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Brief Article

Organometallic Osmium Arene Complexes with Potent Cancer Cell Cytotoxicity

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Iodido osmium(II) complexes $[Os(\eta^6\text{-arene})(XY)I]^+$ (XY = *p*-hydroxy or *p*-dimethylaminophenylazopyridine, arene = *p*-cymene or biphenyl) are potently cytotoxic at nanomolar concentrations toward a panel of human cancer cell lines; e.g., $IC_{50} = 140$ nM for $[Os(\eta^6\text{-bip})(azpy\text{-}NMe_2)I]^+$ toward A2780 ovarian cancer cells. They exhibit low toxicity and negligible deleterious effects in a colon cancer xenograft model, giving rise to the possibility of a broad therapeutic window. The most active complexes are stable and inert toward aquation. Their cytotoxic activity appears to involve redox mechanisms.

Introduction

The success of platinum complexes (e.g., cisplatin and carboplatin) as anticancer drugs is well-known; however, these drugs have a limited spectrum of activity and can have severe toxic side effects and tumors often develop resistance.[1,2] A number of ruthenium-based compounds have been reported to display promising anticancer activity,[3−6] and two octahedral ruthenium(III) complexes have reached clinical trials.[7,8] The Ru^{III} complexes are thought to be reduced to active Ru^{II} species in vivo. Ruthenium(II) can be stabilized by π-bonded arene ligands, and a range of Ru^{II} arene complexes of the type $[Ru(\eta^6\text{-arene})(XY)Z]$, where XY = diamine chelate and Z = Cl, show in vitro and in vivo anticancer activity.[9,10] These complexes can undergo activation via hydrolysis and bind strongly to DNA, a potential target.[11]

Arene complexes of the heavier congener Os^{II} display structures in the solid state similar to those of Ru^{II} but are subtly different with regard to their chemical reactivity. For example, Os^{II} arene ethylenediamine chlorido complexes hydrolyze ~40× more slowly and the related aqua adducts have pK_a values for Os-OH_2/OH, which are ~1.5 pK_a units lower (more acidic) than those of the analogous Ru^{II} complexes.[9,12] Faster ligand exchange in Os^{II} complexes can be achieved by incorporating oxygen-containing chelating ligands, e.g., picolinates.[13,14] Several examples of Os^{II} arene complexes that exhibit cancer cell cytotoxicity have now been reported.[15−19]

The introduction of strong π-acceptor chelating (XY) ligands such as bipyridine or phenylazopyridine (azpy[a]), dramatically changes the properties of the osmium and ruthenium complexes.[11,20,21] For example, ruthenium complexes of the type $[Ru(\eta^6\text{-biphenyl})(azpy)I]^+$ are relatively inert toward ligand substitution and appear to kill tumor cells by ligand-centered redox-mediated mechanisms.[22] Here we report the synthesis of a range of osmium complexes of formula $[Os(\eta^6\text{-arene})(phenylazopyridine\text{-}R)Z]^+$ where arene = *p*-cymene (*p*-cym) or biphenyl (bip), R = hydrogen (H), hydroxyl (OH), or dimethylamino (NMe_2), and Z = chloride or iodide (Chart 1). The X-ray crystal structures of five complexes are reported and their chemical reactivity investigated. Surprisingly, several of these Os^{II} phenylazopyridine complexes are an order of magnitude more potent than the clinically used drug cisplatin toward a range of human cancer cell lines. An evaluation of their toxicity in mice bearing xenografted colon tumors is also reported.

Results and Discussion

Previously we reported the synthesis of the Os^{II} azopyridine complexes $[Os(\eta^6\text{-arene})(azpy\text{-}NMe_2)Cl]PF_6$ with arene = biphenyl (**11**) and *p*-cymene (**12**).[20] Cytotoxicity tests for these complexes were hampered by their low solubility and the occurrence of precipitation under the test conditions used. No activity toward human A549 lung cancer cells was detected up to 100 μM. In the present work we have explored similar Os^{II} azopyridine complexes with iodide replacing chloride (Z) and with azpy-R (R = H, OH, and NMe_2). The complexes remained in solution under the cell testing conditions with no apparent precipitation (vide infra).

Chemistry. Six novel cationic iodido osmium arene complexes containing chelating phenylazopyridine ligands (azpy, azpy-OH, and azpy-NMe_2) were synthesized where PF_6 acts as the counterion. In general, they have poor aqueous solubility (≤0.2 mM), with the biphenyl complexes being less water-soluble than the *p*-cymene complexes. The structures of the iodido complexes with azpy (**2**), azpy-OH (**3**), and azpy-NMe_2 (**5**) (Chart 1) were determined by X-ray crystallography. For comparison, six chlorido analogues were synthesized and the structures of the azpy complex **8** and azpy-NMe_2 complex **11** were also determined by X-ray crystallography. We have reported the X-ray structure of **12** previously.[20] We also determined the structure of the dimer $[Os(\eta^6\text{-}p\text{-cym})I_2]_2$, which is an important synthetic intermediate in this work (Figure S1).

All of the osmium complexes adopt the familiar pseudo-octahedral "piano-stool" structure (Figure 1), with bond lengths and angles within the expected ranges.[20] The crystal structure of

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[a] Abbreviations: NAC, *N*-acetyl-L-cysteine; GSH, glutathione; ROS, reactive oxygen species; azpy, 2-(phenylazo)pyridine; azpy-OH, 4-(2-pyridylazo)phenol; azpy-NMe_2, 4-(2-pyridylazo)-*N*,*N*-dimethylaniline.

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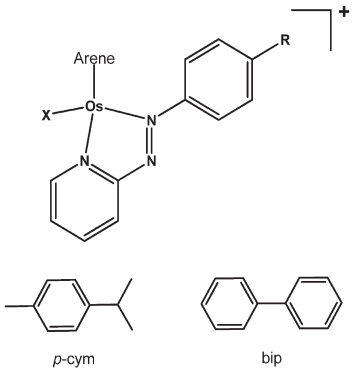
Chart 1. Osmium Phenylazopyridine Arene Complexes Studied in This Work



Complex	Arene	R	X
1	bip	H	I
2	*p*-cym	H	I
3	bip	OH	I
4	*p*-cym	OH	I
5	bip	NMe_2	I
6	*p*-cym	NMe_2	I
7	bip	H	Cl
8	*p*-cym	H	Cl
9	bip	OH	Cl
10	*p*-cym	OH	Cl
11	bip	NMe_2	Cl
12	*p*-cym	NMe_2	Cl

the neutral complex, [Os(η^6-bip)(azpy-O)I]·0.5H_2O (**3***), was obtained by slow evaporation of a methanolic solution of complex **3** ([Os(η^6-bip)(azpy-OH)I]PF_6). **3*** has a relatively short O15−C12 bond length (1.258(4) Å) indicating deprotonation of the OH group, with the iodido ligand balancing the charge on Os^{II} to afford a neutral complex. A water molecule in the lattice forms a short H-bond with the phenoxide group and bridges two phenoxides of neighboring complexes (Figure S2). The pK_a of the azpy-OH ligand in **3** was determined to be ~6.9 (comparable to 6.5 for the ruthenium analogue),[11] and so the complex would be expected to be deprotonated under the conditions of the cytotoxicity assays (pH 7.4), as shown in the X-ray structure. Selected bond lengths and angles for these structures are listed in the Supporting Information (Table S1).

Cytotoxicity. In contrast to previous studies on complexes **11** and **12**,[20] the complexes were soluble in the culture medium at the concentrations tested and no precipitation was observed after 24 h. None of the azopyridine ligands showed cytotoxic activity against A2780 human ovarian cancer cells up to 100 μM. The IC_{50} of the osmium phenylazopyridine complexes (Chart 1) ranged from 0.14 μM for the iodido biphenyl complexes **3** and **5** with azpy-OH and azpy-NMe_2 ligands to >50 μM for the chlorido complex **8** containing azpy in the human ovarian cell line A2780 (Table 1A). From these preliminary results three complexes (**3**, **6**, and **12**) were selected for further evaluation against a cisplatin-resistant subline of A2780, A2780/cis, as well as against a panel of human cancer cell lines of differing histiotype. Complexes **3** and **6** (Table 1B) showed at least 10-fold greater potency than cisplatin against all cell lines tested apart from RT-112 (bladder), which demonstrated a 3-fold difference for complex **3**. Complex **12** showed similar potency to cisplatin over the cell line panel. The highest activity in comparison to the reference cisplatin for all three complexes was seen for the PC-3 (prostate) cell line. Interestingly, complexes bearing electron-donating hydroxyl or dimethylamino substituents on the phenyl ring of the phenylazopyridine ligands are an order of magnitude more active compared to complexes bearing unsubstituted azpy ligands. This may indicate that redox processes associated with the chelated ligand, e.g., involving the azo group,[22] are important for activity. In contrast, octahedral Ru^{II} complexes such as [Ru(azpy)$_2$Cl$_2$] containing two unsubstituted azpy ligands have been reported to be as active as cisplatin against A2780 cells.[23]

The high potency of the organometallic iodido osmium complexes reported here, with IC_{50} within the nanomolar range, is notable. This is unprecedented for cytotoxic organometallic osmium complexes, for which activities generally fall in the micromolar range.[16,18,19] In addition, their ruthenium analogues were significantly less cytotoxic (7 to more than 56 times less active) in the A2780 ovarian cancer cell line.[22] In particular, the Ru analogue of complex **3** ([Ru(η^6-bip)(azpy-OH)I]PF_6) is 35 times less active.

Stability and Hydrolysis. We investigated the hydrolysis (aquation) of the phenylazopyridine complexes, since this is a potential mechanism for activation of halido osmium arene complexes in their interaction with possible biological targets such as DNA.[16,24]

The aqueous behavior of the highly active complexes [Os(η^6-*p*-cym)(azpy-NMe_2)I]PF_6 (**6**, IC_{50} = 0.2 μM), moderately active complex [Os(η^6-*p*-cym)(azpy-NMe_2)Cl]PF_6 (**12**, IC_{50} = 1.8 μM), and inactive complex [Os(η^6-*p*-cym)(azpy)Cl]PF_6 (**8**, IC_{50} > 50 μM) against A2780 was studied at 310 K over 24 h by ^{1}H NMR.

The ^{1}H NMR spectra of a 50 μM solution of inactive complex **8** in 10 mM phosphate buffer 95% D_2O/5% MeOD-d_4 over 24 h showed the disappearance of the peaks corresponding to the bound azopyridine and *p*-cymene ligands (Figure S3), suggesting that this complex is not stable under aqueous conditions. Similar behavior has been observed for the ruthenium analogue [Ru(η^6-*p*-cym)(azpy)Cl]PF_6.[11] In contrast, complexes **6** and **12** did not show ligand loss and neither did they hydrolyze as judged by their ^{1}H NMR spectra, which remained unchanged (Figure S3). In addition, ESI-MS analyses showed ions corresponding to the chlorido and iodido complexes only, and UV−vis spectra in 10% methanol/90% water showed no change over 24 h (Figure S4). An attempt to remove the chloride from the aqueous solution of **12** by reacting it with silver nitrate under reflux overnight did not result in the expected aqua product. The chlorido complex was the only species detected by mass spectrometry (Figure S6), indicating a strong Os−Cl bond.

These observations suggest that active complexes **6** and **12** are stable under biologically relevant testing conditions. In addition, the inertness of the Os−I bond suggests that hydrolysis process is not critical for the activation of these complexes and it is consistent with previous observations on the analogous Ru^{II} azpy complexes,[22] implying that the azpy ligand plays an important role in the activity.

Effect of *N*-Acetyl-L-cysteine (NAC) on Cytotoxicity. Pretreatment of cells with NAC can block cisplatin-dependent caspase-3 activation and apoptosis by inhibiting the accumulation of intracellular reactive oxygen species (ROS) and maintaining intracellular GSH levels.[25] To investigate possible involvement of ROS in the cytotoxicity of azopyridine Os^{II} arene complexes, we investigated the effect of pretreatment of cells with NAC. Cisplatin was used as the positive control, whereas cells not treated with osmium complexes served as the negative control.

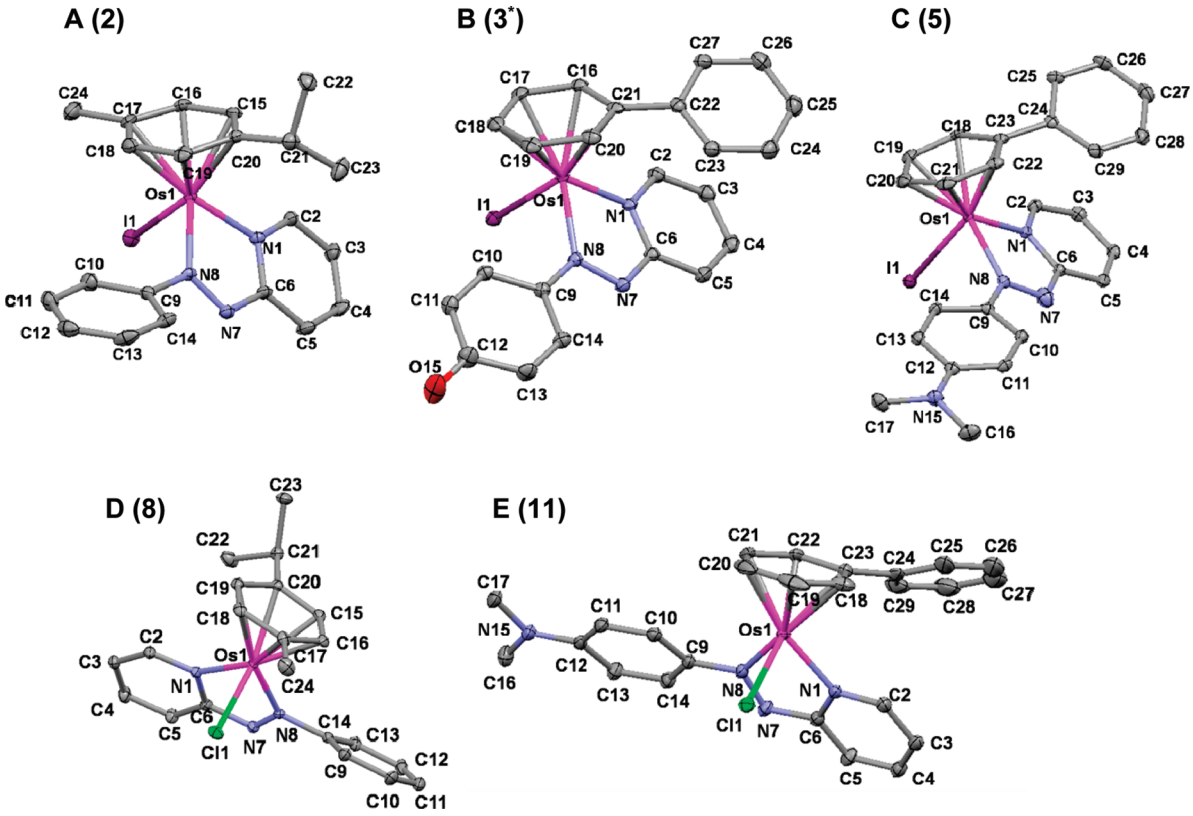


Figure 1. X-ray crystal structures of (A) $[Os(\eta^6\text{-}p\text{-cym})(azpy)I]^+$ (**2**), (B) $[Os(\eta^6\text{-bip})(azpy\text{-}O)I]$ (**3***), (C) $[Os(\eta^6\text{-bip})(azpy\text{-}NMe_2)I]^+$ (**5**), (D) $[Os(\eta^6\text{-}p\text{-cym})(azpy)Cl]^+$ (**8**), and (E) $[Os(\eta^6\text{-bip})(azpy\text{-}NMe_2)Cl]^+$ (**11**), with thermal ellipsoids drawn at 50% probability. The hydrogen atoms, counterions (PF_6), and solvent water molecules have been omitted for clarity.

Table 1. (A) IC_{50} for Complexes **1**−**12** in Human Ovarian Cell Line A2780 and (B) IC_{50} for **3**, **6** and **12** in A2780/cis Ovarian, A549 Lung, HCT-116 Colon, MCF-7 Breast, PC-3 Prostate, and RT-112 Bladder Human Cancer Cell Lines[a]

(A) IC_{50} for **1**−**12** and Cisplatin

complex		IC_{50} (μM)
$[Os(\eta^6\text{-bip})(azpy)I]PF_6$	**1**	5.4 ± 0.8
$[Os(\eta^6\text{-}p\text{-cym})(azpy)I]PF_6$	**2**	10.3 ± 0.1
$[Os(\eta^6\text{-bip})(azpy\text{-OH})I]PF_6$	**3**	0.14 ± 0.01
$[Os(\eta^6\text{-}p\text{-cym})(azpy\text{-OH})I]PF_6$	**4**	0.32 ± 0.20
$[Os(\eta^6\text{-bip})(azpy\text{-}NMe_2)I]PF_6$	**5**	0.14 ± 0.01
$[Os(\eta^6\text{-}p\text{-cym})(azpy\text{-}NMe_2)I]PF_6$	**6**	0.18 ± 0.01
$[Os(\eta^6\text{-bip})(azpy)Cl]PF_6$	**7**	13.9 ± 3.8
$[Os(\eta^6\text{-}p\text{-cym})(azpy)Cl]PF_6$	**8**	> 50
$[Os(\eta^6\text{-bip})(azpy\text{-OH})Cl]PF_6$	**9**	0.84 ± 0.1
$[Os(\eta^6\text{-}p\text{-cym})(azpy\text{-OH})Cl]PF_6$	**10**	1.3 ± 0.2
$[Os(\eta^6\text{-bip})(azpy\text{-}NMe_2)Cl]PF_6$	**11**	3.9 ± 0.3
$[Os(\eta^6\text{-}p\text{-cym})(azpy\text{-}NMe_2)Cl]PF_6$	**12**	1.8 ± 0.1
cisplatin		1.8 ± 0.1

(B) IC_{50} for **3**, **6** and **12** and Cisplatin

	IC_{50} (μM)					
complex	A2780/cis	A549	HCT-116	MCF-7	PC-3	RT-112
3	0.19 ± 0.04	0.42 ± 0.07	0.34 ± 0.02	0.31 ± 0.02	0.37 ± 0.08	0.42 ± 0.06
6	0.23 ± 0.05	0.38 ± 0.07	0.22 ± 0.02	0.20 ± 0.01	0.62 ± 0.12	0.25 ± 0.02
12	1.77 ± 0.68	5.23 ± 1.10	0.93 ± 0.05	1.12 ± 0.81	6.80 ± 0.00	2.17 ± 0.55
cisplatin	4.03 ± 2.08	4.10 ± 2.11	2.58 ± 0.55	3.02 ± 1.11	21.47 ± 4.05	1.35 ± 0.73

[a] Data are the mean of three experiments and are reported as mean ± standard error of the mean (SEM).

A2780 cells were treated with the iodido complexes **3**, **5**, and **6** and chlorido complex **12** at concentrations that were 1.2−1.8× their respective IC_{50} values, and for cisplatin a concentration 2.5× its IC_{50} value was used. These doses reduced cell growth to < 30% of the control value (Figure 2). Pretreatment with 5 mM NAC for 2 h and cotreatment with osmium complexes for 24 h to increase the intracellular glutathione concentration,[22] resulted in the inhibition of the antiproliferation

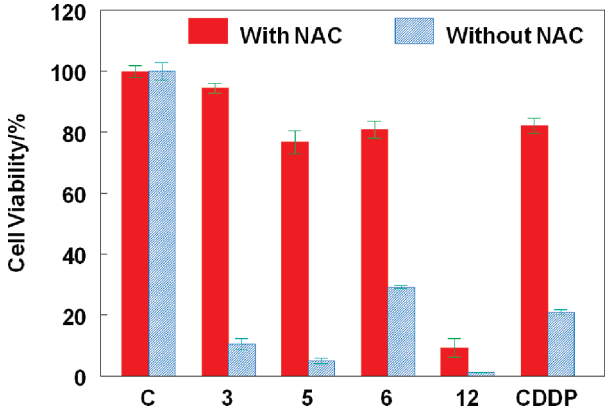


Figure 2. Cell viability of A2780 human ovarian cancer cells after 24 h exposure to osmium complexes or cisplatin followed by 72 h recovery (red bars), and cell viability for cells that were pretreated with 5 mM NAC for 2 h prior to the addition of the osmium complexes or cisplatin (blue bars). Doses of complexes were as follows: [Os(η^6-bip)(azpy-OH)I]PF_6 (**3**), 0.25 μM; [Os(η^6-bip)(azpy-NMe_2)I]PF_6 (**5**), 0.25 μM; [Os(η^6-*p*-cym)(azpy-NMe_2)I]PF_6 (**6**), 0.25 μM; [Os(η^6-*p*-cym)(azpy-NMe_2)Cl]PF_6 (**12**), 2.5 μM; CDDP (cisplatin), 5 μM. The error bars are standard deviations of six replicates. C = control.

effects of iodido complexes **3**, **5**, and **6**. This was shown by their growth levels of >70% when pretreated with NAC. For chlorido complex **12**, the NAC pretreatment and cotreatment had only a small effect on restoring growth (Figure 2). These results suggest that, unlike the azopyridine osmium arene iodido complexes or cisplatin, the cytotoxicity associated with osmium chlorido complex **12** does not appear to depend on the production of reactive oxygen species (ROS) but may be explained by a different mechanism of cytotoxic activity.

To investigate the possibility that the complexes might react directly with NAC and that the effects are not merely due to thiol levels in cells, a solution containing 1.0 mM [Os(η^6-*p*-cym)(azpy-NMe_2)I]PF_6 (**6**) and excess (7.35 mM) NAC in 30% acetone-d_6 and 70% phosphate buffer (10 mM, pH 7.0) was monitored by ^{1}H NMR spectroscopy for 24 h at 310 K. No new peaks appeared in the ^{1}H NMR spectrum (Figure S5), suggesting that complex **6** and probably the other iodido complexes do not readily react with NAC.

Reactions with Glutathione (GSH). Since some phenylazopyridine ruthenium arene complexes appear to oxidize GSH catalytically to form GSSG,[22] similar reactions were studied for the osmium analogues. The ^{1}H NMR spectrum of a solution containing the highly cytotoxic iodido complex [Os(η^6-*p*-cym)(azpy-NMe_2)I]PF_6 (**6**) (100 μM) and a 100× molar excess of GSH (10 mM, to mimic intracellular conditions) showed little change over 24 h. This suggests that complex **6** does not catalytically oxidize GSH, unlike the analogous Ru^{II} complex. The mechanism of cytotoxic activity for these Os^{II} azopyridine complexes therefore appears to be different from that of the Ru^{II} analogues and also different from those bearing hydrolyzable Ru/Os−Cl bonds.

Evaluation of in Vivo Toxicity. Complexes **3** and **6** were selected for further in vivo evaluation based on their promising in vitro activity. On evaluation of their toxicity in a nude mouse tumor HCT-116 xenograft model, the complexes demonstrated negligible deleterious effects at doses up to and including their maximum soluble dose of 40 mg·kg^{-1} (Figure 3). This dose is approximately 6 times higher than the maximum tolerated dose of cisplatin in the same tumor model, and suggests

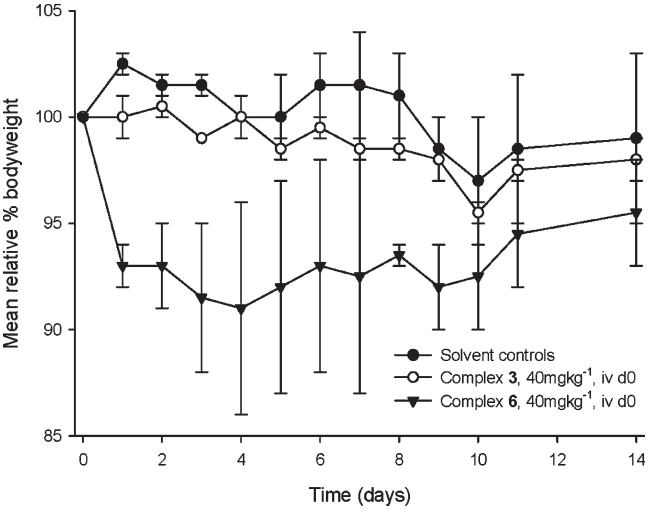


Figure 3. Mean relative body weight curves for complexes **3** and **6** administered intravenously as a single dose on day 0 at their maximum soluble dose of 40 mg·kg^{-1} to mice bearing HCT-116 human tumor xenografts (n = 2). Fluctuations in body weight are well within the normal limits, suggesting that neither complex is toxic.

that with the comparative lack of toxicity of the complexes there is likely to be a much broader therapeutic window. This will be investigated further in extensive in vivo pharmacokinetic and efficacy studies.

Conclusion

Pseudo-octahedral "piano-stool" organometallic osmium arene complexes have potential for exploration as anticancer complexes. They are attractive, since they provide a hydrophobic arene face amenable to a wide variety of substitutions together with three other variable coordination positions. The arene and the other ligands can have a major effect on determining the electron distribution within the complex, controlling their rates of ligand substitution and their redox properties (metal- or ligand-centered). In the present case the chelated azopyridine ligand is a σ-donor and a strong π-acceptor; i.e., there is a strong back-donation of electrons from Os^{II} onto the azopyridine ligand, producing a large effect on overall reactivity. Iodido complexes were more cytotoxic than the analogous chlorido complexes. In addition, iodido complexes containing *p*-hydroxyl or *p*-dimethylamino substituents on the phenylazopyridine chelating ligand (e.g., complexes **3** and **6**) were cytotoxic at nanomolar concentrations toward ovarian, lung, breast, colon, prostate, and bladder human cancer cells, an order of magnitude more potent than cisplatin and (unexpectedly) than their Ru^{II} analogues. These iodido complexes are also inert toward hydrolysis. Interestingly, their cytotoxicity was inhibited by pretreatment of the cells wth *N*-acetyl-L-cysteine, suggesting that reactive oxygen species (ROS) are involved in their mechanism of action, although unlike their Ru^{II} arene azopyridine analogues, the Os^{II} complexes investigated here do not oxidize GSH catalytically. More encouragingly, these Os^{II} complexes exhibited low toxicity and negligible deleterious effects in a HCT-116 tumor xenograft model, indicating that they may exhibit a broad therapeutic window.

Experimental Section

General Information. Details of chemicals and equipment, X-ray crystallography, pH* measurements, cell cultures, *N*-acetyl-L-cysteine (NAC) treatment of cell, determination of IC_{50}

values, and evaluation of in vivo toxicity are in the Supporting Information.

Synthesis. Complexes **1**−**12** were prepared by the same general method: reaction of the appropriate phenylazopyridine derivative with the dimers $[Os(\eta^6\text{-bip})Cl_2]_2$, $[Os(\eta^6\text{-bip})I_2]_2$, $[Os(\eta^6\text{-}p\text{-cym})Cl_2]_2$, or $[Os(\eta^6\text{-}p\text{-cym})I_2]_2$. This is illustrated below for complex **6**. The purities of all compounds prepared were determined to be ≥95% by elemental analysis. The details are in the Supporting Information.

$[Os(\eta^6\text{-}p\text{-cym})(azpy\text{-}NMe_2)I]PF_6$ (6). $[Os(\eta^6\text{-}p\text{-cym})I_2]_2$ (100.0 mg, 0.086 mmol) was dissolved in methanol (50 mL) at 313 K. Azpy-NMe_2 (39.5 mg, 0.175 mmol) in methanol (10 mL) was added dropwise; the solution color changed from orange to blue immediately. The solution was stirred at ambient temperature for 3 h. The volume was reduced to about 10 mL by removal of methanol on a rotary evaporator, and ammonium hexafluorophosphate (141.8 mg. 0.87 mmol) was added. The solution was then left in the freezer for 24 h. Dark colored powder precipitated which was collected by filtration, washed with cold ethanol and diethyl ether, then finally dried in vacuum. Yield: 122.7 mg (87%). ESI-MS calcd for $C_{23}H_{28}IN_4Os$, m/z 679.1; found, 679.0. ^{1}H NMR ($(CD_3)_2CO$): δ 9.43 (d, 1H, J = 6 Hz), 8.65 (d, 1H, J = 8 Hz), 8.29−8.22 (m, 3H), 7.64 (m, 1H), 6.97 (d, 2H, J = 9 Hz), 6.59 (d, 1H, J = 6 Hz), 6.30 (m, 3H), 3.41 (s, 3H), 2.80 (s, 6H), 2.61 (m, 1H), 1.02 (d, 3H, J = 6 Hz), 0.98 (d, 3H, J = 6 Hz) CHN analysis. Found: C, 33.42%; H, 3.28%; N, 6.72%. Calcd for $C_{23}H_{28}F_6IN_4OsP$: C, 33.58% H, 3.43% N, 6.81%.

X-ray Crystallography. X-ray crystallographic data for compounds **2**, **3***, **5**, **8**, **11**, and **14** have been deposited in the Cambridge Crystallographic Data Centre with CCDC references numbers 776271, 776270, 776273, 776268, 776269, and 776272, respectively.

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Supporting Information Available: Details of procedures and experimental data for all synthesized compounds, instrumentation and methods, Table S1, and Figures S1−S6. This material is available free of charge via the Internet at http://pubs.acs.org.

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