CHAPTER 4

4 SUMMARY AND CONCLUSIONS

The development of chloroquine resistance is associated with multiple gene mutations resulting in reduced accumulation of the drug inside the parasite digestive vacuole. The altered drug accumulation is mediated by a PfCRT transporter and an ATP-dependant P-glycoprotein efflux pump (Pgh1) located on the digestive vacuole membrane (Fidock *et al*., 2000). The drug efflux mechanism is capable of rapid extrusion of drugs out of the cell reducing concentrations at the drug target (Plowe, 2005; Grimberg & Mehlotra, 2011). The alkyl amine side chain of chloroquine is the primary recognition motif for chloroquine resistance due to structural specificity of the PfCRT protein (Bray *et al*., 2005). This structural specificity of PfCRT allows for changes in the side chains of drugs to have different affinities for the PfCRT protein (Bray *et al*., 2005). New drugs with structural modifications resulting in reduced affinity for PfCRT may be able to avoid reduced accumulation and retain activity in chloroquine resistant strains. The bisquinoline antimalarial drugs show promise in this regard as many studies on bisquinolines have indicated an increase in activity against chloroquine-resistant strains. This activity against resistant strains has been attributed to their bulky structures and decreased conformational mobility which makes them less efficiently extruded by the PfPgh1 efflux proteins (Gullion *et al*., 2009). Furthermore, the bisquinolines may overcome parasite resistance mechanisms through alteration of the chloroquine side chain, specifically the removal of the chloroquine resistant parasite recognised aliphatic tertiary nitrogen (Vippagunta *et al*., 1999). Chloroquine and other 4-aminoquinoline antimalarials are weak bases which traverse down the pH gradient from the parasite cytosol (pH 7.3) to concentrate inside the acidic food vacuole (pH 4.5 - 5.5). The protonation of the drug inside the vacuole increases its accumulation *via* a pH-trapping mechanism that appears critical to their activity (Solomon *et al.,* 2005). This mechanism explains the hyper concentration of chloroquine from nanomolar- in the plasma to millimolar concentrations in the digestive vacuole (Sullivan *et al.,* 2002).

The aim of this study was to synthesise a series of bisquinoline and bispyrrolo[1,2*a*]quinoxaline compounds containing various polyamines, which may act as potential protonation sites in the hope of increasing their accumulation via pH-trapping. Twelve bisquinoline and five bispyrrolo[1,2*a*]quinoxaline compounds were synthesised by nucleophilic aromatic substitution. The structures of synthesised compounds were confirmed by nuclear magnetic resonance spectroscopy (NMR) and mass spectroscopy (MS). The *in vitro* antimalarial activity of compounds against a chloroquine-sensitive (CQS) D10 strain

and chloroquine-resistant (CQR) Dd2 strain of *Plasmodium falciparum* was determined using a well established method, (Makler *et al.,* 1993; Trager *et al*., 1976). The synthesised compounds were tested alongside chloroquine as a reference drug. The *in vitro* cytotoxicity of the series was determined against a mammalian cell-line, Chinese Hamster Ovarian (CHO) using the MTT-assay and emetine as reference drug. The physicochemical properties of synthesised compounds were experimentally determined. The most potent compounds **4** - **9** were also screened for *in vitro* anticancer activity against TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells.

Series 1 and series 2 containing the same quinoline nucleus and polyamine linkers showed both comparable aqueous solubility (S_W) in the range of 18.94 - 38.86 mM, and lipid solubility (S_{OC}) in the 35.17 - 98.39 mM range. Series 3 compounds **16** - **19** (S_{OC} = 55.55 - 83.57 mM), which contain the pyrroloquinoxaline moiety and therefore a greater aromatic surface was slightly more lipid soluble than series 1 and 2, with the exception of compound **20** (S_{OC} = 30.67 mM) that possessed the lowest lipid solubility in this series. The aqueous solubility of compounds increases with the increasing number of protonation sites in the polyamine bridge, since the secondary nitrogen atoms of the linker can form hydrogen bonds with the surrounding water molecules. However, this effect is alleviated if the C-C chain separating the two intrabridge nitrogen atoms lengthened. The addition of an N-methyl group to the central nitrogen of the polyamine bridge irrespective of the series, increases aqueous- and lipid solubility.

The IC_{50} data revealed that series 1 was the most active of all synthesised compounds. All the bis-(7-chloroquinoline) compounds **4** – **9** were found to be significantly more active than CQ (IC_{50} = 242.30 nM) with IC_{50}-values in the 36 -73 nM range against the Dd2 strain. The compounds **4** - **9** displayed similar activity as CQ (IC_{50} = 48.35 nM) against the D10 strain. Compound **8** (IC_{50} = 35.49 nM) and **9** (IC_{50} = 49.48 nM) were the most active compounds in series 1. These compounds **4** - **9** all concentrated in an almost 1 : 2 ratio between the aqueous and octanol phase, suggesting that a balance between hydrophilicity and lipophilicity, which shifts towards increased lipid solubility enhances activity. Compound **12** was the most active compound in series 2 against both strains. It possessed more potent antimalarial activity than CQ against the Dd2 strain (IC_{50} = 80.22 nM vs. 242.30 nM) and showed activity in the same class as CQ against the D10 strain (IC_{50} = 128.98 nM vs. 48.35 nM). This activity may be as result of the polyamine linker that provides the necessary distance between the identical pharmacophores for optimal configuration for haematin interactions. Compound **14** and **15** was significantly less potent than CQ against both strains and possessed the weakest activity in series 2. The poor antimalarial activity of compound

14 and **15** may suggest that, as result of their high lipid solubility (**14**: S_{OC} = 98.39 mM, **15**: S_{OC} = 73.75 mM), they become trapped in the lipid membranes and do not reach the digestive vacuole in sufficient concentration to inhibit hemozoin formation. In series 3 only compounds **18** and **19** possessed activity in the same range as CQ against both strains. Their activity may be attributed to the relationship between their aqueous- and lipid solubility, which has a ratio of 1 : 2.3 (aqueous:octanol phase) or an optimal configuration that allows for haematin interaction. Compound **20** was the least active in the series with a significantly higher mean IC_{50}-value than CQ against both strains. Presumably this compound is unable to cross the biological membranes because of its increased aqueous solubility that results in its weak antimalarial activity. The bisquinoline compounds **4** - **15** did not show cytotoxicity at the concentrations tested. In the bispyrrolo[1,2*a*]quinoxaline series, compound **17** (SI = 14.22) and **20** (SI = 4.49) were the most cytotoxic. The high toxicity of compound **20** may be attributed to the lengthening of the C-C chain, since an increase in aliphatic chain length is associated with toxicity towards mammalian cells (Caffrey *et al.,* 2007).

The cytotoxic and cytostatic effects of the bis-(7-chloroquinoline) compounds **4** - **9** against the cancer panel consisting of TK10, UACC62 and MCF7 cells revealed potent anticancer activity against all cancer cell lines tested. This may be as result of a polyamine transporter mediated uptake mechanism into cancer cells and/or optimal interactions between the ionized compounds and the negatively charged phosphate molecules and bases in the DNA backbone that results in DNA strand scission. Compound **4**, **6** and **7** exhibited a significantly more potent cytostatic effect on the proliferation of all three cancer cell lines compared to etoposide, with a TGI in the range of 0.6 - 2 μM against TK10 cancer cells, 0.59 - 0.74 μM against UACC62 cancer cells and 0.55 -0.69 μM against MCF7 cancer cells. In view of these results, the series 1 bisquinolines appear to be better anticancer compounds in this study than antimalarials. Thus, these compounds may be worthwhile investigating further in search for more effective anticancer drugs.

Overall, the most active of all synthesised compounds were the series 1 bisquinolines **8** (IC_{50} = 35.49 nM) and **9** (IC_{50} = 49.48 nM). The results of this study showed that the 7-chloro-4-aminoquinoline is a more active antimalarial pharmacophore than the 5-methyl-4-aminoquinoline and pyrrolo[1,2*a*]quinoxaline ones. This activity may be ascribed to the 7-chloro-substituent that contributes to the haematin inhibitory activity, as already established by previous studies (Kaschula *et al.* 2002). The addition of a pyrrolo-ring to the quinoline moiety does not improve antimalarial activity. The ideal linker that provides optimal configuration for haematin interactions could be identified based on *in vitro* activity as containing a three C-C chain that separates the nitrogen atoms of the polyamine bridge, with

or without an N-methyl group attached to the central nitrogen atom. The bisquinolines (Series 1 and 2) were less cytotoxic than the bisquinoxalines. This suggests that the 4-aminoquinoline pharmocophore is less toxic to the mammalian cells that the quinoxaline one. Compound **4**, **6** and **7** possessed the most potent anticancer activity in the series. The ideal linker that provides optimal interaction with the highly polar phosphate backbone and bases of DNA was identified based on the potent cytotoxic and cytostatic effect on cancer cell proliferation as containing a two C-C chain, or three C-C chain that separates the nitrogen atoms of the polyamine bridge, with or without an N-methyl group attached to the central nitrogen atom. This allows for the external binding of drugs to DNA that may pull the AT-rich strains apart and result in the disruption of DNA transcription (McFayden *et al*. 1988).

In conclusion, the formation of biscompounds containing the quinoline pharmacophore with increased protonation sites afforded by polyamine linkers could be a valid strategy to overcome CQ resistance. The bisquinolines **8** and **9** possessed the best drug profile based on antimalarial activity and cytotoxicity and may be good candidates for further investigation in search for antimalarial substitute to chloroquine. The bisquinolines containing the 7-chloro-4-aminoquinoline nucleus also have a potential application in anticancer chemotherapy with potent cytostatic and cytotoxic effects on melanoma (UACC62), renal (TK10) and breast (MCF7) cancer cells. The bisquinolines **4**, **6** and **7** showed the most potent anticancer activity without *in vitro* cytotoxicity towards mammalian cells, these compounds may be good candidates for further investigation in the search for new and more effective anticancer agents.

In order to further understand the activity of the bisquinoline and bispyrrolo[1,2*a*]quinoxaline compounds synthesised, future studies should include β-haematin inhibitory activity assays that can provide more information on their mechanism of action. These studies will be able to determine whether the biscompounds inhibit hemozoin formation or have additional mechanisms of action that result in toxicity for the parasite. Also the determination of compounds' vacuole accumulation ratios (VAR), which will provide information on whether the increased protonation sites enhances drug accumulation, and whether this is in fact responsible for the increased activity against CQR found for bis-(7-chloroquinoline) compounds. The bisquinoline and bispyrrolo[1,2*a*]quinoxaline compounds will be highly lipophilic at a physiological pH (7.1) resulting in a distribution based limitation to their antimalarial activity. Thus, the determination of the series' log D at a physiological pH (7.1) will be worthwhile, since their efficacy as potential antimalarials will be dependend upon the transport of compounds to the site of action.