Electronic Supplementary Information (ESI)

Deubiquitinases as potential anti-cancer targets for gold(III) complexes

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1. General experimental procedure

A. Materials. All chemicals were purchased from Sigma-Aldrich, unless otherwise noted. 2-(3-*n*-butylphenyl)pyridine, and 2-(4-butoxyphenyl)pyridine were purchased from Dieckmann (HK). Chemical Industry Company Limited. 5-Butyl-2-phenylpyridine was purchased from Tractus Company Limited. 2-[4-(Trifluoromethyl)phenyl]pyridine was purchased from Tokyo Chemical Industry Co., Ltd. Sodium di-*n*-butyldithiocarbamate was bought from ABCR Gmblt & Co. KG. 3-[(3-Cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPS) was purchased from USB Corporation. The solvents used for synthesis were of analytical or HPLC grade. Phosphate buffered saline (PBS), Minimum Essential Medium (MEM), and Fetal Bovine Serum (FBS) were purchased from GIBCO. MG132 was purchased from Enzo Life Sciences. Recombinant human ubiquitin Cterminal esterase L5 (UCHL5 Human) was bought from ProSpec. Ubiquitin Cterminal hydrolase L3 (UCHL3), ubiquitin C-terminal hydrolase L1 (UCHL1), USP2_{CD} (UBP41 catalytic domain), 20S proteasome, Ubiquitin C-terminal 7amido-4-methylcoumarin (Ub-AMC), Suc-Leu-Leu-Val-Tyr-AMC (Suc-LLVY-AMC), and Boc-Leu-Arg-Arg-AMC (Boc-LRR-AMC) were bought from Boston Biochem. Anti-p53 antibody was bought from CALBIOCHEM. Cleaved caspase-7, cleave-PARP, and Anti-GAPDH antibodies were purchased from Cell Signaling.

B. Instruments. ¹H-NMR spectra were recorded on a Bruker AVANCE 400 Fourier-Transform NMR spectrometer (chemical shift in ppm). 2-D H-H COSY NMR spectra were recorded on a Bruker AVANCE 600 Fourier-transform NMR spectrometer. Positive-ion FAB (Fast Atom Bombardment) mass spectra were recorded on a Finnigan MAT95 mass spectrometer. Electrospray ionization (ESI) mass spectra were recorded on a Finnigan LCQ mass spectrometer or Waters

(Micromass MS TECHNOLOGIES) Q-Tof PeimierTM mass spectrometer. Liquid chromatography coupled with electrospray ionization mass spectrometry (LC-ESI-MS) analysis were performed on a Waters (Micromass MS TECHNOLOGIES) Q-Tof Peimier TM mass spectrometer combined with Waters ACQuityTM ultra performance LC. Elemental analyses were conducted in the Institute of Chemistry, the Chinese Academy of Sciences, Beijing. UV/Vis absorption spectra were recorded on a Perkin-Elmer Lambda 900 UV-Visible spectrophotometer. Optical and fluorescence micrographs were taken on a Zeiss Axiovert 200M inverted fluorescence microscopy.

C. Synthesis.

[Au^{III}(2-(4-*n*-butylphenyl)pyridyl)(diethyldithiocarbamate)]PF₆ (1). The mixture of [Au^{III}(2-(4-*n*-butylphenyl)pyridyl)Cl₂] (60 mg, 0.126 mmol) and sodium diethyldithiocarbamate trihydrate (25.6 mg, 0.114 mmol) in MeOH (8.0 mL) was stirred at room temperature for 3 hours. After filtration, excess saturated KPF₆ aqueous solution was added to the filtrate. The final mixture was filtered and washed with H₂O, and recrystallized by diffusing ethyl ether to the CH₃CN solution of the crude product. Yield: 65.4 mg, 81.9 %. ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 8.82 (d, *J* = 5.2 Hz, 1 H), 8.42 (m, 2 H), 8.03 (d, *J* = 8.0 Hz, 1 H), 7.64 (ddd, *J* = 1.6 Hz, *J* = 5.8 Hz, *J* = 7.3 Hz, 1 H), 7.38 (dd, *J* = 1.3 Hz, *J* = 7.9 Hz, 1 H), 7.01 (d, *J* = 1.3 Hz, 1 H), 3.90 (p, *J* = 7.2 Hz, 4 H), 2.67 (t, *J* = 7.7 Hz, 2 H), 1.57 (m, 2 H), 1.35 (m, 8 H), 0.90 (t, *J* = 7.3 Hz, 3 H). FAB-MS (+ve, *m*/z): 555.1 [M⁺]. Anal. Calcd for C₂₀H₂₆AuF₆N₂PS₂: C 34.29, H 3.74, N 4.00. Found: C 34.35, H 3.85, N 4.14.

[Au^{III}((2-phenyl)pyridyl)(diethyldithiocarbamate)]PF₆ (2). To the stirring suspension of [Au^{III}((2-phenyl)pyridyl)Cl₂] (20 mg, 0.0474 mmol) in methanol (2.0 mL) was added sodium diethyldithiocarbamate trihydrate (10.7 mg, 0.0475

mmol) in methanol (1.5 mL) drop by drop. After stirring at room temperature overnight, the mixture was filtered and excess saturated KPF₆ aqueous solution was added to the filtrate. The final mixture was filtered and washed with H₂O and methanol. The final product was dried in vacuum. Yield: 25.7 mg, 84.1 %. ¹H-NMR (400 MHz, DMSO-*d*₆): $\delta = 8.86$ (d, J = 5.7 Hz, 1 H), 8.51 (d, J = 8.0 Hz, 1 H), 8.43 (t, J = 7.7 Hz, 1 H), 8.14 (d, J = 7.6 Hz, 1 H), 7.69 (t, J = 6.5 Hz, 1 H), 7.55 (t, J = 7.5 Hz, 1 H), 7.42 (t, J = 7.5 Hz, 1 H), 7.21 (d, J = 7.6 Hz, 1 H), 3.90 (m, 4 H), 1.37 (m, 6 H). FAB-MS (+ve, *m*/*z*): 499.1 [M⁺]. Anal. Calcd for C₁₆H₁₈AuF₆N₂PS₂·H₂O: C 29.01, H 3.04, N 4.23. Found: C 28.29, H 2.66, N 4.19.

[Au^{III}(2-(3-*n*-butylphenyl)pyridine)Cl₂]. To a stirring solution of K[Au^{III}Cl₄] (291.2 mg, 0.775 mmol) in distilled water (20.0 mL) was added 2-(3-*n*-butylphenyl)pyridine (163.6 mg, 0.775 mmol) in acetonitrile (2.0 mL) drop by drop. After stirred for 3.5 h, the final mixture was filtered. The solid was washed with H₂O and hexane, and dried in vacuum. The solid was then heated at 433 K for 1.0 h, and washed with acetone and methanol. Yield: 140.7 mg, 38.07 %. ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 9.50 (d, *J* = 5.5 Hz, 1 H), 8.39 (m, 2 H), 7.82 (d, *J* = 1.8 Hz, 1 H), 7.75 (m, 1 H), 7.68 (d, *J* = 8.3 Hz, 1 H), 7.20 (dd, *J* = 1.8 Hz, *J* = 8.3 Hz, 1 H), 2.63 (t, *J* = 7.7 Hz, 2 H), 1.61 (m, 2 H), 1.33 (m, 2 H), 0.91 (t, *J* = 7.3 Hz, 3 H). FAB-MS (+ve, *m*/z): 442.1 [M-Cl]⁺.

[Au^{III}(2-(3-*n*-butylphenyl)pyridyl) (diethyldithiocarbamate)]PF₆ (3). Sodium diethyldithiocarbamate trihydrate (14.2 mg, 0.0629 mmol) in methanol (2.5 mL) was added dropwise to a stirring suspension of [Au^{III}(2-(3-*n*-butylphenyl)pyridine)Cl₂] (30 mg, 0.0629 mmol) in methanol (3.0 mL). The mixture was stirred at room temperature overnight. After filtration, excess saturated KPF₆ aqueous solution was added to the filtrate. The appeared precipitate was washed with H₂O and methanol. The final product was purified by

recrystallization with ethyl ether diffused to the CH₂Cl₂ solution of the crude product. Yield: 32.4 mg, 73.53 %. ¹H-NMR (400 MHz, DMSO- d_6): $\delta = 8.84$ (d, J = 5.7 Hz, 1 H), 8.53 (d, J = 8.1 Hz, 1 H), 8.42 (t, J = 7.7 Hz, 1 H), 8.00 (s, 1 H), 7.67 (t, J = 6.6 Hz, 1 H), 7.24 (d, J = 7.9 Hz, 1 H), 7.10 (d, J = 7.7 Hz, 1 H), 3.89 (m, 4 H), 2.66 (t, J = 7.4 Hz, 2 H), 1.62 (m, 2 H), 1.34 (m, 8 H), 0.92 (t, J = 7.3 Hz, 3 H). FAB-MS (+ve, m/z): 555.1 [M⁺]. Anal. Calcd for C₂₀H₂₆AuF₆N₂PS₂: C 34.29, H 3.74, N 4.00. Found: C 34.05, H 3.63, N 3.98.

[Au^{III}(2-(4-butoxyphenyl) pyridyl)Cl₂]. To a stirring solution of K[Au^{III}Cl₄] (104.7 mg, 0.277 mmol) in distilled water (7.0 mL) was added 2-(4-butoxyphenyl)pyridine (63.0 mg, 0.277 mmol) in acetonitrile (1.5 mL) drop by drop. After stirred for 3.5 h, the final mixture was filtered. The solid was washed with H₂O and hexane, and dried in vacuum. The solid was then heated at 433 K for 1.0 h, and washed with acetone and methanol. Yield: 49.7 mg, 36.28 %. ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 9.41 (d, *J* = 5.9 Hz, 1 H), 8.30 (t, *J* = 7.5 Hz, 1 H), 8.25 (d, *J* = 7.5 Hz, 1 H), 7.93 (d, *J* = 8.6 Hz, 1 H), 7.63(t, *J* = 6.3 Hz, 1 H), 7.32 (d, *J* = 1.7 Hz, 1 H), 7.06 (dd, *J* = 1.7 Hz, *J* = 8.4 Hz, 1 H), 4.08 (t, *J* = 6.3 Hz, 2 H), 1.73 (m, 2 H), 1.45 (m, 2 H), 0.94 (t, *J* = 7.3 Hz, 3 H). FAB-MS (+ve, *m/z*): 458.1 [M-Cl]⁺.

[Au^{III}(2-(4-butoxyphenyl)pyridyl) (diethyldithiocarbamate)]PF₆ (4). Sodium diethyldithiocarbamate trihydrate (13.68 mg, 0.061 mmol) in MeOH (2.5 mL) was added dropwise to a stirring suspension of [Au^{III}(2-(4-butoxyphenyl) pyridyl)Cl₂] (30 mg, 0.061 mmol) in methanol (3.0 mL). The mixture was stirred at room temperature overnight. After filtration, excess saturated KPF₆ aqueous solution was added to the filtrate. The precipitate was washed with H₂O and methanol, and recrystallized by diffusing ethyl ether to the CH₂Cl₂ solution of the crude product. Yield: 28.9 mg, 66.12 %. ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 8.74 (d, *J* = 5.7 Hz,

1 H), 8.34 (d, J = 4.0 Hz, 2 H), 8.07 (d, J = 8.6 Hz, 1 H), 7.56 (m, 1 H), 7.10 (dd, J = 2.2 Hz, J = 8.6 Hz, 1 H), 6.60 (d, J = 2.3 Hz, 1 H), 4.09 (t, J = 6.3 Hz, 2 H), 3.90 (m, 4 H), 1.72 (m, 2 H), 1.41 (m, 8 H), 0.95 (t, J = 7.4 Hz, 3 H). FAB-MS (+ve, m/z): 571.1 [M⁺]. Anal. Calcd for C₂₀H₂₆AuF₆N₂OPS₂: C 33.53, H 3.66, N 3.91. Found: C 33.46, H 3.61, N 3.97.

[Au^{III}(2-(4-(trifluoromethyl)phenyl)pyridyl)Cl₂]. 2-[4-

(trifluoromethyl)phenyl]pyridine (178.0 mg, 0.798 mmol) in acetonitrile (2.5 mL) was added to a stirring solution of K[Au^{III}Cl₄] (300 mg, 0.798 mmol) in distilled water (20 mL) drop by drop. Around 200 min later, the final mixture was filtered, and washed with H₂O and hexane. After the solid was dried, the solid was further heated at 443 K for 1.0 h, and washed with methanol followed by recrystallized with DMSO. Yield: 126.1 mg, 32.2 %. ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 9.54 (d, *J* = 5.8 Hz, 1 H), 8.54 (d, *J* = 7.8 Hz, 1 H), 8.46 (t, *J* = 7.7 Hz, 1 H), 8.22 (d, *J* = 8.0 Hz, 1 H), 8.07 (s, 1 H), 7.87 (m, 2 H). FAB-MS (+ve, *m/z*): 454.0 [M-Cl]⁺.

[Au^{III}(2-(4-(trifluoromethyl)phenyl)pyridyl) (diethyldithiocarbamate)]PF₆ (5). Sodium diethyldithiocarbamate trihydrate (13.8 mg, 0.0612 mmol) in methanol (2.5 mL) was added dropwise to a stirring suspension of [Au^{III}(2-(4-(trifluoromethyl)phenyl)pyridyl)Cl₂] (30 mg, 0.0612 mmol) in methanol (3.0 mL). The mixture was stirred at room temperature for 2.3 h. After filtration, excess saturated KPF₆ aqueous solution was added to the filtrate. The final mixture was filtered and washed with H₂O and methanol, and recrystallized by diffusing ethyl ether to the CH₂Cl₂ solution of the crude product. Yield: 26.5 mg, 60.8 %. ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 8.94 (d, *J* = 5.6 Hz, 1 H), 8.64 (d, *J* = 7.8 Hz, 1 H), 8.50 (dt, *J* = 1.3 Hz, *J* = 7.9 Hz, 1 H), 8.39 (d, *J* = 8.1 Hz, 1 H), 7.95 (d, *J* = 8.1 Hz, 1 H), 7.79 (m, 1 H), 7.40 (s, 1 H), 3.91 (m, 4 H), 1.37 (m, 6 H). FAB-MS (+ve, *m/z*): 567.0 [M⁺]. Anal. Calcd for C₁₇H₁₇AuF₉N₂PS₂: C 28.66, H 2.41, N 3.93. Found: C 28.75, H 2.21, N 3.97.

[Au^{III}(2-(4-*n*-butylphenyl)pyridyl)(di-*n*-butyldithiocarbamate)]PF₆ (6). To the suspension of [Au^{III}(2-(4-*n*-butylphenyl)pyridyl)Cl₂] (30 mg, 0.0629 mmol) in methanol (3.0 mL) was added sodium di-*n*-butyldithiocarbamate (13.7 µL, 0.0629 mmol, 47 % in H₂O) drop by drop. After stirring overnight, the mixture was filtered, and excess saturated KPF₆ aqueous solution was added to the clear filtrate, and the formed precipitate was collected followed by washing with H₂O. The residue was later washed with small amount of methanol, and the methanol solution was collected. Final product was crystalized by slow evaporation of the methanol solution, and washed with H₂O. Yield: 9.0 mg, 18.7 %. ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 8.83 (d, *J* = 5.6 Hz, 1 H), 8.44 (d, *J* = 7.4 Hz, 1 H), 8.39 (t, *J* = 7.8 Hz, 1 H), 8.04 (d, *J* = 8.0 Hz, 1 H), 7.63 (m, 1 H), 7.38 (d, *J* = 7.8 Hz, 1 H), 1.57 (m, 2 H), 1.36 (m, 6 H), 0.94 (m, 9 H). FAB-MS (+ve, *m*/z): 611.1 [M⁺]. Anal. Calcd for C₂₄H₃₄AuF₆N₂PS₂: C 38.10, H 4.53, N 3.70. Found: C 38.03, H 4.08, N 3.79.

[Au^{III}((2-phenyl)pyridyl)(di-*n*-butyldithiocarbamate)]PF₆ (7). To the suspension of [Au^{III}((2-phenyl)pyridyl)Cl₂] (30 mg, 0.0713 mmol) in methanol (2.5 mL) was added sodium di-*n*-butyldithiocarbamate (15.5 µL, 0.0712 mmol, 47 % in H₂O) drop by drop. After stirring overnight, the mixture was filtered, and excess saturated KPF₆ aqueous solution was added to the clear filtrate, and the formed precipitate was collected followed by washing with H₂O and methanol. The final product was dried in vacuum. Yield: 20.8 mg, 41.7 %. ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 8.86 (d, *J* = 5.6 Hz, 1 H), 8.50 (d, *J* = 8.0 Hz, 1 H), 8.42 (t, *J* = 7.8 Hz, 1 H), 8.13 (d, *J* = 7.6 Hz, 1 H), 7.67 (t, *J* = 6.5 Hz, 1 H), 7.54 (t, *J* = 7.5 Hz, 1

H), 7.41 (t, J = 7.5 Hz, 1 H), 7.21 (d, J = 7.6 Hz, 1 H), 3.84 (m, 4 H), 1.78 (m, 4 H), 1.39 (m, 4 H), 0.96 (m, 6 H). FAB-MS (+ve, m/z): 555.1 [M⁺]. Anal. Calcd for $C_{20}H_{26}AuF_6N_2PS_2$: C 34.29, H 3.74, N 4.00. Found: C 34.27, H 3.69, N 3.96.

[Au^{III}(5-butyl-2-phenylpyridine)Cl₂]. 5-butyl-2-phenylpyridine (168.0 mg, 0.796 mmol) in acetonitrile (2.5 mL) was added to a stirring solution of K[AuIIICl₄] (300 mg, 0.798 mmol) in distilled water (20 mL) drop by drop. Around 200 min later, the final mixture was filtered, and washed with H₂O and hexane. After the solid was dried, the solid was further heated at 443 K for 1.0 h, and washed with acetonitrile followed by recrystallized with DMSO. Yield: 167.8 mg, 44.1 %. ¹H-NMR (400 MHz, DMSO- d_6): $\delta = 9.33$ (s, 1 H), 8.33 (d, J = 8.2 Hz, 1 H), 8.28 (d, J = 8.0 Hz, 1 H), 7.92 (d, J = 7.2 Hz, 1 H), 7.78 (d, J = 8.1 Hz, 1 H), 7.45 (t, J = 7.3 Hz, 1 H), 7.33 (t, J = 7.4 Hz, 1 H), 2.77 (t, J = 7.6 Hz, 2 H), 1.62 (m, 2 H), 1.35 (m, 2 H), 0.92 (t, J = 7.3 Hz, 3 H), FAB-MS (+ve, m/z): 442.1 [M-Cl]⁺.

[Au^{III}(5-butyl-2-phenylpyridine)(diethyldithiocarbamate)]PF₆ (8). Sodium diethyldithiocarbamate trihydrate (14.2 mg, 0.0630 mmol) in methanol (2.5 mL) was added dropwise to a stirring suspension of [Au^{III}(5-butyl-2-phenylpyridine)Cl₂] (30 mg, 0.0629 mmol) in methanol (3.0 mL). The mixture was stirred at room temperature overnight. After filtration, excess saturated KPF₆ aqueous solution was added to the filtrate. The final mixture was filtered and washed with H₂O, and recrystallized by diffusing ethyl ether to the CH₂Cl₂ solution of the crude product. Yield: 29.6 mg, 67.2 %. ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 8.72 (d, *J* = 1.0 Hz, 1 H), 8.42 (d, *J* = 8.4 Hz, 1 H), 8.32 (dd, *J* = 1.3 Hz, *J* = 8.3 Hz, 1 H), 8.09 (d, *J* = 7.2 Hz, 1 H), 7.52 (t, *J* = 7.4 Hz, 1 H), 7.38 (dt, *J* = 1.1 Hz, *J* = 7.6 Hz, 1 H), 7.18 (d, *J* = 7.6 Hz, 1 H), 3.90 (m, 4 H), 2.78 (m, 2 H), 1.61 (m, 2 H), 1.34 (m, 8 H), 0.93 (t, *J* = 7.3 Hz, 3 H). FAB-MS (+ve, *m*/*z*): 555.1 [M⁺]. Anal. Calcd for C₂₀H₂₆AuF₆N₂PS₂: C 34.29, H 3.74, N 4.00. Found: C 34.41, H 3.68, N 4.00.

D. Cell lines and cell cultures. Human cervical epithelial carcinoma (HeLa), melanoma (B16), hepatocellular carcinoma (HepG2), glioblastoma (U87), breast adenocarcinoma (MCF-7), and non-tumorigenic immortalized liver (MIHA) cells were maintained in Minimum Essential Medium (MEM). Breast carcinoma (MDA-MB-231) and lung carcinoma (NCI-H460) cells were maintained with RPMI-1640 Medium. MS1 (CRL-2279) cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM). All the medium contained or were supplemented with 10% FBS, L-glutamine (2 mM), penicillin (100 unites/mL), and streptomycin (100 μ g/mL). All cells were incubated in a humidified atmosphere with 95% air and 5% CO₂ at 37 °C.

E. Deubiquitinases (DUBs) and proteasome activity assays. UCHL1 (10 nM), UCHL3 (0.075 nM), UCHL5 (6.25 nM) and USP2_{CD} (20 nM), or 20S proteasome (0.5 nM) was pre-incubated with the Au(III) complexes for 10 min in assay buffer (50 mM Tris-HCl, 1 mM EDTA, pH 7.6) . The DUBs activities were determined using fluorescent ubiquitin substrate Ub-AMC (0.625 µM). The chymotrypsin-like activity and trypsin-like activity of 20S proteasome were measured with Suc-LLVY-AMC (100 µM) and Boc-LRR-AMC (100 µM), respectively. The initial rates of hydrolysis of the fluorescent substrates were measured using fluorescence plate reader (Perkin-Elmer Fusion α-FP) with 380 nm excitation and 460 nm emission filters. The residual enzyme activity) was determined and expressed as percentage of control (100*k₂/k₁, where k₁ and k₂ are the initial rates of substrate hydrolysis in the absence and presence of complexes, respectively).

For cell-based DUBs activity assay, MCF-7 cells (1.6×10^5) were seeded at 6 well plates. After 24 h, cells were treated with **1** (0.9 µM) for 0, 3, 6, 9, and 24 h, respectively. The cells were lysed in a buffer containing 25 mM HEPES, 5 mM

EDTA, 0.1 % CHAPS, pH 7.3. The DUBs activities of cell extracts containing 0.2 μ g of proteins were assayed with Ub-AMC (0.625 μ M) as described above.

F. Liquid chromatography coupled with electrospray ionization mass spectrometry (LC-ESI-MS) analysis. UCHL1 (1 μ g) was loaded into a SB-C3 column (75 μ m × 60 mm). The LC-ESI-MS analysis was performed on a Waters (Micromass MS TECHNOLOGIES) Q-Tof Peimier TM mass spectrometer combined with Waters ACQuityTM ultra performance LC. Buffer A was 0.1 % formic acid in water, and buffer B was 0.1 % formic acid in acetonitrile. After desalted with a solution of 95 % A and 5 % B, the UCHL1 was detected by increasing the separation gradient from 5 % B to 95 % B in 30 min. For the mixture of UCHL1 (3 μ g) and 1 (excess: 25 times) which had reacted for 10 min at room temperature in buffer (50 mM Tris-HCl, 1 mM EDTA, pH 7.6), the analysis process was similar as that for UCHL1. The separation gradient was increased from 5 % B to 95 % B in 30 min, and held at 95 % B for another 30 min. The data were analyzed with MassLynx software.

G. ESI-MS analysis of the interaction of peptides which contain active sites and present in UCHL1, UCHL3 and UCHL5 with 1. The interaction of peptides that contain active residue present in UCHL1 (TIGNSCGTIGR, DKVNFHFILFR, and HLYELDGRMPR), UCHL3 (TISNACGTIGR) and/or UCHL5 (VINNACATQAR) with 1 was studied by ESI-MS. And an arginine (R) residue was attached to each peptide to achieve positive charge giving better signals. Each peptide was incubated with 1 at the molar ratio of 1:1 in buffer (5 mM NH₄HCO₃, pH 7.6) for 10 min, and the final mixture was analyzed by ESI-MS performed on Waters (Micromass MS TECHNOLOGIES) Q-Tof PeimierTM mass spectrometer. **H. Microarray-based gene expression and Connectivity Map analysis.** MCF-7 cells (1×10^6 cells) were seeded in 10 cm dishes overnight, followed by treatment with or without **1** (0.9 µM) for 24 h. The total RNA of the treated cells was isolated and purified using RNeasy Mini Kit (Qiagen). The microarray-based gene expression analysis was performed on an Affymetrix Human Genome U133 Plus 2.0 Array GeneChip. The data was analyzed with the Agilent GeneSpring GX software (Genome Research Centre, The University of Hong Kong). Those genes with no less than 2-fold changes in expression compared with vehicle control were analyzed with Connectivity Map resources (J. Lamb, *Nat. Rev. Cancer*, 2007, **7**, 54.).

I. Cellular uptake experiment. MCF-7, MDA-MB-231, HeLa and MIHA cells were seeded in 6 well plates with a density of 1.2×10^5 cells/ well. After 24 h, cells were treated with various gold(III) complexes (0.9 µM) for 2 h. Cells were washed with ice-cold PBS for 3 times, and further incubated with milli-Q water (500 µL) on ice. After 15 min, cells were harvested, and 300 µL of the samples were digested with HNO₃ (69 %) overnight at room temperature, while the remaining 200 µL samples were used for protein assay. The digested samples were further diluted by 40 folds with milli-Q water. The gold analysis was performed on an Agilent 7500 inductively-coupled-plasma mass spectrometer (ICP-MS).

J. Cell-cycle analysis. MCF-7 cells (1×10^6) were seeded in 10 cm dishes. After incubated for 24 h, cells were treated with 1 (0.9 µM) for 9 h. Then, cells were trypsinized and washed with cold PBS (3 mL) once, followed by fixing with icecold ethanol (70%, 2 mL) for 1 h at 4 °C. Cells were further washed with PBS containing 1% BSA twice, incubated with RNase A (50 µg/ mL) in PBS at 37 °C for 1 h, and stained with propidium iodide (40 µg/ mL) in PBS at room temperature for 30 minutes in dark. 3×10^4 cells were counted in a Coulter EPICS flow cytometer (Coulter, Miami, FL) equipped with 480 long, 525 band, and 625 long pass mirrors. The data was analyzed with Modfit 5.11 software.

K. Western blotting analysis. MCF-7 and MDA-MB-231 cells (1 \times 10⁶) were cultured in 10 cm dishes overnight and treated with 1 (0.9 μ M) for 24 h. Cells were harvested and washed with ice-cold PBS twice followed by homogenization in icecold lysis buffer (20 mM Tris-HCl, pH 7.2, 150 mM NaCl, 2 mM EDTA, 10% glycerol, 1% Triton X-100, 100 mM dithiothreitol (DTT), protease inhibitors) for 15 min. Then, the lysates were centrifuged at 13,200 rpm for 30 min followed by denaturation at 100 °C for 10 min. The protein content for each sample was quantified by Protein Assay (Bio-Rad) and was normalized. Proteins were fractionated on a 7% or 12% SDS-PAGE in a Tris-Glycine running buffer, and transferred onto a polyvinylidene fluoride (PVDF) membrane (GE Healthcare), which was then blocked in a TBST buffer (10 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.1% Tween-20) containing 5% bovine serum albumin (BSA, USB) at room temperature for 1-2 h. The membrane was incubated with the primary antibodies at 4 °C overnight, washed with TBST 3 times, and further incubated with secondary antibodies at room temperature for another 1-2 h. After washing with TBST, the immunoreactivity was examined with the enhanced chemiluminescence plus kit (GE Healthcare). GAPDH was used as a probe for the equal protein loading.

L. Hoechst 33342 nuclear staining experiment. MCF-7 cells (1.6×10^5) in 2 mL were cultured in 35 mm glass-bottomed microwell dishes (MatTek) overnight followed by treatment with 1 (0.9 μ M, 24 h). Cells were then stained with Hoechst 33342 (5 μ g/mL) for 5 min. The stained cells were observed with a Zeiss Axiovert 200M inverted fluorescence microscopy.

M. Tube-formation assay. The tube-formation assay was performed by using the *In Vitro* Angiogenesis Kit (Millipore Corporation). ECMatrixTM solution was mixed with 10× Diluent Buffer with the ratio of 9:1, and 50 μ L of the mixture was transferred to each well of 96 well plates which were further incubated at 37 °C for 1 h to allow polymerization. Then, MS1 cells (7 × 10⁴) were mixed with **1** (1, 2 and 4 μ M) in 150 μ L DMEM medium containing Endothelial Cell Growth Supplements (ECGS) and added on the top of the polymerized matrix. After incubation at 37 °C for another 2 h, tube formation was examined with a Zeiss Axiovert 200M inverted fluorescence microscopy at a 5× magnification.

N. Cytotoxicity evaluation. The cytotoxicity were determined by using modified naphthol blue black (NBB) staining assay (J. Schulz, S. Dettlaff, U. Fritzsche, U. Harms, H. Schiebel, W. Derer, N. E. Fusenig, A. Hülsen and M. Böhm, *J. Immunol. Methods*, 1994, **167**, 1). After an overnight incubation of various cell lines in 96-well, flat-bottomed microtitre plates at 37 °C in a humidified atmosphere of 5% $CO_2/95\%$ air, different concentrations of complexes were added, and the plates were further incubated for 24–72 h. At the end of incubation, the medium was discarded, a mixture of 50 µL of formaldehyde (3% in PBS) and 50 µL of NBB solution (0.05 % NBB, 0.1 M sodium acetate, 9 % acetic acid) was added. After 30 minutes, the mixture was discarded, and the plates were washed with deionized water for 3 times, dried in air, and 100 µL of NaOH (50 mg/mL) was added to solubilize the NBB-stained cells. The plates were shaken for 1 h. The percentage of survived cells was measured at 580 nm with a microtitre plate reader.

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2. Figures and table.



Fig. S1. The 2-D H-H (COSY) spectrum of 1 in DMSO- d_6 .



Fig. S2. The 2-D H-H (COSY) spectrum of 2 in DMSO- d_6 .

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Fig. S3. The 2-D H-H (COSY) spectrum of 3 in DMSO- d_6 .



Fig. S4. The 2-D H-H (COSY) spectrum of 4 in DMSO- d_6 .



Fig. S5. The 2-D H-H (COSY) spectrum of 5 in DMSO- d_6 .



Fig. S6. The 2-D H-H (COSY) spectrum of 6 in DMSO- d_6 .



Fig. S7. The 2-D H-H (COSY) spectrum of 7 in DMSO- d_6 .



Fig. S8. The 2-D H-H (COSY) spectrum of 8 in DMSO- d_6 .



Fig. S9. The UV-vis spectral changes of **1** (100 μ M) in DMSO/PBS (1:4, v/v), **2** (100 μ M), **3** (50 μ M), **4** (100 μ M), **5** (50 μ M), **7** (50 μ M), and **8** (50 μ M) in DMSO/PBS (1:9, v/v) after incubated for 72 h.



Fig. S10. The UV/vis spectral changes of **1** (100 μ M) in DMSO/PBS (1:4, v/v) in the presence of GSH (2 mM) for 0-72 h. The arrow indicates the absorbance decreased as the time of incubation increased. The inset shows a plot of absorbance at ~260 nm *vs* incubation time.



Fig. S11. (A) The positive-ion ESI (electrospray ionization) mass spectra revealed the peak corresponding to $[Au^{III}(butyl-C^N)(DEDT)]^+$ (555.2), and (B) the negtive-ion ESI mass spectra revealed the peaks corresponding to $[Au^I(butyl-C^N)(DEDT)]^-$ (555.1), and $[Au^{III}(butyl-C^N)(DEDT)+GSH-2H^+]^-$ (860.3) in the mixture of **1** (250 µM) and 2 mM of GSH (24 h) in DMSO/PBS (1:4, v/v).



Fig. S12. Inhibition of various DUBs including UCHL1 (10 nM), UCHL3 (0.075 nM), UCHL5 (6.25 nM) and USP2_{CD} (20 nM) by various gold(III) complexes (0.9 μ M). Ub-AMC (0.625 μ M) was used as the substrate. The proteasome inhibitor MG132 (1.8 μ M) was also tested for comparison. (All values are presented as mean ± s.d. with n ≥ 2.)



Fig. S13. (A) ESI-MS analysis of the interaction of **1** with a peptide of UCHL1 sequence that contains the active cysteine residue (TIGNSCGTIGR, UCHL1-C) at the molar ratio of 1:1 in buffer (5 mM NH₄HCO₃, pH 7.6). The analysis was conducted after the peptide was incubated with **1** for 10 min. (B) ESI-MS spectrum of pure UCHL1-C (1078.20, 539.61). Besides peaks of **1** ([Au^{III}(butyl-C^N)(DEDT)]⁺, 554.93), new peaks corresponding to [Au^{III}(butyl-C^N)(DEDT)(UCHL1-C)-H⁺]⁺ (1632.19) and [Au^{III}(butyl-C^N)(UCHL1-C)-H⁺]²⁺ (742.11) present after UCHL1-C was incubated with **1**. (**C**: active cysteine residue)



Fig. S14. (A) ESI-MS analysis of the interaction of 1 with a peptide of UCHL3 sequence that contains the active cysteine residue (TISNACGTIGR, UCHL3-C) at the molar ratio of 1:1 in buffer (5 mM NH₄HCO₃, pH 7.6). The analysis was conducted after the peptide was incubated with 1 for 10 min. (B) ESI-MS spectrum of pure UCHL3-C (1092.27, 546.65). Besides peaks of 1 ([Au^{III}(butyl-[Au^{III}(butyl- $C^N(DEDT)]^+$, 554.96), corresponding peaks to new $C^N(DEDT)(UCHL3-C)-H^+]^+$ [Au^{III}(butyl-C^N)(DEDT)(UCHL3-(1646.26), C)]²⁺ (823.63) and [Au^{III}(DEDT)(UCHL3-C)-H⁺]²⁺ (718.23) present after UCHL3-C was incubated with 1. (C: active cysteine residue)



Fig. S15. (A) ESI-MS analysis of the interaction of **1** with a peptide of UCHL5 sequence that contains the active cysteine residue (VINNACATQAR, UCHL5-C) with **1** at the molar ratio of 1:1 in buffer (5 mM NH₄HCO₃, pH 7.6). The analysis was conducted after the peptide was incubated with **1** for 10 min. (B) ESI-MS spectrum of pure UCHL5-C (1160.58, 580.79). Besides peaks of **1** ([Au^{III}(butyl-C^N)(DEDT)]⁺, 555.06), new peaks corresponding to [Au^{III}(butyl-C^N)(DEDT)(UCHL5-C)-H⁺]⁺ (1714.67) present after UCHL5-C was incubated with **1**. (**C**: active cysteine residue)

barview	rank	batch 🔊	cmap name ▲▼		dose	cell	score ▲ ▼
	1	506	15-delta prostaglandin J2	-	10 µM	MCF7	1
	2	626	15-delta prostaglandin J2	1	10 µM	MCF7	.951
I	3	755	phenoxybenzamine	1	12 µM	MCF7	.950
I	4	726	phenoxybenzamine	1	12 µM	MCF7	.942
I	5	1068	MG-262	1	100 nM	MCF7	.928
i i	6	758	phenoxybenzamine	1	12 µM	MCF7	.900
Í	7	505	celastrol	1	3 μΜ	MCF7	.898
	8	736	pyrvinium	1	3 µM	MCF7	.894
Í	9	720	withaferin A	1	1 µM	MCF7	.887
	10	757	15-delta prostaglandin J2	1	10 µM	MCF7	.886

Fig. S16. Connectivity map analysis. List of compounds that induce a gene expression profile similar to that of **1.** Compounds with score on top ten are listed.



Fig. S17. The inhibition effect of **1** on 20S proteasome activities. Dose-response curves for the inhibition of the 20S proteasome (0.5 nM) chymotrypsin-like (Suc-LLVY-AMC (100 μ M) as substrate) and trypsin-like activity (Boc-LRR-AMC(100 μ M) as substrate) are showed. The table beneath summarizes the corresponding IC₅₀ values.



Fig. S18. Inhibition of the 20S proteasome (0.5 nM) chymotrypsin-like (Suc-LLVY-AMC, 100 μ M, as substrate) and trypsin-like activity (Boc-LRR-AMC, 100 μ M, as substrate) by various gold(III) complexes (0.9 μ M) and MG132 (1.8 μ M). All values are presented as mean ± s.d. with n ≥ 2.



Fig. S19. Cellular uptake of gold as quantified by ICP-MS experiments for MCF-7, and MIHA cells treated with 2 (0.9 μ M) for 2 h.



Fig. S20. S-phase and G2-phase cell-cycle arrest in MCF-7 cells after treatment with $1 (0.9 \mu M, 9 h)$ as revealed by flow cytometry analysis.



Fig. S21. Induction of apoptosis of MCF-7 cells by treatment with **1**. (A) Western blotting analysis of cleavage of caspase-7 and PARP in MCF-7 cells treated with **1** (0.9 μ M, 24 h); (B) Hoechst 33342 staining of MCF-7 cells treated with **1** (0.9 μ M, 24 h), showing nuclear DNA condensation and shrinkage of apoptotic cells.



Fig. S22. Tube formation assay reveals the anti-angiogenesis effect of **1** (subcytotoxic concentrations) with MS1 cells upon a 2 h incubation. (Black arrows indicate the formed tube).

Table S1. The IC₅₀ values (μ M, 72 h) of **1–8** and cisplatin (cis) against breast adenocarcinoma (MCF-7), breast carcinoma (MDA-MB-231), human cervical epithelial carcinoma (HeLa), melanoma (B16), lung carcinoma (NCI-H460), glioblastoma (U87), hepatocellular carcinoma (HepG2), and non-tumorigenic immortalized liver (MIHA) cells by NBB assay.

	MCF-7	MDA- MD-231	HeLa	B16	NCI- H460	U87	HepG2	MIHA
1	0.15±0.04	0.19±0.05	0.67±0.17	0.56±0.11	1.8±0.9	1.5±0.4	2.3±1.8	2.3±0.9
2	0.12±0.02	0.27 ± 0.07	3.11±0.51	2.2±1.2	2.9±0.6	5.2±0.8	5.7±1.5	> 10
3	0.09 ± 0.05	0.20±0.03	0.50 ± 0.14	0.37±0.01	1.3±0.4	0.96±0.27	1.0±0.2	1.2±0.4
4	0.10±0.03	0.13±0.01	0.73±0.08	0.50±0.18	1.3±0.6	1.1±0.3	1.4±0.2	1.8±0.3
5	0.16±0.04	0.26±0.06	6.2±0.6	2.3±0.4	6.5±0.9	6.3±1.0	6.3±0.6	8.9±0.1
6	0.59±0.16	0.53±0.18	0.95 ± 0.42	0.49±0.02	2.6±0.5	0.89±0.13	1.4±0.3	1.4±0.3
7	0.87 ± 0.06	0.83±0.06	1.8 ± 0.1	0.95±0.15	3.7±0.2	1.7±0.1	2.8±0.2	3.2±0.4
8	0.12±0.02	0.20±0.05	$0.87 {\pm} 0.07$	0.52±0.17	1.3±0.4	1.2±0.3	1.5±0.5	1.6±0.4
cis	30±6	60±2	12±2	33±3	59±11	16±6	55±8	55±0.6