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Supporting Information

for

Fluorescent Method for Platinum Detection in Buffers and Serums for Cancer Medicine and Occupational Hazards

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GENERAL INFORMATION

All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25-mm EMD silica gel plates (60F-254) using a hand-held UV lamp (254 nm or 365 nm).

$Pt(PPh_3)_4$, $PtCl_2$ and $H_2PtCl_6 \bullet 6H_2O$ were purchased from Alfa Aesar and used as received. Cisplatin was purchased from Sigma-Aldrich and used as received. WaterOz Platinum is a product of Kornax Superior Nutrition L.L.C. (Houston, Texas). ImmunoPure® normal human serum was purchased from Thermo Scientific and used after restoration with 2.0 mL distilled water. Sheep serum was obtained from Luminos, LLC. Kimwipes® are a product of Kimberly-Clark. Buffers were purchased from J. T. Baker (pH 7, catalog number 5608-01) and used as received.

EXPERIMENTAL SECTION

Preparation of parent stock solutions used for this study.

Entry	Reagent	Quantity	Solvent (10 mL)	Conc. of stock solution
A	compound **1**	42.7 mg (0.10 mmol)	DMSO	10.0 mM
B	PPh_3	262.3 mg (1.00 mmol)	DMSO	100 mM
C	$PtCl_2$	13.3 mg (50 μmol)	DMSO	5.0 mM
D	$Pt(PPh_3)_4$	62.6 mg (50 μmol)	DMSO	5.0 mM
E	$H_2PtCl_6 \bullet 6H_2O$	25.9 mg (50 μmol)	1% HNO_3 in H_2O	5.0 mM
F	cisplatin	14.5 mg (50 μmol)	pH 7.0 buffer	5.0 mM

Notes:

(1) All the solutions were stored at 24 °C.

(2) Solution **A** was stored in the dark as a precaution.

(3) Solution **B** was freshly prepared every 2 weeks. We found that a 2-month-old solution of PPh_3 was not effective presumably due to air-oxidation.

(4) Each of these stock solutions with the respective solvent was further diluted to prepare 1.0 mM–1.0 μM stock solutions.

Fluorescence spectroscopy. Fluorescence spectra were recorded in a 1 × 1-cm disposable cuvette (VWR; catalog number 58017-880) on a Jobin Yvon FluoroMax-3 spectrometer under the control of a Windows-based PC running FluorEssence software. The samples were excited at 497 nm and the emission intensities were collected at 523 nm. All spectra were corrected for emission intensity using the manufacturer supplied photomultiplier curves.

Determination of reactive oxidation state for platinum. To a solution of compound **1** (5.0 mg, 12 μmol) in THF (150 μL) was added morpholine (2 μL, 13 μmol), and $Pt(PPh_3)_4$ (1.0 mg, 0.6 μmol) at 24 °C. The reaction was stirred for 10 min and TLC analysis (50% EtOAc in hexanes) showed formation of **2**.[1]

Morpholine (2 μL, 13 μmol) and $PtCl_2$ (1.0 mg, 0.6 μmol) were added to a solution of compound **1** (5.0 mg, 12 μmol) in THF (150 μL) at 24 °C. The reaction was stirred for 10 min at the same temperature, and TLC analysis (50% EtOAc in hexanes) showed no reaction (i.e., only starting material). Following addition of $NaBH_4$ (5 mg, 0.13 mmol), the reaction fully proceeded to form **2**.

[1] Song, F.; Garner, A. L.; Koide, K. *J. Am. Chem. Soc.* **2007**, *129*, 12354.

Platinum: buffer screening. $PtCl_2$ solution (25.0 μL of 100.0 μM stock, $[Pt]_{final}$ = 625 nM) and solution **B** (10.0 μL, $[PPh_3]_{final}$ = 250 μM) were added to DMSO/buffer (1:4) solution (pH = 4.0–10.0) (4.0 mL). Solution **A** (5.0 μL, $[\mathbf{1}]_{final}$ = 12.5 μM) was then added to the mixture, and the resulting mixtures were incubated for 1 h at 24 °C before fluorescence measurement.

Platinum: initial rate analysis. $PtCl_2$ stock solution **C** (50.0 μL, 250 nmol), PPh_3 (4.8 mg, 2.34 μmol) and **2** (10.0 mg, 23.4 μmol) were added to DMSO/buffer (1:1) solution (pH = 4.0, 7.0, 10.0) (20 mL) at 24 °C. 1:1 DMSO/buffer was used in this case for the solubility of **1** and PPh_3. The experiment was performed in triplicate. At t = 0.5, 1 and 2 h, 100 μL aliquots were taken from each reaction mixture and diluted to 4.0 mL with DMSO/pH 7.0 buffer (1:4) for fluorescence measurement.

Platinum: TOF. To determine the turnover frequency, a solution containing $PtCl_2$ ($[PtCl_2]_{final}$ = 50 nM), solution **B** (10.0 μL, $[PPh_3]_{final}$ = 250 μM) and solution **A** (5.0 μL, $[\mathbf{1}]_{final}$ = 12.5 μM) was prepared in DMSO/pH 7.0 buffer (1:4). The intensity of this sample was compared to a standard solution containing Pittsburgh Green **2** ($[\mathbf{2}]_{final}$ = 50 nM) in DMSO/pH 7.0 buffer (1:4). The intensity of the Pt^{2+}-containing sample was 2.8×10^6 after 7 h at 24 °C and the intensity of the standard solution of **3** was 8.8×10^4. The turnover frequency is 2.8×10^6 /8.8×10^4 = 31.8/7 h = 4.5 h^{-1}.

Platinum: concentration dependence. Varying amounts of Pt solution and solution **B** (10.0 μL, $[PPh_3]_{final}$ = 250 μM) were added to DMSO/pH 7.0 buffer solution (1:4) (4.0 mL). Solution **A** (5.0 μL, $[\mathbf{1}]_{final}$ = 12.5 μM) was added to the mixture, and the samples were incubated for 20–24 h at 24 °C or 37 °C before fluorescence measurement. Each experiment was performed in triplicate.

Pt detection in WaterOz Platinum. DMSO/pH 7.0 buffer (1:4) (10.0 mL) and solution **B** (20.0 μL, $[PPh_3]_{final}$ = 140 μM) were added to WaterOz Platinum (4.4 mL, 750 nM final concentration). Solution **A** (10.0 μL, $[\mathbf{1}]_{final}$ = 7.0 μM) was added to the mixture, and the samples were incubated for 1 h at 24 °C before fluorescence measurement.

Cisplatin detection in human serum. Varying amounts of cisplatin in pH 7.0 buffer (total volume of pH 7.0 buffer = 100.0 μL) were added to immunopure human serum (50.0 μL). Solution **B** (10.0 μL, $[PPh_3]_{final}$ = 6.0 mM) and solution **A** (5.0 μL, $[\mathbf{1}]_{final}$ = 300.0 μM) were added to the mixture, and the samples were incubated for 24 h at 24 °C before fluorescence measurement. The experiment was performed in triplicate.

Cisplatin detection in sheep serum. Varying amounts of cisplatin in pH 7.0 buffer and 5% HNO_3 (50 μL) were added to sheep serum (50.0 μL). The samples were allowed to sit for 5 min and then heated in a water bath at 100 °C in a fume hood to evaporate the acid. The samples were then diluted with pH 7.0 buffer (total volume of pH 7.0 buffer = 500.0 μL) and treated with solution **B** (10.0 μL, $[PPh_3]_{final}$ = 6.0 mM) and solution **A** (5.0 μL, $[\mathbf{1}]_{final}$ = 300.0 μM). The samples were incubated for 24 h at 24 °C before fluorescence measurement. The experiment was performed in triplicate.

Detection of cisplatin contamination. A solution of cisplatin in pH 7.0 buffer (500 μL, 1.0 mM stock solution) was spilled on an uncleaned floor tile. After 5 min, the spilled area was wiped with a wet paper towel (with water) and subsequently with a Kimwipe® containing a 1% HCl solution. The HCl-soaked Kimwipe® was placed into a 20-mL scintillation vial and additional 1% HCl (2.0 mL) was added. After 10 min, the solution was basified to pH 9.0 with 1.0 N NaOH (<1.0 mL) and diluted to 10.0 mL with DMSO/pH 7.0 buffer (1:4). Solution **B** (20.0 μL, $[PPh_3]_{final}$ = 200 μM) and solution **A** (10.0 μL, $[\mathbf{1}]_{final}$ = 10.0 μM) were then added to the mixture, and the samples were incubated for 3 h at 24 °C before visualization under a UV lamp at 365 nm wavelength. The experiment was performed in triplicate.