Electronic Supporting Information

Osmium(VI) Nitrido Complexes bearing Azole Heterocycles: A New Class of Antitumor Agents†

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Experimental

Materials

The starting materials $[^{n}Bu_4N][Os^{VI}(N)Cl_4]$ and ^{15}N-labelled $[^{n}Bu_4N][Os^{VI}(^{15}N)Cl_4]$ were prepared by literature procedures.[1,2] 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and other chemicals (reagent grade) were purchased from Sigma-Aldrich and used as received. Materials for cell culture medium constituents and phosphate-buffered saline (PBS) (Gibco BRL) were purchased from Invitrogen.

Cell lines used in this work including cervical epithelioid carcinoma (HeLa), hepatocellular carcinoma (HepG2), myelogenous leukemia (K562), and breast ademocarcinoma (MCF-7) were purchased from American-Type Culture Collection (ATCC), and were maintained in tissue culture at 37 $^{\circ}$C in a humidified chamber containing 5% CO_2. HeLa and MCF-7 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco). HepG2 and K562 were cultured in Roswell Park Memorial Institute (RPMI 1640; Gibco), supplemented with 10% fetal bovine serum (FBS) in the presence of antibiotic-antimycotic solution (Gibco). Cyclin D3, cyclin A, Cdk2 and $p19_{skp1}$ (mouse, dilution 1:1000, BD Biosciences), p21 (rabbit, dilution 1:500, Santa Cruz), β-actin (mouse, dilution 1:3000, Sigma-aldrich) were used as primary antibodies and ECL mouse lgG and ECL rabbit lgG (dilution 1:2000, GE Healthcare) were used as secondary antibodies.

Instrumentation

ESI mass spectra were recorded on a PE-SCIEX API 365 triple quadruple mass spectrometer. Elemental analysis was done on an Elementar Vario EL Analyzer. Infrared spectra were obtained from KBr plates using a Nicolet 360 FT-IR spectrophotometer. ^{1}H NMR spectra were obtained on a Bruker Superconducting-Magnet High-Field (400MHz) NMR spectrometer. UV/Vis spectra were recorded with a Perkin-Elmer Lamda 19 spectrophotometer in 1 cm quartz cuvettes. Cyclic voltammetry (CV) was performed with a CH Instrument model 600C series electrochemical analyzer/workstation using a glassy carbon working electrode, a $Ag/AgNO_3$ (0.1 M in CH_3CN) reference electrode, a Pt wire counter electrode with ferrocene (Cp_2Fe) as the internal standard. The $E_{1/2}$ values were reported with reference to $Cp_2Fe^{+/0}$. Conductivity measurements were done on a Cole-Parmer 01481-61 conductivity meter. The osmium contents in the samples were determined by ICP-MS (PE Elan 6100 DRC). Cytotoxicity of the osmium complexes was determined by reading the absorbance at 570 nm on a multiplate reader (Perkin-Elmer FusionTM α-FP). Cell cycle analysis and apoptosis analysis were analyzed by flow cytometry FACS Calibur,

BD. Immunofluorescence images were recorded using Leica TCS SPE confocal laser scanning microscope. The nitrocellulose blotting membrane for western blot analysis was visualized using high performance laser gel and bolt imager, Fujifilm LAS-4000. The gel for DNA migration study was stained with Gel Red and photographed with UV illumination, Bio-Rad Gel Doc.

X-ray crystallography

Suitable single crystals were mounted with glue at the end of a glass fiber. X-ray diffraction data were collected on an Oxford Diffraction Gemini S Ultra 4-circle kappa diffractometer with a 92mm diagonal Sapphire CCD detector using monochromatized Mo-K_α radiation (λ = 0.71073 Å) or Cu-K_α radiation (λ = 1.54178 Å) diffractometer. Absorption corrections were done by the multiscan method. The structures were resolved by the heavy-atom Patterson method or direct methods and refined by full-matrix least-squares using SHELX-97 and expanded using Fourier techniques.[3,4] All non-hydrogen atoms were refined anisotropically. H atoms were generated by the program SHELXL-97. The positions of H atoms were calculated based on riding mode with thermal parameters equal to 1.2 times or 1.5 times that of the associated C atoms and 1.2 times that of the associated N atoms , all these were participated in the calculation of final R-indices. All calculations were performed using the teXsan crystallographic software.[5]

Syntheses of Complexes

$[Os^{VI}(N)Cl_3(Hpz)_2]$ (1) Pyrazole (95 mg, 1.4 mmol) was added to a solution of $[^nBu_4N][Os^{VI}(N)Cl_4]$ (200 mg, 0.34 mmol) in dichloromethane (10 mL) at room temperature. The mixture was stirred for overnight. The resulting pale orange solid was filtered, washed with dichloromethane (1 mL), diethyl ether (10 mL) and then was collected and air-dried. Recrystallization was done by slow diffusion of diethyl ether into a methanol solution of **1**. Yield: 43%. IR (KBr, cm^{-1}): $\nu(Os{\equiv}^{14}N)$ 1070, $\nu(Os{\equiv}^{15}N)$ 1036, ν(C=N) 1484, ν(N–H) 3140; Anal. calcd. for $C_6H_8N_5Cl_3Os$: C, 16.13; H, 1.80; N, 15.68 %. Found: C, 16.43; H, 1.82; N, 15.59 %. ^{1}H NMR (400 MHz, CD_3OD): δ *trans*-form: 8.60 (s, 2H); 8.10 (s, 2H); 6.80 (s, 2H). ESI-MS (acetone): *m/z* = +412, $[M - Cl]^+$.

$[Os^{VI}(N)Cl_3(Hind)_2]$ (2) Indazole (161 mg, 1.36 mmol) was added to a solution of $[^nBu_4N][Os^{VI}(N)Cl_4]$ (200 mg, 0.34 mmol) in ethanol (10 mL) and the mixture was refluxed for 4 h. The resulting light brown precipitate was filtered off, washed with ethanol (1 mL), diethyl ether (10 mL) and then air-dried. Yield:

52%. Single crystals suitable for X-ray crystallography were obtained by slow diffusion of diethyl ether into the methanol solution of **2** at room temperature. IR (KBr, cm^{-1}): ν(Os≡^{14}N) 1080, ν(Os≡^{15}N) 1040, ν(C=N) 1630, ν(N–H) 3130; Anal. calcd. for $C_{13}H_{11}N_2O_3ClOs$: C, 30.75; H, 2.21; N, 12.81%. Found: C, 31.15; H, 2.29; N, 12.83%. ^{1}H NMR (400 MHz, CD_3OD): δ *trans*-form: 9.27 (s, 2H); 8.07（d, J = 8.1 Hz, 2H); 7.87 (d, J = 7.8 Hz, 2H); 7.76（t, J = 7.8 Hz, 2H); 7.47 (t, J = 7.5 Hz, 2H). ESI-MS (MeOH): m/z = +512, $[M - Cl]^+$.

[Os^{VI}(N)Cl$_3$(5-MeHpz)$_2$] (3) To a solution of [^{n}Bu$_4$N][Os^{VI}(N)Cl$_4$] (250 mg, 0.43 mmol) in ethanol (8 mL) was added 3-methylpyrazole (67 μL, 0.77 mmol) and the mixture was stirred for 1 d at room temperature. To the concentrated mixture (ca. 1 mL) was added diethyl ether to give a brick red solid, which was filtered, washed with dichloromethane (1 mL), diethyl ether, and then air-dried. Yield: 38%. Single crystals suitable for X-ray crystallography were obtained by slow diffusion of diethyl ether into the methanol solution of **3**. IR (KBr, cm^{-1}): ν(Os≡^{14}N) 1060, ν(C=N) 1500, ν(N–H) 3220, 3320; Anal. calcd. for $C_8H_{12}N_5Cl_3Os$: C, 20.24; H, 2.55; N, 14.75 %. Found: C, 20.62; H, 2.57; N, 14.76 %. ^{1}H NMR (400 MHz, CD_3OD): δ *trans*-form: 8.49 (d, J = 8.5 Hz, 2H); 6.64 (d, J = 6.6 Hz, 2H); 2.62 (s, 6H). ESI-MS (MeOH): m/z = +440, $[M - Cl]^+$.

[Os^{VI}(N)Cl$_3$(3,5-Me$_2$Hpz)$_2$] (4) This purple-red powder was prepared by a procedure similar to that for **1** instead of using 3,5-dimethylpyrazole and ethanol (10 mL) as solvent. Yield: 58%. Single crystals suitable for X-ray crystallography were obtained by slow diffusion of diethyl ether into the methanol solution of **4**. IR (KBr, cm^{-1}): ν(Os≡N) 1070, ν(C=N) 1470, ν(N–H) 3112; Anal. calcd. for $C_{10}H_{16}N_5Cl_3Os$: C, 23.89; H, 3.21; N 13.93%. Found: C, 23.82; H, 3.25; N, 13.97%. ^{1}H NMR (400 MHz, CD_3OD): δ *trans*-form: 6.17 (s, 2H); 2.84 (s, 12H); *cis*-form I: 6.33 (s, 2H); 2.87 (s, 6H); 2.25 (s, 6H); *cis*-form II: 6.29 (s, 2H); 2.42 (s, 6H); 2.05 (s, 6H). ESI-MS (acetone): m/z = +468, $[M - Cl]^+$.

[Os^{VI}(N)Cl$_3$(Him)$_2$] (5) Imidazole (23 mg, 0.34 mmol) was added to a solution of [^{n}Bu$_4$N][Os^{VI}(N)Cl$_4$] (200 mg, 0.34 mmol) in ethanol (10 mL) and the mixture was refluxed for overnight. The resulting light brown precipitate was filtered off, washed with ethanol (1 mL), diethyl ether (1 mL), and then air-dried. Yield: 32%. IR (KBr, cm^{-1}): ν(Os≡N) 1070, ν(C=N) 1484, ν(N–H) 3321; Anal. calcd. for $C_6H_8N_5Cl_3Os$: C, 16.13; H, 1.80; N, 15.68%. Found: C, 16.24; H, 1.80; N, 15.47%. ^{1}H NMR (400 MHz, CD_3OD): δ *trans*-form: 8.88 (s, 2H); 7.50 (s, 4H). ESI-MS (acetone/H_2O): m/z = +412, $[M - Cl]^+$.

[Os^{VI}(N)Cl$_3$(1-Meim)$_2$] (6) 1-Methylimidazole (67 μL, 0.82 mmol) was added to a solution of [^{n}Bu$_4$N][Os^{VI}(N)Cl$_4$] (268 mg, 0.46 mmol) in ethanol (10 mL) and the mixture was stirred for 2 d. The resulting dark red precipitate was filtered off, washed with ethanol (1 mL), diethyl ether (1 mL), and then

air-dried. Yield: 76%. IR (KBr, cm^{-1}): *v*(Os≡N) 1080, *v*(C=N) 1520; Anal. calcd. for $C_8H_{12}N_5Cl_3Os$: C, 20.24; H, 2.55; N, 14.75%. Found: C, 20.26; H, 2.53; N, 14.37%. ^1H NMR (400 MHz, CD_3OD): δ *trans*-form: 8.98 (s, 2H); 8.04 (s, 2H); 7.57 (s, 2H); 4.00 (s, 6H); *cis*-form I: 8.87 (d, *J* = 8.9 Hz, 1H); 8.41 (d, *J* = 8.4 Hz, 1H); 8.0 (s, 1H); 7.59 (s, 1H), 7.46 (d, *J* = 7.4 Hz, 1H); 7.33 (d, *J* = 7.3 Hz, 1H); 3.97 (s, 3H); 3.967 (s, 3H); *cis*-form II: 8.71 (d, *J* = 8.7 Hz, 1H); 8.58 (d, *J* = 8.6 Hz, 1H); 7.85 (s, 1H); 7.69 (s, 1H); 7.51 (s, 1H); 7.4 (s, 1H); 3.964 (s, 3H); 3.93 (s, 3H). ESI-MS (MeOH): *m/z* = +440, $[M - Cl]^+$.

$[Os^{VI}(N)Cl_3(2\text{-MeHim})_2]$ (7) The brown powder was prepared by a procedure similar to that for **1** using 2-methylimidazole. Yield: 32%. IR (KBr, cm^{-1}): *v*(Os≡N) 1074, *v*(C=N) 1487, *v*(N–H) 3235; Anal. calcd. for $C_8H_{12}N_5Cl_3Os$: C, 20.24; H, 2.55; N, 14.75%. Found: C, 20.32; H, 2.50; N, 14.86%. ^1H NMR (400 MHz, CD_3OD): δ *trans*-form: 7.40 (s, 4H); 2.63 (s, 6H); *cis*-form I: 7.34 (d, *J* = 7.3 Hz, 2H); 7.31 (d, *J* = 7.3 Hz, 2H); 2.88 (s, 6H). ESI-MS (MeOH): *m/z* = +440, $[M - Cl]^+$.

$[Os^{VI}(N)Cl_3(1\text{-Mebzim})_2]$ (8) The brown solid was prepared by a procedure similar to that for **1** using 1-methylbenzimidazole. Yield: 62%. Single crystals suitable for X-ray crystallography were obtained by slow diffusion of diethyl ether into the methanol/dichloromethane solution of **8**. IR (KBr, cm^{-1}): *v*(Os≡N) 1070, *v*(C=N) 1460; Anal. calcd. for $C_{16}H_{16}N_5Cl_3Os$: C, 33.43; H, 2.81; N 12.18%. Found: C, 33.36; H, 2.97; N, 11.97%. ^1H NMR (400 MHz, CD_3OD): δ *trans*-form: 9.28 (s, 2H); 8.12 (t, *J* = 8.1 Hz, 2H); 7.30 (t, *J* = 7.3 Hz, 2H); 7.65 (m, 4H); 4.14 (s, 6H); *cis*-form I: 9.01 (s, 1H); 8.63(s, 1H); 7.65 (m, 8H); 4.15 (s, 3H); 4.12 (s, 3H); *cis*-form II: 8.74 (s, 1H); 8.47(s, 1H); 7.65 (m, 8H); 4.18 (s, 3H); 4.02 (s, 3H). ESI-MS (MeOH): *m/z* = +540, $[M - Cl]^+$.

Cellular uptake ICP-MS analysis

Cellular uptake of complexes **1**–**8** in HeLa cells was measured as follow. The cells were seeded in 6-well tissue culture plates. After overnight incubation, the cells were treated with the corresponding complexes (30 μM) for 6 h in the growing medium. The attached cells were washed twice with PBS. The whole cell pellets were then digested with 32.5% HNO_3 (2 mL), and the samples were transferred into V-Vials, which were heated at 100 °C for overnight. After cooling, each sample was diluted to 10 mL using milli-Q water. The osmium contents in the samples were determined by ICP-MS. Data is the mean of three experiments and reported as mean ± SD.

Cell viability assay

Cytotoxicity was determined by the colorimetric MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). Cells were seeded into 96-well microculture plates (5×10^3 cells per well) (MCF-7, HeLa, HepG2) and 8×10^3 (K562) viable cells per well in order to ensure exponential growth throughout drug exposure. After an overnight incubation for cells attachment, they were exposed to the solutions (100 μL per well) of the test compounds for 48 h, which were first dissolved in DMSO and then diluted in culture medium to the desired concentrations, (final DMSO concentration < 1% in all cases). At the end of exposure, drug solutions were added 10% MTT solution in phosphate-buffered saline (5 mg/ml PBS). After incubation for 1 h at 37º C, medium was removed, and the reduced formazan products formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in DMSO (100 μL per well). Optical densities at 570 nm were measured with a microplate reader. The quantity of vital cells was expressed in terms of T/C values by comparison to untreated control microcultures, and IC_{50} values were calculated from concentration-effect curves by logarithmic interpolation. Evaluation is based on means from at least three independent experiments, each comprising four replicates per concentration level.

Soft agar clonogenic assay

HeLa cells were seeded into 6-well plates (5×10^3 cells per well) in 0.3 % top agarose, which was added on the top of 0.6 % base agarose. Complexes **1** and **3** were mixed with the top agarose at final concentration of 20 and 40 *u*M. HeLa cells were allowed to grow for 14 d, and colonies stained with crystal violet (0.005%, Sigma) were counted. The total numbers of the colonies were recorded as average of three counts. The experiment was repeated twice, each comprising three replicates per concentration level.

Flow cytometric analysis of cell cycle and apoptosis

For the cell cycle analysis, HeLa cells were treated with complexes for 12, 24, 36 and 48 h at 37 ^{o}C in 6-well plates. Appropriate controls, in which cells were mock-treated by DMSO (< 1%) for the same durations, were also set up. After being treated, all cells were trypsinized and collected, washed twice with PBS, added with –20 $^{\circ}$C 70% ethanol (10 mL) and stored overnight at 4 $^{\circ}$C. After this, cells were spun down at 1500 rpm for 5 min, washed once with PBS, and then treated with the mixture of 10 μL RNase (10 mg/mL), 50 μL propidium iodide (1 mg/mL) and 940 μL 1 × PBS, incubated at room temperature for 30 min in the dark. The stained cells were analyzed by flow cytometry (BD FACS Calibur). For the apoptosis determination,

after 24 and 48 h treatment with complexes **1** and **3**, HeLa cells were harvested, re-suspend in 100 μL annexin-binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4) at the cell density of about 10^6 cells/mL and stained with 5 μL annexin V and 5 μL propidium iodide for 15 min at room temperature. 400 μL annexin-binding buffer was added for flow-cytometric analysis.

EdU incorporation

HeLa cells were grown on coverslips and treated with 40 μM **3** for 24 h and added 10 uM EdU into medium for 2 h incubation. After fixation, permabilization, Click-iT^{TM} Edu Alexa Fluor® 488 Imaging Kit (Invitrogen) was used for staining. H33342 (1:2000) in PBS was added in the absence of light for 15 min and then washed twice with PBS. The slides were mounted and images were recorded using confocal laser scanning microscope (Leica TCS SPE).

Western blot analysis

After treatment of **3** (40 μM), HeLa cells were washed (PBS) and collected at different time intervals. Cells were lysed in 2% sodium dodecyl sulfate (SDS) in PBS supplemented with protease inhibitor cocktail (Roche). Protein concentrations were measured using the Coomassie protein assay reagent according to manufacturer's instruction (Thermo Scientific). Equal amounts of cellular proteins were mixed with sample buffer containing 2% SDS, 50 mM Tris-HCl (pH 6.8), 0.2 mg/ml bromophenol blue, 0.1 M dithiothreitol (DTT), 0.1% glycerol, boiled at 95 $^{\circ}$C for 5 min, and run on 12% Bis-Tris Novex precast gels (invitrogen). Protein was transferred to nitrocellulose blotting membrane (Pall Corporation), which was incubated with 5% nonfat milk (Nestle) in PBS-T buffer (1×PBS, 0.05% Tween-20) for 1 h to block non-specific binding, and then probed with primary antibody followed by secondary antibody. The ECL detection kit (GE Healthcare) was added to catalyze oxidation of luminol to emit chemiluminescence, and the membrane was visualized using high performance laser gel and bolt imager (Fujifilm LAS-4000).

Reaction of complex with pBR322 plasmid DNA

The reaction of nitridoosmium complexes with DNA was conducted using plasmid pBR322 (Invitrogen) and analyzed by gel electrophoresis. The plasmid DNA concentration per nucleotide was determined by absorption spectroscopy, using the molar extinction coefficient 6600 M^{-1} cm^{-1} at 260 nm. The plasmid DNA (0.55 ug) was incubated with varying concentrations of the nitridoosmium compound at room temperature for 24 h. After incubation, samples were analyzed by agarose gel electrophoresis (1% agarose, 1 × TAE),

and then the gel was stained with Gel Red (Biotium) and photographed with UV illumination.

References

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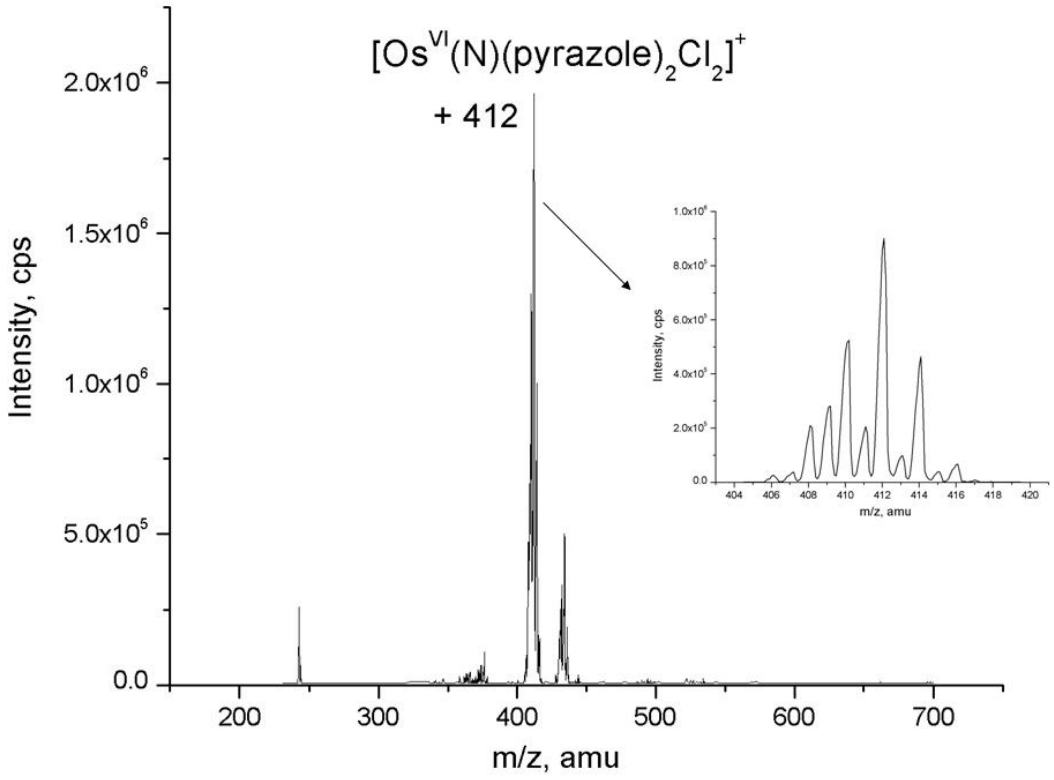
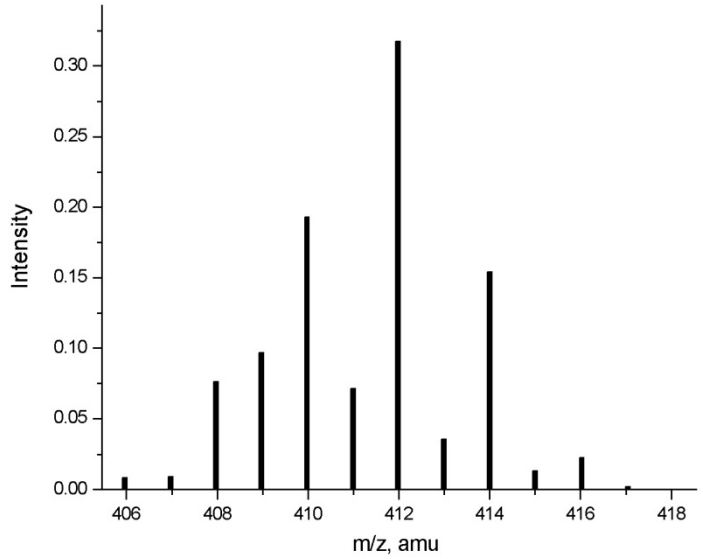


(A)



(B)

Fig. S1 (A) ESI mass spectrum (+ve mode) of **1** and experimental isotopic distribution pattern (inset) of the peak at *m/z* 412; (B) Simulated isotopic distribution pattern of $[Os(N)(Hpz)_2Cl_2]^+$.

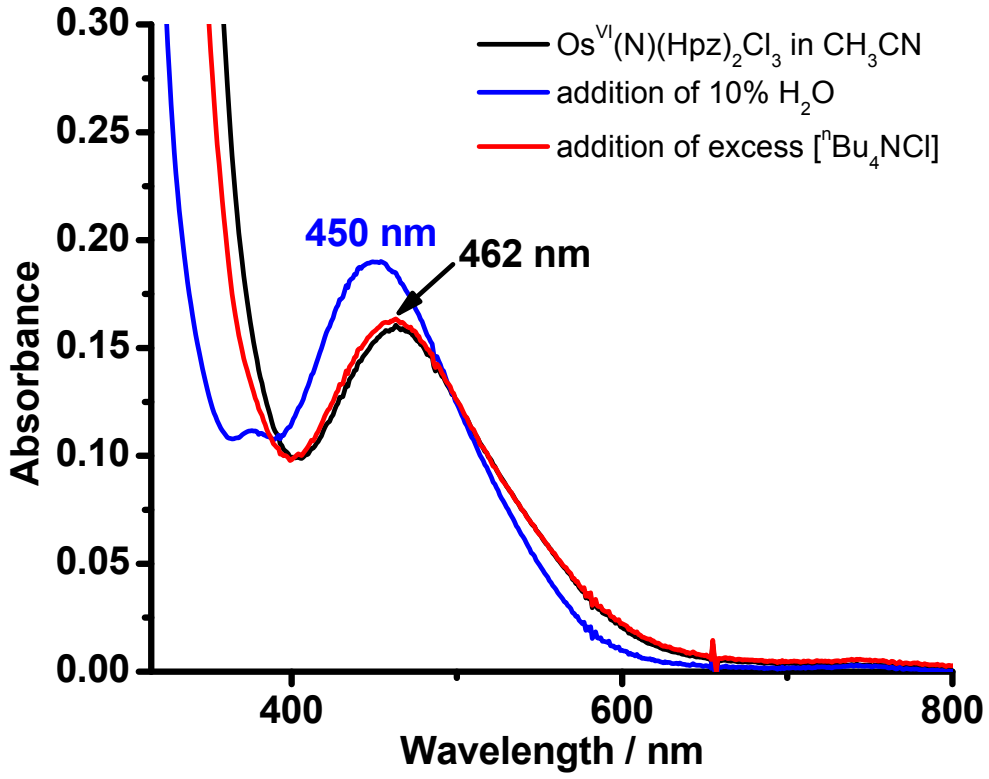


Fig. S2 UV-Vis spectra of 1.20 mM $Os^{VI}(N)(Hpz)_2Cl_3$ (**1**) in CH_3CN (black line); immediately after adding 10%L H_2O (blue line); immediately after adding excess [$^nBu_4NCl$] (red line).

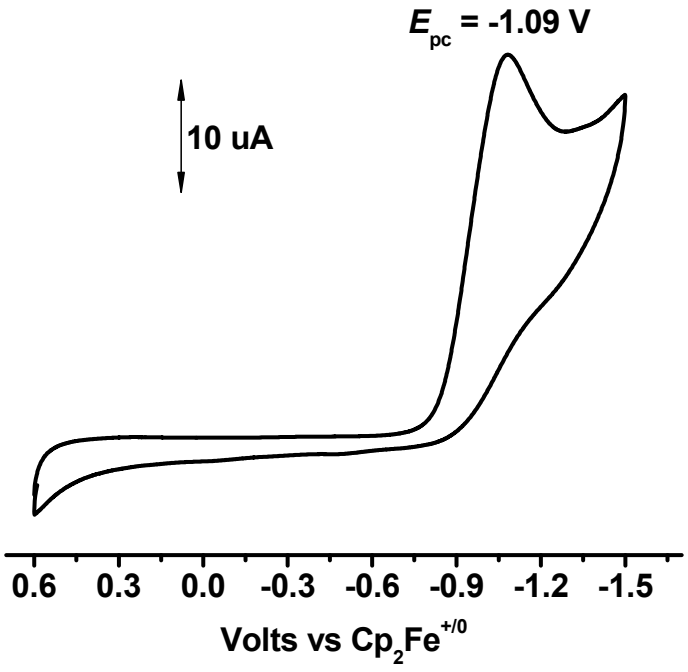
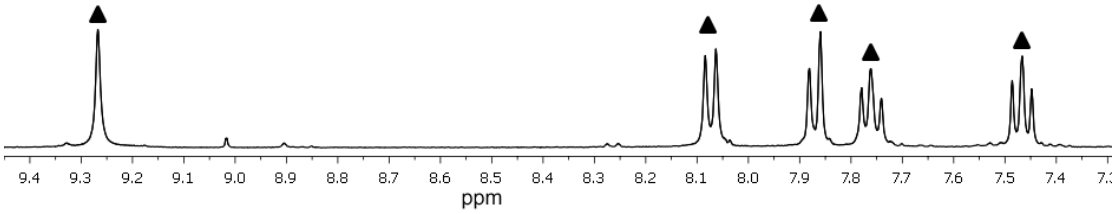
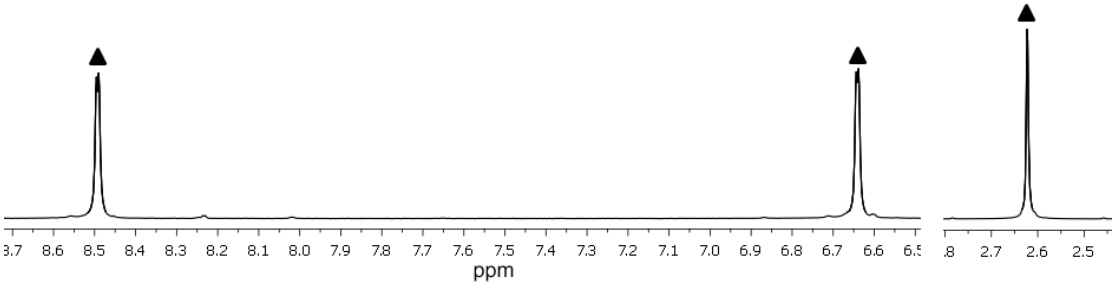


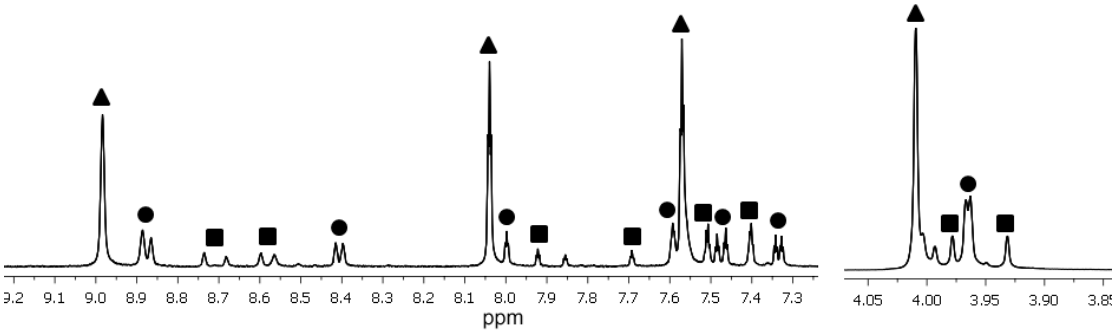
Fig. S3 CV of [$Os^{VI}(N)(5\text{-}MeHpz)_2Cl_3$] (**3**) in 0.1 M [$^nBu_4N$]$PF_6$ in CH_3CN.



Complex **2**



Complex **3**



Complex **6**

Fig. S4 ^{1}H NMR (400 MHz) spectral of complexes **2**, **3** and **6** in CD_3OD solution. The signals corresponding to *trans*-form (▲), *cis*-form I or II (●) or (■) are labeled.

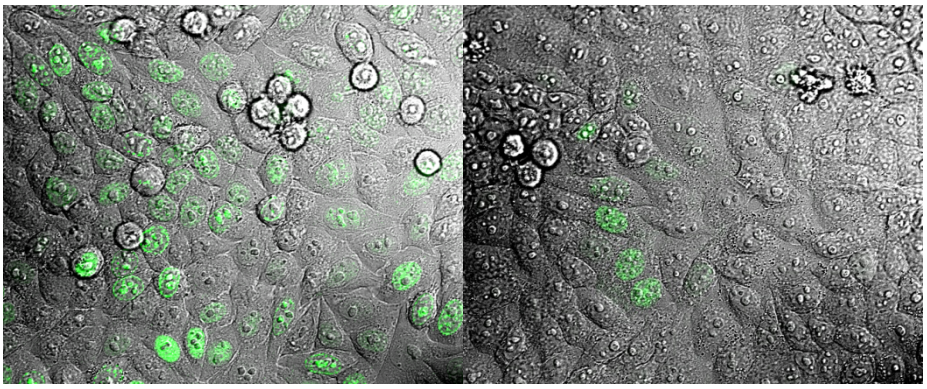
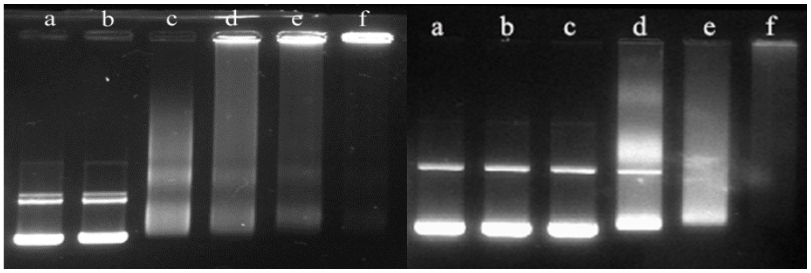


Fig. S5 HeLa cells were mock-treated (left) or treated (right) with compound **3** (40 *u*M) for 24 h. Cells were labeled by EDU incorporation (Green). Bars: 50 *u*m.



Complex **1** Complex **3**

Fig. S6 Gel-migration studies of plasmid DNA pBR322 which had been separately treated with increasing amount of **1** and **3** for 24 h. Concentrations (in mM) of complex are as follows: (lane a) 0, (lane b) 0.15, (lane c) 0.73, (lane d) 1.46, (lane e) 1.75, (lane f) 2.19. After gel electrophoresis, the DNA were stained by Gel-Red and visualized under UV illumination.

Table S1 Crystal data and structure refinement details for **2**, **3**, **4** and **8**

	2	**3**	**4**	**8**
Formula	$C_{14}H_{12}Cl_3N_5Os$	$C_8H_{12}Cl_3N_5Os$	$C_{10}H_{16}Cl_3N_5Os$	$C_{16}H_{16}Cl_3N_5Os$
M_r	546.84	474.78	502.83	574.89
T / K	293(2)	293(2)	173(2)	193(2)
Crystal system	Monoclinic	Monoclinic	Monoclinic	Triclinic
Space group	*C2/c*	*C2/c*	*C2/c*	*P–1*
a (Å)	14.4225(11)	10.314(5)	10.466	10.4137(3)
b (Å)	13.2370(12)	15.450(6)	14.595	11.0728(3)
c (Å)	9.0745(8)	8.562(5)	10.063	11.4646(3)
α / º	90.00	90.00	90.00	87.628(2)
β / º	102.821(8)	96.39(5)	92.79	70.662(2)
γ / º	90.00	90.00	90.00	66.250(2)
V /Å^3	1689.2(2)	1355.8(11)	1535.3	1135.25(5)
Z	4	4	4	2
D_c / Mg m^{-3}	2.150	2.326	2.175	1.682
F(000)	1032	888	952	548
Collected refl.	2750	5771	3882	19842
Unique refl.	1496	1196	1372	3980
R(int)	0.0277	0.0417	0.0144	0.0242
Final R indices,	R_1(obs) = 0.0316,	R_1(obs) = 0.0192,	R_1(obs) = 0.0181,	R_1(obs) = 0.0174,
$I > 2\sigma(I)$ R^a	wR(all) = 0.0915	wR(all) = 0.0423	wR(all) = 0.0459	wR(all) = 0.0500
GOF	1.081	1.089	1.194	1.126
No. of parameter	106	80	90	228

Table S2 Selected bond lengths (Å) and bond angles (°) of **2**.

Os(1)–N(1)	1.619(9)	Os(1)–Cl(1)	2.553(2)
Os(1)–N(2)	2.074(5)	Os(1)–Cl(2)	2.3589(16)
N(1)–Os(1)–N(2)	97.35(15)	N(2)–Os(1)–Cl(2)	88.39(15)
N(1)–Os(1)–Cl(2)	95.90(5)	N(2)–Os(1)–N(2A)	165.3(3)
N(1)–Os(1)–Cl(1)	180.0(1)	Cl(1)–Os(1)–Cl(2)	84.10(5)
N(2)–Os(1)–Cl(1)	82.65(15)	Cl(2)–Os(1)–Cl(2A)	168.21(9)

Symmetry transformations used to generate equivalent atoms: A –x+1,y,–z+1/2

Table S3 Selected bond lengths (Å) and bond angles (°) of **3**.

Os(1)–N(1)	1.615(5)	Os(1)–Cl(1)	2.5307(16)
Os(1)–N(2)	2.063(3)	Os(1)–Cl(2)	2.3491(17)
N(1)–Os(1)–N(2)	96.33(9)	N(2)–Os(1)–Cl(2)	89.65(10)
N(1)–Os(1)–Cl(2)	96.18(3)	N(2)–Os(1)–N(2A)	167.35(17)
N(1)–Os(1)–Cl(1)	180.0(1)	Cl(1)–Os(1)–Cl(2)	83.82(3)
N(2)–Os(1)–Cl(1)	83.67(9)	Cl(2)–Os(1)–Cl(2A)	167.63(6)

Symmetry transformations used to generate equivalent atoms: A –x+1,y,–z+3/2

Table S4 Selected bond lengths (Å) and bond angles (°) of **4**.

Os(1)–N(1)	1.643(4)	Os(1)–Cl(1)	2.5072(10)
Os(1)–N(2)	2.090(3)	Os(1)–Cl(2)	2.3613(8)
N(1)–Os(1)–N(2)	95.53(8)	N(2)–Os(1)–Cl(2)	91.29(8)
N(1)–Os(1)–Cl(2)	95.56(2)	N(2)–Os(1)–N(2A)	168.94(15)
N(1)–Os(1)–Cl(1)	180.0(1)	Cl(1)–Os(1)–Cl(2)	84.44(2)
N(2)–Os(1)–Cl(1)	84.47(8)	Cl(2)–Os(1)–Cl(2A)	168.89(4)

Symmetry transformations used to generate equivalent atoms: A –x+2,y,–z+1/2

Table S5 Selected bond lengths (Å) and bond angles (°) of **8**.

Os(1)–N(1)	1.675(3)	Os(1)–Cl(3)	2.3389(9)
Os(1)–N(2)	2.085(3)	Os(1)–Cl(2)	2.3526(8)
Os(1)–N(4)	2.089(3)	Os(1)–Cl(1)	2.5793(9)
N(1)–Os(1)–N(2)	92.79(13)	N(2)–Os(1)–Cl(2)	89.56(8)
N(1)–Os(1)–N(4)	93.93(13)	N(4)–Os(1)–Cl(2)	169.77(8)
N(2)–Os(1)–N(4)	89.59(11)	Cl(3)–Os(1)–Cl(2)	89.37(3)
N(1)–Os(1)–Cl(3)	99.42(11)	N(1)–Os(1)–Cl(1)	174.68(10)
N(2)–Os(1)–Cl(3)	167.78(8)	N(2)–Os(1)–Cl(1)	82.31(8)
N(4)–Os(1)–Cl(3)	89.31(8)	N(4)–Os(1)–Cl(1)	83.98(8)
N(1)–Os(1)–Cl(2)	96.30(10)	Cl(3)–Os(1)–Cl(1)	85.47(3)
Cl(2)–Os(1)–Cl(1)	85.80(3)		