

Synthesis and testing of Palladium and Platinum phosphine complexes with potential mitochondrial targeting anti-cancer properties

By

Patricia W. Gitari (MSc. Chemistry)

Submitted in fulfilment of the requirements for the degree

PHILOSOPHIAE DOCTOR

In the Faculty of Health Sciences
UNIVERSITY OF PRETORIA
PRETORIA

Supervisor: Professor Connie E. Medlen

Co-supervisor: Professor Simon Lotz

October 2007

Declaration

The work described in this thesis was carried out at the Departments of Pharmacology and Chemistry, University of Pretoria, South Africa from February 2004 to December 2006 under the supervision of Prof Connie Medlen and co-supervision of Prof Simon Lotz. I declare that this thesis is my own, unaided work submitted for the degree of Philosophiae Doctor, and it has not been submitted previously for a degree or examination at this or any other university.

.....

(Patricia W. Gitari)

..... day of, 2007.

Acknowledgements

First and foremost, I would like to thank Almighty God for enabling me to complete this degree. I would also like to thank my supervisor Prof. Constance Medlen for accepting to supervise a chemist who was venturing into a new area of research. I am also grateful to my co-supervisor, Prof. Simon Lotz for his assistance in solving difficult synthetic problems. I would like to extend my gratitude to Dr. Richard Bowen for encouraging me to change disciplines and venture into the world of pharmacology. It is also a pleasure to acknowledge my colleagues in the Department of Pharmacology (Ms M. Nell, Dr G. Joone, Dr D. Cromarty) for their patience and guidance especially in the area of instrumentation and cell culture work.

I would also like to extend my gratitude to Mr Dave Liles for his assistance in x-ray crystallography. I would also like to thank Mr. Patrick Selahle-for carrying out animal studies, Ms Judith Wagener and Dr. J. R. Zeevaart -synthesis of radiolabelled compounds and carrying out *in vivo* experiments. Others are Dr C. Durandt for providing training in flow cytometric techniques and Mr E. Dombodzvuku for editing the final manuscript. The completion of this degree has relied heavily on the assistance I received from the people mentioned above. Hence it confirms the Kikuyu proverb “ kaara kamwe gatiūragaga ndaa” which translates to “ one finger cannot kill a louse”.

Lastly, I would like to thank MINTEK (Pty) for financial assistance that enabled me to study for this degree.

Dedication

To

My dearest mum, Mary Mumbi Gitari (1947-12.10.2007)

My father, A. Gitari Herman

My siblings, nieces and nephews

And lastly,

My dearest daughter Erica Mumbi

Summary

The main theme of this thesis focuses on the preparation of palladium and platinum phosphine complexes that possess the potential to act as anti-cancer agents. The design of the complexes was based on the known compound, $[\text{Au}(\text{dppe})_2]\text{Cl}$ which was shown to have an anti-mitochondrial mode of action on cancer cells. Major problems were experienced in the synthesis of these novel palladium and platinum compounds as the five phosphine ligands required diverse reaction conditions. Instability was the major hindrance as decomposition occurred during purification. This led to the substitution of the counter-ion (Cl^-) with PF_6^- . The complexes prepared in this study were varied in lipophilicity as the gold complex was found to be non-selective due to high lipophilicity. In total, six compounds were prepared, purified and tested for potency against a panel of cancer cell lines as well as normal cells.

The most lipophilic compound, $[\text{Au}(\text{dppe})_2]\text{Cl}$, was non-selective as it exhibited the highest toxicity to both cancerous and normal cells. In general, *in vitro* studies showed that palladium complexes were more toxic than the platinum analogues. These novel compounds were also non-toxic to both resting and stimulated lymphocytes signifying high selectivity for cancer cells. Three compounds, **Pg 3**, **Pg 4a** and **Pg 8** exhibited high toxicity and were hence tested as such on murine cancer cell lines. **Pg 8**, with intermediate lipophilicity, showed toxicity against a larger number of cancer cell lines and this led to further investigations in an attempt to determine its mode of action.

Analysis of the effects of **Pg 8** on the mitochondria showed that it did not depolarise the mitochondrial membrane potential. A seven day analysis showed that while it did not have any effect on the mitochondrial membrane potential, it depolarised the plasma membrane potential from

day 4. In contrast, $[\text{Au}(\text{dppe})_2]\text{Cl}$ depolarised the mitochondrial membrane potential as expected. **Pg 8** was shown to induce apoptosis and necrosis on Jurkat cells after exposure for 48 h. It was also shown to induce cell cycle arrest (after 48 h) as it caused blockade in the S-phase. In contrast, $[\text{Au}(\text{dppe})_2]\text{Cl}$ caused a blockade in the G_0/G_1 phase.

Uptake studies with radiolabelled **Pg 8**, $^{103}\text{Pd}(\text{d}2\text{pyrpe})_2[\text{PF}_6]_2$, showed that it accumulated significantly in Jurkat cells. Biodistribution studies in Wistar rats demonstrated that it was mostly taken up in the spleen followed by the liver. However, it was excreted faster than $^{198}\text{Au}(\text{dppe})_2\text{Cl}$ as this latter compound accumulated significantly in the lungs followed by the spleen, small intestine and liver. Acute toxicity studies in Balb/c mice showed that **Pg 8** was less toxic than $[\text{Au}(\text{dppe})_2]\text{Cl}$. The latter compound (at 3 and 6 μM) caused a significant reduction of total body weight over a 5-day period. Toxicity was evident as it was also shown to cause elevation of liver enzymes (AST and GGT), contrary to the results obtained from the mice treated with **Pg 8** (at 3, 6, 12 and 15 μM).

Preparation of a patent for the synthesis as well as anti-cancer properties of the novel compound, $[\text{Pd}(\text{d}2\text{pyrpe})_2][\text{PF}_6]_2$ (**Pg 8**) is currently in progress.

Table of contents

Declaration	ii
Acknowledgements	iii
Dedication	iv
Summary	v
List of abbreviations.....	xi
List of compounds	xii
Chapter I.....	1
Introduction.....	1
1.1 Cancer	2
1.2 Cancer incidences	2
1.3 Cancer treatment	4
1.4 Limitations of current cancer treatment.....	7
1.5 The need for new anti-cancer drugs	8
1.6 Drug design and targets.....	9
1.7 Metal based drugs	10
1.8 Metal phosphines as anti-tumour agents	16
1.9 Hypothesis	19
1.10 Aim of study	19
1.11 Objectives of the study.....	20
Chapter II.....	22
Phosphines and metal phosphine complexes.....	22
2.1 Phosphine ligands	23
2.2 Pyridylphosphines.....	27
2.3 Metal pyridylphosphines	29
2.4 Metal complexes of diphenylphosphine ligands.....	32
Chapter III.....	34
Preparation and characterisation of Pt and Pd phosphine complexes.....	34
3.1 Synthesis of metal phenylphosphine complexes	35
3.2 Synthesis of metal pyridylphosphine complexes.....	41
3.3 General discussion on the behaviour of the 2, 3 and 4-pyridylphosphine ligands	58
3.4 Structural analysis of compounds 3b, 4e 3f, 1h and 2h	59



Chapter IV	75
Experimental details	75
4.1 General	76
4.2 Synthesis of phenyl phosphine complexes	77
4.3 Preparation of pyridyl phosphine ligands	80
4.4 Preparation of 2-pyridyl phosphine complexes	81
4.5 Preparation of 3-pyridyl phosphine complexes	83
Chapter V	85
Stability, Lipophilicity and Cytotoxicity	85
5.1 Stability	86
5.2 Nuclear Magnetic Resonance Spectroscopy (NMR).....	87
5.3 Evaluation of stability of the test compounds by ³¹ P NMR spectroscopy ...	88
5.4 Materials and methods.....	88
5.5 Results.....	89
5.6 Lipophilicity	96
5.7 Aim of the experiment.....	98
5.8 Materials and methods.....	98
5.9 Results and discussion	99
5.10 Cytotoxicity	101
5.11 Determination of cytotoxicity	102
5.12 Materials and methods.....	102
5.13 General procedure	103
5.14 Statistical analysis	104
5.15 Results and discussion	105
Chapter VI	109
Analysis of mitochondrial function	109
6.1 The Mitochondria	110
6.2 Mitochondria and cancer.....	111
6.3 Mitochondria as cancer drug targets.....	111
6.4 Analysis of mitochondrial membrane potential.....	115
6.5 Objective of this experiment.....	116
6.6 Materials and methods.....	117
6.7 Results and discussion	119
6.8 Plasma membrane Potential.....	124



6.9	Analysis of plasma membrane changes by flow cytometry.....	125
6.10	Materials and methods.....	125
6.11	Results and discussion	127
Chapter VII		134
Apoptosis.....		134
7.1	Introduction	135
7.2	Apoptosis and cancer	139
7.3	Apoptosis and chemotherapy	140
7.4	Detection of apoptosis by flow cytometry.....	142
7.5	Materials and methods.....	143
7.6	Results and discussion	144
Chapter VIII		149
The cell cycle.....		149
8.1	Introduction	150
8.2	Cell cycle and carcinogenesis.....	152
8.3	Cell cycle and chemotherapy	153
8.4	Determination of the effect of Pg 8 and [Au(dppe) ₂]Cl on cell cycle of Jurkat cells.....	155
8.5	Materials and methods.....	155
8.6	Results and discussion	157
Chapter IX		164
Uptake studies.....		164
9.1	Introduction	165
9.2	Preparation of ¹⁰³ Pd labelled [Pd(d2pyrpe) ₂][PF ₆] ₂	166
9.3	Preparation of ¹⁹⁸ Au labelled [Au(dppe) ₂]Cl	167
9.4	Uptake of [¹⁰³ Pd(d2pyrpe) ₂][PF ₆] ₂ and [¹⁹⁸ Au(dppe) ₂]Cl by Jurkat cells ...	168
9.5	Materials and methods.....	168
9.6	Results and discussion	170
9.7	Biodistribution of [¹⁰³ Pd(d2pyrpe) ₂][PF ₆] ₂ and [¹⁹⁸ Au(dppe) ₂]Cl in rats	171
9.8	Results and discussion	173
Chapter X		175
Acute toxicity studies		175
10.1	Introduction	176
10.2	Motivation for the study.....	177

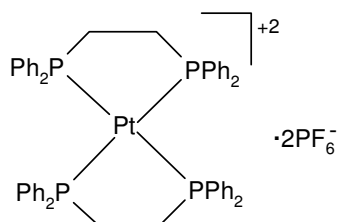


10.3	Aim.....	177
10.4	Materials and Methods:.....	177
10.5	Results and discussion	181
Chapter XI	189
Conclusions	189

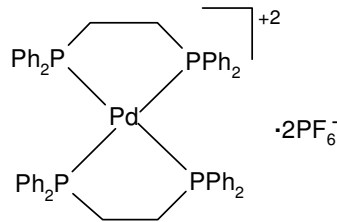
List of abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATCC	American Type Culture Collection
CDCl ₃	Deuterated chloroform
D ₂ O	Deuterated H ₂ O
DKFZ	German Cancer Research Center
DMF	Dimethylformamide
DMSO	Dimethylsulphoxide
d ₆ -DMSO	Deuterated dimethylsulphoxide
Dppa	1,2-bis-(diphenylphosphino)acetylene
Dppe	1,2- bis-(diphenylphosphino)ethane
Dppen	1,2-bis-(diphenylphosphino)ethylene
D2pyrpe	1,2-bis-(di-2-pyridylphosphino)ethane
d(pyr)pcp	1,5-bis-(di-2-pyridylphosphino)cyclopentane
ECACC	European Collection of Animal Cell Cultures
GGT	Gamma-glutamyltransferase
Gy	The international system (SI) unit of radiation dose
MS-FAB	Mass Spectrometry Fast Atomic Bombardment
NMR	Nuclear Magnetic Resonance
NRBM	Netherlands Reference Laboratory for Bacterial Meningitis
NSCLC	Non-Small-Cell Lung Cancer
PHA	Phytohaemagglutinin
Pyr	Pyridyl group
THF	Tetrahydrofuran

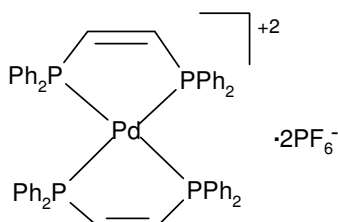
List of compounds



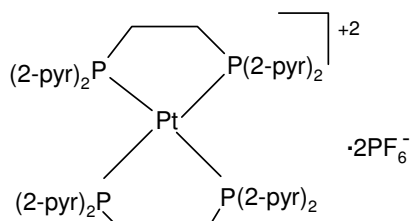
Pg 1



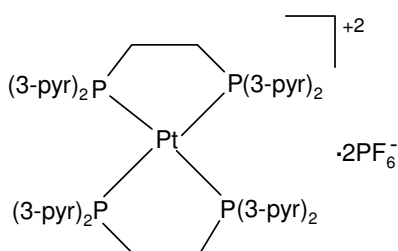
Pg 3



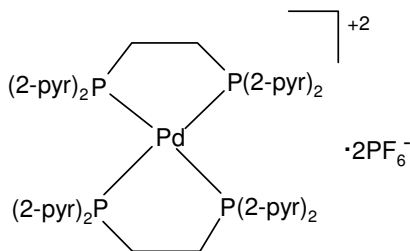
Pg 4a



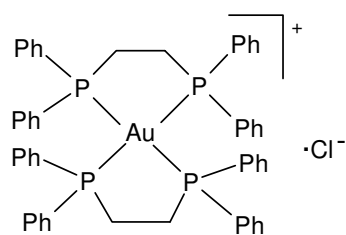
Pg 5



Pg 6



Pg 8



[Au(dppe)₂]Cl

Key

