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Gold compounds with anti-HIV and immunomodulatory activity

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***Philosophiae* Doctor Biochemistry**

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University of Pretoria

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SUBMISSION DECLARATION

I Pascaline N. Fonteh hereby declare that this thesis which is herewith submitted to the Faculty of Natural and Agricultural Sciences for the degree of *Philosophiae* doctor Biochemistry at the University of Pretoria is my own work and has not been previously submitted for a degree at this or any other tertiary institution.

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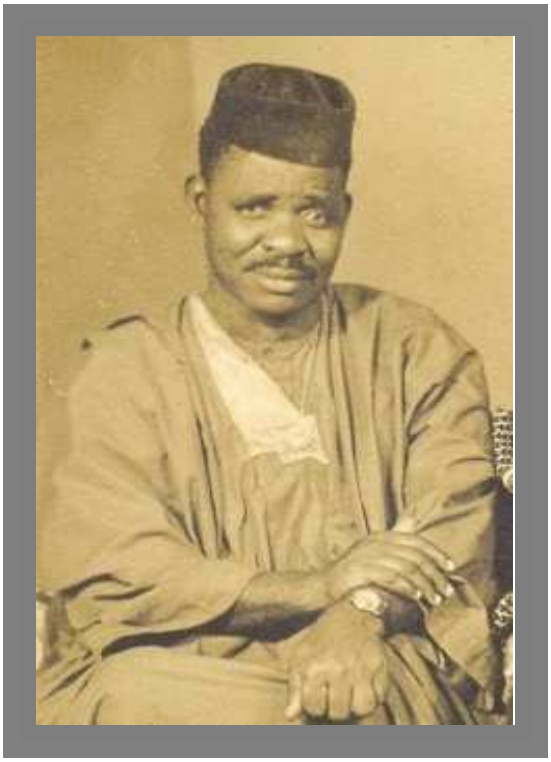
DEDICATION

To my **GOD** and **LORD ALMIGHTY** for giving me the strength, supreme guidance, the means and the understanding to have pursued this project to this point.

To my late dad **Papa Lucas Che Fonteh** (1927 - Jan 1981); for your believe in the education of your children and for being our “Fo-nteh” or main rock. It’s been 30 years since you left us to be with the LORD and despite the fact that I vaguely remember you, the zeal to keep your legacy alive still burns in me and I always wish I had known you better. RIP daddy.



Papa Lucas Che Fonteh (1927 - 1981)

And

To my late niece/sister/champion/adviser **Isabella A. Ade-Tamungang** (Nov. 1974 - Feb 2011): your zeal for success and passion in everything you did made you my role model. I know your next project was pursuing a Ph.D, by dedicating this to you, I hope it somehow fulfils that plan you had on earth. I will forever miss you. Words can’t express how hurt I was finding out that your death could have been avoided. Only the Good LORD knows why and I continue to praise HIM even in my pain. RIP Issa.

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Pascaline Fonteh

PREFACE

Portions of this thesis have been published in peer reviewed journals, and presented at both local and international conferences while other sections are under preparation for submission for peer review.

Publications

- Fonteh P., Keter K., Meyer D. (2010). HIV therapeutic possibilities of gold compounds. *Biometals*, 23; 185-196. **Review paper.**
- Fonteh P, Keter K, Meyer D., (2011). New bis(thiosemicarbazonate) gold(III) complexes inhibit HIV replication at cytostatic concentrations: potential for incorporation into virostatic cocktails. *Journal of Inorganic Biochemistry*, 105; 1173-1180. **Original Paper**.

NB: Please see copies of the review and original paper at the end of this thesis.

- Fonteh P. and Meyer D. (2011). The inhibition of HIV-1 infectivity of TZM-bl cells by gold(I) phosphine compounds is related to cytostasis, In preparation.

Awards

The following are awards received by Fonteh P. in the course of this study:

- Travel award by the World Gold Council to attend the Gold2009 Conference in Heidelberg, Germany (April 2009).
- L'ORÉAL/UNESCO fellowship award for Women in Science in Sub-Saharan Africa in March 2010 (awarded on the 30th of March 2010, in Johannesburg, South Africa)
- Scholarship award (conference registration) by the South African AIDS organising committee (April 2011) sponsored by SIDA, CDC and the Bill and Melinda gates Foundation to attend the 5th SA AIDS conference, Durban, South Africa, 7th-10th of June 2011.
- Travel support from NIH through the Infectious Disease Society of America (IDSA) and HIV Medical Association to attend the 49th IDSA meeting in Boston, MA, USA (20th-23rd October 2011).
- WhiteScience Travel support (16th August 2011) to attend the IDSA meeting in Boston, MA, USA.

Conferences

- Fonteh P. and Meyer D. 2009. Poster presentation entitled: Chrysotherapy: evaluating the anti-HIV activity of novel gold(I) compounds. Gold2009 Conference (26th-29th July 2009, Heidelberg, Germany).
- Fonteh P, 2010. Overview of research project on gold compounds as anti-HIV agents/Challenges of being a female scientist. Network of UNESCO Chairs "Women, Science and Technology" conference (20th-30th March 2010, Johannesburg, South Africa).
- Fonteh P., Meyer D., 2010. Poster presentation titled: Cytostasis: a novel anti-HIV mechanism of gold(III) bisthiosemicarbazonate compounds to remedy drug resistance. The 5th SA AIDS Conference (7th-10th June 2011, Durban, South Africa).
- Fonteh P. 2011. Experiences as a L'ORÉAL/UNESCO fellow: impact on the realisation of the PhD project. Workshop on the promotion of women in science in Africa (29th-30th June 2011).
- Fonteh P., Meyer D. Poster presentation entitled: The inhibition of HIV-1 infectivity of TZM-bl cells by gold(I) phosphine compounds is related to cytostasis. The 49th IDSA meeting, Boston, MA, USA (20th-23rd October 2011).

SUMMARY

GOLD COMPOUNDS WITH ANTI-HIV AND IMMUNOMODULATORY ACTIVITY
by
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Supervisor: **Prof. Debra Meyer**

Department: **Biochemistry**

Degree: **Ph.D Biochemistry**

The human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) that subsequently develops remain major health concerns even after three decades since the first cases were reported. Successful therapeutic measures to address HIV/AIDS consist mostly of combinations of drugs targeting viral enzymes including reverse transcriptase (RT), protease (PR) and integrase (IN) as well as entry steps of the viral life cycle. The remarkable benefits (e.g. improved quality of life) derived from the use of these agents are unfortunately limited by toxicity to the host and the development of drug resistant viral strains. Drug resistance limits the repertoire of drug combinations available. Unfortunately, because latent forms of the virus exists, therapy has to be life-long and with new infections occurring every day, resistant strains tend to spread. To circumvent these problems, new drugs that inhibit resistant strains or work against new viral targets have to be developed. The history of gold compounds as potential inhibitors of HIV prompted this study in which twenty seven compounds consisting of gold(I), gold(III) and precursors from five classes were tested for drug-likeness, anti-HIV and immunomodulatory effects using wet lab and *in silico* methodologies. Cytotoxicity determination was done using viability dyes and flow cytometry. Cell proliferation profiles were monitored using the carboxyflourescein succinimidyl ester dye dilution technology and a real time cell analyser for confirming viability dye findings. The compounds' effects on viral enzymes was determined using direct enzyme assays and *in silico* molecular modelling techniques. ^{1}H and ^{31}P nuclear magnetic resonance spectroscopy studies for determining stability revealed that the backbone chemical shifts of the compounds were relatively unchanged after one week (-20 and 37 ºC) when dissolved in dimethylsulfoxide. Eight of the gold compounds had drug-like properties comparable to clinically available drugs when *in silico* predictions were performed. The 50% cytotoxic dose of the compounds in human cells was between 1 and 20 µM (clinically relevant concentrations for gold compounds). Three gold(I) compounds inhibited viral infectivity at non-toxic concentrations and two gold(III) compounds did so at cytostatic (anti-proliferative mechanism that is also anti-viral) concentrations. In the immunomodulatory assay, cytokine levels were altered by five compounds with one gold(I) and a gold(III) compound significantly reducing the frequency of CD4+ cells (an anti-viral function) from HIV+ donors ($p= 0.005$ and 0.027 respectively) when multi-parametric flow cytometry was performed. Inhibition of RT activity was predicted in *in silico* studies to be through interactions with the ribonuclease (RNase) H site although with poor stereochemical orientation while favourable binding predictions with the IN cofactor binding site were observed for some gold(III) complexes. Compounds predicted to interact with the RNase H site of RT and the IN cofactor site require structural modification to improve drug-likeness and binding affinity. The drug-like compound(s) which inhibited viral infectivity and lowered CD4+ cell frequency have potential for incorporation into virostatic cocktails (combination of cytostatic and directly anti-viral agent). Cytostatic agents are known to be less prone to drug resistance and because they lower CD4+ cell frequency, such compounds can potentially limit HIV immune activation.

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LIST OF IMPORTANT ABBREVIATIONS

3’	3 prime
5’	5 prime
3’P	3 prime Processing
5-CITEP	1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)-propenone
Å	Armstrong
ADMET	Adsorption, Distribution, Metabolism, Excretion, Toxicity
AIDS	Acquired Immune Deficiency Syndrome
AlogP	Atom-based logarithm of Partition coefficient
ART	Antiretroviral Therapy
ARV	Antiretroviral
BBB	Blood Brain Barrier
BPH	Bis(Phosphino) Hydrazine
CC_{50}	50% Cytotoxic Concentration
CCD	Catalytic Core Domain
CCR5	Chemokine Receptor 5
CD4	Cluster of Differentiation 4
CD8	Cluster of Differentiation 8
cDNA	Complementary Deoxyribonucleic Acid
CDOCKER	CHARMm-based Docker
CFSE	CarboxyFlourescein Succinimidyl Ester
CHARMm	Chemistry at Harvard Macromolecular Mechanics
CI	Cell Index
CTLs	Cytotoxic T Lymphocytes
CXCR4	CXC chemokine receptor 4
CYP	Cytochrome P450
d6-DMSO	Deuterated Dimethysulfoxide
DMEM	Dulbecco’s Modified Essential Medium
DMSO	Dimethylsulfoxide
dNTPs	Deoxynucleotide Triphosphates
DS	Discovery Studio
ELISA	Enzyme Linked ImmunoSorbent Assay
FACS	Fluorescence-Activated Cell Sorter
FCS	Fetal Calf Serum
FITC	Flourescein Isothiocyanate
FMO	Fluorescent Minus One
GS	Gentamycine Sulphate
HAART	Highly Active Antiretroviral Therapy
H-bond	Hydrogen bond
HIA	Human Intestinal Absorption
HIV	Human Immunodeficiency Virus
HTS	High Throughput Screening
HU	Hydroxyurea
hu-PBL-SCID	human-Peripheral Blood Lymphocytes-Severe Combined Immunodeficiency
IC_{50}	Inhibitory Concentration 50%
ICC	Intracellular Cytokine
ICCS	Intracellular Cytokine Staining
IFN-γ	Interferon gamma
IL	interleukin
IN	Integrase
ION	Ionomycin
kcal/mol	kilocalorie per mole
LDH	Lactate Dehydrogenase

LEDGF	Lens Epithelium Derived Growth Factor
Log P	Logarithm of Partition coefficient
LTR	Long Terminal Repeat
Mabs	Monoclonal Antibodies
MD	Molecular Dynamics
MHC	Major Histocompatibility Complex
MTS	3-(4,5-dimethylthiazol-2-yl)-5-3(3-car oxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
MTT	3-(4,5-dimethylythiazol-2-yl)-2,5-diphenyltetrazolium bromide.
NF-κB	Nuclear Factor Kappa Beta
NMR	Nuclear Magnetic Resonance
NNRTIs	Non Nucleoside Reverse Transcriptase Inhibitors
NRTIs	Nucleos(t)ide Reverse Transcriptase Inhibitors
P value	Probability value
PBMCs	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PDB	Protein Data Bank
PHA-P	Phytohemagglutinin-Protein
PI	Propidium Iodide
PMA	Phorbol Myristate Acetate
PPB	Plasma Protein Binding
ppm	parts per million
PR	Protease
PSA	Polar Surface Area
QUANTUMm	Quantum Mechanics and Molecular Mechanics
RA	Rheumatoid Arthritis
RNase H	Ribonuclease H
RNR	Ribonucleotide Reductase
RPMI	Rosewell Park Memorial Institute
RT	Reverse Transcriptase
RT-CES	Real Time Cell Electronic Sensing
SAR	Structure Activity Relationship
sdf	Structural Data Files
SIV	Simian Immunodeficiency Virus
ssRNA	Single Stranded Ribonucleic Acid
ST	Strand Transfer
TNF-α	Tumour Necrosis Factor alpha
tRNA	transfer Rinbonucleic Acid
Tscs	Thiosemicarbone(s)
U	Units
U3	Untranslated 3’
U5	Untranslated 5’
UNAIDS	Joint United Nations Programme on HIV/AIDS
UV	Ultraviolet