Synthesis and *in vitro* antimalarial activity of novel chalcone derivatives

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Solemn Declaration

NORTH-WEST UNIVERSITY
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Preface

This thesis is submitted in an article format in accordance with the General Academic Rules (A.13.7.3) of the North-West University. Three articles, two of which have been submitted, are included in this thesis:

Chapter 3: Article 1

Synthesis, *in vitro* antimalarial activity and cytotoxicity of novel chalcone-quinoline amides

Chapter 4: Article 2

Synthesis, antimalarial and cytotoxic activity of novel aminoferrocenyl-chalcone amides

Chapter 5: Article 3

Synthesis and biological evaluation of dihydroartemisinyl-chalcone esters

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Abstract

Malaria is endemic in 106 countries worldwide. This disease is caused by a parasite from the genus *Plasmodium*. Of the five species that infect humans, *Plasmodium falciparum* is the most virulent, with over three billion people at risk and around 660 000 deaths reported in 2011. Of these deaths, 91% were in the African region, while 86% were children under the age of five. In light of the widespread development of resistance by malaria parasites against the classic antimalarial drugs, such as chloroquine (CQ) and now the established tolerance towards the widely used artemisinins, an immense need exists for identifying and developing new and effective antiplasmodial drugs. In search for such new antimalarial drugs, three chalcone based series of compounds were prepared and investigated during this study.

The first series (Chapter 3) comprised 4-aminoquinolinyl-chalcone amides, which were synthesized through amidation of carboxylic acid-functionalised chalcone with aminoquinolines, using 1,1'-carbonyldiimidazole (CDI) as coupling agent. These compounds were screened alongside CQ against the CQ sensitive (3D7) and CQ resistant (W2) strains of *P. falciparum*. Cytotoxicity was assessed against the WI-38 cell line. The amide, featuring the 1,6-diaminohexane linker, was found the most active of all these new novel compounds tested. It was found to be as potent as CQ against 3D7, while displaying a two-fold higher activity than CQ against the W2 strain, coupled with good selective antimalarial activity (SI = 435) towards the parasitic cells.

The second series (Chapter 4) consisted of aminoferrocenyl-chalcone amides, synthesized through condensation of a chalcone with an aminoferrocenyl. These compounds were screened against the 3D7, and antifolate- and CQ resistant (FCR3) strains of *P. falciparum* and cytotoxicity was determined against the WI-38 line. The most active compound of this series was the amide, containing the 1,2-diaminoethane linker, which showed 130- and 42 times less potency than CQ against the 3D7 and W2 strains, respectively.

The third series of antimalarials (Chapter 5) involved dihydroartemisinyl-chalcone esters, synthesized through esterification of chalcones with DHA. These compounds were screened against 3D7 and W2 strains of *P. falciparum*, while the cytotoxicity was determined against the WI-38 line. Those esters featuring oxygenated aryl rings were three- to four-fold more potent than current clinically used artesunate against both *P. falciparum* strains. They were also screened *in vitro* against a panel of three cancer cell lines consisting of TK-10, UACC-

62 and MCF-7. Thermogravimetric analysis revealed that the targeted hybrids were all thermally more stable than DHA as a result of the presence of the chalcone moiety in their structures. This could prove beneficial to the high temperature storage conditions that prevail in most malaria endemic countries.

This study resulted in a number of compounds with varying antiplasmodial activity ranges. The compounds in series 3 were overall the most active, due to the incorporation of the highly active dihydroartemisinin pharmacophore. The chalcone moiety, especially, demonstrated a large scope for future development, owing to the ease of synthesis and the relatively low costs involved. The most active compounds of the three series could serve as potential lead compounds in the future development of more effective antimalarial drugs.

Keywords: Plasmodium falciparum, malaria, chalcone, amino quinoline, aminoferrocenyl, dihydroartemisinin

Opsomming

Malaria is endemies aan 106 lande regoor die wêreld. Hierdie siekte word deur ’n parasiet uit die genus *Plasmodium* veroorsaak. Uit die vyf spesies wat mense kan besmet, is *Plasmodium falciparum* die mees virulente, met meer as drie miljard lewens wat in gevaar is, terwyl ongeveer 660 000 sterftes in 2011 voorgekom het. Uit hierdie gerapporteerde sterftes het 91% in die Afrika-streek voorgekom, waarvan 86% kinders jonger as vyf jaar was. In die lig van die wydverspreide weerstand van die malariaparasiete teen die klassieke antimalariamiddels, soos chlorokien (CQ), en nou die gevestigde toleransie teen die algemeen-gebruikte artemisiniene, bestaan daar ’n geweldige behoefte om nuwe, doeltreffende anti-malariamiddels te identifiseer en te ontwikkel. In ’n soektog na nuwe antimalariamiddels is drie chalkoon-gebaseerde reekse verbindings tydens hierdie studie voorberei en ondersoek.

Die eerste reeks middels (Hoofstuk 3) het 4-aminokinoliniel-chalkoon-amiede behels, wat deur die amidasie van karboksielsuur-gefunksionaliseerde chalkoon met aminokinoliene, deur middel van 1,1'-karbonieldiimiedasool as koppelmiddel, gesintetiseer is. Hierdie verbindings is saam met CQ teen die CQ-sensitiewe (3D7) en CQ-weerstandige (W2) stamme van *P. falciparum* getoets. Sitotoksisiteit is teen die WI-38-sellyn geëvalueer. Die amied met die 1,6-diaminoheksaan-skakel was die aktiefste van almal. Daar is bevind dat dit so effektief soos CQ teen 3D7 was, terwyl dit twee keer hoër aktiwiteit teen die W2-stam getoon het, tesame met goeie selektiewe antimalaria-aktiwiteit (SI = 435) teen die parasitiese selle.

Die tweede reeks (Hoofstuk 4) het uit aminoferroseniel-chalkoon-amiede bestaan, wat deur kondensasie van ’n chalkoon met ‘n aminoferroseniel gesintetiseer was. Hierdie verbindings is teen die 3D7-, en antifolaat- en CQ-weerstandige (FCR3) stamme van *P. falciparum* getoets en sitotoksisiteit is teen die WI-38-lyn bepaal. Die aktiefste verbinding uit hierdie reeks is die amied met die 1,2-diaminoëtaan-skakel, wat 130- en 42 keer minder aktief as CQ teen die 3D7- en W2-stamme, onderskeidelik, was.

Die derde reeks antimalariamiddels (Hoofstuk 5) het dihidroartemisiniel-chalkoon-esters behels, wat deur die verestering van chalkone met DHA gesintetiseer was. Hierdie verbindings is teen die 3D7- en W2-stamme van *Plasmodium falciparum* getoets, terwyl die sitotoksisiteit teen die WI-38-sellyn geëvalueer is. Daardie esters met geoksigeneerde

arielringe is drie tot vier keer meer aktief as die artesunaat wat tans klinies teen albei *P. falciparum*-stamme gebruik word. Hierdie verbindings is ook teen drie kanker sel-lyne, opgemaak uit TK-10, UACC-62 and MCF-7 kanker selle, geëvalueer. Termogravimetriese ontleding het getoon dat al die geteikende hibriede, as gevolg van die aanwesigheid van die chalkoon-gedeelte in hulle strukture, termies meer stabiel as DHA was. Dit mag tot voordeel van die hoë temperatuurstoorkondisies, soos wat in die meeste malaria-endemiese lande heers, bevind word.

Hierdie studie het tot ’n aantal verbindings met wisselende aktiwiteitsvlakke aanleiding gegee. Die verbindings in reeks 3 was oorkoepelend die aktiefste, weens die inkorporasie van die uiters aktiewe dihidroartemesinien-farmakofoor. Die chalkoon-komponent, veral, het geweldige potensiaal vir toekomstige ontwikkeling getoon, as gevolg van die gemak waarmee dit gesintetiseer word en die relatief lae koste betrokke. Die aktiefste verbindings uit al die drie reekse kan as potensiële leidraadverbindings vir die ontwikkeling van doeltreffender antimalariamiddels in die toekoms dien.

Sleutelwoorde: Plasmodium falciparum, malaria, chalkoon, aminokinolien, aminoferroseniel, dihidroartemesinien

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List of Abbreviations

ACT	Artemisinin based combined therapy
ARF	Acute renal failure
AS	Artesunate
^{13}C NMR	Carbon NMR
CBV	Cerebral blood volume
CM	Cerebral malaria
COSY	Correlation spectroscopy
CQ	Chloroquine
CQR	Chloroquine resistant
CQS	Chloroquine sensitive
Cyt bc1	Cytochrome bc1
DDT	Dichlorodiphenyltrichloroethane
DEPT	Distortionless enhancement by polarization transfer
DHA	Dihydroartemisinin
DHF	Dihydrofolate
DHFR	Dihydrofolate reductase
dhfr-ts	Dihydrofolate reductase-thymidylate synthase
DHPS	Dihydropteroate synthase
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
FDA	Food and Drug Administration
G6PD	Glucose-6-phosphate dehydrogenase
^{1}H NMR	Proton NMR
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
IC_{50}	50% inhibitory concentration
IL-4	Interleukin-4
IPT	Intermittent preventative treatment
IR	Infrared spectroscopy
IRS	Indoor residual spraying
ITN	Insecticide treated nets
NADPH	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance

npRBCs	Non-parasitized red blood cells
PABA	Para-aminobenzoic acid
*Pf*crt	*Plasmodium falciparum* chloroquine resistance transporter
*pf*mdr1 gene	*Plasmodium falciparum* multi-drug resistant gene
Pgh1 efflux pump	P-glycoprotein efflux pump
pRBCs	Parasitized red blood cells
RBM	Roll Back Malaria
RDTs	Rapid diagnostic tests
ROS	Reactive oxygen species
SCD	Sickle cell disease
SCT	Sickle cell trait
SERCA	Sarcoplasmic/endoplasmic reticulum calcium ATPase
SP	Sulfadoxine/pyrimethamine
TGA	Thermogravemetric analysis
THF	Tetrahydrofolate
WHO	World Health Organization