Electronic Supporting Information

Platinum (II) Compounds Bearing Bone-targeting Group: Synthesis, Crystal Structure and Antitumor Activity

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Experimental

Materials and Reagents: Anhydrous dichloromethane was prepared by refluxing available solvent in the presence of calcium hydride for several hours. All other reagents available from commercial vendors were used as received without further purification. Double distilled water was used to prepare water solutions. Bromotrimethylsilane (TMSBr) was purchased from Aldrich. Tetraethyl methylenediphosphonate[1], Tetraethyl ethene-1,1-diyldiphosphonate[2] and *cis*-$Pt(DMSO)_2Cl_2$[3] were synthesized according to previous literature, respectively. Human osteosarcoma cells MG-63 were purchased from Institute of Biochemistry and Cell Biology (Shanghai Institute for Biological Science, Chinese Academy of Sciences). Human ovarian cancer cells COC1 were purchased from China Center for Type Culture Collection (CCTCC).

Instrumental Measurements: ^{1}H, ^{13}C and ^{31}P NMR experiments were performed on a Bruker DRX-500 spectrometer or a Bruker DRX-300 spectrometer at 298 K using

standard pulse sequences. Elemental analyses were performed on a Perkin-Elmer 240C analytic instrument. Electrospray mass spectra were recorded using an LCQ electron spray mass spectrometer (ESMS, Finnigan). The isotopic distribution patterns for the complex were simulated using the Isopro 3.0 program. Circular dichroism (CD) spectra were recorded on a Jasco J-810 spectropolarimeter in a 1 cm path length cylindrical quartz cell at room temperature.

X-ray Crystallographic Analysis and Data Collection: Suitable crystals of **2** and **3** were grown from ethyl acetate/acetone (1:3). Crystals of **7•D2O** were obtained from D_2O. The single crystals were mounted on glass fibers, and the X-ray diffraction intensity data were measured at 291(2) K on a Bruker Smart Apex CCD area detector with graphite-monochromated $Mo_{K\alpha}$ radiation (λ = 0.71073 Å). The data reduction was made with the Bruker SAINT package. An absorption correction was performed with the SADABS program. The structure was solved by direct methods and refined on F^2 by full-matrix least squares by using SHELXTL-2000 with anisotropic displacement parameters for all non-hydrogen atoms. For complexes **2** and **3**, H atoms were positioned geometrically and refined using a riding model with Uiso(H) = 1.2 Ueq(C，N). For complex **7**, the D atoms bonded to N and O atoms were located in a difference map and refined with Uiso(D) = 1.2Ueq(N,O). The H atoms bonded to C were positioned geometrically and refined using a riding model with Uiso(H) = 1.2 Ueq(C). All computations were carried out with the SHELXTL-2000 program package.

Syntheses.

Preparation of the ligands.[4,5]

General method for **L1** (m=1, n=0) and **L3** (m=2, n=0).

L1 and **L3** were prepared by heating a mixture of 2-aminomethanpydine (2.16 g, 20 mmol) or 2-aminoethanpydine (2.44 g, 20 mmol) with diethyl phosphate (5.52 g, 40 mmol) and triethyl orthoformate (2.96 g, 20 mmol) at 100 °C in the atmosphere of N_2 for 12 h. After that, the ethanol formed was evaporated. The crude was purified on a silica gel column (CH_3OH:CH_2Cl_2=1:30). Yellow oil was obtained.

Data for L1.

^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.61 (d, J=4.2Hz, 1H), 7.71(t, J=7.6Hz, 1H), 7.49(d, J=7.6Hz, 1H), 7.24 (t, J=6.1HZ, 1H), 4.39 (d, J=21Hz, 1H), 4.11-4.37 (m, 8H), 2.99 (t, J=13.1Hz, 1H), 2.90 (t, J=13.2Hz, 1H), 1.20-1.36 (m, 12H); ^{31}P NMR (500 MHz, $CDCl_3$) δ: 21.14(d, 1P), 24.93(d, 1P); ESI^+ MS: m/z 395.08($L1+H^+$)，417.17($L1+Na^+$)，810.83($2L1+Na^+$).

Data for L3.

^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.53 (d, J=3.9Hz, 1H), 7.63(t, J=7.3Hz, 1H), 7.26(d, J=7.7Hz, 1H), 7.15 (t, J=5.4Hz, 1H), 4.18-4.26 (m, 8H), 3.36 (m, 1H), 3.28 (m, 2H), 2.99 (t, J=6.6Hz, 2H), 1.23-1.40 (m, 12H); ^{31}P NMR (500 MHz, $CDCl_3$) δ: 22.13; ESI^+ MS: m/z 409.00($L1+H^+$)，431.08($L1+Na^+$)，838.75($2L1+Na^+$).

General method for **L2** (m=2, n=1) and **L4** (m=1, n=1).

To a solution of 2-aminomethanpydine (0.54 g, 5 mmol) or 2-aminoethanpydine (0.61 g, 5 mmol) was added a solution of Tetraethyl ethene-1,1-diyldiphosphonate (1.5 g, 5

mmol) in 5 mL dry THF. The mixture was refluxed for 8 h. After that, the solvent was distilled off and the yellow oil was obtained.

Data for L2.

^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.53 (d, J=3.5Hz, 1H), 7.60(t, J=7.8Hz, 1H), 7.14(d, J=8.0Hz, 1H), 7.12 (t, J=6.0Hz, 1H), 4.12-4.21 (m, 8H), 3.13-3.23 (m, 1H), 3.02 (m, 2H), 2.98 (m, 2H), 2.65(m, 1H), 1.23-1.40 (m, 12H); ^{31}P NMR (500 MHz, $CDCl_3$) δ: 23.56-23.98(m); ESI^+ MS: m/z 422.92 ($L1+H^+$)，445.25($L1+Na^+$)，867.00($2L1+Na^+$).

Data for L4.

^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.55 (d, J=4.5Hz, 1H), 7.66(t, J=7.4Hz, 1H), 7.40(d, J=7.9Hz, 1H), 7.16 (t, J=7.0Hz, 1H), 4.15-4.24 (m, 8H), 3.95 (s, 2H), 3.18 (td, 2H), 2.72 (tt, 1H), 1.32-1.38 (m, 12H); ^{31}P NMR (500 MHz, $CDCl_3$) δ: 23.62-24.06(m); ESI^+ MS: m/z 409.00($L1+H^+$)，431.00($L1+Na^+$)，838.33($2L1+Na^+$).

Preparation of the platinum complexes.

To a solution of *cis*-$Pt(DMSO)_2Cl_2$ (84 mg, 0.2 mmol) was added L1-4 (0.2 mmol) dissolved in 5 mL anhydrous dichloromethane. The mixture was stirred in the dark at room temperature for 1 day. The resulting yellow solution was concentrated to 2 mL and added to 30 mL diethyl ether. The white precipitate was collected and washed by diethyl ether. Purified solid was obtained by slow evaporation of ethyl acetate/acetone (1:3) solution.

Data for complex 1. ($C_{15}H_{28}O_6N_2P_2PtCl_2$)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.40 (d, 1H), 8.06(t, 1H), 7.54(d, 1H), 7.45 (t, 1H), 4.80-4.90 (m, 1H), 4.58-4.67 (m, 1H), 4.12-4.19 (m, 8H), 3.98 (td, 1H), 1.31-1.50 (m, 12H); ^{31}P NMR (500 MHz, $CDCl_3$) δ: 16.05(1P), 14.84(1P); ESI^+ MS: m/z

683.00(**1**+Na^+), 1342.67(2×**1**+Na^+). Elemental analysis: calcd (%) for **1**, C 27.27, H 4.24, N 4.24; found, C 27.28, H 4.43, N 4.50.

Data for complex 2. ($C_{17}H_{32}O_6N_2P_2PtCl_2$)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.140(d, 1H), 7.876(t, 1H), 7.36-7.28(m, 2H), 4.38-4.11(m, 8H), 3.74-3.70(m, 2H), 3.36-3.20(m, 3H), 3.05-2.85(m, 1H), 2.41-2.25(m, 1H), 1.43-1.30(m, 12H); ^{31}P NMR (500 MHz, $CDCl_3$) δ: 23.26 (d, J=25Hz, 1P), 21.68 (d, J=25Hz, 1P); ESI^+ MS: m/z 710.92(**2**+Na^+), 1398.58(2×**2**+Na^+). Elemental analysis: calcd (%) for **2**, C 29.65, H 4.65, N 4.07; found, C 29.38, H 4.22, N 4.10.

Data for complex 3. ($C_{16}H_{30}O_6N_2P_2PtCl_2$)

^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.16 (d, 1H), 7.83(t, 1H), 7.31-7.25 (m, 2H), 4.67 (t, 1H), 4.19-4.44 (m, 8H), 3.80 (t, 1H), 3.34-3.46 (m, 1H), 3.05-3.15 (m, 1H), 1.38-1.50 (m, 12H); ^{13}C NMR(500 MHz) δ: 158.785, 153.920, 139.251, 124.561, 123.932, 64.605($O\underline{C}H_2CH_3$), 64.200($O\underline{C}H_2CH_3$), 56.629(P-$\underline{C}$-P), 44.196($N\underline{C}H_2CH_2N$), 40.612($NCH_2\underline{C}H_2N$), 16.842($OCH_2\underline{C}H_3$); ^{31}P NMR (500 MHz, $CDCl_3$) δ: 17.65(1P), 16.20(1P); ESI^+ MS: m/z 696.92(**3**+Na^+), 1370.75(2×**3**+Na^+). Elemental analysis: calcd (%) for **3**, C 28.49, H 4.45, N 4.15; found, C 28.02, H 4.46, N 4.00.

Data for complex 4. ($C_{16}H_{30}O_6N_2P_2PtCl_2$)

^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.229(d, 1H), 7.999(t, 1H), 7.537(d, 1H), 7.348(t, 1H), 4.992(dd, 1H), 4.421-4.074(m, 9H), 3.995-3.821(m, 1H), 3.585-3.485(m, 1H), 3.299-3.136(m, 1H), 1.412-1.308(m, 12H);^{31}P NMR (500 MHz, $CDCl_3$) δ: 24.64(1P), 21.40(1P); ESI^+ MS: m/z 696.92(**4**+Na^+), 1370.67(2×**4**+Na^+). Elemental analysis: calcd (%) for **4**, C 28.49, H 4.45, N 4.15; found, C 28.79, H 4.32, N 4.05.

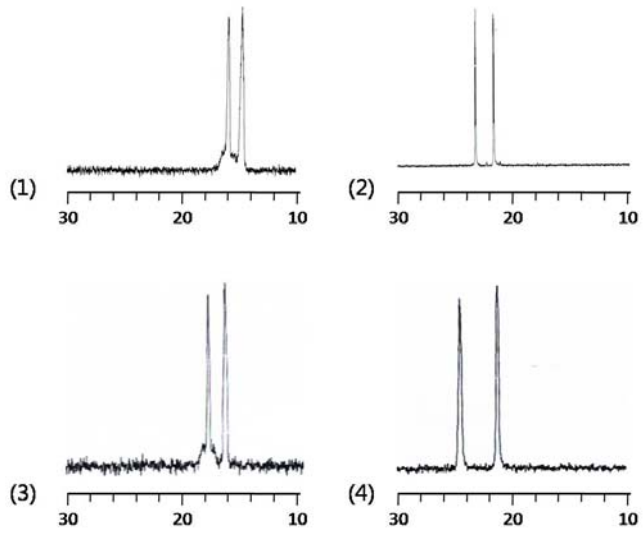

Fig. S1 The ^{31}P NMR spectra of platinum complexes **1**,**2**,**3**,**4**.

Preparation of the de-ester platinum complexes.[6]

To a solution of compounds **1** or **4** (2 mmol) in dry dichloromethane was added 400 μL bromotrimethylsilane, and the mixture was allowed to react for 3 days at room temperature. Discard the insoluble black solid, the solvent was evaporated. 5 mL CH_3CN was added and distilled off for several times. The solid was washed by CH_3Cl for several times. Hydroscopic yellow compounds **5** and **6** were obtained.

Data for 5.

($C_7H_{12}O_6N_2P_3PtCl_2$) ESI^- MS: m/z 547.00($M-H^+$).

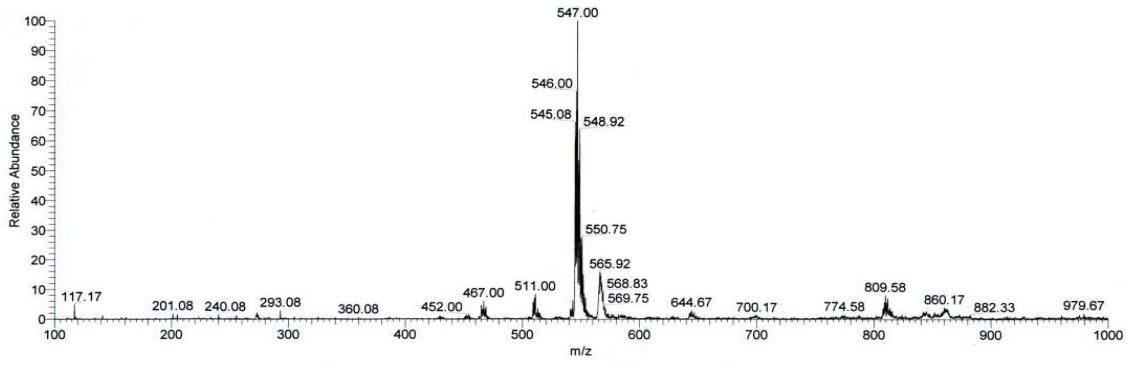

Fig. S2 The ES-MS spectrum of compound **5** in H_2O.

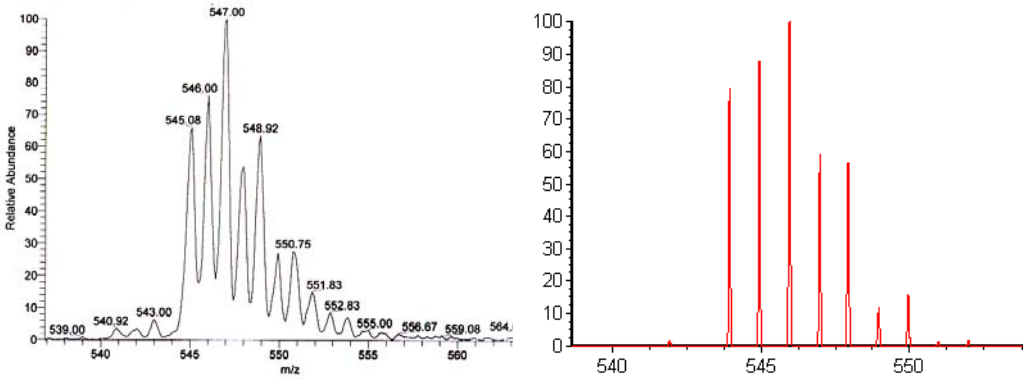

Fig. S3 The expanded ES-MS spectrum peaks of complex **5** in H_2O. The highest peak at 547.00 was assigned to $[\mathbf{5} - H]^+$ (left). The right part is the simulated isotopic distribution pattern (right).

Data for 6.

$(C_8H_{14}O_6N_2P_3PtCl_2)$ ESI^- MS: m/z 561.08$(M\text{-}H^+)$.

Cell lines, Culture Conditions, and Cytotoxicity Assay (MTT assay): Human osteosarcoma cells (MG-63) were cultured in Eagle's Minimum Essential Medium (Gibco) with heat-inactivated fetal bovine serum to a final concentration of 10%. Human ovarian cancer cells (COC1) were maintained in RPMI medium 1640 (Gibco) with 10% heat-inactivated fetal bovine serum. These cells were cultured at 37°C in an atmosphere of 5% CO_2 and 95% air and 100% relative humidity.

The cytotoxicity of the platinum compounds was measured by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay in 96-well microtiter plates at a predetermined cell density depending on the doubling time of

individual cell lines. After cell inoculation, the plates were incubated over night. Then the platinum compounds were added to the culture medium to give the indicated final concentrations, and cells were then incubated for additional 48 hours. MTT assays were performed as described previously.[7] The absorbance of each well was measured at 570 nm.

Quantitative Apoptosis Assays by Flow Cytometry: The ability of these platinum complexes to induce apoptosis is evaluated in COC1 cell line using Annexin V conjugated with FITC and propium iodide (PI) counterstaining by flow cytometry. COC1 cells of exponential growth were inoculated in 12-well plates and cultured for 12 hours before the platinum compounds were added to give the indicated final concentrations. After 48-hour incubation, cells were harvested, washed twice in phosphate-buffered saline (PBS), and resuspended in 100 μL binding buffer (including 140 mmol/L NaCl, 2.5 mmol/L $CaCl_2$ and 10 mmol/L Hepes / NaOH, pH 7.4) at a concentration of 1×10^6 cells/mL. Then cells were incubated with 5 μL of Annexin V- FITC (in buffer including 10mmol/L NaCl, 1% bovine serum albumin, 0.02% NaN_3 and 50 mmol/L Tris, pH 7.4) and 10 μL PI (20 μg/mL) for 15 min at room temperature in the dark. Cells were kept shielded from light before being analyzed by flow cytometry using a Becton-Dickinson FACSCalibur.

Circular Dichorism Study: CT-DNA was dissolved in buffer solution (5 mM Tris–HCl, 50 mM NaCl, pH 7.40) as a stock solution, which was stored at 4°C and used within 3 days. The solution gives a UV absorbance ratio (A260/A280) of ca 1.8, indicating that

CT-DNA was sufficiently protein-free. The concentration of CT-DNA was determined by measuring the UV absorption at 260 nm, taking 6600 $M^{-1}cm^{-1}$ as its molar absorption coefficient. The stock solutions of complex **1** and **3** used for the CD experiment were prepared with Tris–HCl buffer (5 mM Tris–HCl, 50 mM NaCl, pH 7.40). The CD spectra for CT-DNA were recorded as follows. Each sample of CT-DNA (1.0×10^{-4} M) was incubated with different concentrations of complex **1** or **3** (concentration ratios of platinum complex to DNA of 0, 0.1, 0.2, 0.4, 0.6, and 0.8, respectively) at 37 °C for 24 h in the dark and was scanned in the wavelength range of 220–320 nm at a speed of 10 nm/min and the buffer background was subtracted.

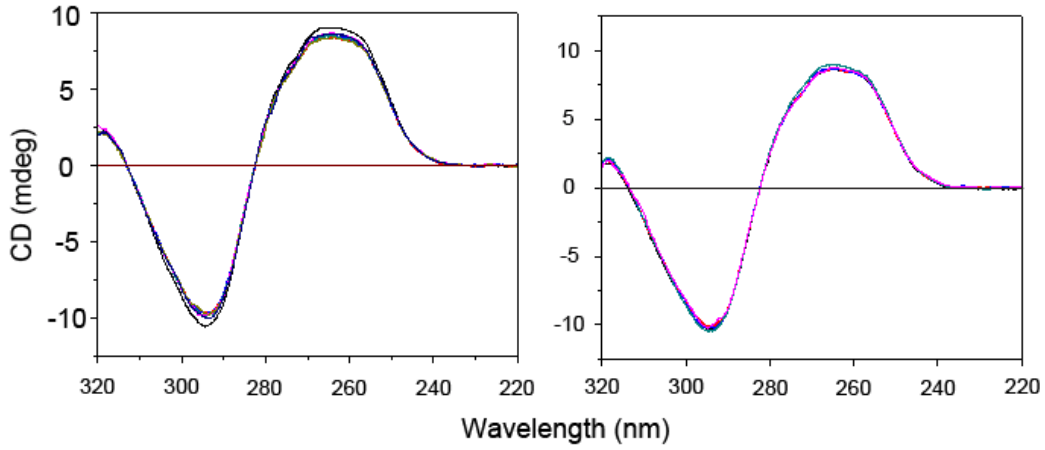

Fig. S4 CD spectra of CT-DNA (1.0×10^{-4} M) in the absence and presence of compounds **1** or **3** in buffer (5 mM Tris-HCl/40mM NaCl, pH 7.40) at room temperature

DNA binding: pUC19 plasmid DNA (200 ng) was incubated with each platinum complex at 37 °C for 24 h with different input drug-to-nucleotide ratios. Samples were examined by electrophoretic mobility shift assays through 1% agarose gel with TAE buffer (40 mM Tris acetate, 1 mM ethylenediaminetetraacetic acid (EDTA)) for 2 h at 70

V. The resultant gels were stained with 0.5 μg/mL ethidium bromide in TAE buffer and visualized under UV light.

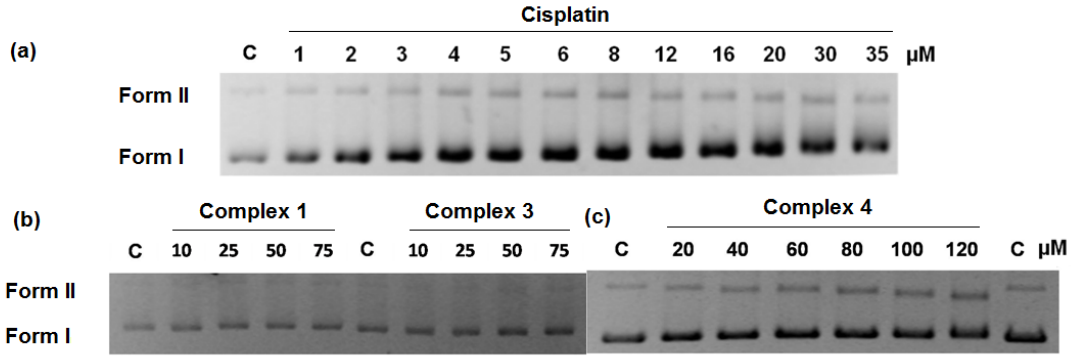

Fig. S5 Electrophoresis in agarose gel of pUC19 plasmid DNA (0.02 mg/mL, 30 μM base pair) incubated for 24 h at 37 °C with (a) cisplatin (b) complex **1**, complex **3**, and (c) complex **4** respectively. "C" represents control; the concentration of each platinum complex is indicated in the picture.

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