Imaging Investigations of the Ruthenium(III) Anti-Cancer Drugs, NAMI-A and KP1019, and Novel Analogues

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March 2014

A thesis submitted in total fulfilment of the requirements for the degree of Doctor of Philosophy

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Acknowledgements

First and foremost, I would like to thank Assoc. Prof. Hugh H. Harris, The University of Adelaide for giving me an opportunity to do PhD under his guidance. I acknowledge his motivation, patience, constant support and optimistic outlook whilst my PhD and I could not imagine a better advisor and mentor to pursue my PhD. I sincerely appreciate his guidance and willingness to show faith and drive me back on right track when needed.

I have been fortunate enough to work under the guidance of Prof. Leone Spiccia, Monash University and I am indebted to him for giving me an opportunity to continue my research in his group. I would like to thank him wholeheartedly as he was excessively kind with his time and profound feedback.

I would like to thank Dr. Ian Musgrave, The University of Adelaide and Assoc. Prof. Tracey Brown, Monash University, for their time, tremendous support and for authorising access to their cell culture laboratory as well as for their willingness to discuss research. I am extremely grateful to Dr. Jade Aitken for her valuable teachings in synchrotron-related work. I would like to express my sincere thanks to Dr. Aviva Levina and Assoc. Prof. Paul Witting, University of Sydney, for the constant guidance and support with cell culture studies.

My PhD journey could not have been completed without the support from the staff of School of Chemistry and Physics, University of Adelaide and School of Chemistry, Monash University. I am thankful to Dr. Chris Sumby, The University of Adelaide and Prof. Jonathan C. Morris, The University of New South Wales, for their prerequisite help to endure my research. I would like to thank all the staff of Monash University especially, Dr Peter Nicholas for the help with NMR measurements, Ms Sally Duck for mass spectrometry and Dr. Craig Forsyth for X-ray crystallography. I also would like to thank all the staff

of Adelaide Microscopy and Monash Micro Imaging for their enormous support and guidance whilst fluorescent microscopy experiments.

A big thank you to all my friends in The University of Adelaide, members of the Harris group especially Claire M. Weekly – I have been lucky enough to work in her company and friendship. I am also thankful to Dr. Ashok Pehere for his generous help and suggestions with synthetic techniques, Dr. Marcus and all other members of Prof. Abell's group for sharing their lab facilities in the beginning of my PhD. Members of the past and present Spiccia group – Jenny, Dominique, Michelle, Yan, D'Souza, Leena, Archana, Tanmaya, Monica, Bopha, Solmas and all others – thank you all for your company while working in the lab, office and making this PhD journey a great learning experience. I would like to express my sincere gratitude towards Vera and Andrian, for their help and support in cell culture studies.

Last but not least, I am indebted to my whole family for their constant support and encouragement. My great motivation was my mom and dad, I am thankful for their blessings and love which uphold me when obstacles came to my passage throughout my education. I may not be achieving this higher degree without their prayer support, sacrifices and dedication. I do not have enough words to express my gratitude towards Vino and Angie for their unwavering support – emotional and moral – during PhD and this thesis would certainly not have existed without them. My deepest gratitude to my siblings – Smitha, Julie, Anto, Anoop – for their unconditional love and support, which gave me enough inspiration to complete my studies. I would like to dedicate this thesis to my beloved Parents, Vino and Angie.

Thank you all once again, for making this PhD a great success.

Abbreviations

aq	aqueous
BSA	bovine serum albumin
br	broad (spectroscopic)
calcd	calculated
conc	concentrated
°C	degrees Celsius
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
d	doublet
ESI	electrospray ionisation
eV	electron volt
FTIR	Fourier transform infrared
g	gram(s)
h	hour(s)
HPLC	high-performance liquid chromatography
Hz	Hertz
IR	infrared
J	coupling constant
MS	mass spectrometry
m/z	mass-to-charge ratio
MP	melting point
mg	milligram(s)
min	minute(s)
mL	millilitre(s)
mmol	millimole(s)
M	molarity
m	multiplet
NMR	nuclear magnetic resonance
q	quintet

Rf	retention factor
RT	room temperature
s	singlet
SAR	structure activity relationship
SRIXE	synchrotron radiation induced X-ray emission
t	triplet
TLC	thin layer chromatography
TMS	tetramethyl silane
UV	ultraviolet
XRF	X-ray fluorescence
NMR	nuclear magnetic resonance
δ	chemical shift (in ppm)
μM	micromolar

Abstract

The success of platinum-based anti-cancer agents, such as cisplatin, has led to further investigation of the utilisation of other non-platinum metal compounds in cancer therapy. The research has generated particular interest in ruthenium-based chemotherapeutics, as ruthenium species display numerous characteristics which make them appropriate for drug design. Numerous small-molecule ruthenium complexes e.g., NAMI-A (new anti-metastatic inhibitor; 'A' stands for first compound in the series; [ImH][*trans*-$RuCl_4$(DMSO)(Im)]; Im = imidazole) and KP1019 (Keppler compound 1019; [IndH][*trans*-$RuCl_4Ind_2$]; Ind = indazole), have been discovered and found to exhibit promising anti-cancer properties without complete knowledge about the mechanism of their activity.

To develop more targeted drugs with reduced toxicity, it is important to have an accurate knowledge about the mechanism of anti-metastatic activity of these drugs in a complete biological system. Our specific interest in this research has been concerned with the investigation of anti-metastatic activity of analogues of NAMI-A and KP1019 by advanced techniques such as synchrotron-based X-ray fluorescence imaging (XRF) and fluorescent microscopy imaging.

A series of ruthenium(III) complexes, which are analogous to either NAMI-A or KP1019, have been synthesised and structurally characterised and their pharmacological activities have been investigated *in vitro*. The intracellular uptake and distribution of these complexes in human lung cancer cell line (A549) was explored by synchrotron-based X-ray fluorescence microscopy and optical fluorescent microscopy. The intracellular distribution of ruthenium in individual A549 cells treated with KP1019 was revealed by XRF and the results demonstrate an accumulation of ruthenium inside the cytosol and the nucleus. On the contrary, NAMI-A treated cells are devoid of any alteration in intracellular distribution of elements, indicating a membrane-binding mechanism for the cytotoxic activity of NAMI-A. The results in turn

demonstrate a different cellular destiny for the complexes, NAMI-A and KP1019.

The selective aggregation or intracellular speciation of ruthenium inside the cancer cells, treated with iodine substituted KP1019 analogues, was investigated by synchrotron-based μ-XRF studies. The addition of an iodinated ligand to the parent complex significantly altered the overall distribution of ruthenium across the cell from that observed for the parent complex. The 'double-tag' approach, tagging the N-heterocyclic ligand on KP1019 analogues with iodine and then tracking the cellular distribution of both ruthenium and iodine, demonstrated that the Ru-N bond in the treatment compound remained intact inside the cells. A significant increase in the concentration of both ruthenium and iodine inside the nucleus compared to that of control cells proved that the complexes selectively targeted and aggregated inside the nucleus of the treated cells.

The distinct cellular pathways of the ruthenium(III) complexes have been investigated by the functionalisation of the imidazole ligand of NAMI-A with other optically fluorescent functionalities. With the aim of eliminating the fluorescence arising from the counter cations, tetramethylammonium analogues were synthesised and characterised. The observed optical properties thus reflected the contribution from the metal complex anion only. The *in vitro* investigation of the synthesised complexes in lung cancer cells (A549) demonstrated that the functionalisation of NAMI-A does not significantly change the cytotoxic properties of the synthesised analogues. Numerous analogues of NAMI-A with enhanced imaging and targeting functionalities could be synthesised to investigate the biotransformation of ruthenium containing metabolites inside the cancer cell.

In general, a number of new biologically active ruthenium(III) complexes, which are analogous to the model complexes NAMI-A and KP1019, have been synthesised and characterised. Their intracellular speciation, aggregation and the optical fluorescent properties have been investigated. The acquired knowledge on the cytotoxicity, intracellular distribution and speciation of treated NAMI-A

and KP1019 analogues can contribute to the investigation of the mechanism of anti-metastatic activity of these drugs inside the cancer cells. It may lead to the further development of new ruthenium chemotherapeutics having wide spectrum of activity when compared to platinum-based drugs.

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