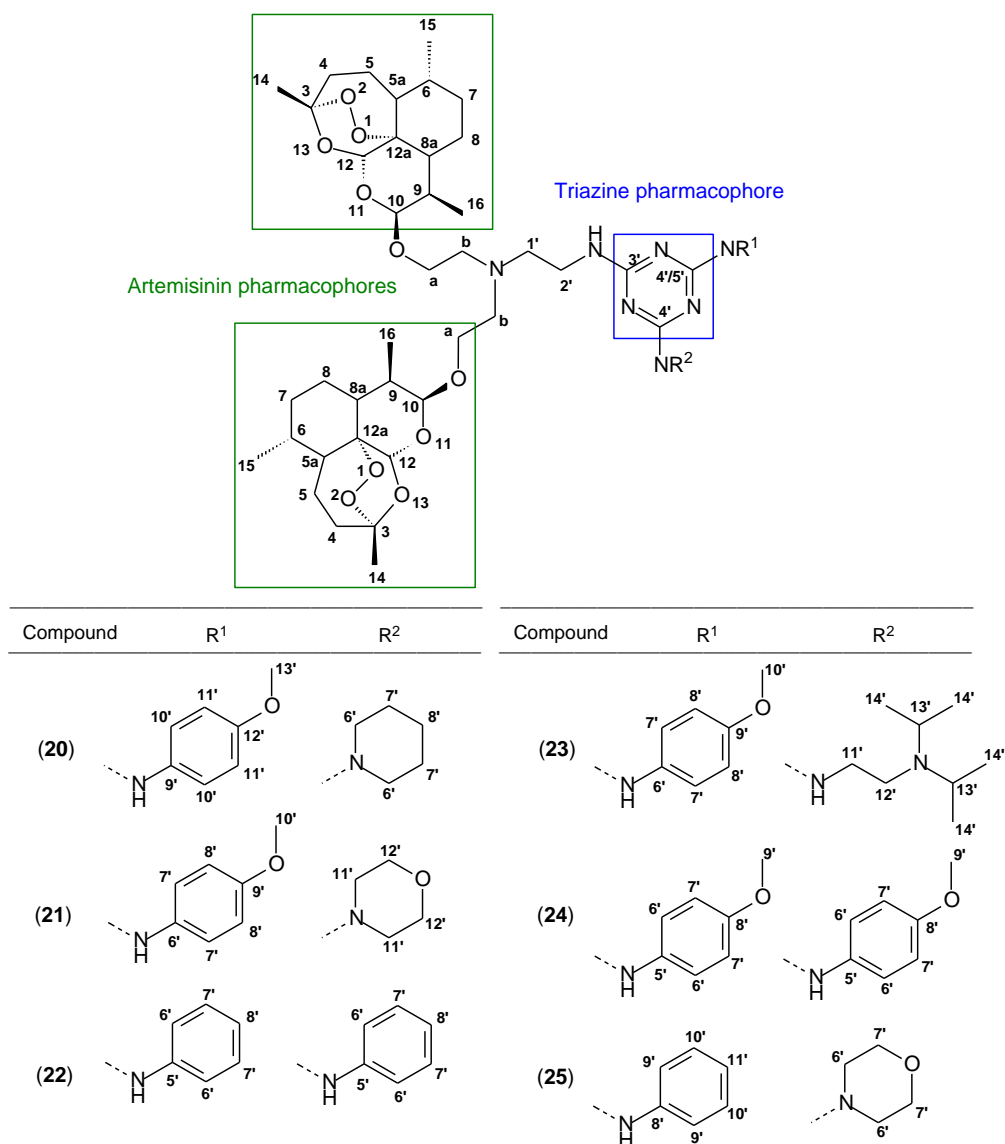


Figure 1.4: Target compounds of series 3 (see Chapter 6).