

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

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degree of Doctor of Philosophy*



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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
<i>J</i>	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

Abbreviations

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*-RuCl₄(DMSO)(Im)]; Im = imidazole) and KP1019 (Keppler compound 1019; [IndH][*trans*-RuCl₄Ind₂]; 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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