Synthesis and *in vitro* antimalarial activity of series of bisquinoline and bispyrrolo[1,2*a*]quinoxaline compounds

Lezanne van Heerden

(B.Pharm.)

Thesis submitted in the partial fulfilment of the requirements for the degree

MAGISTER SCIENTIAE

in the

Department of Pharmaceutical Chemistry, School of Pharmacy

Faculty of Health Sciences

at the

North-West University

Supervisor: Dr. D.D. N'Da

Assistant-supervisor: Mr. T.T. Cloete

Potchefstroom

2012

ABSTRACT

Every year an estimated 1 million people die of malaria, with the majority of these deaths reported in Africa. The disease caused by *Plasmodium falciparum* affects an approximate 250 million people annually and with the emergence of monodrug and multidrug resistance it has seen resurgence in the last decade. The decline in effectiveness of chloroquine in the treatment of drug resistant malaria has contributed to the doubling of malaria specific mortality in the last fifteen years. Since the quinoline drug family represents the basis of malaria chemotherapy for much of the past 50 years. This spread of resistance to existing antimalarial drugs such as chloroquine, mefloquine, sulfadoxine and pyrimethamine has driven the search for new drugs that might circumvent parasite resistance mechanisms. The mechanism of chloroquine resistance is associated with reduced accumulation of the drug inside the digestive vacuole, which is connected to a *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) or ATP-dependant P-glycoprotein efflux pump (Pgh1). The PfCRT protein demonstrates a structural specificity for the chloroquine side chain, which allows for changes in the structures of drugs to have different affinities for the transporter. New drugs with structural modifications that result in reduced affinity for PfCRT may be able to avoid reduced drug accumulation. Despite resistance, the aminoquinoline pharmacophore remains an attractive scaffold in the design of new drugs, since it demonstrates a unique affinity for haematin. This is a desirable feature since the quinoline antimalarial drugs inhibit conversion of haematin to hemozoin. The 4-aminoquinoline antimalarial drugs are also weak bases which traverse down the pH gradient to concentrate inside the acidic food vacuole. The protonation of these drugs inside the vacuole makes them membrane impermeable and increases their accumulation, which allows for the high concentrations required for hemozoin inhibition.

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. In order to achieve this aim twelve bisquinolines **4** - **15** and five bispyrrolo[1,2*a*]quinoxalines **16** - **20** were synthesised and their structures confirmed by nuclear magnetic resonance spectroscopy (NMR) and mass spectroscopy (MS). The aqueous solubility (S_w) and distribution coefficients (logD) were experimentally determined in phosphate buffered saline (pH 5.5) to mimic the parasitic digestive vacuole environment. The compounds were screened for antimalarial activity alongside chloroquine (CQ) against chloroquine-sensitive (CQS) D10 and the moderately chloroquine-resistant (CQR) Dd2 strains of *P. falciparum.* The series were also tested for cytotoxicity against Chinese Hamster Ovarian (CHO) cells,

using emetine as reference drug. The most active compounds against *P. falciparum* were screened for anticancer activity against the TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells.

The bisquinoline- and bispyrrolo[1,2a]quinoxaline compounds were found to be more hydrophilic than chloroquine (S_W = 0.033 mM) itself with aqueous solubility varying in the 18.94 - 38.86 mM range. Irrespective of the series, the aqueous solubility increases with the increase in potential protonation sites (N atoms) in the polyamine bridge. However, this effect is overruled if the carbon-carbon chain separating two nitrogen atoms in the polyamine also increases.

The *in vitro* data revealed seven of the twelve bisquinoline compounds to be significantly more potent against the CQR (Dd2) strain compared to chloroquine. Compounds **8** (*7-chloro-4-[10-(7-chloroquinolin-4-yl)-1,4,7,10-tetraazadecan-1-yl]quinoline*) (IC_{50} = 35.49 nM) and **9** (*7-chloro-4-[12-(7-chloroquinolin-4-yl)-1,5,8,12-tetraazadodecan-1-yl]quinoline*) (IC_{50} = 49.48 nM) featuring the triethylenetetramine or *N,N'*-bis(3-aminopropyl)ethylenediamine linkers respectively, were the most active of all synthesised compounds. They were found significantly more potent than CQ (IC_{50} = 242.3 nM) against the Dd2 strain. However, they were as potent as CQ (IC_{50} = 48.35 nM) against the D10 strain. This potent activity against the CQR strain could possibly be as result of enhanced pH-trapping inside the digestive vacuole, since they contain increased protonation sites that also enhance their hydrophilicity. These compounds also displayed the best drug profile based on toxicity and antimalarial activity, both demonstrating good selectivity towards parasitic cells with a selectivity index of greater than 90.

The bis-(7-chloroquinoline)-series displayed the most potent antimalarial activity and were subsequently screened for potential anticancer activity. The series showed potent growth inhibitory activity against all 3 cancer cell lines. Presumably the polyamine bridges of bisquinoline compounds provide increased ionisation of structures that allows for increased van der Waals interactions with the highly polar phosphate backbone of the parasite DNA. These interactions possibly interfere with cell replication and cause DNA strand scission, since bisquinolines are known to bind by external attachment to the AT-rich sequences of DNA, which is less stable and easier to pull apart. Compound **4** (*7-chloro-N-[2-({2-[(7-chloroquinolin-4-yl)amino]ethyl}amino)ethyl]quinolin-4-amine)*, **6** (*7-chloro-N-[3-({3-[(7-chloroquinolin-4-yl)amino]propyl}amino)propyl]quinolin-4-amine)* and **7** (*bis({3-[(7-chloroquinolin-4-yl)amino]propyl})(methyl)amine*) showed significantly more potent growth inhibition efficacy against breast (MCF7) cancer cells compared to etoposide (TGI > 100 μM)

with TGI-values in the range of 0.55 - 0.69 μM. Compounds **4**, **6** and **7** were also the most potent against TK10 (renal) and melanoma (UACC62) cancer cells with TGI-values of 0.6, 2.05 and 1 μM against TK10 cells respectively, compared to etoposide (TGI = 43.33 μM). Against melanoma cells the TGI values were 0.59 for **4**, 0.74 for **6** and 0.64 μM for **7**, compared to 4 μM for etoposide. The results reveal that a two C-C chain, and a three C-C chain with or without methyl substitution is the optimal linker to separate the identical non-intercalating pharmacophores for potent anticancer activity. All of the compounds in the series warrant further investigation in search of more potent anticancer agents.

OPSOMMING

Daar sterf elke jaar ’n beraamde een miljoen mense aan malaria, waarvan die meerderheid gevalle in Afrika aangemeld word. *Plasmodium falciparum* veroorsaak die meeste malariaverwante sterftes en ongeveer 250 miljoen mense per jaar word daardeur aangetas. In die afgelope dekade het die ontwikkeling van enkel- en veelvuldige-geneesmiddelweerstandigheid ’n toename in malariavoorkoms veroorsaak. Die afname in chlorokien se effektiwiteit in die behandeling van geneesmiddelweerstandige malaria het malariaverwante sterftes in die laaste vyftien jaar laat verdubbel, aangesien die kinolien-antimalariamiddels die basis van malariabehandeling gedurende die afgelope vyftig jaar verteenwoordig. Hierdie toename in *P. falciparum* geneesmiddelweerstandigheid teen chlorokien, meflokien, sulfadoksien en pirimetamien is die dryfkrag agter nuwe geneesmiddelontwikkeling wat poog om parasietweerstandigheid te oorkom. Chlorokienweerstandigheid word veroorsaak deur verlaagde akkumulasie van die geneesmiddel in die parasiet se voedselvakuool. Hierdie meganisme hou verband met ’n PfCRT-proteïen of ’n ATP-afhanklike P-glikoproteïen, wat geneesmiddels aktief uit die voedselvakuool vervoer en die syketting van chlorokien herken. Hierdie strukturele spesifisiteit van die betrokke proteïen veroorsaak dat veranderinge aan die struktuur van geneesmiddels verskillende affiniteite vir die proteïen vertoon. Dus kan geneesmiddels met nuwe strukture en gevolglik ’n verlaagde affiniteit vir die PfCRT-proteïen moontlik die chlorokienweerstandigheidsmeganisme oorkom. Die aminokinolienfarmakofoor is steeds ’n aantreklike templaat vir geneesmiddelontwikkeling ten spyte van die bogenoemde weerstandigheid, weens die kern se unieke affiniteit vir hematien. Laasgenoemde is ’n voordelige eienskap aangesien die kinolienantimalariamiddels die omskakeling van hematien na hemosoien voorkom. Die aminokinolienklas antimalariamiddels is verder swak basisse wat deur middel van ’n pH-gradiënt in die parasiet se voedselvakuool konsentreer. Die protonering van dié strukture in die voedselvakuool veroorsaak dat hulle membraanondeurlaatbaar word en verhoog hul akkumulasie in die voedselvakuool, wat die verhoogde konsentrasies daarstel wat benodig word vir hemosoien-inhibisie.

Die doel van hierdie studie was om ’n reeks biskinolien- en bispirrolo[1,2*a*]kinoksalienverbindings, wat verskeie poliamiene bevat, te sintetiseer wat potensiële protoneerbare setels bevat met die oogmerk om hul akkumulasie *via* ’n pH-vangsmeganisme te verhoog. Om hierdie doel te bereik is twaalf biskinolien- **4-15** en vyf bispirrolo[1,2*a*]kinoksalienverbindings **16-20** gesintetiseer, en hul strukture met kernmagnetieseresonansiespektroskopie (KMR) en massaspektrometrie (MS) bevestig. Die wateroplosbaarheid (Sw) en verdelingskoëffisiënte (log D) is eksperimenteel in

fosfaatgebufferde soutoplossing (pH 5.5) bepaal om die suuromgewing in die parasiet se voedselvakuool na te boots. Die verbindings se antimalaria-aktiwiteit teen die chlorokiensensitiewe (CQS) D10 en chlorokienweerstandige (CQR) Dd2 stamme van *P. falciparum* is bepaal en vergelyk met die aktiwiteit van chlorokien (CQ). Die sitotoksisiteit van die reekse is uitgevoer op die "Chinese Hamster Ovarian" (CHO) soogdier sellyn teenoor emetien as verwysingstandaard. Die verbindings met die hoogste aktiwiteit teenoor *P. falciparum* se antikankeraktiwiteit teen TK10 (renale-), UACC62 (melanoom-) en MCF7 (borskanker) sellyne is ook bepaal.

Die biskinolien- en bispirrolo[1,2*a*]kinoksalienverbindings, met wateroplosbaarheidswaardes wat strek van 18.94-38.86 mM, was meer hidrofiel as chlorokien (Sw = 0.033). By albei reekse word 'n toename in die wateroplosbaarheid waargeneem met 'n vermeerdering in potensiële protoneerbare setels (N-atome) in die poliamienbrug. Hierdie verskynsel is egter nie meer geldig nie indien die koolstofketting, wat die stikstof atome in die brug skei, ook verleng word.

Volgens die *in vitro* data was sewe uit die twaalf biskinolienverbindings beduidend meer aktief as chlorokien teenoor die CQR Dd2 stam. Verbinding **8,** (*7-chloro-4-[10-(7-chlorokinolien-4-iel)-1,4,7,10-tetraäsaandekaan-1-iel]kinolien*) (IC_{50} = 36 nM) en **9,** (*7-chloro-4-[12-(7-chlorokinolien-4-iel)-1,5,8,12-tetraäsaandodekaan-1-iel]kinolien*) (IC_{50} = 49 nM), wat onderskeidelik 'n triëtileentetramien- en *N,N'*-bis(3-aminopropiel)etileendiamienbrug bevat is die aktiefste verbindings in die reeks. Hierdie verbindings het beduidende groter aktiwiteit as CQ (IC_{50} = 48.35 nM) teenoor die weerstandige Dd2 stam getoon, maar hul aktiwiteit teenoor die sensitiewe D10 stam was naastenby dieselfde as dié van chlorokien. Hierdie goeie antimalaria-aktiwiteit teenoor die CQR-stam kan moontlik toegeskryf word aan die verbindings se groter akkumulasie in die parasiet se voedselvakuool, danksy hul verhoogde protonering in 'n suuromgewing wat hul hidrofiliteit verhoog. Hierdie verbindings toon dus die beste geneesmiddelprofiel gebaseer op antimalaria-aktiwiteit en aangesien hulle beide 'n selektiwiteitsindeks van groter as 90 het.

Die bis-(7-chlorokinolien)-reeks het oor die algemeen die hoogste aktiwiteit getoon van die gesintetiseerde verbindings en is vervolgens getoets vir potensiële antikankeraktiwiteit. Die reeks het merkwaardige toksisiteit teen die groei van al drie kankersellyne getoon. Hierdie aktiwiteit kan waarskynlik toegeskryf word aan die geïoniseerde poli-amienbrûe in die biskinolien-strukture wat bydra tot van der Waals interaksies met die polêre fosfaat-groepe van die parasiet-DNA. Hierdie interaksies kan moontlik selreplikasie inhibeer en DNA-stringbreking teweegbring, aangesien dit reeds bekend is dat die biskinoliene ekstern aan

die AT-ryke (adenien-tiamien) volgordes van DNA bind, wat minder stabiel is en dus makliker ontrafel word. Verbindings **4** (*7-chloro-N-[2-({2-[(7-chlorokinolien-4-iel)amino]etiel}amino)etiel]kinolien-4-amien)*, **6** (*7-chloro-N-[3-({3-[(7-chlorokinolien-4-iel)amino]propiel}amino)propiel]kinolien-4-amien)* en **7** (*bis({3-[(7-chlorokinolien-4-iel)amino]propiel})(metiel)amien*), met TGI-waardes tussen 0.55 – 0.69 μM, het beduidende groter aktiwiteit teenoor die groei van borskankerselle (MCF7) as etoposied (TGI> 100 μM) getoon. Verbindings **4**, **6** en **7** was ook die mees potente teen TK10 (renale) en UACC62 (melanoom-) kankerselle met TGI-waardes van 0.6, 2.05 en 1 μM teen TK10-selle onderskeidelik, vergeleke met etoposied (TGI = 43.33 μM). Teen melanoomselle was die TGI-waardes 0.59 vir **4**, 0.74 vir **6** en 0.64 μM vir **7**, vergeleke met 4 μM vir etoposied. Die resultate toon dat 'n 2-C- en 'n 3-C-ketting, met of sonder metielsubstitusie, die optimale bindingslengte tussen die identiese nie-interkalerende farmakofore, vir optimale antikankeraktiwiteit is. Al die verbindings in die reeks behoort verder nagevors te word in die soeke na kragtiger antikankermiddels.

ACKNOWLEDGEMENTS

I would like to thank the following people and institutions for their support and contributions;

Dr. D.D. N'Da, my supervisor, for his guidance, support and enthusiasm throughout this study.

Theunis Cloete, my assistant-supervisor and lab partner, for his encouragement and unfailing optimism during my study, especially the frequent coffee breaks at the SS.

Prof. JC Breytenbach for his precious support.

Prof. Peter Smith from the University of Cape Town for the *in vitro* antimalarial activity and cytotoxicity of my synthesised compounds.

Natasha Kolesnikova from the CSIR for the anticancer activity screening of my compounds.

Prof. Jan du Preez for the HPLC separation of the compounds.

André Joubert for the skilled recording of NMR spectra.

Marnitz Verwey for his friendship and sense of humour throughout the past six years.

Melanie van Heerden for all her advice and encouragement, especially in times of distress. I was truly blessed to have had an older sister on the floor to go to for support and who understood the challenges of a Masters.

My parents, Leon and Marlize, my brother, Louis and my sister, Vanessa for all their love and support during this journey.

Daniel for all his love and patience, who got me through the difficult times and kept me going. Thank you for being so understanding and always listening, even if you don't speak chemistry.

The National Research Foundation for financial support.

Members of the Department of Pharmaceutical Chemistry for their assistance.

And most importantly, to God.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES