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

By

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Supervisor: Professor Connie E. Medlen
Co-supervisor: Professor Simon Lotz

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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.

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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, $[Au(dppe)_2]Cl$ 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, $[Au(dppe)_2]Cl$, 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 mitochodria 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, $[Au(dppe)_2]Cl$ 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, $[Au(dppe)_2]Cl$ caused a blockade in the G_0/G_1 phase.

Uptake studies with radiolabelled **Pg 8**, $[^{103}Pd(d2pyrpe)_2][PF_6]_2$, 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 $[^{198}Au(dppe)_2]Cl$ 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 $[Au(dppe)_2]Cl$. 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).

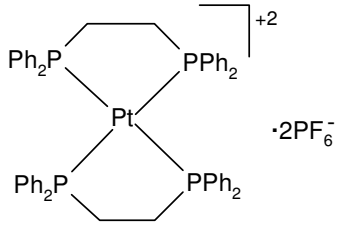
Preparation of a patent for the synthesis as well as anti-cancer properties of the novel compound, $[Pd(d2pyrpe)_2][PF_6]_2$ (**Pg 8**) is currently in progress.

Table of contents

List of abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATCC	American Type Culture Collection
$CDCl_3$	Deuterated chloroform
D_2O	Deuterated H_2O
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



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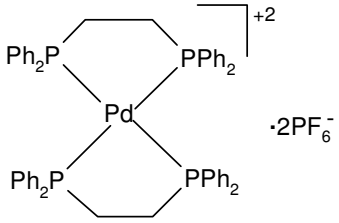

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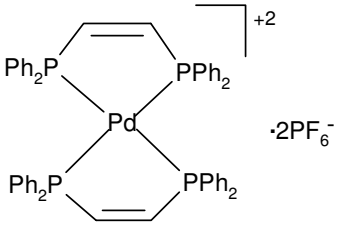

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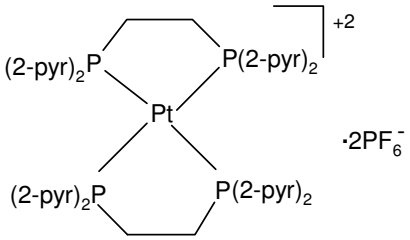

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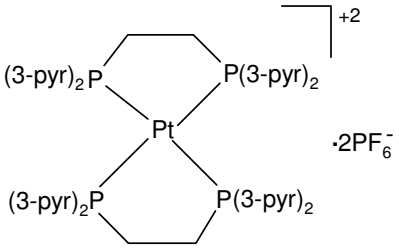

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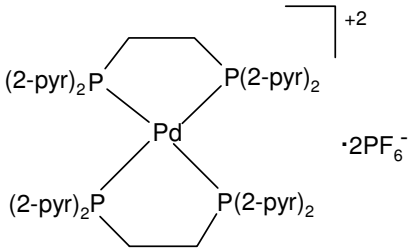

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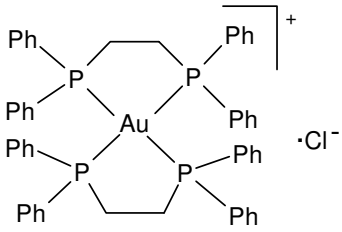
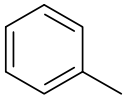
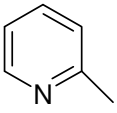
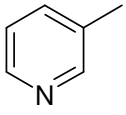


[Au(dppe)$_2$]Cl

Key

Ph = 

2-pyr = 

3-pyr = 

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