# Synthesis and antimalarial activity of amine derivatives of artemisinin

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Thesis submitted in fulfilment of the requirements for the degree

***Philosophiae Doctor***

in the

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Supervisor: Prof. D.D. N'Da

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# Declaration

This thesis is submitted in fulfilment of the requirements for the degree of the Philosophiae Doctor in Pharmaceutical Chemistry, at the School of Pharmacy, North-West University.

I, Theunis Theodorus Cloete, hereby declare that the dissertation with the title:

**Synthesis and antimalarial activity of amine derivatives of artemisinin**

is my own work and has not been submitted at any other University either in whole or in part.

Signed at Potchefstroom on the 30th day of November, 2012.

Theunis Theodorus Cloete

November, 2012

# 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. Four articles, two of which have been published, are included in this thesis:

**Chapter 3- Article 1:**

CLOETE, T.T., BREYTENBACH, J.W., DE KOCK, C., SMITH, P.J., BREYTENBACH, J.C., N'DA, D.D. 2012. Synthesis, antimalarial activity and cytotoxicity of 10-aminoethylether derivatives of artemisinin. *Bioorganic & medicinal chemistry*, 20(15):4701–4709, Aug.

**Chapter 4- Article 2:**

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**Chapter 5- Article 3:**

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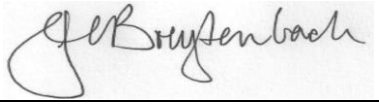
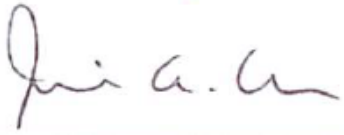
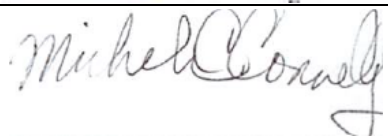
**Chapter 6- Article 4:**

CLOETE, T.T., CLARK, J.A., CONNELLY, M.C., MATHENY, A., SIGAL, M.S., GUY, R.K., N'DA, D.D. 2012. Synthesis, antimalarial activity and cytotoxicity of dimeric artemisinin triazine hybrids. *Bioorganic & medicinal chemistry letters*, To be submitted.

The contributions and consent to submit the articles for degree purposes from all the co-authors are given in the table below. Both articles 1 and 2 were published by Elsevier, which grants the author the right to include the article(s) in a thesis. Permission from Elsevier, as per the website: http://authors.elsevier.com/definitions.html?journal_name= Remote%20 Sensing%20of%20Environment&lang=English&dc=OCTDEF

# Table of consent

| Author | Contribution | Consent |
|---|---|---|
| Theunis T. Cloete | Responsible for the planning and design of the study. Carried out the synthetic procedures. Wrote all four articles as first author. |  |
| David D. N'Da | As promoter he assisted in all aspects of the study. | |
| Jaco C. Breytenbach | Co-author of article 1. Gave critical reviews of articles 2-4. |  |
| J. Wilma Breytenbach | Co-author of article 1. Planned and carried out statistical analysis of data. Assisted with the interpretation of data. |  |
| Peter J. Smith | Oversaw the antiplasmodial procedures in article 1. Gave critical reviews of article 1. |  |
| Carmen de Kock | Conducted *in vitro* antiplasmodial experiments in article 1 and analysed the data. |  |
| R. Kiplin Guy | Oversaw the antiplasmodial procedures in articles 2-4. Gave critical reviews of articles 2-4. |  |
| Julie A. Clark | Conducted *in vitro* antiplasmodial experiments in articles 2-4 and analysed the data. |  |
| Martina S. Sigal | Conducted *in vitro* antiplasmodial experiments in articles 2-4 and analysed the data. |  |
| Amy Matheny | Conducted *in vitro* antiplasmodial experiments in articles 2-4 and analysed the data. |  |
| Michele C. Connelly | Conducted *in vitro* antiplasmodial experiments in articles 2-4 and analysed the data. |  |
| Henk J. Krebs | Designed and synthesised compounds **11**-**18** of article 2. |  24/11/2012 |

[#] 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 Theunis Theodorus Cloete.

Dedicated to my wife, Anke Cloete.

# Abstract

Malaria has since antiquity been a leading cause of morbidity and mortality throughout the world having serious health, sociological and financial implications. Today, the parasite kills an approximate 655 000 people annually, with most deaths being amongst pregnant women and children under the age of 5 in Africa. *Plasmodium falciparum* is the species that accounts for 91% of all case fatalities and predominates in Africa and Asia. The parasite is aggressive in its means of acquiring resistance, nullifying the majority of drugs used against it.

Artemisinin, a sesquiterpene lactone with a peroxide bridge, was discovered in 1971 and was found to possess remarkable antimalarial activity. Together with its semi synthetic derivatives, artemisinin not only lack cross-resistance with other antimalarials but also has the remarkable ability to induce a 10 000 fold reduction in parasitemia.

The artemisinin class of compounds is currently the basis of treatment favoured by the World Health Organisation (WHO) for the treatment of uncomplicated *P. falciparum* infection. Regrettably, resistance has started to emerge even against this class of compounds, characterised by a significantly longer *in vivo* parasite clearance time.

The global impact of this disease and its ability to circumvent most efforts to counter it justifies the search for new treatment methods and drugs.

The aim of this study was to synthesise three series of artemisinin-amine derivatives, to evaluate their antimalarial activity against both sensitive and resistant strains of *P. falciparum* and to determine their toxicity against mammalian cells. This may lead to new compounds with favourable properties and increased activity which can be used in the fight against malaria.

Chapter 3 describes the synthesis of eleven 10-aminoethylether derivatives of artemisinin, confirmation of their structures by physical means and the determination of their *in vitro* antimalarial activity against the chloroquine sensitive (D10) and resistant (Dd2) strains of *P. falciparum* as well as their toxicity against Chinese hamster ovarian (CHO) cells. All derivatives were active against both strains of the parasite, with no mentionable toxicity. The highest activity was displayed by compound **8**, a short chain aromatic derivative containing only one nitrogen atom, which was found to have comparable activity to artesunate (AS).

Long chain polyamine derivatives had the lowest activity against both strains. An interesting correlation between the $IC_{50}$, $pK_a$ values and resistance index (RI) was found.

Chapter 4 compares the same 10-aminoethylether derivatives of artemisinin discussed in chapter 3 with eight 10-*n*-alkyl/aryl/aroyl ester derivatives previously synthesised in our group. The *in vitro* antimalarial activity of these nineteen compounds was determined against both the chloroquine sensitive (3D7) and resistant (K1) strains of *P. falciparum*, whilst their cytotoxicity was determined against both human embryonic kidney cells (HEK 293) and hepatocellular carcinoma cells (Hep G2). Both series of compounds showed activity versus the 3D7 and K1 strains, with the majority of compounds possessing potency either comparable with or higher than that of AS. None of the synthesised derivatives had any mentionable toxicity against the mammalian cells. The 10$\alpha$-*n*-propyl and 10$\alpha$-benzyl ester derivatives, **11** and **18** respectively, were the most active compounds against both strains, whilst the other ester derivatives also showed a slightly higher degree of activity than the aminoethers. Compound **29**, featuring an isobutylamine substituent, was the most active of all aminoethers.

Chapter 5 entails the synthesis of seven artemisinin-triazine hybrids, confirmation of their structures and the determination of their *in vitro* antimalarial activity against the 3D7 and K1 strains of *P. falciparum*, whilst their cytotoxicity was determined against HEK 293, Hep G2, B-lymphocyte cells (Raji) and human fibroblast cells (BJ). The synthesised hybrids all showed activity against both strains and were found to be non-toxic to all mammalian cells. Compound **17**, featuring *p*-anisidine and 2-(diisopropylamino)ethylamine substituents on the triazine ring, was the most active of all synthesised compounds and had comparable activity to that of AS and artemether (AM), while being significantly more potent than chloroquine (CQ).

Chapter 6 describes the synthesis and structure determination of six dimeric artemisinin triazine hybrids and the determination of their antimalarial activity and toxicity against the same strains of *P. falciparum* and mammalian cells as in chapter 5. All dimers showed activity against both strains and no noticeable toxicity towards any of the mammalian cell lines used. All synthesised compounds showed higher activity than CQ, irrespective of the *P. falciparum* strain considered. Compound **15**, featuring aniline and morpholine substituents on the triazine ring, was not only meaningfully more potent than CQ but was also found to possess activity comparable to those of AS and AM against both malaria strains. Compounds **10**, **11**, **12** and **13** all had corresponding monomer equivalents as have been reported in chapter 5. Against both strains of *P. falciparum*, most dimer compounds were slightly more active than their monomer counterparts.

This study delivered a number of compounds that exhibited activity comparable to that of potent antimalarial drugs currently on the market, and showed that there is ample scope for developments in the chemotherapy of malaria. These compounds stand as good candidates for *in vivo* and pharmacokinetic studies and may serve as leads for further investigations.

# Opsomming

Sedert die vroegste tye is malaria 'n groot oorsaak van siektes en sterftes regoor die wêreld, met ernstige gesondheidsprobleme en sosiologiese en finansiële implikasies. Die parasiet veroorsaak tans jaarliks 'n geskatte 655 000 sterftes waarvan die meeste swanger vroue en kinders onder die ouderdom van 5 jaar in Afrika is. *Plasmodium falciparum* is die spesie wat vir 91% van alle sterfgevalle verantwoordelik is en is die belangrikste een in Afrika en Asië. Die parasiet bou op ’n aggressiewe wyse weerstand teen geneesmiddels op en maak die meeste daarvan sodoende nutteloos.

Artemisinien, 'n seskwiterpeenlaktoon met 'n peroksiedbrug, is in 1971 ontdek en besit merkwaardige aktiwiteit teen malaria. Soos sy semi-sintetiese derivate, toon artemisinien nie net geen kruisweerstand met ander antimalariamiddels nie, maar het ook die merkwaardige vermoë om 'n 10 000-voudige verlaging in parasitemie teweeg te bring.

Die artemisinienklas van verbindings is tans die basis van middels wat deur die Wêreld-gesondheidsorganisasie (WGO) vir die behandeling van ongekompliseerde infeksie deur *P. falciparum* verkies word. Ongelukkig het weerstand selfs teen hierdie klas van verbindings begin verskyn, wat gekenmerk word deur 'n aansienlik langer tyd vir die *in vivo*-opklaring van die parasiet.

Die wêreldwye impak van hierdie siekte en sy vermoë om die meeste pogings om dit teen te werk te omseil, regverdig die soeke na nuwe metodes en middels vir behandeling.

Die doel van hierdie studie was om drie reekse amienderivate van artemisinin te sintetiseer, hulle aktiwiteit teen beide sensitiewe en weerstandbiedende stamme van *P. falciparum* te evalueer en hulle toksisiteit teen soogdierselle te bepaal. Dit kan tot nuwe verbindings met gunstige eienskappe en beter aktiwiteit lei wat in die stryd teen malaria gebruik kan word.

Hoofstuk 3 beskryf die sintese van elf 10-aminoetieleterderivate van artemisinien, bevestiging van die strukture daarvan met fisiese metodes en die bepaling van hulle *in vitro*-aktiwiteit teen die chlorokiensensitiewe (D10) en -weerstandige (Dd2) stamme van *P. falciparum*, sowel as hul toksisiteit teen ovariumselle van die Chinese hamster (CHO). Alle derivate was aktief teen beide stamme van die parasiet, met geen noemenswaardige toksisiteit nie. Verbinding **8**, 'n aromatiese derivaat met ’n kort ketting en slegs een stikstofatoom, het die hoogste aktiwiteit vertoon, wat vergelykbaar met dié van artesunaat

(AS) is. Poliamienderivate met lang kettings het die laagste aktiwiteit teen albei stamme getoon. 'n Interessante korrelasie tussen die $IC_{50}$, $pK_a$-waardes en weerstandsindeks (WI) is gevind.

Hoofstuk 4 vergelyk dieselfde 10-aminoetieleterderivate van artemisinin van hoofstuk 3 met agt 10-*n*-alkiel/arielesterderivate wat voorheen in ons groep gesintetiseer is. Die *in vitro*-aktiwiteit van hierdie negentien verbindings is teen beide die chlorokiensensitiewe (3D7) en weerstandige (K1) stamme van *P. falciparum* bepaal, terwyl hulle sitotoksisiteit teen menslike embrioniese nierselle (HEK 293) en lewerkarsinoomselle (HepG2) bepaal is. Albei reekse verbindings toon aktiwiteit teenoor die 3D7- en K1-stamme en die meeste het ’n werking óf vergelykbaar met óf hoër as dié van AS. Geeneen van die gesintetiseerde derivate het enige noemenswaardige toksisiteit teen die soogdierselle nie. Die 10$\alpha$-*n*-propiel- en 10$\alpha$-bensielesters, **11** en **18** onderskeidelik, was die mees aktiewe verbindings teen albei stamme, terwyl die ander esters ook 'n effens hoër mate van aktiwiteit as die aminoeters het. Verbinding **29**, met 'n isobutielamiensubstituent, was die mees aktiewe van alle aminoeters.

Hoofstuk 5 behels die sintese van sewe artemisinien-triasien hibriede, bevestiging van hulle strukture en die bepaling van die *in vitro*-aktiwiteit teen die 3D7- en K1-stamme van *P. falciparum*, terwyl hulle sitotoksisiteit teen HEK 293, Hep G2, B-limfosietselle (Raji) en menslike fibroblastselle (BJ) bepaal is. Die gesintetiseerde hibriede toon almal aktiwiteit teen beide stamme en is nie toksies teenoor die soogdierselle nie. Verbinding **17**, met 'n *p*-anisidien- en ’n 2-(diisopropielamino)etielamiensubstituent op die triasienring, was die mees aktiewe van alle gesintetiseerde verbindings en het aktiwiteit soortgelyk aan dié van AS en artemeter (AM), terwyl dit beduidend meer potent as chlorokien (CQ) is.

Hoofstuk 6 beskryf die sintese en struktuurbepaling van ses dimeriese artemisinien-triasien hibriede en die bepaling van hulle aktiwiteit en toksisiteit teen dieselfde stamme van *P. falciparum* en soogdierselle as in hoofstuk 5. Alle dimere toon aktiwiteit teen albei stamme en geen waarneembare toksisiteit teenoor enige van die gebruikte soogdiersellyne nie. Al die gesintetiseerde verbindings het hoër aktiwiteit as CQ, ongeag van die betrokke stam van *P. falciparum*. Verbinding **15**, met ’n anilien- en ’n morfoliensubstituent op die triasienring, was nie net betekenisvol meer potent as CQ nie, maar het ook aktiwiteit wat vergelykbaar is met dié van AS en AM teen beide malariastamme. Verbindings **10**, **11**, **12** en **13** het elk 'n ooreenstemmende monomeer soos in hoofstuk 5 gerapporteer is. Teen beide stamme van *P. falciparum* was die meeste dimere effens meer aktief as hulle ekwivalente monomere.

Hierdie studie het 'n aantal verbindings gelewer met aktiwiteit soortgelyk aan dié van kragtige malariamiddels wat tans op die mark is, en het getoon dat daar ruimte vir die ontwikkeling in die chemoterapie van malaria is. Hierdie verbindings is goeie kandidate vir *in vivo*- en farmakokinetiese studies en kan as leidrade vir verdere navorsing dien.

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Lief jou!

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## Article 1



## Article 2



## Article 3

# Table of tables and schemes

# Abbreviations

| | |
|---|---|
| ACT | Artemisinin-based combination therapy |
| AE | Arteether |
| AM | Artemether |
| AQ | Amodiaquine |
| ARDS | Acute respiratory distress syndrome |
| ARF | Acute renal failure |
| ART | Artemisinin |
| AS | Artesunate |
| CDC | Centre for disease control |
| CGN | Cycloguanil |
| CHO | Chinese hamster ovarian |
| CQ | Chloroquine |
| CyCl | Cyanuric chloride |
| DCM | Dichloromethane |
| DHA | Dihydroartemisinin |
| DHF | Dihydrofolate |
| DHFR | Dihydrofolate reductase |
| DMF | N,N-dimethylformamide |
| DMSO | Dimethyl sulfoxide |
| DVS | Dominant vector species |
| EDA | Ethylenediamine |
| EPO | Erythropoietin |
| EtOAc | Ethyl acetate |
| HF | Halofantrine |
| MDR | Multidrug resistant |
| MeOH | Methanol |
| MQ | Mefloquine |
| NADPH | Adenine dinucleotide phosphate |
| NAI | Naturally acquired immunity |
| $NH_4OH$ | Ammonium hydroxide |
| PABA | *p*-Aminobenzoic acid |
| PC | Phosphatidylcholine |

PE ..... Petroleum ether
PGN ..... Proguanil
PL ..... Phospholipid
PQ ..... Primaquine
PYR ..... Pyrimethamine
QN ..... Quinine
RBC ..... Red blood cell
RBM ..... Roll back malaria initiative
RH ..... Relative humidity
RI ..... Resistance index
ROS ..... Reactive oxygen species
SDX ..... Sulfadoxine
SI ..... Selectivity index
SP ..... Sulfadoxine and pyrimethamine
STP ..... Staurosporine
THF ..... Tetrahydrofuran
WHO ..... World Health Organization