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Selective Cytotoxic Ru(II) Arene Cp* Complex Salts [R-PhRuCp*]$^+X^-$ for X = BF_4^-, PF_6^-, and BPh_4^-

Bradley T. Loughrey,[†] Peter C. Healy,[†] Peter G. Parsons,[‡] and Michael L. Williams*[,†]

Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, Australia, and Drug Discovery Group, Queensland Institute of Medical Research, Brisbane, Australia

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A novel series of ionic Ru(II) arene Cp* sandwich complexes has been synthesized and characterized. Screening results for cytotoxicity against a range of human tumor cell lines and normal human cells indicate that the complexes show promising anticancer activity, which varies with changes in the arene ligand and the anionic counterion.

The significant involvement of metal ions and complexes in biological processes and systems has, over recent times, led to the realization that considerable scope exists for the design of metal-based therapeutics.[1] While considerable work in this area has been in the field of coordination complexes, the large diversity of structure and unique bonding modes of organometallic complexes also suggests that these systems may find use as therapeutic agents.[2]

Recently, ruthenium(II) organometallic complexes have been gaining popular interest as potential anticancer agents.[3,4] The Ru(II) arene half-sandwich complexes [(R-Ph)Ru(Y-Z)L] (R-Ph = substituted arene, Y-Z = bidentate ligand, and L = monodentate anion) have proven to be potent cytotoxic agents against a range of tumor cell lines while retaining good stability and aqueous solubility.[3] The RAPTA series of compounds [(R-Ph)Ru(YZ)PTA], which incorporate a 1,3,5-triaza-7-phospha-adamantane (PTA) ligand and two monodentate ligands (YZ), have proven to be effective antimetastatic agents with comparable biological effects to the Ru(III)-based antimetastatic drug NAMI-A.[4]

The promising biological effects displayed by both of these classes of Ru(II) organometallic complexes both *in vitro* and *in vivo* has prompted our research group to synthesize and structurally characterize a series of ionic Ru(II) arene Cp* (η^5-$C_5(CH_3)_5$) complexes [R-PhRuCp*]$^+X^-$ in which the ligands (YZ)(L) of the above systems are replaced by the Cp* anion. These highly stable, nonlabile complexes have no readily available leaving groups, potentially indicating that this series of compounds could display its own unique mechanism of action.

The aim of this study was to investigate how changes in arene size and hydrophobicity impact the overall biological activity of these complexes. Potential applications of this work include, but are not limited to, complexation of the RuCp* moiety to known aromatic drugs such as chloroquine and tamoxifen. These clinical therapeutics have previously been tethered to ferrocene yielding ferroquine and ferrocifen, respectively.[5,6] Ferroquine is highly active against chloroquine-resistant strains of the malaria parasite, while the ferrocifens were shown to inhibit proliferation of both hormone-dependent and hormone-independent forms of breast cancer.[5,6]

A second aim of the study was to determine if the presence of differing counterions could effect any noticeable changes in the biological activity of the compounds, as literature data obtained on a number of cytotoxic cations indicate that variation in the chemical properties of the anion can result in significant changes in biological activity.[7−14] To study this, we prepared each complex as the $[BF_4]^-$, $[PF_6]^-$, and $[BPh_4]^-$ salts. The complexes were prepared through a combinatorial synthetic approach (Scheme 1), yielding a small focused library of functional groups incorporated on the arene ring, including monosubstituted esters (**1a**−**c**), ketones (**2a**−**c**), alkyl side chains (**3a**−**c**), and amines (**4a**−**c**) (**a** = $[BF_4]^-$, **b** = $[PF_6]^-$, **c** = $[BPh_4]^-$). Synthetic details and compound characterization (nuclear magnetic resonance, electrospray ionization mass spectrometry, Fourier transform infrared spectroscopy, microanalysis, and X-ray crystal-

* Author to whom correspondence should be addressed. Phone: +61 07 373 57728. E-mail: michael.williams@griffith.edu.au.
† Griffith University.
‡ Queensland Institute of Medical Research.

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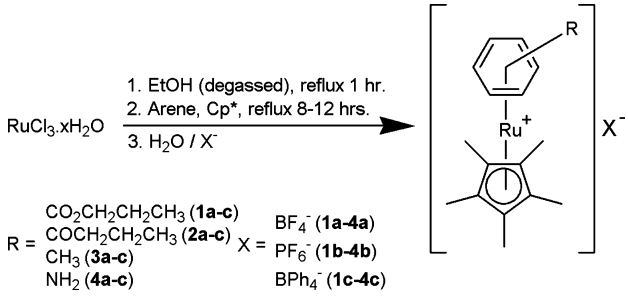
Scheme 1. Synthesis of the Ru(II) Arene Cp* Sandwich Complexes



lography for **2c**) are supplied within the Supporting Information of this communication. An ORTEP plot of complex **2c** is presented in Figure 1.

The cytotoxicities of **1**−**4** (Table 1) were investigated using a sulphorhodamine B colorimetric assay of cell number following drug treatment in microlite wells for 6 days.[15] The cell lines chosen for study were MCF7 (hormone-dependent breast cancer), MDA-MB-231 (hormone-independent breast cancer), MM96L (human melanoma), and normal human cells (NFF, neonatal foreskin fibroblasts). Each of these cell lines is susceptible to a variety of applied chemotherapeutics and also displays different mechanisms of cross-resistance to such chemotherapies.

Our results indicate that each of the complexes, **1**−**4**, is biologically active with respect to all three tumor cell lines, while displaying a moderate level of selectivity toward NFF (Table 1, Figures 2 and 3). The ammonium salts of the counterions were also screened for toxicity. Each anion obtained IC50 values >1000 μM against all four cell lines and was considered nontoxic when not in the presence of the organometallic cation.

Selectivity of the complexes was found to be dependent on the nature of the monosubstituted arene functional group with the counterion prompting little to no difference (Figure 3). The most selective functional groups were the nonpolar monosubstituted methyl complex **3** and the monosubstituted amine complex **4**. In relation to the MM96L human melanoma cell line, this series of complexes achieved selectivity ratios of 34.9 (**3a**), 35.1 (**3b**), 40.4 (**3c**), 30.0 (**4a**), 20.5 (**4b**), and 28.1 (**4c**), respectively. These results can be compared to cisplatin, which achieved a selectivity ratio of only 1.94 against the same tumor cell line.

Toxicity of the organometallic complexes is shown to change not only with variation of the monosubstituted arene ligand but also with variation of the counterion (Figure 2).

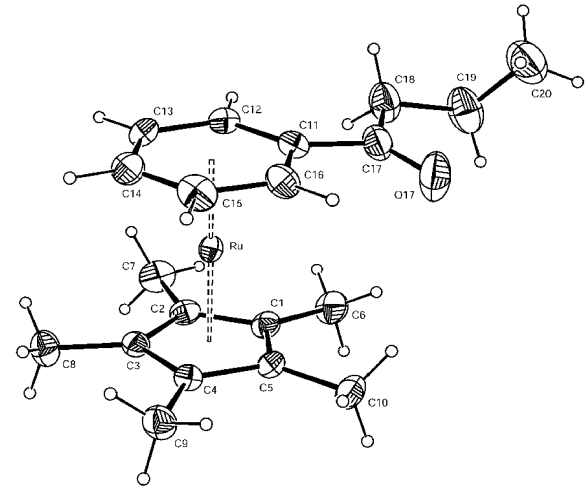


Figure 1. Representative view of the Ru(II) propiophenone Cp* tetraphenylborate complex (**2c**), anion omitted for clarity.

The results show that the complexes incorporating the tetraphenylborate (TPB) anion (**1c**−**4c**) are significantly more toxic than those with the tetrafluoroborate (**1a**−**4a**) and hexafluorophosphate (**1b**−**4b**) anions. The TPB organometallic salts are nearly 3 times more active than their BF_4^- and PF_6^- counterparts against all three of the tumor cell lines investigated and yielded IC50 values comparable in magnitude to that of cisplatin (Table 1).

It is postulated that hydrophobic interactions may occur between the arene ligand of the organometallic cation and the aromatic hydrocarbons of TPB. These interactions could prompt strong ion pairing, allowing the TPB to potentiate transport of the organometallic cation across the cell membrane and into various organelles within the cell.

Ion-pair formation of this kind has been proposed to account for a number of physiochemical phenomena in which lipophilic anions modulate the lipid solubility of cationic species and vice versa.[7−14,16−26]

Studies carried out on a range of structurally diverse organic hydrophobic amines such as 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP),[8−12] 4,4′-diethylaminoethoxyhexestrol,[13] and 2-[3-chloro-8-(4-chlorophenyl)-1,7-diazabicyclonona-2,4,6,8-tetraen-9-yl]-N,N-dipropyl-acetamide (Alpidem)[14] concluded that TPB was capable of modulating both drug uptake and toxicity through hydrophobic ion-pairing interactions.[8−14] These studies also found

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Table 1. Inhibitory Concentration That Limits Proliferation by 50% (IC50) and Drug Selectivity Ratios of Ru(II) Arene Cp* Sandwich Complexes against a Range of Cancer Cell Lines and a Normal Human Cell Line

	IC50 values (μM)[a]				selectivity ratios[b]		
complex #	MCF7	MDA-MB-231	MM96L	NFF	IC50 (NFF)/ IC50 (MCF7)	IC50 (NFF)/IC50 (MDA-MB-231)	IC50 (NFF)/ IC50 (MM96L)
1a	12.0	16.9	7.60	48.4	4.03	2.86	6.37
1b	14.3	17.8	6.15	55.6	3.89	3.12	9.04
1c	2.33	3.36	2.54	10.6	4.55	3.15	4.17
2a	11.3	32.0	6.76	134	11.9	4.19	19.8
2b	16.8	29.5	7.18	136	8.10	4.61	18.9
2c	3.00	9.14	2.85	32.7	10.9	3.58	11.5
3a	8.55	13.7	4.62	161	18.8	11.8	34.9
3b	13.6	20.8	4.53	159	11.7	7.64	35.1
3c	4.99	5.20	2.28	92.2	18.5	17.7	40.4
4a	13.1	24.9	6.10	183	14.0	7.35	30.0
4b	10.9	30.9	9.30	191	17.5	6.18	20.5
4c	4.73	12.0	3.50	98.2	20.8	8.18	28.1
5 − cisplatin	1.80	N/A	1.70	3.30	1.83	N/A	1.94

[a] Errors within the range of ±5−10% of the reported value. Results are the average of three separate experiments. [b] Selectivity ratios are the direct comparison of drug cytotoxicity between the normal human cell line (NFF) and the annotated tumor cell line.

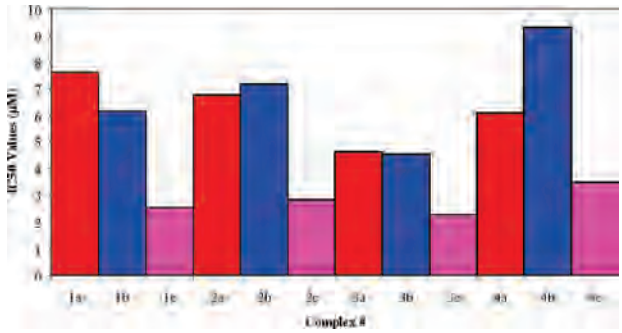

Figure 2. Complex IC50 values for the MM96L cell line. Comparison of the effect of the arene ligand and counterion choice on cytotoxicity for the BF_4 (a), PF_6 (b), and $B(C_6H_5)_4$ (c) derivatives of the complexes (**1**−**4**).

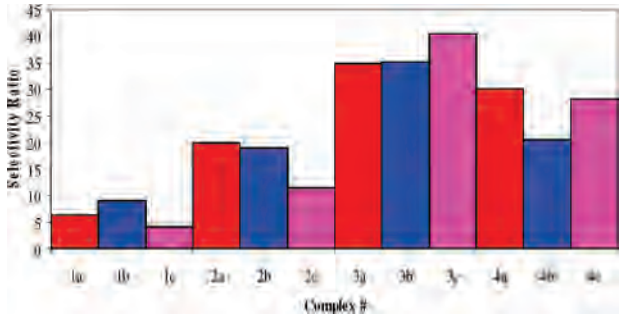

Figure 3. Complex selectivity ratios witnessed against the MM96L cell line. Comparison of the effect of arene ligand and counterion choice on tumor specificity for the BF_4 (a), PF_6 (b), and $B(C_6H_5)_4$ (c) derivatives of the complexes (**1**−**4**).

that the toxicity of these hydrophobic amines arose from their capacity to inhibit cellular mitochondria, and that TPB alone exhibited little to no effect on the health of intact mitochondria.[7−14] Coadministration of TPB with each hydrophobic amine, however, significantly increased both mitochondrial drug uptake and subsequent inhibition of the organelle.[8−14]

The series of *in vitro* and *in vivo* studies carried out on TPB and MPTP found that TPB highly potentiated the rate of ATP depletion of neuroblastoma X glioma hybrid NG 108-15 cells induced by MPTP.[8,9] TPB also accelerated the onset of respiratory inhibition on intact mitochondria as well as increased the overall level of mitochondrial inhibition.[10−12] This enhanced activity was considered to be a consequence of TPB ion pairing with MPP+ to facilitate penetration of the cytotoxic material into the mitochondrial organelle as well as allow MPP+ access to hydrophobic inhibition sites on NADH hydrogenase.[12] Experiments carried out *in vivo* indicated that, although anion exchange reactions would be expected, TPB successfully modulated the dopaminergic neurotoxic effects of MPTP within male SWISS-webster mice.[9]

The present results obtained on the prepared focused library of ruthenium(II)-based organometallic complexes indicate that TPB's ability to potentiate the biological effects of cytotoxic compounds is not limited to just organic hydrophobic amines.

In summary, this manuscript details the first recorded biological study of Ru(II) arene Cp* ionic complexes. The results show that these complexes exhibit potent antiproliferative effects against all screened tumorigenic cell lines while retaining moderate to good selectivity toward the normal human cell line NFF. Cytotoxicity exhibited by the complexes was found to be dependent on both the nature of the arene ligand and the anionic counterion. In particular, the large hydrophobic counterion tetraphenylborate was shown to significantly increase the toxic effects exhibited by the complexes *in vitro*, with the TPB ionic salts achieving IC50 values comparable to that of cisplatin. Of significance is the potential that these organometallic complexes may hold for optimization, both through the attachment of the RuCp* moiety to an established aromatic therapeutic and also through the incorporation of counterions that aid in both complex solubility and transport.

Supporting Information Available: Experimental details and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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