Metals in Medicine: Revealing Cellular Targets for Osmium and Iridium Organometallic Anticancer Agents

Jessica M Hearn[$], Ana M Pizarro[*], Peter J Sadler[*]

[$]Department of Systems Biology, University of Warwick [*]Department of Chemistry, University of Warwick,

1. Introduction

In 2008, 12.7 million cancer cases and 7.6 million cancer deaths were estimated. Cisplatin is a well-established, successful anticancer drug used in the clinic worldwide. However, increasing cases of metallodrug resistance highlights the importance of cancer research and in novel drug development. At the University of Warwick, Os and Ir organometallic complexes (Figure 1) have shown cancer cell cytotoxicity towards ovarian cell line, A2780 and towards 60 tumour cell lines in the National Cancer Institute (NCI) NCI60 cell screening panel. These complexes are thought to damage DNA, to direct cancer cells into apoptosis. We explore the potential for multi-targeted modes of activity, comparing results to the Pt-based DNA binding drugs (Cisplatin, Oxaliplatin, Carboplatin) currently in the clinic.

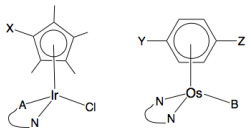


Figure 1. Typical piano-stool complex structures used in this project; transition metal centre coordinated in an octahedral geometry to a cyclopentadiene/arene (X = H or Ph; Y = Ph or 2-methylpropane; Z = Me or H), an A-N or N-N chelating ligand (A = C) and a halogen (B = Cl or I).

Table 1. List of metal complexes.

Complex	Molecular formula
1	$[Os(\eta^6\text{-bip})(azpy\text{-}NMe_2)I]^+$
2	$[Os(\eta^6\text{-}p\text{-cym})(azpy\text{-}NMe_2)I]^+$
3	$[Os(\eta^6\text{-}p\text{-cym})(azpy\text{-}NMe_2)Cl]^+$
4	$[Os(\eta^6\text{-bip})(F\text{-azpy})I]^+$
5	$[Os(\eta^6\text{-bip})(azpy\text{-}OH)I]^+$
6	$[Ir(\eta^5\text{-}Cp^{xbiph})(phen)Cl]^+$
7	$[Ir(\eta^5\text{-}Cp^{xph})(phen)Cl]^+$
8	$[Ir(\eta^5\text{-}Cp^{xbiph})(bpy)Cl]^+$
9	$[Ir(\eta^5\text{-}Cp^{xbiph})(ppy)Cl]$

Abbreviations: bip = biphenyl; azpy = azopyridine; *p*-cym = para-cymene; Cp*, pentamethylcyclopentadienyl; Cp^{xph}, tetra-methyl-(phenyl)-cyclopentadienyl; Cp^{xbiph}, tetramethyl(biphenyl)-cyclopenta-dienyl; phen, 1,10-phenanthroline; bpy, 2,2'bipyridine; ppy, 2-phenylpyridinato

2. Aims

- Use data provided by the NCI60 screen to construct mean graphs and analyse complex activity and cell line susceptibility
- Apply the COMPARE algorithm to determine the similarity in mean graphs between the Os/Ir complexes and other drugs on the NCI database to infer other potential modes of activity
- Compare and contrast these findings to those obtained for clinically used DNA binding anticancer agents
- Perform TEM analysis of A2780 cells to assess morphological changes through activation of apoptosis.

3. Methods

Mean graphs

Graphs are constructed from *in vitro* data collected by the NCI for each of the organometallic drugs across ca. 60 tumour cell lines.

1. For a given complex the GI_{50}, TGI and LC_{50} are interpolated after 48 h drug exposure for each cell line.
2. Values are represented as $\log_{10}GI_{50}$(TGI or LC_{50})
3. Mean graphs are constructed with a central line (mean $\log_{10}GI_{50}$) and projections to the left and right (δs)
4. δ = (cell line $\log_{10}GI_{50}$ – mean $\log_{10}GI_{50}$)
5. Left projecting δ values suggest the cell line has a higher GI_{50} compared to the mean – cell line is less susceptible.
6. Right projection δ values suggest cell line has a lower GI_{50} compared to the mean – cell line is more susceptible

The COMPARE algorithm

This calculates the pairwise Pearson's correlation coefficient (*r*) between two mean graphs, producing a value on the interval [-1 1].

- 1 denotes a positive correlation
- 0 denotes no correlation
- -1 denotes an inverse correlation

This process is repeated iteratively between a given compound and ca. 65,000 other compounds on the NCI database. Correlated agents are listed in descending values of *r.*

4. Results & Discussion

Mean graphs

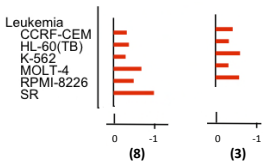


Figure 2. Mean graphs for leukemia cell lines after exposure to Ir complex **8** (mean GI_{50} 4.17 μM) and Os complex **3** (mean GI_{50} 0.31 μM). δ values are highly positive in each case, shown by the log scale axis and demonstrating a high cell line susceptibility.

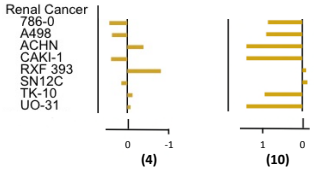


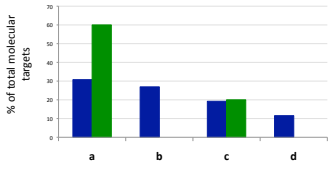
Figure 3. Mean graphs for renal cell lines after exposure to Ir complex **8** (mean GI_{50} 4.17 μM) and Os complex **4** (mean GI_{50} 4.68 μM). δ values are highly positive for **8** and significantly more negative for **4** shown by the log scale axis and demonstrating the increased growth inhibition of renal cells exposed to **4**.

COMPARE analysis

Table 2. Correlations of cytotoxic profiles of metal complexes **1**-**9** (See Figure 1 and Table 1 for structures) with clinically used drugs. All correlations are made using GI_{50} cytotoxic profiles

Cellular target	Clinical drugs	Correlated complexes (*r* > 0.6)
DNA	Olivomycin[a], Ellipticine[b], Rhodium dimer[c], Chromomycin[d], Bisantrene[e], Isobaccharin[f]	1^{abcdef}, 2^{abcde}, 3^{abcd}, 6^{acde}, 7^{abc}, 8^{abcde}, 9^{c}
Protein synthesis	Phyllanthoside[g], Aurantomycin B[h], Bouvardin[i], Undulatone[j], Harringtonine[k], Tubulosine[l]	1^{ghijkl}, 2^{ghil}, 3^{ghij}, 6^{ghik}, 7^{gi}, 8^{gh}, 9^{i}
Topoisomerases	Ellipticine[m], Bisantrene[n], Berberine[o], Daunomycin[p]	1^{mn} ,2^{mn} ,3^{mp} ,6^{n} ,7^{o} ,8^{mn} ,9^{p}
Mitosis	Taxol[q], Malformin A[r], Vinblastine[s]	1^{qrs} ,2^{qrs} ,3^{rs} ,6^{qrs} ,7^{r} ,8^{qr} ,9^{s}

Figure 4. Bar chart showing the molecular targets for the Os and Ir complexes **1** to **9** (green) and Pt drugs Cisplatin, Oxaliplatin and Carboplatin (blue); (a) DNA binders (b) Protein synthesis inhibitors (c) Topoisomerase inhibitors (d) Mitotic inhibitors



TEM analysis

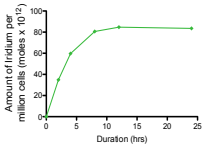


Figure 5. Uptake of Ir by A2780 cells after exposure to 100 μM $[Ir(\eta^5\text{-}Cp^*)(phen)Cl]^+$ for 24 h. Maximum uptake occurs after ca. 12 h. Os drugs have shown the same uptake curve suggesting similar uptake mechanisms.

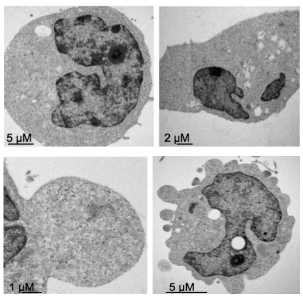


Figure 6. TEM images of A2780 cells after exposure to Ir complex **6. Top:** Drug build up in the nucleus followed by nuclear break down; **Bottom**: Apoptotic body (left); Membrane blebbing (right)

5. Conclusion

- Confirmed the DNA binding nature of the compounds sharing similarity to the Pt drugs; Cisplatin, Oxaliplatin and Carboplatin.
- Identified protein synthesis, topoisomerase and mitotic inhibition as other potential modes of action for the Os and Ir drugs.
- Leukemia cell lines are in all cases susceptible to these complexes
- Identified $[Os(\eta^6\text{-bip})(F\text{-azpy})I]^+$ (**4**) with a potentially novel mode of action, in particular with differential activity towards renal cancer cell lines.
- Future research will analyse cell targets on a genomics level to assess the regulation of gene networks in cells exposed to these complexes.

References

1. Zhe Liu, Abraha Habtemariam, Ana M. Pizarro, Sally A. Fletcher, Anna Kisova, Oldrich Vrana, Luca Salassa, Pieter C. A. Bruijnincx, Guy J. Clarkson, Viktor Brabec, and Peter J. Sadler. **Organometallic Half-Sandwich Iridium Anticancer Complexes.** *J. Med. Chem.*, ***2011, 54 (8), pp 3011–3026***
2. Ying Fu, Abraha Habtemariam, Ana M. Pizarro, Sabine H. van Rijt, David J. Healey, Patricia A. Cooper, Steven D. Shnyder, Guy J. Clarkson, and Peter J. Sadler. **Organometallic Osmium Arene Complexes with Potent Cancer Cell Cytotoxicity.** *J. Med. Chem.*, ***2010, 53 (22), pp 8192–8196***
3. NCI-DTP: *http://dtp.nci.nih.gov*

SYSTEMS BIOLOGY DOCTORAL TRAINING CENTRE
THE UNIVERSITY OF WARWICK
BBSRC bioscience for the future