Artemisinin-quinoline hybrids:

Design, synthesis and antimalarial activity

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Promoter: Prof. David D. N'Da

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This thesis is a sign of God's grace and mercy, to Him be the glory!

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ABSTRACT

Introduction

Malaria is a major global health problem, with more than 500 million reported cases and at least 1 million deaths each year. The main problem with malaria control is the emerging drug resistance. *Plasmodium falciparum* (*P. falciparum*) developed widespread resistance to antimalarial drugs such as chloroquine (CQ) and mefloquine, but not to the artemisinins. The World Health Organization (WHO) recommended artemisinin combination therapy (ACT) for the treatment of uncomplicated malaria in all chloroquine resistance areas. However, *P. falciparum* has recently started to display resistance to these ACTs, highlighting the need for new chemotherapeutic approaches for the treatment of *P. falciparum* infections.

Aims

The aims of this study were: (i) to design and synthesise a new series of antimalarial hybrid drugs, consisting of dihydroartemisinin (DHA) and aminoquinoline moieties bound covalently through different, very distinctive linkers; (ii) to determine the *in vitro* antiplasmodial activity and cytotoxicity of the synthesised series; (iii) to ascertain whether the *in vitro* antiplasmodial activity of the promising compounds would be carried over *in vivo* against *Plasmodium vinckei (P. vinckei)*; and, (iv) to obtain an indication of the pharmacokinetic properties of this class of antimalarial drugs by performing snapshot pharmacokinetic analysis.

Methods

DHA was coupled *via* an aminoethylether bond to various aminoquinolines to give hybrids and hybrid-dimers. CQ-susceptible (D10 and 3D7) and CQ-resistant (Dd2) strains of *P. falciparum* were used to determine the *in vitro* antiplasmodial activity. *In vitro* cytotoxicity was assessed using a mammalian cell-line (Chinese Hamster Ovarian, CHO). The antiproliferative activity of the hybrid-dimers was tested against three cell lines; renal adenocarcinoma (TK-10), breast adenocarcinoma (MCF-7) and melanoma (UACC-62). *P. vinckei*-infected mice were treated with

the hybrid drugs for four days at a dosage of 0.8 mg/kg, 2.5 mg/kg, 7.5 mg/kg or 15 mg/kg intraperitoneally (ip) or orally (po), with 2.7 mg/kg, 8.3 mg/kg, 25 mg/kg or 50 mg/kg, in order to determine their antimalarial activity. A snapshot oral and intravenous (IV) pharmacokinetic study was performed.

Results

All compounds were obtained as the 10-β-isomers and were isolated as the oxalate salts. Low nanomolar *in vitro* antiplasmodial activities were displayed by several compounds in this series, with IC_{50} values ranging from 5.15 to 29.5 nM, in comparison with the values of 2.09–5.11 nM and 21.54–157.90 nM for each of DHA and CQ respectively. All compounds displayed good selectivity towards *P. falciparum in vitro* (selectivity index (SI) ≥ 20). Two of the hybrids, featuring non-methylated and methylated two-carbon diaminoalkyl linkers, exerted potent *in vivo* antimalarial activities, with ED_{50} values of 1.1 and 1.4 mg/kg by ip route and 12 and 16 mg/kg po, respectively. Long-term monitoring of parasitaemia showed a complete cure of mice (without recrudescence) at 15 mg/kg ip and at 50 mg/kg po for these two hybrids, whereas artesunate was able to provide a complete cure only at 30 mg/kg ip and 80 mg/kg po.

Conclusions

These compounds may provide a lead into a new class of antimalarial drugs so badly needed for treatment of resistant strains. Despite shorter half-lives and moderate oral bioavailability in comparison with DHA, two of the compounds of this series were able to cure malaria in mice at very low dosages, implicating extremely active metabolites. The optimum linker length for antimalarial activity was found to be a diaminoalkyl linker consisting of two carbon atoms, either unmethylated or bearing a single methyl group.

Keywords

Malaria, artemisinin, quinoline, hybrid, pharmacokinetics, *in vitro* and *in vivo* activity.

ARTEMISINIEN-KINOLIEN HIBRIEDE:
Ontwerp, sintese en anti-malaria aktiwiteit

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OPSOMMING

Inleiding

Malaria is 'n massiewe wêreldwye gesondheidsprobleem, met meer as 500 miljoen aangemelde gevalle en ten minste 1 miljoen sterftes jaarliks. Die belangrikste probleem met die beheer van malaria is die verspreiding van geneesmiddelweerstandigheid. *Plasmodium falciparum* (*P. falciparum*) het reeds wydverspreide weerstand teen antimalariamiddels soos chlorokien (CQ) en meflokien ontwikkel, maar nog nie teen die artemisiniene nie. Die Wêreldgesondheidsorganisasie stel artemisinien-kombinasieterapie, vir die behandeling van ongekompliseerde malaria in alle CQ weerstandige areas, voor. *P. falciparum* het wel onlangs weerstand getoon teen artemisinien-kombinasieterapie, wat die dringende noodsaaklikheid vir nuwe chemoterapeutiese benaderings teen malaria beklemtoon.

Doelwitte

Die doelwitte van hierdie studie was: (i) om 'n nuwe reeks antimalaria-hibriedgeneesmiddels te ontwerp en te sintetiseer wat uit 'n dihidroartemisinien- (DHA) en aminokinoliengedeelte bestaan, wat kovalent deur verskillende bindingsgroepe gebind is; (ii) om die *in vitro* antiplasmodiese-aktiwiteit en sitotoksisiteit van die gesintetiseerde reeks te bepaal; (iii) om vas te stel of die *in vitro* antiplasmodiese aktiwiteit van die mees belowende verbindings ook *in vivo* teen *Plasmodium vinckei (P. vinckei)* sou geld, en (iv) om 'n aanduiding te kry van die farmakokinetiese eienskappe van hierdie klas antimalariamiddels deur die uitvoering van 'n beperkte farmakokinetiese analise.

Metodes

DHA is *via* 'n amino-etieleter binding aan verskillende aminokinoliene gekoppel om hibriede en hibried-dimere te vorm. CQ-sensitiewe (D10 en 3D7) en CQ-weerstandige (Dd2) rasse van *P. falciparum* is gebruik om die *in vitro* antiplasmodiese aktiwiteit te bepaal. *In vitro* sitotoksisiteit is bepaal deur 'n soogdiersellyn (Chinese Hamster Ovaria, CHO) te gebruik. Die antiproliferatiewe aktiwiteit van die hibried-dimere is teen drie sellyne getoets nl.; renale adenokarsinoom (TK-10),

borsadenokarsinoom (MCF-7) en melanoom (UACC-62). *P. vinckei* geïnfekteerde muise is vir vier dae met die hibriede behandel met dosisse van 0.8 mg/kg, 2.5 mg/kg, 7.5 mg/kg en 15 mg/kg intraperitoneaal (ip), of oraal (po) met 2.7 mg/kg, 8.3 mg/kg, 25 mg/kg en 50 mg/kg, om die antimalaria-aktiwiteit daarvan te bepaal. 'n Beperkte farmakokinetiese studie is oraal en intraveneus uitgevoer.

Resultate

Alle verbindings is as die 10-β-isomere verkry en is as die oksalaatsoute geïsoleer. Verskeie verbindings in hierdie reeks het lae nanomolêre, *in vitro,* antiplasmodiese aktiwiteite getoon, met IC_{50}-waardes van 5.15 tot 29.5 nM, in vergelyking met 2.09–5.11 nM en 21.54–157.90 nM vir DHA en CQ onderskeidelik. Alle verbindings het baie goeie *in vitro* selektiwiteit teenoor *P. falciparum* getoon (selektiwiteitsindeks (SI) ≥ 20). Twee van die hibriede, wat 'n diaminoalkielbindingsgroep, bestaande uit twee koolstofatome wat gemetileerd of nie-gemetileerd is nie, bevat het, het 'n baie hoë *in vivo* antimalaria-aktiwiteit getoon met ED_{50}-waardes van 1.1 en 1.4 mg/kg ip en 12 en 16 mg/kg po, onderskeidelik. Langtermynmonitering van die parasitemie dui op 'n volledige herstel van die muise (sonder enige toename van die parasiet) teen 15 mg/kg ip en 50 mg/kg po, vir hierdie twee hibriede. Hierteenoor kon artesunaat slegs teen 30 mg/kg ip en 80 mg/kg po volledige herstel bewerkstellig.

Gevolgtrekkings

Hierdie verbindings het die potensiaal om 'n nuwe klas antimalariamiddels daar te stel, wat tans so dringend benodig word. Ten spyte van hul korter halfleeftye en beperkte orale beskikbaarheid, vergeleke met DHA, was twee van die verbindings in hierdie reeks daartoe in staat om malaria by baie lae dosisse te genees, wat dui op besonderse aktiewe metaboliete. Die optimale bindingslengte vir antimalaria-aktiwiteit was 'n diaminoalkielgroep wat uit twee koolstofatome bestaan, wat of gemetileerd was, of nie.

Sleutelwoorde

Malaria, artemisinien, kinolien, hibried, farmakokinetika, *in vitro* en *in vivo* aktiwiteit.

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. Five published articles are included in this thesis, which were still written under my maiden name Lombard:

I. **Lombard, M.C.**, Fernandes, M.A., Breytenbach, J.C., N'Da, D.D. 2010, "1-Bromo-2-(10b-dihydroartemisinoxy)-ethane", *Acta Crystallographica,* Section E: E66, pp. 2182-2183.

II. **Lombard, M.C.**, N'Da, D.D., Breytenbach, J.C., Smith, P.J., Lategan, C.A. 2010, "Artemisinin–quinoline hybrid-dimers: Synthesis and *in vitro* antiplasmodial activity", *Bioorganic & Medicinal Chemistry Letters*, vol. 20, pp. 6975-6977.

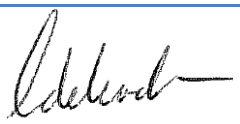
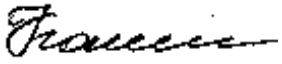
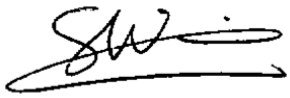
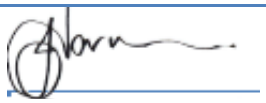
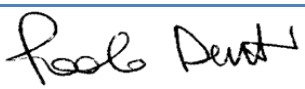
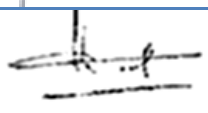
III. **Lombard, M.C.**, N'Da, D.D., Breytenbach, J.C., Smith, P.J., Lategan, C.A. 2011, "Synthesis, *in vitro* antimalarial and cytotoxicity of artemisinin-aminoquinoline hybrids", *Bioorganic & Medicinal Chemistry Letters*, vol. 21, pp. 1683-1686.

IV. **Lombard, M.C.**, N'Da, D.D., Breytenbach, J.C., Kolesnikova, N.I., Tran Van Ba, C., Wein, S., Norman, J., Denti, P., Vial, H., Wiesner, L. 2012, "Antimalarial and anticancer activities of artemisinin-quinoline hybrid-dimers and pharmacokinetic properties in mice", *European Journal of Pharmaceutical Sciences*, vol. 47, pp. 834-841.

V. **Lombard, M.C.**, N'Da, D.D., Tran Van Ba, C., Wein, S., Norman, J., Wiesner, L., Vial, H. 2013, "Potent *in vivo* antimalarial activity and representative snapshot pharmacokinetic evaluation of artemisinin-quinoline hybrids", *Malaria Journal*, vol. 12:71

The contributions by the co-authors and consent from all the co-authors to submit the articles for degree purposes are given in the table below. Permission was granted on behalf of the International Union of Crystallography (IUCr) to include Article I as part of this thesis. Articles II–IV and V were published by Elsevier and Malaria Journal, respectively, which grant the author the right to include the article(s) in a thesis. Proof thereof is given in Annexure A.

Table 1: Contributions and consent of all the co-authors

Author	Contributions	Consent[#]
Marli C. Vlok	Responsible for the planning, design and collaborations of the study. Carried out the synthetic procedures. Wrote all five articles as first author.	
David D. N'Da	As promoter he planned and designed the study. He assisted in all aspects of carrying it out.	
Jaco C. Breytenbach	Contributed to the design of the study. Gave a critical review of articles I – IV.	
Manual A. Fernandes	Conducted the X-ray crystallography procedures for article I. Contributed greatly towards the article, especially in terms of technical guidance.	
Carmen A. Lategan	Conducted *in vitro* antiplasmodial experiments in articles II and III, analysed the data and critically reviewed the articles.	
Peter J. Smith	Oversaw the antiplasmodial procedures in article II and III.	
Natasha I. Kolesnikova	Conducted the anticancer experiments and critically reviewed article IV.	
Christophe Tran Van Ba	Conducted the antimalarial experiments of article IV and V and critically reviewed the articles.	
Sharon Wein	Conducted the antimalarial experiments of article IV and V.	
Jennifer Norman	Analysed the pharmacokinetic data of articles IV and V and critically reviewed the articles.	
Paolo Denti	Gave a critical review of the statistical analysis of the pharmacokinetic data in manuscript IV.	
Lubbe Wiesner	Designed and conducted pharmacokinetic experiments. Gave a critical review of manuscripts IV and V.	
Henri Vial	Designed and conducted *in vivo* antimalarial experiments. Gave a critical review of manuscripts IV and V.	

[#] I declare that I have approved the article(s) and that my role in the study was as indicated above. I hereby give my consent that the article(s) may be published as part of the thesis of Marli C. Vlok.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACT	Artemisinin Combination Therapy
AUC	Area Under Curve
CHO	Chinese Hamster Ovarian
CL	Clearance
C_{max}	Maximum Concentration
CQ	Chloroquine
CYP	Cytochrome P450
DHA	Dihydroartemisinin
ED_{50}	Effective Dose at 50%
ED_{90}	Effective Dose at 90%
GI_{50}	Growth Inhibition at 50%
HR	High Resolution
IC_{50}	Inhibition Concentration at 50%
ip	Intraperitoneal
IV	Intravenous
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LLOQ	Lower Limit of Quantification
MMV	Medicines for Malaria Venture
MS	Mass Spectrometry
NCI	National Cancer Institute
NMR	Nuclear Magnetic Resonance
P. berghei	*Plasmodium berghei*
P. falciparum	*Plasmodium falciparum*
PK	Pharmacokinetic
po	Per os (by mouth, oral)

P. vinckei	*Plasmodium vinckei*
RBC	Red Blood Cells
ROS	Reactive Oxygen Species
sc	Subcutaneous
SD	Standard Deviation
SI	Selectivity Index
TCM	Traditional Chinese Medicine
TGI	Total Growth Inhibition
T_{max}	Maximum Time
$T_{½}$	Half Life
UGT	UDP Glucuronosyl Transferases
V	Volume of Distribution
WHO	World Health Organization