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Gold compounds with anti-HIV and immunomodulatory activity

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In the Faculty of Natural and Agricultural Sciences

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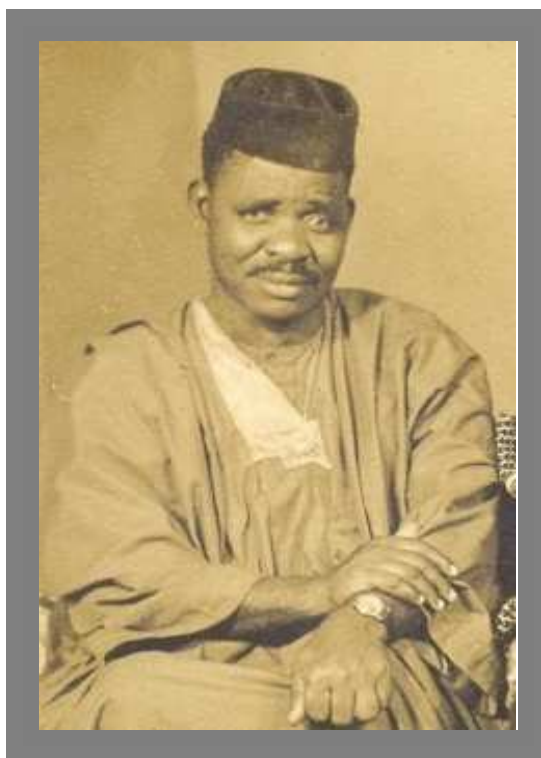
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DEDICATION

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Papa Lucas Che Fonteh (1927 - 1981)

And

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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

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 - Fonteh P, Keter K, Meyer D., (2011). New bis(thiosemicarbazone) 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.

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- 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).
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SUMMARY

GOLD COMPOUNDS WITH ANTI-HIV AND IMMUNOMODULATORY ACTIVITY

by

PASCALINE N. FONTEH

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 carboxyfluorescein 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. ^1H 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 °C) 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
d ₆ -DMSO	Deuterated Dimethylsulfoxide
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-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
NF- κ B	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 Ribonucleic Acid
TsCs	Thiosemicarbone(s)
U	Units
U3	Untranslated 3'
U5	Untranslated 5'
UNAIDS	Joint United Nations Programme on HIV/AIDS
UV	Ultraviolet