Synthesis and antimalarial activity screening of artemisinin-acridine hybrids

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ABSTRACT

Malaria endemic areas not only pose a public health threat, but affects 3.3 billion people worldwide. In 2011, estimated malaria related deaths amounted to 660 000 out of 219 million reported cases, with 81% of these and 91% of malaria related mortality occurred in the African region. Those most affected were pregnant women, children under the age of five and immuno-compromised individuals. Malaria is the fifth deadliest disease worldwide and accounts for the second highest death rate in Africa, following HIV/Aids.

To combat this parasitic infection of antiquity, the ideal malaria pharmacotherapy would be a cost effective and easily obtainable monotherapy. The malaria parasite, however, has an intrinsic ability to develop drug resistance through various mechanisms. Widespread resistance towards antimalarial drugs has rendered traditionally used drugs therapeutically ineffective, hence accentuating the efficacy of the artemisinins as first line treatment option for uncomplicated *Plasmodium falciparum* (*P. falciparum*). A devastating reality of the challenging battle against malaria is the confirmed prolonged parasitic clearance times of the artemisinins, despite adequate drug exposure, which emphasises the urgent need for identifying and developing new, effective and safe therapies.

During this study, 9-aminoacridines and artemisinin-acridine hybrids were successfully synthesised through nucleophillic substitution and their chemical structures confirmed by means of nuclear magnetic resonance spectroscopy (NMR), high resolution mass spectroscopy (HRMS) and infrared spectroscopy (IR). The hybrid compounds were synthesised through microwave assisted radiation, by covalently linking the artemisinin- and amino-functionalised acridine pharmacophores by means of a liable aminoethyl ether chain.

The target compounds were screened *in vitro* for antimalarial activity against both the chloroquine sensitive (NF54) and chloroquine resistant (Dd2) strains of *P. falciparum.* Their cytotoxicities were assessed against various mammalian cells of different origins, *viz.* the Chinese hamster ovarian cells (CHO) from animal origin, and from human origin, hepatocellular- (HepG2), neuroblastoma- (SH-SY5Y) and cervical cancer (HeLa) cells.

The synthesised hybrids exhibited antimalarial activity against both *Plasmodium* strains. Compound **7**, featuring an ethylenediamine moiety in the linker, was the most active hybrid, with 50% inhibitory concentration (IC_{50}) values of 2.6 nM and 35.3 nM against the NF54 and Dd2 strains, respectively. It had gametocytocidal activity against the NF54 strain, comparable to dihydroartemisinin (DHA) and artesunate (AS) and it is significantly more potent than

chloroquine (CQ), whilst possessing a resistance index value of 14, indicative of a significant loss of activity against the CQ resistant strain.

Contrary, the promising hybrid **10**, containing a 2-methylpiperazine linker, had gametocytocidal activity, comparable to CQ and was found to be six-fold more potent than CQ against the Dd2 strain, with a resistance index (RI) value of 2, whilst it further showed highly selective action towards the parasitic cells. Compound **10** was also found to possess anticancer activity against the HeLa cell line, comparable to DHA and AS, but fivefold higher than that of CQ, with the same levels of hepatotoxicity and neurotoxicity.

The artemisinin-acridine hybrids displayed superior antimalarial activity, compared to the derived 9-aminoacridines against both the *Plasmodium* strains. They, however, did not have the ability to overcome resistance, reduce the toxicity of acridine, nor induce synergistic activity. The hybrids, indeed displayed promising anticancer activity against HeLa cells. It is anticipated that these compounds may stand as drug candidates for further investigation in the search for new anti-cervical cancer drugs, rather than as antimalarials.

Keywords: Malaria, artemisinin, acridine, hybrids, *Plasmodium falciparum*, cytotoxicity

OPSOMMING

Malaria in endemiese gebiede bly nie net 'n openbare gesondheidskommer nie, maar raak 3.3 biljoen mense wêreldwyd. In 2011 het beraamde malaria-verwante sterftes 660 000 uit 219 miljoen aangemelde gevalle beloop, waarvan 81% van hierdie gevalle en 91% van die malaria verwante sterftes, in die Afrika-streek voorgekom. Diegene wat die meeste hierdeur geraak was, was swanger vroue, kinders onder die ouderdom van vyf jaar en immuunonderdrukte individue. Malaria is tans die vyfde dodelikste siekte wêreldwyd en is naas MIV/Vigs vir die tweede hoogste sterftesyfer in Afrika verantwoordelik.

Om hierdie parasitiese infeksie van ouds te bekamp, sou die ideale malaria-farmakoterapie 'n koste-effektiewe en maklik verkrygbare monoterapie moes wees. Die malaria-parasiet het egter die intrinsieke vermoë om weerstand teen medikasie deur verskeie meganismes te ontwikkel. Wydverspreide weerstand teen malaria-geneesmiddels het tradisioneel-voorgeskrewe medikasie terapeuties oneffektief gelaat, wat die effektiwiteit van artemisinien as eerste linie behandeling vir ongekompliseerde *Plasmodium falciparum* (*P. falciparum*) beklemtoon. Die ontstellende werklikheid van die uitdagende stryd teen malaria is die bevestigde verhoging van die parasiet se opruimingstye, ten spyte van genoegsame blootstelling aan artemisinin, wat dus die dringende noodsaaklikheid om nuwe, effektiewe en veilige behandelings te identifiseer, beklemtoon.

Tydens hierdie studie is 9-aminoakridiene en artemisinien-akridienhibriede suksesvol deur middel van nukleofiliese vervanging gesintetiseer en is hul onderskeie chemiese strukture deur middel van kernmagnetiese resonansspektroskopie (KMR), hoë resolusie massa-spektroskopie (HRMS) en infrarooi-spektroskopie (IR) bevestig. Die hibriedverbindings is deur middel van mikrogolfbestraling gesintetiseer, deur die artimisinin- en die 9-aminoakridiene-gefunksionaliseerde kovalent met behulp van ’n aminoetiel-eter-ketting te koppel.

Die teikenverbindings is *in vitro* vir malaria-aktiwiteit teen beide die chlorokiensensitiewe (NF54) en chlorokienweerstandbiedende (Dd2) stamme van *P. Falciparum* getoets. Hul sitotoksisiteit teen verskeie soogdierselle is ondersoek, naamlik Sjinese hamster ovaria-selle van dierlike oorsprong, en van menslike oorsprong, hepatosellulêre selle (HepG2), neuroblastomaselle (SH-SY5Y) en servikale kankerselle (HeLa). Die gesintetiseerde hibriedverbindings het teen-malaria aktiwiteit teen beide *Plasmodium*-stamme getoon.

Verbinding **7**, wat ’n etileendiamienstruktuur in die skakel bevat, was die mees aktiefste hibried met 50% inhiberende konsentrasie (IC_{50}) waardes van 2.6 nM en 35.3 nM teen die NF54 en

Dd2 stamme, onderskeidelik. Die verbinding het ook gametositositale aktiwiteit teen NF45 getoon, vergelykbaar met dihidroartemisinien (DHA) en artesunaat (AS) en was dit aansienlik sterker as chlorokien (CQ), terwyl dit 'n weerstandsindeks-waarde van 14 getoon het, wat aanduidend van 'n beduidende verlies in aktiwiteit teen die CQ weerstandige stam was.

Hierteenoor het die belowende hibriedverbinding **10**, met ‘n 2-metielpiperasien-skakel, gametositositale aktiwiteit, vergelykbaar met CQ getoon. Die verbinding is ook 6-maal sterker as CQ teen die Dd2 stam bevind, met ’n weerstandsindeks-waarde van 2, terwyl dit voorts hoogs selektiewe aktiwiteit teenoor die parasitiese selle getoon het. Daar was ook bevind dat verbinding **10** teen-kanker aktiwiteit teen die HeLa sellyn getoon het, vergelykbaar met DHA en AS, maar vyfkeer hoër as dié van CQ, met dieselfde vlakke van hepatotoksisiteit en neurotoksisiteit.

Die artemisinien-akridien hibriedverbindings het beter teen-malaria aktiwiteit as die 9-aminoakridiene teenoor beide die *Plasmodium*-stamme vertoon. Hulle het egter nie die vermoë besit om weerstand te oorkom nie, of om die toksisiteit van die akridien te verminder nie, of om sinergistiese aktiwiteit te weeg te bring nie. Die hibriedverbindings het egter belowende teen-kanker aktiwiteit teen HeLa selle vertoon. Daar word verwag dat hierdie verbindings dus as kandidaat-geneesmiddels mag staan vir verdere navorsing in die soeke na teen-servikale kankermiddels, eerder as teen-malaria-middels.

Kernwoorde: Malaria, artemisinien, akridien, hibriede, *Plasmodium falciparum*, sitotoksisiteit

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.

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CHAPTER 3: ARTICLE FOR SUBMISSION

LIST OF SCHEMES

LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
APCI	Atmospheric pressure chemical ionization
AS	Artesunate
BBB	Blood brain barrier
CDC	Centre for disease control
CHO	Chinese hamster ovarian
CQ	Chloroquine
CQR	Chloroquine resistant
CQS	Chloroquine sensitive
DCM	Dichloromethane
DHA	Dihydroartemisinin
DHF	Dihydrofolate
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
DMF	N,N-dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
ESI	Electrospray ionization
EtOAc	Ethyl acetate
HepG2	Human hepatocellular cells
HIV	Human immunodeficiency virus
HRMS	High resolution mass spectrometry
IR	Infrared
IRS	Indoor residual spraying
MeOH	Methanol
NADH	Nicotinamide adenine dinucleotide
NADPH	Adenine dinucleotide phosphate
NH_4OH	Ammonium hydroxide
NMR	Nuclear magnetic resonance
PABA	*p*-Aminobenzoic acid
PfATP6	*Plasmodium falciparum* chloroquine resistance transporter
PfHRP	*Plasmodium falciparum* histidine rich protein
PfMDR1	*Plasmodium falciparum* multidrug resistance 1
RDT’s	Rapid diagnostic tests

RI	Resistance index
ROS	Reactive oxygen species
SD	Standard deviation
SERCA	Sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase
SH-SY5Y	Human neuroblastoma cells
SR	Sarcoplasmic reticulum
TGA	Thermal gravimetric analysis
THF	Tethrahydrofuran
WHO	World Health Organisation
WRAIR	Walter Reed Army Institute of Research