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Cancer Cell Cytotoxicity of Cyclometalated Compounds Obtained with Osmium(II) Complexes

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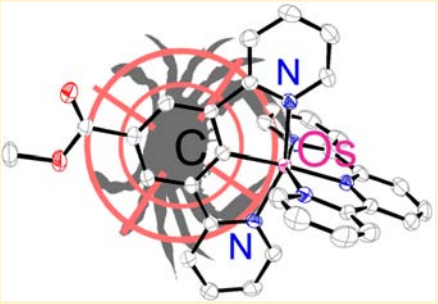
Supporting Information

ABSTRACT: A library of 29 organoosmium compounds has been built up with known and novel cyclometalated compounds obtained with C−N, N^C^N, and C^N^N ligands. All compounds have been tested for their in vitro cytotoxic properties against A172, a tumor cell line derived from a human glioblastoma, this affording a contrasted picture of the activities of the compounds gathered in this study. Some compounds displayed good to excellent activities, some of them showing IC_{50} in the nanomolar range. The level of activity was tentatively correlated to several physicochemical properties of the compounds such as their $E^0_{1/2}(Os^{III/II})$ redox potential and their lipophilicity (log $P_{o/w}$). A parallel with related ruthenium derivatives was tentatively proposed.



INTRODUCTION

The antiproliferative effect against tumor cells of transition metal containing complexes is now well established. Among these complexes, several organometallic compounds obtained via the cyclometalation reaction of N-containing ligands have been shown to be rather efficient as many Au(III),[1] Pd(II),[2] and Pt(II)[3] compounds have been shown to have high cytotoxic activities against several cell lines. We have been involved in this field since we found that several organoruthenium compounds derived from cycloruthenated nitrogen containing ligands are also good candidates for becoming anticancer drugs[4] as they showed most of the usual required properties (in vitro and in vivo) for such purpose. Since 2006, osmium compounds have become a field of growing interest as several studies on osmium complexes showed that these latter compounds can offer interesting alternative to their ruthenium analogues.[5] Inter alia, these osmium-containing compounds also display in vitro antitumor activity as well as interesting reactivity toward DNA. As very recently more and more reports from other groups showed that organometallic compounds obtained via the cyclometalation of N-containing ligands performed via Ru(II) or Ir(III) complexes are indeed good candidates for this antitumor activity,[6] we decided to disclose our own results that we obtained with a library of cyclometalated osmium(II) compounds obtained with such ligands.

EXPERIMENTAL SECTION

Experiments were carried out under an argon atmosphere using a vacuum line. Diethyl ether and pentane were distilled over sodium/benzophenone, dichloromethane, and acetonitrile over calcium hydride and methanol and ethanol over magnesium under argon immediately before use. Chromatography columns were carried out on Merk aluminum oxide 90 standardized. The other starting materials were purchased from Sigma Aldrich, Alfa Aesar, or Strem Chemicals and used as received without further purification.

The dimeric complexes $[(\eta^6\text{-bz})OsCl_2]_2$[7] and $[(p\text{-cym})OsCl_2]_2$[8] were synthesized according to reported procedure. The ligands listed hereafter were synthesized following reported procedures: *N,N*-dimethyl-4-(pyridin-2-yl)benzenamine,[9] 1,3-di(pyridin-2-yl)benzene (N^C(H)^N),[10,11] methyl-3,5-di(2-pyridyl)benzoate (MeO_2C−N^C-(H)^N),[12a−c] 3,5-di(2-pyridyl)toluene (Me-N^C(H)^N),[12c] 6-phenyl-2,2′-bipyridine (C(H)^N^N),[13,14] 4-ethoxycarbonyl-6-phenyl-2,2′-bipyridine (EtO_2C−C(H)^N^N),[12d,e] 4,4′-di(methoxycarbonyl)-6-phenyl-2,2′-bipyridine ($(MeO_2C)_2$-C(H)^N^N).[12e] Osmium complexes listed hereafter were synthesized following reported procedures **1a** $[Os(o\text{-}C_6H_4py\text{-}\kappa C,N)(\eta^6\text{-}C_6H_6)(NCMe)]PF_6$, **2** $Os(o\text{-}C_6H_4py\text{-}\kappa C,N)(\eta^6\text{-}C_6H_6)Cl$, **5a** $[Os(o\text{-}C_6H_4py\text{-}\kappa C,N)(phen)(NCMe)_2]PF_6$, and **9a** $[Os(o\text{-}C_6H_4py\text{-}\kappa C,N)(phen)_2]PF_6$.[15]

The NMR spectra were obtained at room temperature on Bruker spectrometers. ^1H NMR spectra were recorded at 300.13 MHz (AC-300) or 400.13 MHz (AM-400) and referenced to $SiMe_4$. $^{13}C\{^1H\}$ NMR spectra were recorded at 75.48 MHz (AC-300) or 100.62 MHz (AC-400) and referenced to $SiMe_4$. The NMR assignments were supported by COSY spectra for ^1H NMR. The chemical shifts are referenced to the residual solvent peak. Chemical shifts (δ) and coupling constants (J) are expressed in parts per million and hertz, respectively. Multiplicity: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet.

The infrared spectra were recorded on an alpha ATR spectrometer from Bruker Optics and analyzed with OPUS software. UV/vis spectra (absorption spectroscopy) were recorded with a Kontron Instruments UVIKON 860 spectrometer at RT.

ES-MS spectra and elemental analyses were carried out by the corresponding facilities at the Institut de Chimie, Université de Strasbourg, and at the Service Central d'Analyze du CNRS, Vernaison.

Synthesis of the Compounds. We only report below typical synthesis of some of the product described in this paper. The

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syntheses of all other new compounds are given in the Supporting Information (SI).

[Os(η^6-C_6H_6)(NCMe)](2-C_6H_4-2′,5′-C_5H_3N-NMe$_2$-κC,N)PF_6, 1b. To a suspension of $[OsCl(\mu\text{-}Cl)(\eta^6\text{-}C_6H_6)]_2$ (599 mg, 0.881 mmol), NaOH (70 mg, 1.762 mmol) and KPF_6 (649 mg, 3.525 mmol) in 50 mL of acetonitrile was added 4-amino-phenylpyridine (300 mg, 1.762 mmol). The mixture was stirred at 40 °C for 48 h. The solvent was evaporated under vacuum, and the dark residue was dissolved in 20 mL of CH_2Cl_2. The solution was filtered through Al_2O_3, using a 10:1 CH_2Cl_2/NCMe mixture as eluent. The bright yellow fraction was collected and concentrated to about 5 mL. Addition of 50 mL of diethyl ether caused precipitation of a yellow solid (768 mg, 70%). Anal. Calcd for $C_{19}H_{18}F_6N_3POs$: C, 36.60; H, 2.91; N, 6.74. Found: C, 36.32; H, 2.87; N, 6.69. MS (ES, *m*/*z*). Calcd for $C_{19}H_{18}N_3{}^{192}Os$: 480.1116 (M). Found: 480.110. IR (cm^{-1}): 2289 (weak, $\nu_{N\equiv C}$), 836 (strong, ν_{PF}), 565 (medium, ν_{PF}). ^1H NMR (300 MHz, CD_3CN, 300 K), 8.58 (d, 1H, $^3J_{HH}$ = 6.6), 8.01 (d, 1H, $^3J_{HH}$ = 8.8, $^4J_{HH}$ = 2.0), 7.60 (d, 1H, $^3J_{HH}$ = 8.8, $^4J_{HH}$ = 2.0), 7.04−7.14 (m, 3H), 6.43 (dd, 1H, $^3J_{HH}$ = 6.6, $^3J_{HH}$ = 2.0), 5.67 (s, 6H), 5.45 (s, 6H), 2.22 (s, 3H). ^{13}C {^1H} NMR (78 MHz, CD_3CN, 300 K), 167.4, 158.3, 157.3, 156.9, 147.3, 140.9, 131.2, 124.9, 124.7, 110.3, 104.5, 80.6, 45.8.

[Os(terpy)(NCMe)(2-C_6H_4-2′-C_5H_4N-κC,N)]PF_6, 7a. A solution of $[Os(o\text{-}C_6H_4py\text{-}\kappa C,N)(\eta^6\text{-}C_6H_6)(NCMe)]PF_6$ (20 mg, 0.033 mmol) with 2,2′;6′,2″-terpyridine (7.28 mg, 0.031 mmol) in acetonitrile (5 mL) was refluxed for 24 h. The solvent was evaporated under vacuum, and the dark residue was dissolved in 10 mL of CH_2Cl_2. The solution was filtered through Al_2O_3 using a 90:10 CH_2Cl_2/NCMe mixture as eluent. The dark purple fraction was collected and evaporated to dryness under vacuum. Crystallization from acetone/pentane or dichloromethane/pentane (slow diffusion) gave dark purple microcrystals (20 mg, 80%), which were washed with pentane and dried under vacuum. Anal. Calcd for $C_{28}H_{22}F_6N_5POs$: C, 44.04; H, 2.90; N, 9.17. Found: C, 43.81; H, 2.89; N, 8.97. MS (ES, *m*/*z*). Calcd for $C_{28}H_{22}N_5{}^{192}Os$: 620.1490 (M). Found: 620.154. IR (cm^{-1}): 2287 (weak, $\nu_{N\equiv C}$), 830 (strong, ν_{PF}), 562 (medium, ν_{PF}). ^1H NMR (400 MHz, CD_3CN, 300 K): 8.44 (d, 2H, $^3J_{HH}$ = 8.1), 8.28 (d, 2H, $^3J_{HH}$ = 8.2), 8.24 (d, 1H, $^3J_{HH}$ = 7.5), 7.98 (d, 1H, $^3J_{HH}$ = 7.5), 7.82−7.87 (m, 3H), 7.68 (td, 2H, $^3J_{HH}$ = 8.2, $^4J_{HH}$ = 1.4), 7.6 (dd, 1H, $^3J_{HH}$ = 8.1), 7.35 (td, 1H, $^3J_{HH}$ = 7.5, $^4J_{HH}$ = 1.2), 7.24 (td, 1H, $^3J_{HH}$ = 7.5, $^4J_{HH}$ = 1.5), 7.15 (td, 2H, $^3J_{HH}$ = 8.2, $^4J_{HH}$ = 1.5), 6.96 (td, 1H, $^3J_{HH}$ = 7.5, $^4J_{HH}$ = 1.5), 6.63 (d, 1H, $^3J_{HH}$ = 7.5), 6.42 (td, 1H, $^3J_{HH}$ = 7.5, $^4J_{HH}$ = 1.5), 2.11 (s, 3H). ^{13}C {^1H} NMR (100.62 MHz, CD_3CN, 300 K): 156.2, 149.0, 135.5, 135.2, 135.0, 132.8, 129.4, 128.5, 123.6, 120.8, 120.7, 119.9, 3.8.

[Os(NCMe)$_3$(MeO$_2$C−N^C^N)]PF_6, 11a. To a suspension of $[OsCl(\mu\text{-}Cl)(\eta^6\text{-}C_6H_6)]_2$ (100 mg, 0.147 mmol), NaOH (12 mg, 0.294 mmol) and KPF_6 (91 mg, 0.588 mmol) in 10 mL of acetonitrile was added methyl-3,5-(2-pyridyl)benzoate (85 mg, 0.294 mmol). The mixture was refluxed for 72 h under an incandescent lamp irradiation (60 W). The solvent was evaporated under vacuum, and the dark residue was dissolved in 10 mL of CH_2Cl_2. The solution was filtered through Al_2O_3, using a 10:1 CH_2Cl_2/NCMe mixture as eluent. The dark yellow fraction was collected and concentrated to about 1 mL. Addition of 10 mL of diethyl ether caused precipitation of a dark yellow solid (164 mg, 75%). Anal. Calcd for $C_{24}H_{22}F_6N_5O_2POs$: C, 38.55; H, 2.97; N, 9.37. Found: C, 38.54; H, 3.01; N, 9.43. MS (ES, *m*/*z*). Calcd for $C_{24}H_{22}N_5O_2{}^{192}Os$: 604.1388 (M). Found: 604.143. IR (cm^{-1}): 2258 (medium, $\nu_{N\equiv C}$), 1686 (medium, $\nu_{C=O}$), 830 (strong, ν_{PF}), 565 (medium, ν_{PF}). ^1H NMR (300 MHz, CD_3CN, 300 K): 8.98 (d, 2H, $^3J_{HH}$ = 5.5), 8.43 (s, 2H), 8.19 (d, 2H, $^3J_{HH}$ = 8.0), 7.84 (td, 2H, $^3J_{HH}$ = 8.0, $^4J_{HH}$ = 1.5), 7.33 (td, 2H, $^3J_{HH}$ = 5.5, $^4J_{HH}$ = 1.5), 3.93 (s, 3H), 1.98 (s, 3H), 1.97 (s, 6H). ^{13}C {^1H} NMR (78 MHz, CD_3CN, 300 K): 226.2, 168.9, 168.3, 154.7, 146.6, 137.5, 123.7, 123.6, 122.7, 120.3, 52.4, 3.27.

[Os(terpy)(MeO$_2$C−N^C^N)]PF_6, 14a. A solution of $[Os(MeO_2C\text{−}N^C^N)(NCMe)_3]PF_6$ (55 mg, 0.074 mmol) with 2,2′;6′,2″-terpyridine (17.0 mg, 0.074 mmol) in methanol (5 mL) was refluxed for 48 h. The solvent was evaporated under vacuum, and the dark brown residue was dissolved in 10 mL of CH_2Cl_2 and filtered through Al_2O_3 using a 10:0.5 CH_2Cl_2/NCMe mixture as eluent. The purple fraction was collected and evaporated to dryness under vacuum. Crystallization from CH_2Cl_2/pentane or acetone/pentane (slow diffusion) gave dark purple crystals, which were washed with diethyl ether and dried under vacuum (39 mg, 65%). Anal. Calcd for $C_{33}H_{24}F_6N_5O_2POs$: C, 46.21; H, 2.82; N, 8.16. Found: C, 46.05; H, 2.83; N, 8.06. MS (ES, *m*/*z*). Calcd for $C_{33}H_{24}N_5O_2{}^{192}Os$: 714.1545 (M). Found: 714.155. IR (cm^{-1}): 1698 (medium $\nu_{C=O}$), 834 (strong, ν_{PF}), 565 (medium, ν_{PF}). ^1H NMR (300 MHz, CD_3CN, 300 K): 8.91 (s, 2H), 8.73 (d, 2H, $^3J_{HH}$ = 8.1), 8.42 (d, 2H, $^3J_{HH}$ = 8.1), 8.30 (d, 2H, $^3J_{HH}$ = 8.1), 7.81 (t, 1H, $^3J_{HH}$ = 8.1), 7.50−7.59 (m, 4H), 7.06 (d, 2H, $^3J_{HH}$ = 5.9), 6.91 (d, 2H, $^3J_{HH}$ = 5.9), 6.85 (td, 2H, $^3J_{HH}$ = 5.9, $^4J_{HH}$ = 1.5), 6.64 (td, 2H, $^3J_{HH}$ = 5.9, $^4J_{HH}$ = 1.5), 4.04 (s, 3H). ^{13}C {^1H} NMR (78 MHz, CD_3CN, 300 K): 155.4, 151.9, 135.3, 134.7, 131.0, 127.1, 124.3, 123.7, 122.1, 120.9, 120.1, 51.5.

Electrochemical Measurements. Electrochemical experiments were performed with a three-electrode system consisting of a platinum working electrode, a platinum wire counter electrode, and a silver wire as a pseudo- reference electrode. The potentials were referenced to SCE using ferrocene $FeCp_2$ or $[Os(tterpy)_2](PF_6)_2$ as an internal reference. The measurements were carried out under argon, in degassed CH_3CN at 298 K (scan rate: 0.100 $V{\cdot}s^{-1}$) using 0.1 M $[(nBu)_4N](PF_6)$ as the supporting electrolyte. An EG&G Princeton Applied Research Model 273A potentiostat connected to a computer was used for the cyclic voltammetry experiments.

Cell Proliferation Assays. The osmium samples for in vitro tests were obtained from 50 mM stock solutions of the osmium complexes in neat DMSO. The stock solutions were then sequentially diluted with the required amount of cell culture media in order to obtain the studied solutions whose concentration varies from 0.1 to 50 μM. A172 cells were obtained from American Type Cell Culture Collection. Cells were grown in 96-well plates and treated at 70% confluence. After 48 h, the medium was removed and MTT (0.5 mg mL^{-1}) in DMEM was added for 1 h.[16] The medium was removed again and 0.04% HCl in isopropanol was added to dissolve the crystals. Absorption differences were quantified using an Elisa plate reader (Metertech USA) at 490−650 nm. The experiments were repeated at least twice, the mean deviation was determined by considering the extreme values found over all experiments.

Log($P_{o/w}$) Determination. Hydrophobic properties measurements were performed by determining the octanol/water partition $\log(P_{o/w})$ thanks to the method described by Minick et al.[17] and completed by Pomper et al.[18]

The aqueous portion of the mobile phase was prepared by first dissolving the buffer agent 4-morpholinepropanesulfonic acid MOPS (0.02 M) and *n*-decylamine amino modifier (0.15% v/v) in deionized 1-octanol saturated water and then adjusting the pH of this solution to 7.4. The organic portion was prepared by diluting 1-octanol (0.20% v/v) into analytical grade methanol. All mobile-phase solvents were filtered through 0.45 μm filters before use and disgazed continuously during the experiment.

Measurements were performed on a Varian prostar 210 HPLC equipped with a Prostar 335 photodiode array detector and a Prostar 410 autosampler. The stationery phase was a 250 mm × 4.6 mm column packed with 10 Å Kromasil C-8. The different compounds were dissolved into methanol (5.10^{-5} M) and then injected onto the column (5 μL). Column void volume was estimated from the retention time of uracil, which was included as a nonretained internal reference for each injection.[19] The $\log(k'_w)$ was determined by linear extrapolation of $\log(k'_\Phi)$ vs $\Phi_{methanol}$ data acquired in the region $0.50 \leq \Phi_{methanol} \leq 0.85$. The measurements were repeated at least twice, and the mean deviation was determined by considering the extreme values found over all experiments.

RESULTS AND DISCUSSION

Synthesis. Herein we briefly present the synthesis of several novel osmium(II) compounds which all contain a C−Os σ bond that was obtained via a cyclometalation reaction. Since several cycloosmated derivatives have been obtained earlier with 2-phenylpyridine (2-PhPy) (see below), we checked that

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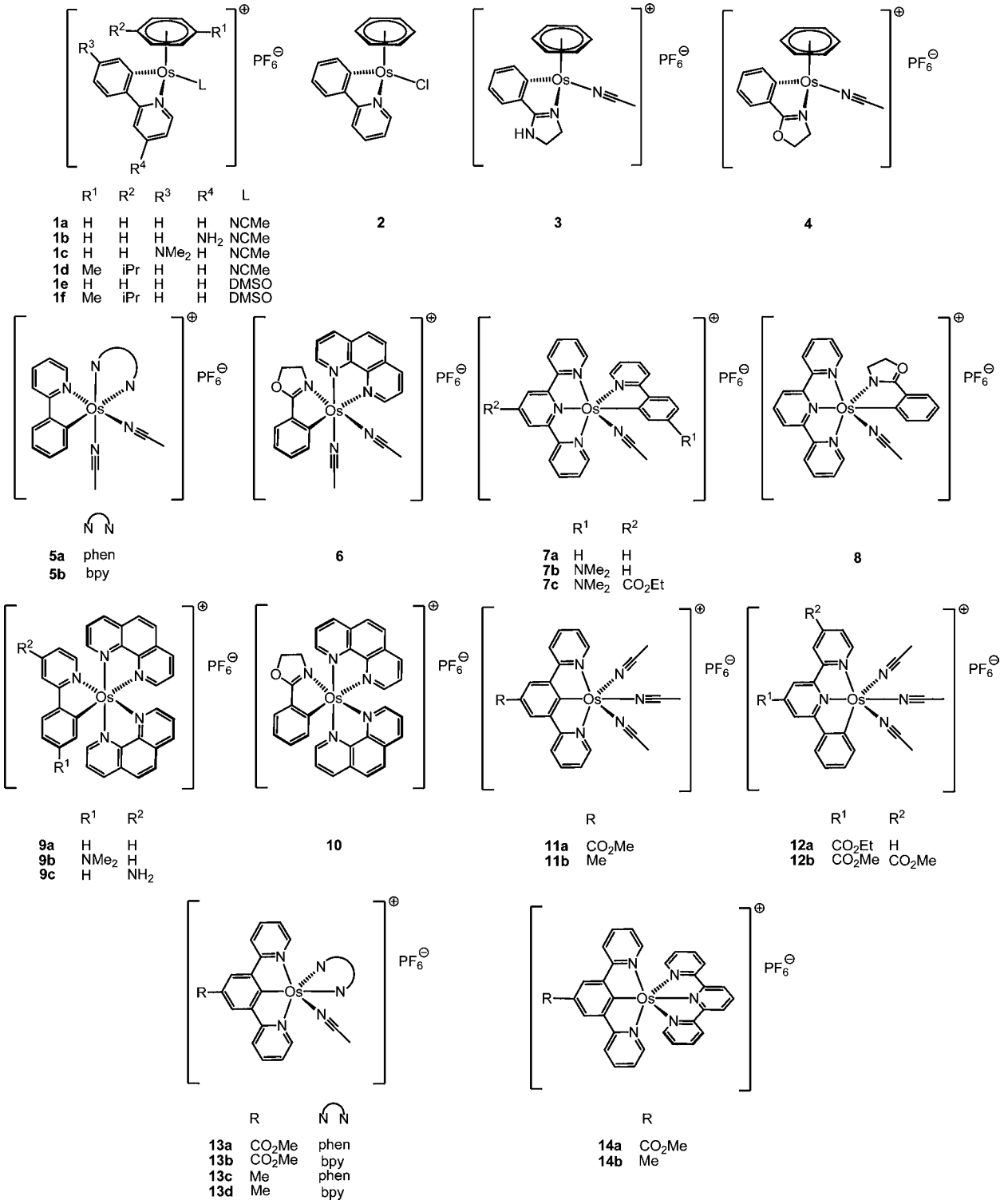
Scheme 1. Chemical Library of Studied Osmacycles



2-phenyl-2-oxazoline (2-PhOx), 2-phenyl-2-imidazoline (2-PhIm), and so-called pincer ligands (N^C(H)^N or C-(H)^N^N) could be cyclometalated by Os(II) accordingly. The coordination to these cycloosmated compounds of a small series of ancillary ligands, which are known to play an important role for the biological properties of related compounds,[4,20] was also investigated this leading to the library of compounds shown in Scheme 1.

Using the same procedure described for ruthenium derivatives,[21] Ryabov et al. have recently successfully cyclometalated 2-phenylpyridine with osmium(II) by C(sp^2)−H activation reactions. These metalacycles have been studied to establish their bioelectrochemical properties with respect to glucose oxidase.[22] Complexes **1a**, **2**, **5a**, and **9a** were prepared according to this procedure and NMR data as well as cyclic voltamperometry data agreed well with those previously reported.[15]

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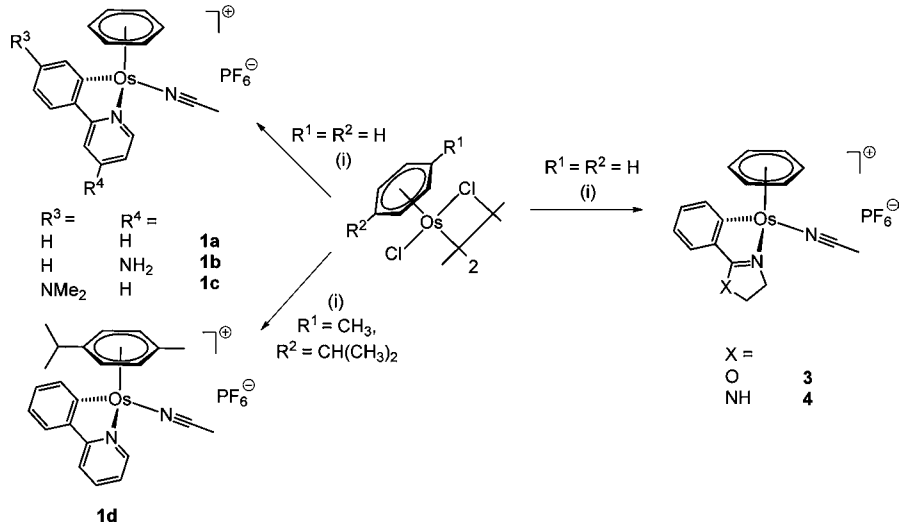
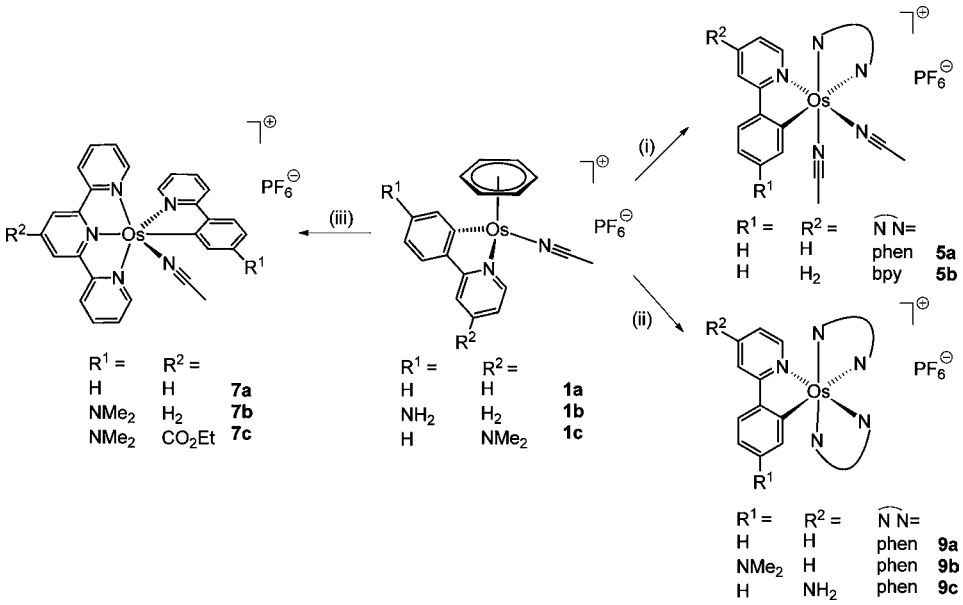
Scheme 2. Synthesis of Cycloosmated Os(II) Piano-Stool Complexes[a]



[a](i) N^C(H), NaOH (2 equiv), KPF_6 (4 equiv), CH_3CN 45 °C, 48 h.

Scheme 3. Synthesis of Os(II) Complexes Containing a 2-Phenylpyridine Cyclometalating Unit[a]



[a](i) N^N (1 equiv), CH_3CN reflux, 24 h. (ii) N^N (2 equiv), CH_3OH reflux, 48 h. (iii) N^N^N, CH_3CN reflux, 72 h.

The synthesis of piano-stool type compounds (**1b–d**) was achieved with the dimer $[Os(\eta^6\text{-arene})Cl(\mu Cl)]_2$ according to Scheme 2. These compounds were obtained in good to excellent yield through the reaction between the appropriate 2-arylpyridine, and the corresponding dimeric starting material in the presence of KPF_6 and NaOH at 40 °C for 48 h. The electrophilic $C(sp^2)$–H cyclometalation of this species by the osmium(II) dimer under mild conditions in acetonitrile afforded stable tetrahedral piano-stool Os(II) complex $[Os(\eta^6\text{-arene})(NCMe)(N^C)]PF_6$ whose η^6-arene ligand was not substituted by acetonitrile, in marked contrast with the ruthenium analogues whose η^6-benzene ligands was readily displaced by acetonitrile. Similarly, cyclometalation of more electron rich heterocyclic ligands such as 2-phenyloxazoline or 2-phenylimidazoline led to tetrahedral piano-stool complexes (**3–4**) without changing the synthetic conditions (Scheme 2). Hence, this synthetic method is probably applicable to a large selection of ligands of this type. However, cyclometalation of even more electron rich ligands such as 2-phenylpyrimidine did not lead to the desired compound.

The piano-stool type complexes **1e** and **1f** could be obtained by the substitution of the MeCN ligand by treating respectively complexes **1a** and **1d** with DMSO (see stability studies). Note that complex **1e** could also be obtained from the previously reported complex **2**.[15] Rapid abstraction of the halide at room temperature was undertaken by treating the precursor **2** with silver hexafluorophophate ($AgPF_6$) in dichloromethane and 1 equiv of DMSO to afford the desired air stable **1e** complex. Using the same method, complex **1f** was synthesized in good yield. Despite the fact that we did not obtain X-ray quality crystals of **1f** to ascertain the mode of coordination of DMSO on the Os center, we believe that this ligand is bound to the

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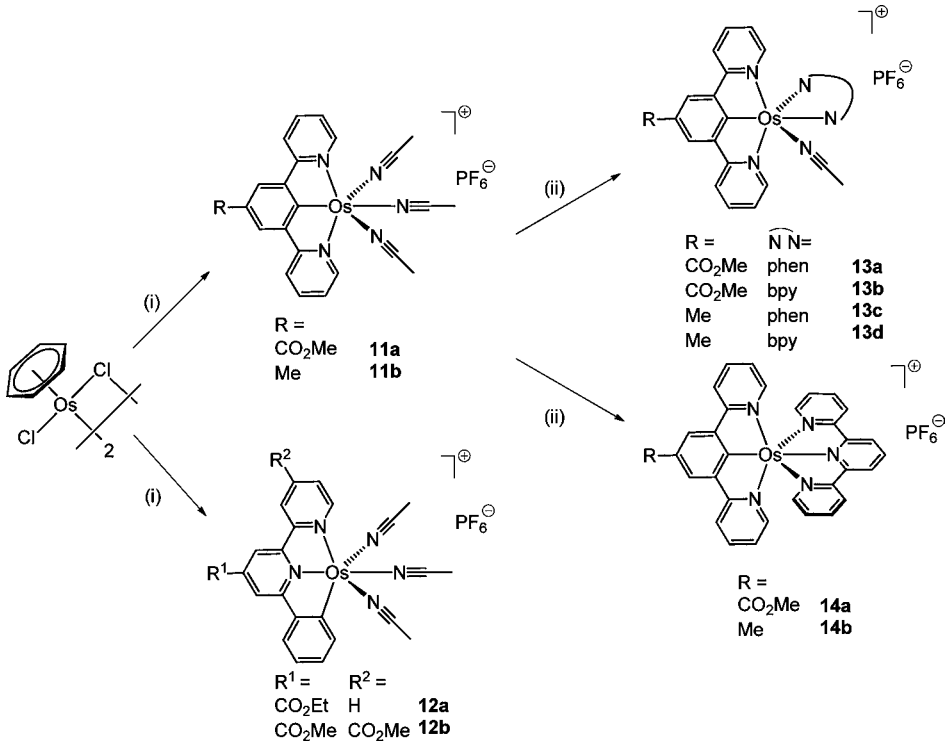
Scheme 4. Synthesis of Os(II) Complexes Containing a N^C^N or N^N^C Cyclometalating Unit[a]



[a](i) N^(R)−C(H)^N, or (R²)−N^(R¹)−N^C(H), NaOH (2 equiv), KPF_6, CH_3CN reflux, *hν* 60 W, 72 h. (ii) N^N or tpy, MeOH, reflux 48 h.

metal via its S atom, as shown by the crystal structure of its ruthenium analogue, **1f-Ru**, see the SI.

Complexes **5** and **6** are genuine octahedral complexes bearing the above-mentioned bidentate cyclometalated ligands and one or two a priori weakly bound acetonitrile ligands. Complexes **9** and **10** consist of octahedral complexes in which three bidentate chelates (one mono anionic N−C chelate and two neutral N−N chelates) are found on the Os center.

Using similar conditions as for the synthesis of the already described **5a** and **9a** complexes,[15] the further reactions of piano-stool complexes with 1 or 2 equiv of 1,10-phenanthroline or 2,2′-bipyridine (N^N) was possible (Scheme 3) and led to the substitution of the η^6-bound benzene to produce octahedral species $[Os(N^C)(N^N)(NCMe)_2]PF_6$ (**5**−**6**) or $[Os(N^C)(N^N)_2]PF_6$ (**9**−**10**), respectively, in MeCN or MeOH. In contrast to the results observed by Ryabov et al., we succeeded in coordinating 1 equiv of 2,2′-bipyridine forming complex **5b** in good yield despite the fact that the coordination of 2,2′-bipyridine occurred less readily than that of 1,10-phenanthroline as already discussed.[23] As observed for ruthenium compounds, the two remaining acetonitrile ligands trans to two sp^2-hybridized N atoms in $[Os(N^C)(N^N)(NCMe)_2]PF_6$ (**5**−**6**) were not labile and hence were difficult to substitute. Once **5a** was obtained, further coordination with one bidentate ligand such as 1,10-phenanthroline did not lead to products **9a**, whatever the conditions. This allowed us to conclude that the compound **5a** was not an intermediate in the synthesis of **9a**. In the synthesis of **9a**, the coordination of the two 1,10-phenanthroline on the Os center should happen simultaneously. Therefore, the two 1,10-phenanthroline ligands should intermediary coordinate in a monodentate manner to **1a** in order to form $[Os(N^C)(N^N)_2(NCMe)_2]PF_6$. From this hypothetical, and somewhat provocative, intermediate, decoordination of the acetonitrile ligands followed by the coordination of the two remaining nitrogen from the two monodentate coordinated 1,10-phenanthroline should occur quickly, affording complex **9a**. Treating the piano-stool complexes **1a** and **4** with 1 equiv of tridentate terpy N^N^N ligand in methanol at reflux afforded, in good yield, osmium complexes bearing mono-, bi-, and tridentate ligands on the same metallic center (**7**−**8**) (Scheme 3).

To achieve the cyclometalation of pincer type ligands (N^C^N) with the same osmium dimeric complexes as above, we had to face 2 major problems: (i) the use of 1,3-di(pyridin-2-yl)benzene led to unselective ortho-metalation (i.e., at the positions −2− or −6−) and (ii) the stronger binding of the η^6-arene ligand to the osmium center might prevent the coordination of the 2 N atoms in trans position to each other as requested for genuine cyclometalated pincer ligands. We solved these two burdens to synthesize **11a,b** by (i) using 1,3,5-substituted arenes such as 3,5-di(pyridyl-2-yl)-toluene and methyl-3,5-di(pyridin-2-yl)benzoate to avoid metalation at the −2− position (ii) by using harsher reaction conditions, i.e., refluxing a solution of the dimeric starting material in acetonitrile for 72 h in the presence of a 60 W lamp.[24]

Using the latter reaction conditions allowed us to cyclometalate substituted 6-phenyl-2′,2″-bipyridine to obtain good yields of **12a,b** (Scheme 4).

Treating tris-acetonitrile complexes **11a**−**b** with 1 equiv of N^N or N^N^N ligand such as 1,10-phenanthroline, 2,2′-bipyridine, and terpy in methanol at reflux temperatures afforded the expected osmium complexes in good yields (**13**−**14**) (Scheme 4). Note that under the same conditions, the substitution of the acetonitrile ligands in complexes **12** by either bidentate N^N or tridentate N^N^N ligands led to

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Table 1. Crystallographic Data for Compounds 1a, 1d, 3, and 4

	1a	1d	3	4
chemical formula	$C_{19}H_{17}N_2Os{\cdot}C_3H_6O{\cdot}F_6P$	$C_{23}H_{25}N_2Os{\cdot}F_6P$	$C_{17}H_{18}N_3Os{\cdot}F_6P$	$C_{17}H_{17}N_2OOs{\cdot}F_6P$
formula mass	666.59	664.62	599.51	600.50
crystal system	monoclinic	monoclinic	orthorhombic	orthorhombic
a/Å	12.8418(4)	26.1320(12)	11.7105(2)	11.6190(3)
b/Å	9.2865(2)	10.2475(3)	9.8464(2)	9.6830(3)
c/Å	21.8939(6)	9.0981(4)	16.3400(4)	16.5337(4)
α/deg	90.00	90.00	90.00	90.00
β/deg	116.439(2)	106.497(2)	90.00	90.00
γ/deg	90.00	90.00	90.00	90.00
unit cell volume/Å^3	2337.88(11)	2336.06(16)	1884.10(7)	1860.15(9)
temperature/K	193(2)	173(2)	173(2)	173(2)
space group	$P2_1/c$	C_2	$Pna2_1$	$Pna2_1$
no. of formula units per unit cell, Z	4	4	4	4
no. of reflections measured	17380	11979	15483	12323
no. of independent reflections	5329	5214	3834	4141
R_{int}	0.0498	0.0485	0.0438	0.0386
final R_1 values ($I > 2\sigma(I)$)	0.0376	0.0343	0.0430	0.0463
final $wR(F^2)$ values ($I > 2\sigma(I)$)	0.0957	0.0906	0.1248	0.1322
final R_1 values (all data)	0.0456	0.0388	0.0470	0.0499
final $wR(F^2)$ values (all data)	0.1143	0.1002	0.1282	0.1355

complex mixtures of products whose structure could not be determined precisely.

X-ray Structural Studies. Structures of selected complexes were confirmed by three-dimensional structure determinations using X-ray diffraction on single crystals of **1a**, **1d**, **3**, **4**, **14a**, and **14b**. The crystallographic data are summarized in Table 1 for piano-stool type complexes and in Table 2 for N^C^N type complexes. The molecular structure of piano-stool complexes **1a**, **4**, and N^C^N complexes **14a** and **14b** are depicted respectively in Figures 1 and 2. Details about selected length and angle of all complexes as well as molecular structure of **1d** and **3** are respectively displayed in Tables S1 and S2 and Figure S1 (SI). The data integration was done using a monoclinic unit cell for **1a**, **1d**, **14b**, a triclinic one for **14a**, and an orthorhombic one for **3** and **4**. In complexes **1a**, **1d**, **3**, and **4**, the Os metal is in the center of a tetrahedron (in which the centroid of the η^6-arene is the fourth coordination site at Os) with bond distances and angles within the usual range for such compounds. The Os−C distances are respectively 2.077(5), 2.074(6), 2.076(12), and 2.095(12) Å larger than their Ru^{II} counterparts with an η^6-coordinated *p*-cymene instead of a benzene.[20] No marked structural differences between the corresponding osmium and ruthenium analogues were observed as already noted by Sadler et al. for Ru- and Os-analogues with ligands different from ours.[25] However, the metal−ligand bond distances are by 0.1−0.2 Å longer in Os complexes because of the larger ionic radius of the latter metal. Note also that in complex **4**, the coordination of the 2-phenyl-2-oxazoline occurs through the nitrogen N1 atom.

Table 2. Crystallographic Data for Compounds 14a and 14b

	14a	14b
chemical formula	$C_{33}H_{24}N_5O_2Os{\cdot}F_6P{\cdot}CH_2Cl_2$	$C_{32}H_{24}N_5Os{\cdot}F_6P{\cdot}CH_2Cl_2$
formula mass	942.67	898.66
crystal system	triclinic	monoclinic
a/Å	9.0078(2)	17.4514(7)
b/Å	13.9936(6)	20.7576(8)
c/Å	14.0300(5)	24.9930(7)
α/deg	77.509(2)	90.00
β/deg	84.338(2)	134.063(2)
γ/deg	73.697(2)	90.00
unit cell volume/Å^3	1655.86(10)	6505.8(4)
temperature/K	173(2)	173(2)
space group	P-1	$P2_1/c$
no. of formula units per unit cell, Z	2	8
no. of reflections measured	16533	105006
no. of independent reflections	7549	19035
R_{int}	0.0577	0.0289
final R_1 values ($I > 2\sigma(I)$)	0.0393	0.0313
final $wR(F^2)$ values ($I > 2\sigma(I)$)	0.0956	0.0549
final R_1 values (all data)	0.0489	0.0516
final $wR(F^2)$ values (all data)	0.1112	0.0659

In the case of complexes **14a** and **14b**, each of the structure displays the expected geometry, with both ligands coordinated in a tridentate, meridional fashion to the Os(II) ion. Each ligand molecule is essentially planar, with the metal atom lying almost in the plane. The central C−Os distance in **14a** (1.971(5) Å) and **14b** (1.96(3) Å) is short while the peripheral N4 and N5-to-osmium bonds in cyclometalated ligand are in the range expected for tridentate terpy ligand (2.060 ± 0.010 Å).[26] Similar effect is observed in the terpy ligand: the Os−N(2) distance of the central pyridine are shorter than the Os−N(1) and Os−(N3) which is typical for coordination of conjugated terpy. Both bond angles and Os−N distances are also comparable to those reported for systems in which either one[27] or two terpy[26] molecules are coordinated to osmium center.

Stability Studies. As the compounds described above were synthesized for evaluating their in vitro cytotoxicity toward cancer cell lines, we checked whether their solutions in DMSO were stable toward substitution reactions. In previous studies, we found that ruthenium arene-type complexes showed

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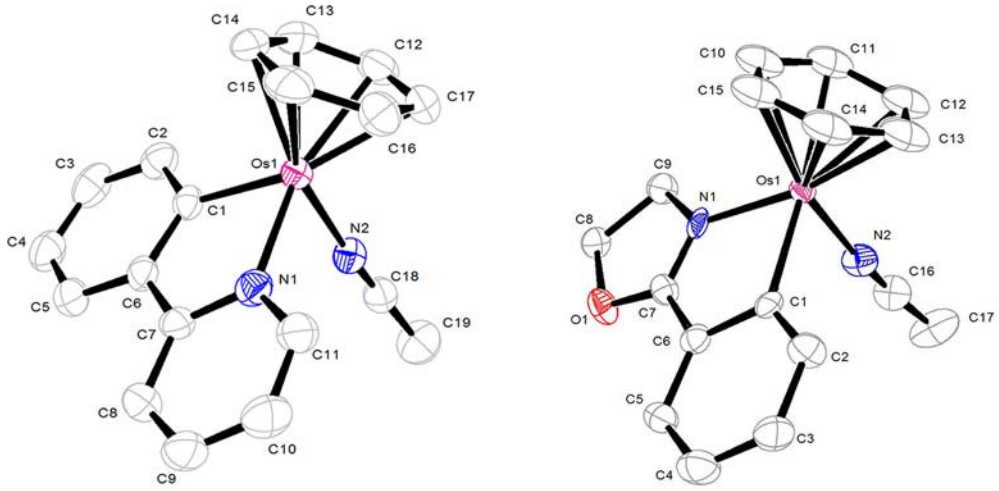

Figure 1. ORTEP diagram of the molecular structure of **1a** (left) and **4** (right). Displacement ellipsoids are drawn at a probability level of 50%. Hydrogen atoms, counteranions and solvent have been omitted for clarity.

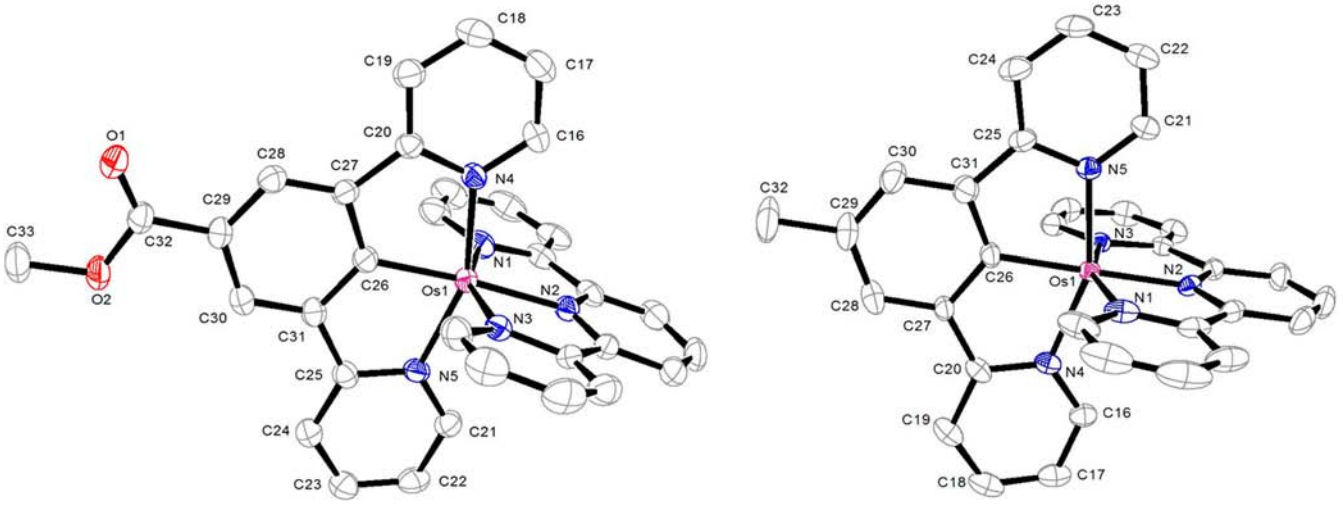

Figure 2. ORTEP diagram of the molecular structure of **14a** (left) and **14b** (right). Displacement ellipsoids are drawn at a probability level of 50%. Hydrogen atoms, counteranions, and solvent have been omitted for clarity.

obvious instability in solution as their ^{1}H NMR spectra in DMSO-d_6 were significantly modified after 3 h.[20] It was thus useful to study the kinetics of substitution of the MeCN ligands by DMSO in osmium and to compare these data with those of their ruthenium analogues. We therefore studied the reactivity of four osmium compounds vs that of their ruthenium analogues by investigating the evolution of these pairs of compounds in DMSO-d_6 by ^{1}H NMR. The first class of complexes which have been studied includes piano-stool complexes (**1d** vs **1d-Ru**) bearing one a priori labile MeCN ligand. Similarly, we examined other types of complexes including cyclometalated N^C^N pincer derivatives displaying one MeCN ligand (**13a** vs **13a-Ru**), cyclometalated C^N compounds complexes comprising two MeCN ligands (**5a** vs **5a-Ru**), and complexes whose coordination sphere is completely saturated (**9a** vs **9a-Ru**).

Considering the first class of piano-stool complexes (**1d** vs **1d-Ru**), we initially checked that the UV/vis spectra of a 10^{-4} M solution in pure CH_3CN did not change with time (after 48 h). This somewhat predictable result proved that neither the N−C nor the *p*-cymene ligands are labile in that medium. In contrast, similar solutions in pure DMSO showed a rapid evolution in the UV region (see SI Figure S2). The ^{1}H NMR spectrum of **1d** and **1d-Ru** in DMSO-d_6 showed the formation of new products after 30 and 6 h, respectively, which could be assigned to the substitution of the MeCN ligand by DMSO-d_6 or by H_2O (present as impurities in DMSO-d_6). Following these first results, kinetic studies were undertaken on samples of **1d** and **1d-Ru** at 10 mM concentration in DMSO-d_6. ^{1}H NMR spectra of **1d** and **1d-Ru** were recorded at room temperature and at −15 °C in the dark on a 500 MHz spectrometer at regular time intervals (depicted in SI Figures S3 and S4). The amount of water contained in DMSO-d_6 did not have any impact, as standard addition of water (0%, 10%, and 25% in volume) did not affect the substitution kinetics thus illustrating the fact that water did not substitute the MeCN ligand. The resonance peaks of the new product corresponded to those of **1f** and **1f-Ru**. Hence, the evolution of **1d** and **1d-Ru** in DMSO-d_6 respectively led to the formation of air stable **1f** and **1f-Ru** complexes bearing a deuterated DMSO ligand. Using mass balance equation, the kinetic law can be rapidly integrated. Supposing a first-order reaction, the evolution of $\ln([\mathbf{1d}]) = f(t)$ and $\ln([\mathbf{1d\text{-}Ru}]) = f(t)$ can be determined by following the kinetics of $\ln([\text{coordinated MeCN}]) = f(t)$ (Figures 3 and 4).

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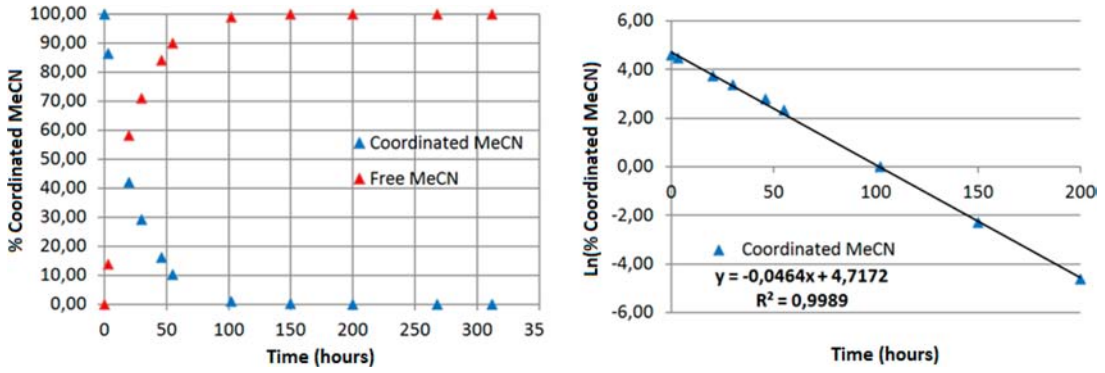

Figure 3. Evolution of **1d** in dry DMSO-d_6 monitored by ^{1}H NMR spectroscopy at room temperature (rt).

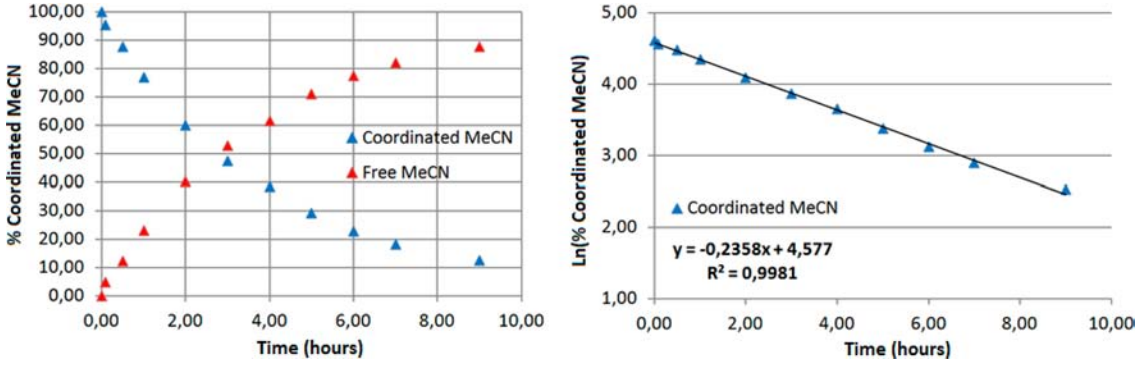

Figure 4. Evolution of **1d-Ru** in dry DMSO-d_6 monitored by ^{1}H NMR spectroscopy at rt.

In all cases, regression on the data gives a straight line with good correlation factor ($R^2 > 0.99$). Hence the speed constant k can be directly extrapolated from the slope of regression line and the value of half-life time can be determined (Table 3). At

Table 3. Recapitulative Table of k and $t_{1/2}$ Values for 1d and 1d-Ru

	1d	1d-Ru
k (min^{-1} rt)	*2.80* ± 0.5	*14.15* ± 0.1
$t_{1/2}$ (at rt)	*14 h 56 min*	2 h 56 min
$t_{1/2}$ (at −15 °C)	*433 h 13 min*	41 h 45 min

room temperature, the kinetics of substitution of one MeCN ligand appears to be 5 times slower for the osmium complexes by comparison to its ruthenium analogue suggesting that the osmium compounds are more inert toward substitution reactions.

Following the same strategy, it was a tempting challenge to perform similar studies on complexes **13a** vs **13a-Ru** bearing one a priori weakly bound MeCN. The exchange of MeCN by DMSO for this latter compound was slower than for cyclometalated piano-stool type complexes, hence indicating that here also the MeCN ligand is strongly bound to the metal center. Regression on the data ln([coordinated MeCN]) = $f(t)$ gave a straight line suggesting a first-order reaction. At room temperature, the mean value of the kinetic constant was approximately $k = 0.186 \pm 0.001$ min^{-1}, and the value of half-life time was approximately $t_{1/2} = 9$ days and 19 h. The same product kept at −15 °C for 15 days did not show any modification of its ^{1}H NMR spectrum. ^{1}H NMR spectra of **13a** in DMSO-d_6 at room temperature did not show any modification after 9 days.

Previous studies from our laboratory[20] on **5a-Ru** that were now extended to the osmium analogue **5a** (see above) indicated that the two acetonitrile ligands are relatively strongly bound to the metal center because the substitution of these two MeCN ligands by 1,10-phenanthroline in refluxing methanol to afford **9a-Ru** and **9a**, respectively, did not occur. In **5a-Ru**, the exchange of MeCN by DMSO was relatively slow, this demonstrating that the MeCN ligands were indeed strongly bound to the metal center. At room temperature, the mean value of the kinetic constant was approximately $k = 0.078 \pm 0.001$ min^{-1} and the value of half-life time was approximately $t_{1/2} = 22$ days. The same product kept at −15 °C for 15 days did not show any modification of its ^{1}H NMR spectra. Therefore, in this bis-MeCN type complexes, the MeCN ligand trans to sp^2-hybridized N atoms are not labile. ^{1}H NMR spectra of **5a** in DMSO-d_6 at room temperature did not show any modification after 15 days, suggesting again that the substitution is much slower for the heavier osmium congeners.

All other complexes whose coordination sphere is completely saturated (especially **9a** vs **9a-Ru**) showed excellent stability toward ligand substitution.

In vitro Cell Growth Inhibition. To evaluate the antitumor potential of the various osmium-derived compounds, we analyzed their effect on cell proliferation. We have chosen a glioblastoma cell line as these cancers represent yet one of the most difficult challenge for chemotherapy treatment. Although there are several gold standards for glioblastoma treatment, such as Temozolomide, the survival rate at 5 years remains particularly low (around 20%). Previous studies in our laboratories indicated that ruthenium derived compounds were more efficient on glioblastoma compared to cisplatin. Therefore, we decided to asses whether osmium-derived compounds would retain similar properties. Interestingly, in

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our condition the IC_{50} of temozolimide was in the range of 50 μM on the glioblastoma cell lines tested in vitro, an efficacy much lower compared to our osmium compounds.

A172 cells derived from a human glioblastoma were treated with different doses of complexes from our library or with cisplatin and the cell viability was determined by measuring a specific cellular enzymatic activity of the remaining living cells after 48 h (MTT test). The results obtained with this cell line are gathered in Table 4. Comparing these data with those of the cycloruthenated compounds,[4,20] we found that exchanging the metal center for osmium afforded complexes displaying good to very good cytotoxicities against the A172 cell line. We first tested the complexes **1**−**4** that are structurally related to piano-stool complexes. The antiproliferative activity of **1d** is equivalent to its ruthenium counterpart **1d-Ru**.

Substitution of MeCN ligand in **1a** and **1d** by DMSO led to noncytotoxic compounds **1e** and **1f**. This loss of activity may be imputable to an incresase of the redox potential (+237 mV both for **1e** and **1f**). Replacement of 2-phenylpyridine by more electron-rich ligands in **3**−**4** led to less stable compounds that are more likely to be oxidized and/or undergo exchange of the MeCN ligand with DMSO. The IC_{50} of **3**−**4** are quite high, however, these data may not be representative of the initial composition of the solution.

Octahedral compounds **5**−**10** bearing previously mentioned bidentate cyclometalated ligands show promising effects for their in vitro activities displaying IC_{50} in the order of magnitude of their ruthenium counterparts. Indeed, **5a** and **9a** display respectively similar behaviors toward A172 cell line as their corresponding ruthenium analogues **5a-Ru** (IC_{50} = 5 μM ± 0.5) and **9a-Ru** (IC_{50} = 0.5 μM ± 0.1). However, modifying the structure of the 2-phenylpyridine with an electro-donating group (**7b**, **9b**), exchanging the 2-phenylpyridine for a more electron-rich 2-phenyloxazoline (**6**) or by substituting the 1,10-phenanthroline and a MeCN ligands by 2,2′;6′,2″-terpyridine (**7**−**8**) did not allow us to rationalize the in vitro results through simple structure−activity relationship (SAR). This class of organometallic osmium complexes seems to have new and unusual features, worthy of further exploration for the design of novel anticancer compounds whose activity relationships will be based on their physicochemical properties (PAR = property−activity relationship) rather than on their structures.

Complexes **11** and **12** deriving from pincer ligands in conjunction to three a priori labile monodentate acetonitrile ligands displayed weak or no cytotoxic activity at all. The absence of activity in complexes **11** can be assigned to the substitution of one or several acetonitrile ligands (especially those trans to the metal−carbon bond), which may, again, not be representative of the initial composition of the solution. Nevertheless, the other complexes (**13** and **14**) bearing a tridentate N^C^N in conjunction to a bidentate 1,10-phenanthroline or a tridentate 2,2′;6′,2″-terpyridine displayed good to very good cytotoxicity passing, for two of them, the symbolic barrier of the nanomolar range for their IC_{50}. Note that substitutions of the N^C^N cyclometalated ligand had little effect on the cytotoxicity of the complexes. For instance, the exchange of CO_2Me group by Me group on the cyclometalated ring did not show any meaningful effect.

Physico-chemical Properties. Given that we did not find an obvious relationship between the structure of the complexes and their cytotoxicity, we checked whether the redox potential and the lipophilicities (Table 4) of our complexes could be somehow correlated with their in vitro activity.

Table 4. Cytotoxicity, $Os^{III/II}$ Redox Potential, and Lipophilicity of the Compounds of the Library

complexes	IC_{50} (μM) A172	$E^{\circ}_{1/2}$ Os(III/II) (±5 mV)	$E^{\circ}_{1/2}$ L^+/L^- (±5 mV)	$Log(P_{o/w})$
1a	**10** ± 0.5	**1250**		
1b	**90** ± 1.0	**1600**	1164 (NH_2^+/NH_2)	
1c	**60** ± 5.0	**1493**	739 (NMe_2^+/NMe_2)	
1d	**10** ± 0.5	**1196**		**0.74** ± 0.03
1e	**300** ± 5.0	**1487**		
1f	**300** ± 5.0	**1433**		
2	**22** ± 1.0	**722**		
3	**100** ± 5.0	**1030**		
4	**90** ± 5.0	**1234**		
5a	**2.0** ± 0.2	**280**		**1.15** ± 0.05
5b	>25	**245**		**0.50** ± 0.03
6	**18** ± 2.0	**176**		**1.13** ± 0.07
7a	**14** ± 0.5	**195**		**1.42** ± 0.05
7b	**3.5** ± 5.0	**131**	886 (NMe_2^+/NMe_2)	**1.78** ± 0.05
7c	**2.25** ± 0.1	**143**	884 (NMe_2^+/NMe_2)	**2.23** ± 0.06
8	**5.0** ± 0.2	**279**		**1.52** ± 0.06
9a	**0.4** ± 0.2	**300**		**2.43** ± 0.04
9b	**1.25** ± 1.0	**151**	885 (NMe_2^+/NMe_2)	**2.64** ± 0.05
9c	**8.5** ± 5.0	**143**	1305 (NH_2^+/NH_2)	**0.80** ± 0.05
10	**0.75** ± 0.3	**160**		**2.50** ± 0.06
11a	**320** ± 0.1	**359**		**0.45** ± 0.08
11b	**375** ± 0.1	**213**		**0.85** ± 0.08
12a	**21** ± 0.1	**536**		**1.91** ± 0.05
12b	**62** ± 0.1	**602**		**2.10** ± 0.05
13a	**1.6** ± 0.2	**330**		**1.77** ± 0.05
13b	**3.8** ± 0.3	**330**		**1.20** ± 0.05
13c	**3.6** ± 0.2	**204**		**2.48** ± 0.04
13d	**4.1** ± 0.2	**203**		**2.10** ± 0.05
14a	**0.8** ± 0.1	**454**		**1.95** ± 0.03
14b	**0.3** ± 0.1	**302**		**2.64** ± 0.03
cisplatin	**5.0** ± 0.5			

To emphasize the importance of the presence of a C−Os bond and that of $Os^{III/II}$ redox potential in the cytotoxicity of the compounds, we measured and compared the electrochemical properties of the different compounds by cyclic

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voltammetry in a 0.1 M $[n\text{-}Bu_4N][PF_6]$ electrolyte. The electrochemical data for the different complexes are summarized in Table 4.

Osmium piano-stool complex **1**−**4** displayed an irreversible single electron oxidation wave assigned to $Os^{II} \rightarrow Os^{III}$ process with $E^{\circ}_{1/2}$ potential values comprised between 1.0 and 1.7 V (vs SCE). In all other cases (**5**−**14**), the anodic region of the cyclic volt-amperograms was dominated by reversible waves corresponding to the one-electron oxidation of the Os(II) state with $E^{\circ}_{1/2}$ potential values between 0.13 and 0.65 V (vs SCE). However, cathodic region exhibited sometimes poorly defined or irreversible waves resulting from the reduction of the ligands (L^+/L). The data clearly showed that molecules having a red-ox potential in the range 0.3−0.6 V (vs SCE) displayed the lowest IC_{50}. Hence, the red-ox properties may be involved in the biological activity given that both oxidation states of osmium (II and III) are accessible under physiological conditions.

In pursuing the long-term goal of elucidating the relationship between $Os^{III/II}$ redox potential and cytotoxicity, and hence establishing a property activity relationship for osmium complexes, Keppler et al. reported several osmium(III) complexes comprising azole heterocycles[28] with no evidence of a correlation between antiproliferative activity and redox potential.

Other key factors influencing the cytotoxicity such as the lipophilicity, cellular uptake, and interaction with molecular target should also be taken into account in order to develop and assess a PAR model suitable to predict the cytotoxicity of the future drugs from their biophysicochemical properties. In order to see if small variations of other physicochemical parameters can allow to assess a possible relationship between the biological activity and the lipophilicity, the partition coefficients $\log(P_{o/w})$ were determined by HPLC using *n*-octanol/water partition. The measurements were repeated at least twice, and the mean deviation was determined by considering the extreme values found over all experiments. The $\log(P_{o/w})$ data for the different complexes are summarized in Table 4. Twelve compounds, (**5a**, **7a**−**b**, **8**, **9a**−**b**, **10**, **13a**−**d**, and **14a**−**b**), showed good to very good activities with IC_{50} below 5.0 μmol, and it appeared that most of the most active have $\log(P_{o/w})$ close to or above 2, a value which is in line with that of the most active cytotoxic ruthenium derivatives or cytotoxic related iridium complexes[6,20,29] whereas the compounds having a $\log(P_{o/w})$ value <1 displayed most of the time weak or no cytotoxic activities at all.

CONCLUSION

This study has shown that large libraries of cycloosmated compounds are indeed accessible via the straightforward intramolecular CH activation reaction of a selection of N-ligands. Many modifications on either the C−N ligands or the other ancillary ligands are thus available in order to modify the properties of the compounds susceptible to be checked for their biologic activity. The osmium-derived compounds are more stable than their ruthenium analogues with respect to the substitution reactions of apparently weakly bound ligands such as acetonitrile or η^6-arenes. Despite the fact that their $E^{\circ}_{1/2}(Os^{III/II})$ potential is lower than that of their ruthenium counterparts, we never observed their oxidation in air either as solids (at solid state) or even in solutions. A majority of the synthesized compounds show good to very good in vitro cytoxicities against the tumor cell line we have used for this study. The compounds displaying an $E^{\circ}_{1/2}$ redox potential in the range 0.2−0.5 V and a $\log(P_{o/w})$ around 2 are among the most active of the series. It also appeared clearly several times that the compounds, which were shown to be the more reactive toward substitution reactions of a ligand such as acetonitrile were the least cytotoxic of the series. This result is in line with what we observed earlier for cycloruthenated derivatives, and it indicates that the most likely species that are active are those in which the coordination sphere of the metal has not changed prior to their penetration in the cells. It is obviously too early to try to rationalize further our data and to speculate about their mode of action on tumor cells. Our results about the importance of the $E^{\circ}_{1/2}(Os^{III/II})$ potential, however, lead us to believe that the mechanism involved for the tumor cell deaths is akin to that of the ruthenium species, i.e. that they probably strongly modify the metabolism of the cells while interacting with several oxido-reductase enzymes. This hypothesis is further supported by our recent finding that cycloruthenated compounds induced radical oxidized species in cancer cells.[30]

ASSOCIATED CONTENT

Supporting Information

Ortep diagrams of the molecular structure of **1d** and **3**, selected bond lengths and angles for **1a**, **1d**, **3**, **4**, **14a**, and **14b**, the evolution of **1d-Ru** and **1d** in DMSO monitored by UV/vis, kinetic studies of **1d-Ru** and **1d** in DMSO monitored by NMR, and the protocol used for the synthesis of the new compounds not described in the paper.This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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