Supporting Information

Synthesis and antitumor activity of a series of osmium(VI) nitrido complexes bearing quinolinolato ligands

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Experimental

General

The complexes $[^{n}Bu_4N][Os^{VI}(N)Cl_4]$ and ^{15}N-labeled $[^{n}Bu_4N][Os^{VI}(^{15}N)Cl_4]$ were prepared by a literature method.[1] Complexes **1**–**4** were synthesized according to a literature procedure.[2] The 8-hydroxyquinoline ligands (Sigma-Aldrich) were used as received. All other chemicals were of reagent grade and used without further purification.

Human cancer cell lines used in this work, including cervical epithelioid carcinoma (HeLa), hepatocellular arcinoma (HepG2), lung carcinoma (A549) and colorectal carcinoma (HCT 116) were purchased from American Type Culture Collection (ATCC, Manassas, VA). Other reagents and materials include cell culture medium and phosphate-buffered saline (PBS) (Gibco BRL), cell proliferation kit I (MTT) (Roche). HeLa and A549 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco). HepG2 and HCT 116 cells were grown in Roswell Park Memorial Institute (RPMI 1640; Gibco), they both contain 10 % fetal bovine serum (FBS), 1% glutamine, 1% antibiotic-antimycotic (Gibco) and were maintained in tissue culture incubator at 37 ºC (95 % relative humidity, 5% CO_2).

IR spectra were obtained from KBr discs using a Bomen MB-120 FTIR spectrophotometer. UV-visible spectra were recorded on either a Perkin-Elmer Lamda 19 or a Shimadzu UV3100 spectrophotometer in 1 cm cuvettes. ^{1}H NMR spectra were recorded on a Varian (300 MHz) FT NMR spectrometer. The chemical shifts (δ ppm) were reported with reference to tetramethylsilane (TMS). Elemental analysis was done on an Elementar Vario EL Analyzer.

Synthesis of osmium(VI) nitrido complexes

$[Os^{VI}(N)(SQ)_2Cl]$ (5). A solution of 8-thiolquinoline (55 mg, 0.34 mmol) in methanol (5 mL) was slowly added to a solution of $[^{n}Bu_4N][Os^{VI}(N)Cl_4]$ (100 mg, 0.17mmol) in methanol (10 mL). The mixture was stirred for 1 h to give a dark green solid which was filtered, washed with methanol, diethyl ether and then air-dried. The

solid was recrystallized from dichloromethane/n-hexane. Yield 77 mg (81%). Single crystals suitable for X-ray crystallography were obtained by slow diffusion of n-hexane into a dichloromethane solution of **5** at room temperature. IR (KBr, cm^{-1}): 1071 ν(Os≡^{14}N), 1035 (Os≡^{15}N). ^{1}H NMR (δ/ppm, $CDCl_3$-d_1): 7.03 (1H, m), 7.20 (1H, d), 7.44 (1H, t), 7.55 (2H, t), 8.15 (1H, d), 8.10 (1H, d), 8.32 (1H, d), 8.40 (1H, d), 8.75 (1H, d), 10.74(1H, d). Found: C, 38.47; H, 2.41; N, 7.73. $C_{18}H_{12}S_2N_3Cl_3Os$ requires C, 38.60; H, 2.16; N, 7.50. UV-Vis (CH_2Cl_2) λ_{max}, nm (ε, M^{-1} cm^{-1}): 408 (6660).

[^{n}Bu$_4$N][Os^{VI}(N)(Q)Cl$_3$] (6). A mixture of 8-hydroxyquinoline (25 mg, 0.17 mmol) and [^{n}Bu$_4$N][Os^{VI}(N)(Cl)$_4$] (100 mg, 0.17 mmol) in acetone (15 mL) was refluxed for 15 min. After cooling to room temperature, 0.5 mL of 2,6-dimethyl pyridine was added and the mixture was refluxed for another 20 min. The resulting red solution was cooled to room temperature and diethyl ether (ca. 35 mL) was slowly added to precipitate unreacted ligand which was filtered. On standing the filtrate for 2 d, red rod-shaped crystal was obtained. Yield 71 mg (60%). IR (KBr, cm^{-1}): 1066 ν(Os≡N). ^{1}H NMR (δ/ppm, $CDCl_3$-d_1): 1.00 (12H, t), 1.47 (8H, m), 1.69 (8H, m), 3.30 (8H, m), 6.88 (1H, d), 7.20 (1H, d), 7.47 (2H, m), 8.20 (1H, d), 8.78 (1H, d). Found C, 43.27; H, 6.34; N, 6.10. $C_{25}H_{41}N_4OCl_3Os$ requires C, 43.07; H, 6.07; N, 6.03. UV-Vis (CH_2Cl_2) λ_{max}, nm (ε, M^{-1} cm^{-1}): 410 (4070), 325 (4670).

[^{n}Bu$_4$N][Os^{VI}(N)(Me-Q)Cl$_3$] (7). This compound was prepared by a procedure similar to that for **8** using 8-hydroxyquinaldine. Yield 70 mg (58%). IR (KBr, cm^{-1}): 1066 ν(Os≡N). ^{1}H NMR (δ/ppm, $CDCl_3$-d_1): 0.94 (12H, t), 1.41 (8H, m), 1.63 (8H, m), 2.20 (3H, s), 3.45 (8H, m), 6.83 (1H, d), 6.92 (1H, d), 7.32 (1H, t), 7.47 (1H, d), 7.98 (1H, d). Found C, 44.00; H, 6.21; N, 5.90. $C_{25}H_{41}N_4OCl_3Os$ requires C, 43.91; H, 6.24; N, 5.91. UV-Vis (CH_2Cl_2) λ_{max}, nm (ε, M^{-1} cm^{-1}): 419 (3070), 324 (3460).

[^{n}Bu$_4$N][Os^{VI}(N)(NO$_2$-Q)Cl$_3$] (8). This compound was prepared by a procedure similar to that for **8** using 8-hydroxy-5-nitroquinoline. Yield 79 mg (63%). IR (KBr, cm^{-1}): 1073 ν(Os≡N). ^{1}H NMR (δ/ppm, $CDCl_3$-d_1): 1.10 (12H, t), 1.46 (8H, m), 1.68 (8H, m), 3.28 (8H, m), 6.76 (1H, d), 7.77 (1H, m), 8.72 (1H, d), 8.93 (1H, d), 9.63 (1H, d). Found C, 41.24; H, 5.43; N, 7.28. $C_{25}H_{41}N_4O_3Cl_3Os$ requires C, 40.46; H,

5.57; N, 7.55. UV-Vis (CH_2Cl_2) λ_{max}, nm (ε, M^{-1} cm^{-1}): 436 (21900), 346 (6390).

[^{n}Bu$_4$N][Os^{VI}(N)(Cl-Q)Cl$_3$] (9). This solid was prepared by a procedure similar to **8** using 5-chloro-8-hydroxyquinoline. Yield 78 mg (63%). IR (KBr, cm^{-1}): 2960, 1498, 1380, 1326, 1066 ν(Os≡N). ^{1}H NMR (δ/ppm, $CDCl_3$-d_1): 1.00 (12H, t), 1.46 (8H, m), 1.69 (8H, m), 3.30 (8H, m), 6.79 (1H, d), 7.53 (1H, d), 7.62 (1H, m), 8.54 (1H, d), 8.85 (1H, d). Found C, 40.90; H, 5.74; N, 5.58. $C_{25}H_{41}N_3OCl_4Os$ requires C, 41.04; H, 5.65; N, 5.74. UV-Vis (CH_2Cl_2) λ_{max}, nm (ε, M^{-1} cm^{-1}): 433 (4150), 333 (4750).

X-Ray Crystallography

Diffraction data for compounds **5**, **8** and **9** were collected on a Bruker SMART 1000 CCD area-detector diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Details of the intensity data collection and crystal data are given in Table S1. The collected frames were processed with the software SAINT+[3] and an empirical absorption correction (SADABS or multi-scan)[4] was applied to the collected reflections. The structures were solved by Direct or Patterson methods (SHELXTL)[5] in conjunction with standard difference Fourier techniques and subsequently refined by full-matrix least-squares analyses on F^2. Hydrogen atoms were generated in their idealized positions and all non-hydrogen atoms were assigned with anisotropic displacement parameters.

Cytotoxicity Tests

HeLa, HepG2, A549 and HCT 116 cells were harvested from culture dishes by trypsinization and seeded into 96-well microculture plates with supplemented culture medium (100 μL / well) at a density of 4×10^3 cells for 24h. Thereafter, the medium was replaced by solutions (100 μL/well) containing various concentrations (ranging from 0.16 to 100 μM) of complexes **1~9** for 72h. Cytotoxicity was evaluated by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, in which drug solutions were removed and 100μL/well medium with 10 % MTT solution were added to each well, followed by a 1-hour incubation. Solution was removed and

100 μL DMSO was added per well, then measured at 570 nm using a microplate reader (BioTek, Power Wave XS). The quantity of vital cells was expressed by comparison to untreated cells as control and IC_{50} values were calculated from concentration-effect curves by logarithmic interpolation. Evaluation is based on means from at least two independent experiments, each comprising four replicates per concentration level.

Cellular-uptake experiment

HepG2 cells were seeded onto 6-well plates at a density of 8×10^5 cells/well 24 h prior to the uptake experiment. After washing with 1X PBS twice, HepG2 cells were incubated with the culture medium RPMI in the presence of drugs (10 μM concentration). After 6 h, the cells were rinsed three times with PBS to completely remove all the dead cells and complexes. The cells were then detached from the wells with trypsin-EDTA, centrifuged and then digested with 2 mL of HNO_3 (32.5 %) and heated overnight at 100 °C. After cooling, each sample was diluted to 10 mL using milli-Q water. The solution was analyzed by inductively coupled plasma mass spectrometry (ICP-MS). All analyses were done in triplicate.

Flow Cytometric Studies

Flow cytometric analysis was performed with a BD FACS Calibur flow cytometer. Fluorescence signals were manipulated with Cell Quest software (Coulter) and analyzed by Modfit 2.0 software (Verity Software House, Topsham, ME).

For cell cycle evaluation, HepG2 cells were cultured in 6-well plates (5×10^5 cells/well) with 2 mL of freshly growth medium. After incubation for 24 h, 10 μM complex **3** in freshly growth medium was added and the plates were further incubated for 24, and 48 and 72 h. Appropriate controls, in which cells were mock-treated by DMSO (< 1%) for the same durations, were also set up. At the end of each incubation period, cells were trypsinized and washed with PBS. Cells were then fixed with ice-cold 70% ethanol and kept at -20 ºC before analysis. Before staining, cells were centrifuged at 1500 rpm for 5 min, washed with PBS. After being incubated with 950

μL 1X PBS, 10 μL RNase (10 mg/mL) and 40 μL propidium iodide (1 mg/mL) at room temperature for 30 minutes in the dark, cells were ready for analysis.

For the apoptosis determination, after 24, 48 and 72 h treatment with 10 μM complexes **3**, HepG2 cells were collected, re-suspended in 100 μL annexin-binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4) 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. For each experiment, 10000 events per sample were recorded.

References

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Table S1 Crystal data and structure refinement details for compounds **5**, **8** and **9**.

	5	**8**	**9**
Formula	$C_{18}H_{12}ClN_3O_2OsS_2$	$C_{25}H_{41}Cl_3N_4O_3Os$	$C_{25}H_{41}Cl_4N_3OOs$
M_r	560.08	742.17	731.61
T/K	295(2)	293(2)	293(2)
Crystal system	monoclinic	monoclinic	monoclinic
Space group	$P21/c$	$P21/n$	$P21/n$
a/Å	15.1083(4)	10.1945(5)	10.2298(6)
b/Å	8.5584(2)	14.7147(8)	14.3648(9)
c/Å	13.7695(4)	21.1777(11)	21.7600(13)
β, deg	100.3605(6)	90.7940(10)	95.4690(10)
V/ Å^3	1751.41(8)	3176.5(3)	3183.1(3)
Z	4	4	4
ρ_{calcd}, Mg m^{-3}	2.124	1.552	1.527
F(000)	1064	1480	1456
Collected refl.	4016	7012	7162
Unique refl.	3235	6131	4853
R(int)	0.0308	0.0357	0.0349
Final R indices, $I > 2\sigma(I)$ R^a	$R1$(obs) = 0.0656 wR(all) = 0.1747	$R1$(obs) = 0.0283 wR(all) = 0.0786	$R1$(obs) = 0.0320 wR(all) = 0.0814
GOF	1.030	1.025	0.926
No. of parameter	226	329	311

Table S2 Selected bond distances (Å) and angles (deg) of **5**.

Os(1)–N(1)	1.716(10)	Os(1)–S(1)	2.332(3)
Os(1)–N(2)	2.127(10)	Os(1)–S(2)	2.413(3)
Os(1)–N(3)	2.406(9)	Os(1)–Cl(1)	2.329(4)
Os(1)–N(1)–N(2)	95.0(4)	Os(1)–N(1)–S(1)	103.6(3)
Os(1)–N(1)–N(3)	165.9(4)	Os(1)–N(1)–S(2)	91.1(3)
Os(1)–N(1)–Cl(1)	100.1(4)		

Table S3 Selected bond distances (Å) and angles (deg) of **8** and **9**.

	8	**9**
Os(1)–N(1)	1.646(3)	1.642(3)
Os(1)–N(2)	2.102(2)	2.098(2)
Os(1)– O(1)	2.146(2)	2.1216(19)
Os(1)–Cl(1)	2.3601(9)	2.3668(8)
Os(1)–Cl(2)	2.3558(10)	2.3203(9)
Os(1)–Cl(3)	2.3195(10)	2.3568(8)
Os(1)–N(1)–N(2)	91.52(12)	90.93(11)
Os(1)–N(1)–O(1)	167.04(12)	167.23(10)
Os(1)–N(1)–Cl(1)	95.77(12)	95.28(9)
Os(1)–N(1)–Cl(2)	95.14(12)	103.70(10)
Os(1)–N(1)–Cl(3)	103.69(11)	94.49(9)

Table S4 Cellular Osmium Concentrations in HepG2 cells with 10 μM Os complexes. Each value represents the mean ± SD for three independent experiments.

Compound	Cellular uptake (ppb Os/10^6 cell)
1	109 ± 33
2	176 ± 38
3	202 ± 44
4	195 ± 39
5	67 ±10
6	10 ± 2
7	17 ± 4
8	16 ± 2
9	33 ± 8

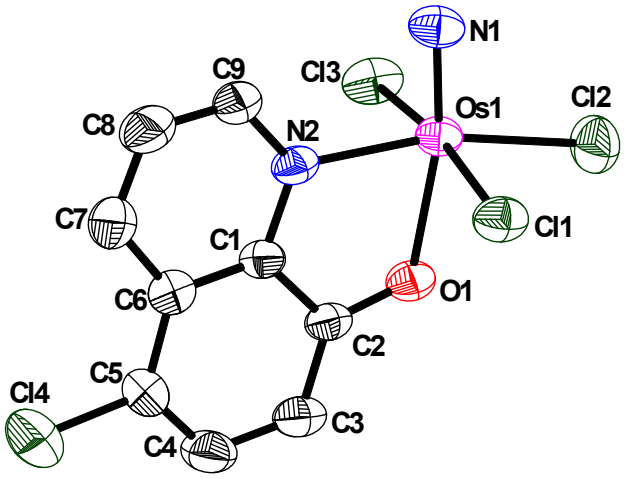

Figure S1. Molecular structure of anion of **9** with atomic labelling. Thermal ellipsoids are drawn at 30% probability. H atoms are omitted for clarity.