Electronic Supplementary Information (ESI) for Chemical Science

A dinuclear cyclometalated gold(III)-phosphine complex targeting thioredoxin reductase inhibits hepatocellular carcinoma *in vivo*†

Raymond Wai-Yin Sun, Chun-Nam Lok, Tommy Tsz-Him Fong, Carrie Ka-Lei Li, Zhen Fan Yang, Taotao Zou, Annie Fung-Ming Siu, and Chi-Ming Che*

Materials and Methods

Materials

Analytical grade organic solvents were used in all experiments unless otherwise stated. Gold(III) complexes **2**, **Au1**−**Au6** and platinum(II) complex **5** have been reported previously [*Chem*. *Eur*. *J*., 2006, **12**, 5253]. (C^N^C)AuCl (where C^N^C = 2,6-diphenylpyridine), 1,3-dibutyl-1*H*-imidazol-3-ium bromide and 1,1'-(propane-1,3-diyl)bis(3-butyl-1*H*-imidazol-3-ium) bromide were synthesized according to the literature procedure [*Chem*. *Sci*., 2011, **2**, 728]. Fast Atom Bombardment (FAB) mass spectra were obtained on a Finnigan Mat 95 mass spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on DPX 300, 400 M Bruker FT-NMR spectrometers relative to the signal of tetramethylsilane. Elemental analysis was performed by the Institute of Chemistry at the Chinese Academy of Science, Beijing. Soluble human recombinant TRAIL/APO2L was obtained from PeproTech. Other chemicals unless otherwise stated were purchased from Sigma-Aldrich Co.

Synthesis and characterization of the gold(III)-carbene complexes

Complex 3. A mixture of 1,1'-(propane-1,3-diyl)bis(3-butyl-1*H*-imidazol-3-ium) bromide (30 mg, 0.071 mmol) and KOBu (23.9 mg, 0.213 mmol) in 30 mL methanol was heated to reflux for 0.5 h; then (C^N^C)AuCl (72.0 mg, 0.156 mmol) was added to the reaction mixture and were refluxed for 12 h. Excess lithium trifluoromethanesulfonate was added and the reaction mixture was further refluxed for 0.5 h. After cooling to room temperature, methanol was removed by rotavapor and the residue was washed with water and hence diethyl ether. Light yellow product was obtained after silica-gel chromatography separation. Yield: 45 %. ^{1}H NMR (400 MHz, CD_3CN, 25 °C): 8.15 (t, J = 8.0 Hz, 2 H), 7.75 (d, J = 8.1 Hz, 4 H), 7.68 (d, J = 6.8 Hz, 4 H), 7.51 (s, 4 H), 7.22 (t, J = 7.6 Hz, 4 H), 7.12 (t, J = 7.3 Hz, 4 H), 6.82 (d, J = 7.3 Hz, 4 H), 4.17 (t, J = 7.3 Hz, 4 H), 4.05 (t, J = 7.2 Hz, 4 H), 2.46 (m, 2 H), 1.66 (m, 4 H), 1.13 (m, 4 H), 0.67 (t, J = 7.4 Hz, 6 H); ^{13}C NMR (400 MHz, CD3CN, 25 °C): 165.2, 152.7, 150.7, 145.6, 136.4, 133.2, 128.9, 127.5, 125.0, 123.8, 120.6, 119.5, 51.9, 49.5, 32.9, 31.5, 20.0, 13.6; positive FAB-MS: m/z 1141 $[M\text{-}2OTf+H]^+$, 1290 $[M\text{-}OTf]^+$. Elemental Analysis, calcd: C, 44.24; H, 3.50; N, 5.84; found: 44.51, H 3.69, N 5.66

Complex 4. The procedure is similar to **3** except 1,3-dibutyl-1*H*-imidazol-3-ium bromide was used. Yield: 50 %. ^{1}H NMR (300 MHz, CD_3CN, 25°C): 8.14 (t, J = 8.1 Hz, 1 H), 7.82 (m, 4 H), 7.57 (s, 2 H), 7.27 – 7.38 (m, 4 H), 6.99 (dd, J = 7.1 Hz, 1.2 Hz, 2 H), 4.17 (t, J = 7.2 Hz, 4 H), 1.78 (m, 4 H), 1.22 (m, 4 H), 0.75 (t, J = 7.4 Hz, 6 H); ^{13}C NMR (300 MHz, CD_3CN, 25 °C): 145.3, 136.7, 133.2, 128.9, 127.4, 124.3, 119.3, 52.0, 33.0, 20.1, 13.7; positive FAB-MS: m/z 606 $[M\text{-}OTf]^+$. Elemental Analysis: calcd: C, 46.10; H, 4.14; N, 5.56; found: 46.16, 4.37, 5.41.

Electrochemical measurements

Cyclic voltammetric measurements were recorded on a Princeton Applied Research Model 273 A Potentiostat/galvanostat coulometer and Model 270/250 universal programmer, using a three-electrode cell system with a glassy carbon disk as the working electrode, an Ag/AgNO3 (0.1 M), electrode in CH_2Cl_2 as the reference electrode, and a

platinum wire as the counter electrode. Tetrabutylammonium hexafluorophosphate (0.1 M) was used as the supporting electrolyte.

Localization of 5 in HeLa cells.
HeLa cells (1×10^4 cells/mL) were cultured in glass-bottomed dishes with supplemented culture medium and incubated for 24 h. Cells were treated with DMSO or **5** (30 μM) for 15 min and stained with MitoTracker Red (50 nmol/L, Molecular Probes) and LysoTracker Red (50 nmol/L, Molecular Probes) for 2 min at 37 °C. After washing with PBS, cell images were examined under a fluorescence microscope.

Cell cultures
All cell lines were obtained from American Type Culture Collection (ATCC). Human cervical epithelial carcinoma (HeLa), human hepatocellular carcinoma (HepG2 and PLC), normal human hepatocytes (MiHA), and human normal lung fibroblast (CCD-19Lu) were maintained in Eagle's minimum essential medium. All cell culture media were supplemented with 10% (v/v) fetal bovine serum, L-glutamine (2 mM) and penicillin/streptomycin (100 U/mL). Cells were incubated in 5 % CO_2 humidified air atmosphere at 37°C and subcultured when 80% confluence was reached.

Cytotoxicity assay
The cytotoxic properties of the complexes were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [*J. Immunol. Methods*, 1983, **65**, 55]. Briefly, cells were seeded in 96-well plates and incubated for overnight prior to be examined. All of the complexes were dissolved in DMSO. The concentration of the complexes was calculated according to the elemental composition of the complexes determined by the elemental analyses. Media in the presence of the tested complexes were added and serially diluted to various concentrations (from 50 μM to 0.2 μM), and media containing cisplatin were used as the positive control. The maximum concentration of DMSO in media did not exceed 0.1 % (v/v). The cells were incubated for 72 h and followed by the addition of MTT solution. The cells were further incubated for 3 h and solubilization buffer (100 μL, 10 % SDS in 0.01 M HCl) was subsequently added. The absorption intensities at 580 nm in each well were measured by a Perkin-Elmer Fusion™ α-FP plate reader. The IC_{50} values of the complexes (concentrations at which could inhibit cellular growth by 50 % compared to the negative control) were determined from the plots of the cell viability percentage *versus* the complex concentration. For each set of data, at least three independent experiments have been done.

In some experiments, cytotoxicity assay was conducted by Naphthol blue black (NBB) method. After drug treatment, the culture medium was removed, and cells were fixed with 10 % formalin (50 μL) in acetate buffer for 15 min. Naphthol blue black (50 μL, 0.05 %; Aldrich) in acetate buffer was then added to stain the cells. After 30 min, the stain was poured off and the plates were washed with distilled water. The dye was then eluted by adding NaOH (50 mM; 150 μL per well). Absorbance of each well was read at 580 nm using a Perkin-Elmer Fusion ™ α-FP plate reader.

Human HCC tumor xenograft in nude mice.

Six week old BALB/c nude mice (Laboratory Animal Unit, The University of Hong Kong) were maintained under standard conditions and cared for according to the Institutional guidelines for animal care. All the animal experiments were approved by the Committee on the Use of Live Animals in Teaching and Research, the University of Hong Kong. Nude mice were inoculated subcutaneously with 2×10^6 PLC cells. Tumor nodules were macroscopically observed 20 days after inoculation. Mice were randomly divided into four treatment groups with n = 5 of each group: (1) 10 % PET in PBS (vehicle control); (2) 10 mg/kg of **Au3**; (3) 3 mg/kg cisplatin (4) 5 mg/kg doxorubicin. All drugs were dissolved in 10 % PET in PBS. Treatment of i.p. administration was started 21 days after inoculation. Tumor growth was monitored twice per week by caliper, and tumor volumes were calculated according to a standard formula: (mm^3) = L × W2 / 2, where L is the length and W is the width. The weight was also measured. All mice were sacrificed on day 28 after treatment.

H22 (hepatocarcinoma) and S180 (sarcoma) tumor xenograft in nude mice.
This experiment was conducted in Tianjin Institute of Pharmaceutical Research (TIPR) and the nude mice were maintained under standard conditions and cared for according to the State Food and Drug Administration (SFDA) guidelines for animal care. Nude mice were inoculated subcutaneously with H22 or S180 cells. Tumor nodules were macroscopically observed a week after inoculation. Mice were randomly divided into three treatment groups with *n* = 12 or 14 of each group: (1) 10 % PET in PBS (vehicle control); (2) 4 mg/kg of **Au3**; (3) 10 mg/kg cisplatin. All drugs were dissolved in 10 % PET in PBS. Treatment of i.v. administration was started 8 days after inoculation. Tumor growth was monitored twice per week by caliper, and tumor volumes were calculated according to a standard formula: (mm^3) = L × W2 / 2, where L is the length and W is the width. The weight was also measured.

Histology study.
When the animals were killed, tumor tissues were obtained and fixed in 10% buffered formalin for 16 h, followed by embedding in paraffin for histological studies. Paraffin-embedded, 4-μm sections were deparaffinized, rehydrated, and subjected to hemoatoxylin and eosin (H & E) (Roche, Basel, Switzerland) for detection of necrotic cells and in situ Cell Death Detection Kit for (Roche, Penzberg, Germany) for detection of apoptotic cells. Expression of von-Wille-brand factor, a marker for angiogenesis was measured using Blood Vessel Staining Kit. The tissue sections were viewed under an inverted microscope at 100× magnification.

McA-RH7777 hepatoma cell transfection.
McA-RH7777 cells were transfected with luciferase expression plasmid (Promega). Twenty-four hours after transfection, stable clones were selected by continuous G418 incubation at a dose of 800 μg/mL for about 30 days.

Orthotopic HCC model in rat liver.
Male Buffalo rats, weighing 250−320 g were purchased from Charles River Labs (Wilmington, MA). The rats were raised under standard conditions according to the institutional (HKU) guidelines for animal care. McA-RH7777 cells stably transfected

with luciferase (McA-RH7777-luc, 2×10^6) were injected into the left lobe of the liver. Seven days after tumor induction, the rat livers were exposed to confirm the formation of tumor nodules. All the experimental rats had developed a single tumor nodule with size ranging from 1×2 to 2×3 mm^2. The rats were then divided into the following four groups with n = 5 of each group: (1) saline (vehicle control); (2) 3, 0.25 mg/kg; (3) 3, 0.5 mg/kg; (4) 3, 0.75 mg/kg. Different doses were injected intratumorally at the first instance, followed by intraperitoneal injection twice weekly until they died. The volume of DMSO injected was kept ≤ 3 μL in all rats. Viable tumor cells (MCA-RH7777-luc) were assessed in rat using the IVIS imaging system (Xenogen Corporation, Alameda, CA). Fifteen minutes before imaging, rats were injected with 150 mg/kg luciferin (Xenogen) intraperitoneally, followed by anesthesia with isoflurane.

Acute toxicity test of Au3 in mice.

This experiment was conducted in Tianjin Institute of Pharmaceutical Research (TIPR) and the nude mice were maintained under standard conditions and cared for according to the State Food and Drug Administration (SFDA) guidelines for animal care. 60 mice (30 male and 30 female) of 18−22 g were picked and divided into six groups for i.v. injection of **Au3** at five different doses. **Au3** was prepared in a solution of 90 % PBS/ 10 % PET (v/v) (composition of PET: 60 % Polyethylene glycol 400, 30 % ethanol and 10 % tween-80). The health status of the mice was observed for 14 days after injection. On day 15, all mice were anatomized and different organs including heart, lung, liver, kidney, intestine, spleen, stomach, testis and ovary were examined. The median lethal dose was calculated by Bliss method.

Acute and sub-chronic toxicity test of Au3 in beagle dogs.

These experiments were conducted in Tianjin Institute of Pharmaceutical Research (TIPR) and the beagle dogs were maintained under standard conditions according to the State Food and Drug Administration (SFDA) guidelines for animal care. At the beginning of the predose period, each Beagle dog was identified by ear tattoo and assigned a predose number, which was indicated on its cage card. After assignment to dosage groups, each Beagle dog was assigned a unique study identification number (which was indicated on its cage card). Beagle dogs were individually housed in stainless steel, slat floor cages. Study rooms were maintained on a 12-hour light/dark cycle (light/dark cycle wasn't be interrupted for study-related activities), within a temperature range of 16 to 26°C (60.8 to 78.8°F), and a relative humidity range of 40 to 70%.

For acute toxicity study, 10 beagle dogs (5 male and 5 female) of 8.0−9.0 kg were picked and divided into four groups for i.v. injection of **Au3** at five different doses (0, 4.0, 6.0, 9.0 and 13.5 mg/kg). All Beagle dogs assigned to the study were injected intravenously via peripheral vein. The health status of the beagle dogs was observed for 14 days after the first injection. On day 15, all beagle dogs were anatomized and different organs including heart, lung, liver, kidney, intestine, spleen, stomach, testis and ovary were examined. The median lethal dose was calculated by Bliss method.

For sub-chronic toxicity, 32 beagle dogs (16 male and 16 female) of 7.0−9.0 kg were picked and divided into five groups for i.v. injection of **Au3** at 4 different doses (0, 2.0,

4.0 and 8.0 mg/kg). All Beagle dogs assigned to the study were injected intravenously via peripheral vein twice a week for up to 4 weeks (total 8 administrations). Mortality checks were performed twice daily. Clinical observations were performed at least once predose and at least three times during the dosing days, and twice a day during the non-dosing days through 4 weeks. Body weight was recorded twice during the predose period and once a week through 4 weeks. The health status of the beagle dogs was observed for 28 days after the first injection. On day 29, all beagle dogs were anatomized and different organs including heart, lung, liver, kidney, intestine, spleen, stomach, testis and ovary were examined.

Mutagenicity test

This experiment was conducted in Tianjin Institute of Pharmaceutical Research (TIPR) and the nude mice were maintained under standard conditions and cared for according to the State Food and Drug Administration (SFDA) guidelines for animal care. Using ICR mice as the experimental subjects, the frequency of micronucleated polychromatic erythrocytes (PCE) was determined as the indicator to evaluate the **Au3** induced genotoxicity. 30 mice (15 male and 15 female) of 18−22 g were picked and divided into five groups for i.v. injection of Au3 at four different doses (0, 3.2, 8 and 20 mg/kg), and of cyclophosphamide (50 mg/kg) as positive control. For each mouse, 2000 polychromatic erythrocytes (PCE) were examined to determine the frequency of micronucleated PCE.

Total RNA extraction

Total RNA was isolated using Trizol protocol. HepG2 cells were washed with PBS and homogenized in Trizol after **Au3** (1.5 or 3 μM) treatment for 6 h. Chloroform was added followed by vigorous shaking for 15 s. The aqueous layers were collected after centrifugation (13200 rpm, 5 min). Equal volumes of isopropanol were added and the solutions were incubated at on ice for 2 h to allow complete RNA precipitation. The supernatants were discarded after centrifugation. The remaining portions were washed twice with ethanol (70%), re-dissolved in RNAase-free water and purified by an RNeasy® Mini Kit. The purity of the RNA samples was examined by using Agilent RNA 6000 Pico Kit (Agilent, Santa Clara, CA) in Agilent 2100 Bioanalyzer (Agilent).

Microarray and bioinformatics analysis

The gene expression profiles of **Au3**-treated and untreated samples were compared by Affymetrix Human Genome U133 Plus 2.0 microarray. All of the samples were assayed in triplate. Hydridization and fluorescence labeling of extracted RNA were performed at Centre for Genome Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong. The data analysis was performed by the statistical package BRB-ArrayTools using MAS-normalization. The statistically significant genes were identified by SAM algorithm. The gene lists were submitted to connectivity map analysis to identify the drug with similar gene signatures.

Reserve transcriptase-polymerase chain reaction (RT-PCR)

Primers were purchased from Tech Dragon Limited and Genome Research Centre, The University of Hong Kong. Reverse transcription and PCR of extracted RNA were

performed using SuperScript™ III One-Step RT-PCR System with Platinum® *Taq* (Invitrogen). The following parameters were used: cDNA synthesis and pre-denaturation (323 K for 30 min followed by 367 K for 2 min), PCR amplification (denatured at 367 K for 20 s, annealed at 328 K for 1 min and extend at 341 K for 1 min) and final extension (345 K for 7 min). The sequences of the primers were shown as follows: *CHOP*: 5'-TCT GGC TTG GCT GAC TGA GGA GG A -3' (forward), 5'- TGG TGC AGA TTC ACC ATT CGG TCA -3' (reverse); *DR5*: 5'-AAG ACC CTT GTG CTC GTT GTC-3' (forward)), 5'- GAC ACA TTC GAT GTC ACT CCA -3' (reverse); *GAPDH*: 5'-ACC ACA GTC CAT GCC TAC AC -3' (forward), 5'-TTC ACC ACC CTG TTG CTG TA -3' (reverse)

Small interfering RNA.

The *DR5* and *CHOP* siRNA duplexes have 21 bp including a 2-base deoxynucleotide overhang (synthesized by Dharmacon Research, Lafayette, CO). The sequence of the *DR5* siRNA oligos was 5'- AAG ACC CUU GUG CUC GUU GUC -3', and the sequence of *CHOP* siRNA oligos was 5'- AAG AAC CAG CAG AGG UCA CAA -3'. The control siRNA used was 5'- AAC GUA CGC GGA AUA CUU CGA -3' against LacZ. Cells were transfected with siRNAs by using Lipofectamine 2000 (Invitrogen) following the manufacturer's instruction.

Western blotting analysis

HeLa cells (5×10^5) were cultured on 60 mm dish and incubated overnight before experiments. HepG2 cells were treated with **Au3** for various incubation periods (4, 8, 16, and 24 h). At the end of each incubation period, cells were harvested and lysed using the lysis buffer (150 mM NaCl, 100 mM Tris-HCl pH 7.4, 10 % glycerol, 1 % Triton X-100, 10 mM NaF, 5 mM sodium pyrophosphate, 5 mM sodium orthovanadate, 0.1 % SDS) with protease inhibitor cocktail. Total protein extracts (40 μg) were loaded onto 12.5 % SDS-polyacrylamide gel, separated by electrophoresis followed by transfer of proteins from the gel to polyvinylidene fluoride (PVDF) membranes. After the transfer of protein, the membrane was then blocked with 3 % BSA in TBST buffer and incubated with corresponding primary antibodies at 4 ^{o}C overnight. Primary antibodies of anti-CHOP (1:1000), anti-GRP78, anti-phospho-elF2α (1:1000), anti-DR4 (1:500), anti-DR5 (1:500), anti-P-70S6K T389 (1:1500), anti-P-Akt-S473 (1:1500), anti-P-Akt-T308 (1:1500), anti-cleaved caspase 3 (1:1500), anti-cleaved caspase 7 (1:1500), anti caspase 8 (1:1500), anti-cleaved caspase 9 (1:1500), anti-cleaved PARP (1:1500), mcl-1 (1:1500) were obtained from Cell Signaling Technology. After washing, the membrane was incubated with secondary antibody conjugated with horseradish peroxidase (1:5000) for 90 min. The immunoreactive signals were detected using enhanced chemiluminenscene kit (GE healthcare) following the procedures given in the user manual. Equal loading of each lane was confirmed by the intensity of β-actin.

Cellular thioredoxin reductase assays

HepG2 cells were seeded at 2×10^5/well in 6-well plates and incubated for 24 h. Complexes **Au3** and **5** (16 to 1 μM) were serially diluted and added to the cells (final DMSO concentrations ≤ 1 %). After incubation for 1 h, the cells were washed three times with PBS, and 100 μL of ice-cold lysis buffer (50 mM phosphate buffer, pH 7.4, 1 mM

EDTA, 0.1 % Triton-X 100) were added to the cell layer. Cell lysis was carried on ice for 5 min and the cell lysates were collected and stored at -80 ^{o}C or assayed immediately. Cell lysates (10 µg proteins) were added to a buffer (100 µL) containing 100 mM potassium phosphate, pH 7.4, 1 mM EDTA and 0.2 mM NADPH. Reaction was initiated by adding 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, 3 mM final) and the TrxR activities were determined as increases in $O.D._{412\ nm}$ in 10 min.

Purified TrxR enzyme assay

Recombinant rat TrxR1 (ICMO Corp, Sweden; 1 nM) was reduced with NADPH (0.2 mM) and then incubated with complexes (1 nM – 1 µM) (Table S4) for 30 min in a 100 mM potassium phosphate buffer, pH 7.4 and 1 mM EDTA. The residual activities (initial rates of increases in $O.D._{412\ nm}$) were measured using 3 mM DTNB.

Table S1. *In vivo* anti-tumor activities of **Au3** towards human H22 (hepatocarcinoma) bearing nude mice (*n*=14).

	Dose (mg/kg)	*n*	Weight of mice (g)	Weight of tumor (g)	Tumor inhibition (%)
vehicle control	/	14	25.3±2.9	1.598±0.411	/
cisplatin	10	14	26.4±3.3	1.070±0.412**	33.0
Au3	4	14	24.8±2.9	0.982±0.292***	38.6

** $P<0.01$, *** $P<0.001$

Table S2. *In vivo* anti-tumor activities of **Au3** towards human S180 (sarcoma) bearing nude mice (*n*=12).

	Dose (mg/kg)	*n*	Weight of mice (g)	Weight of tumor (g)	Tumor inhibition (%)
vehicle control	/	12	23.8±4.0	1.985±0.395	/
cisplatin	10	12	21.9±2.9	1.457±0.568*	26.6
Au3	4	12	25.7±3.6	1.013±0.480***	48.9

* $P<0.05$, *** $P<0.001$

Table S3. Effect of i.v. administration of **Au3** on micronuclei formation of bone marrow polychromatic erythrocyte in mice.

Group	Dose (mg/kg)	Number of sample (*n*)	PCE (total)	MNPCE (sum)	MNPCE/PCE % Mean ±SD	PCE/NCE Mean ±SD	PCE/(PCE+NCE) Mean ±SD
vehicle control	/	6	12379	9	0.73±0.26	2.62±0.85	0.71±0.08
cisplatin	50	6	12189	155	12.70±3.74	1.08±0.56	0.49±0.12
Au3	3.2	5[a]	10351	12	1.16±0.26	1.24±0.51	0.54±0.10
Au3	8	6	12249	12	0.98±0.32	1.86±0.56	0.64±0.08
Au3	20	6	12188	15	1.23±0.51	1.66±1.10	0.57±0.15

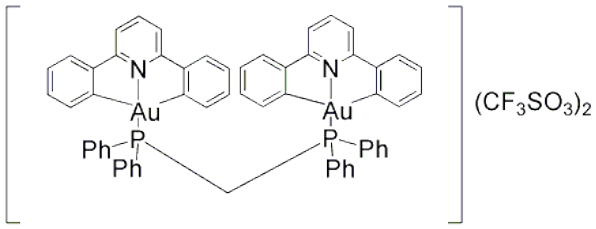
[a]one mouse was dead accidentally.

Table S4. Inhibition of **Au3**, [Au(C^N^C)]Cl, 1,2-bis(diphenylphosphino)propane (dppp), **2**, **3**, and **4** on purified TrxR (upper) and TrxR in HepG2 cells (lower).

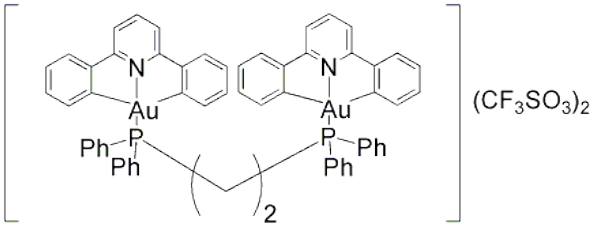
purified TrxR enzyme	IC_{50} (nM)
Au3	7.50±0.99
2	19.27±2.12
3	>100
4	>100
Au(C^N^C)Cl	6.96±0.62
dppp	>100

TrxR in HepG2 cells (1 h treatment)	IC_{50} (μM)
Au3	7.32±0.51
2	13.37±0.89
3	>32
4	22.50±1.15
Au(C^N^C)Cl	>32
dppp	>32

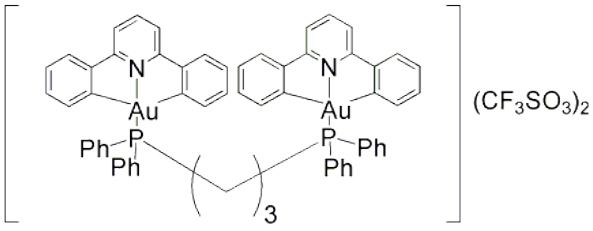
Fig. S1 Chemical structures of **Au1**–**Au6**.



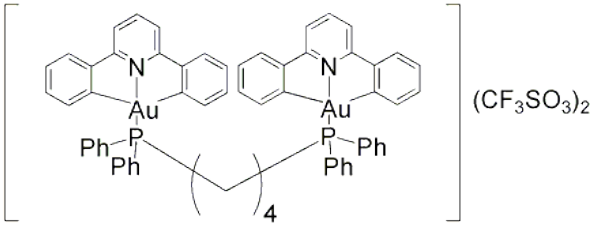
$[Au_2(C^N^C)_2(\mu\text{-dppm})](CF_3SO_3)_2$ (**Au1**)



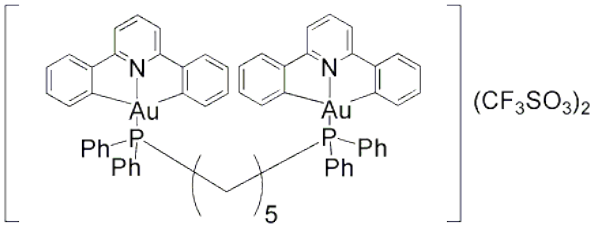
$[Au_2(C^N^C)_2(\mu\text{-dppe})](CF_3SO_3)_2$ (Au2)



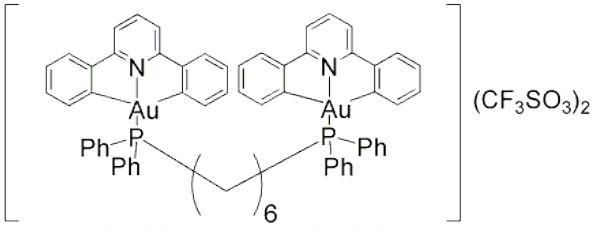
$[Au_2(C^N^C)_2(\mu\text{-dppp})](CF_3SO_3)_2$ (**Au3**)



$[Au_2(C^N^C)_2(\mu\text{-dppb})](CF_3SO_3)_2$ (**Au4**)

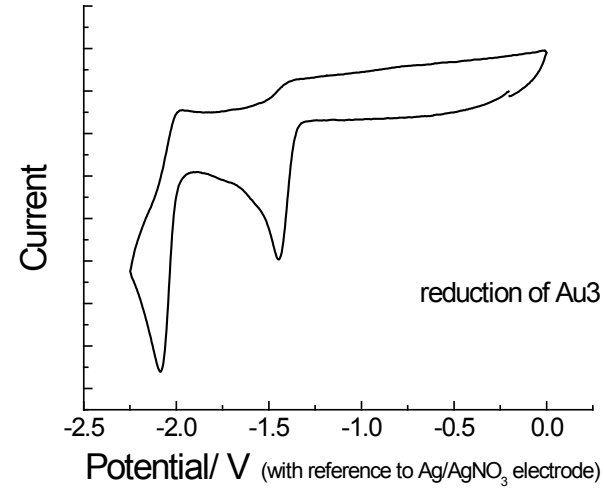


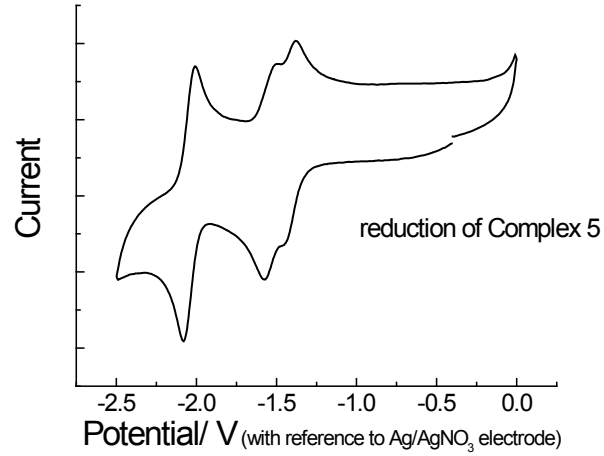
$[Au_2(C^N^C)_2(\mu\text{-dpppe})](CF_3SO_3)_2$ (Au**5**)



$[Au_2(C^N^C)_2(\mu\text{-dpph})](CF_3SO_3)_2$ (Au**6**)

Fig. S2 Cyclic voltammogram of **Au3**, **5** and $Pt_2(C^N^C)_2$dppp in CH_2Cl_2 containing n-Bu_4NPF_6 (0.1 M) at 298 K.





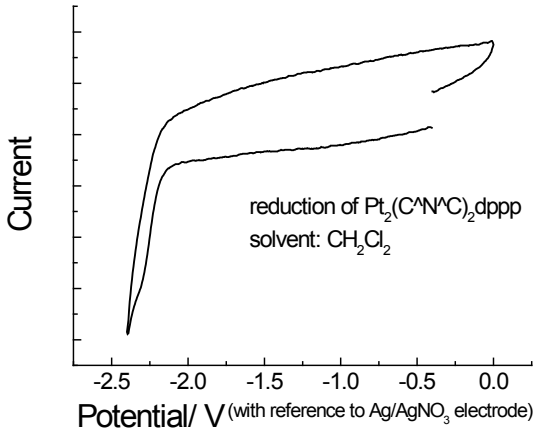


Fig. S3 Fluorescence microscopic examination of HeLa cells treated with **5** and stained with MitoTracker Red (left) or Lysotracker Red (right).

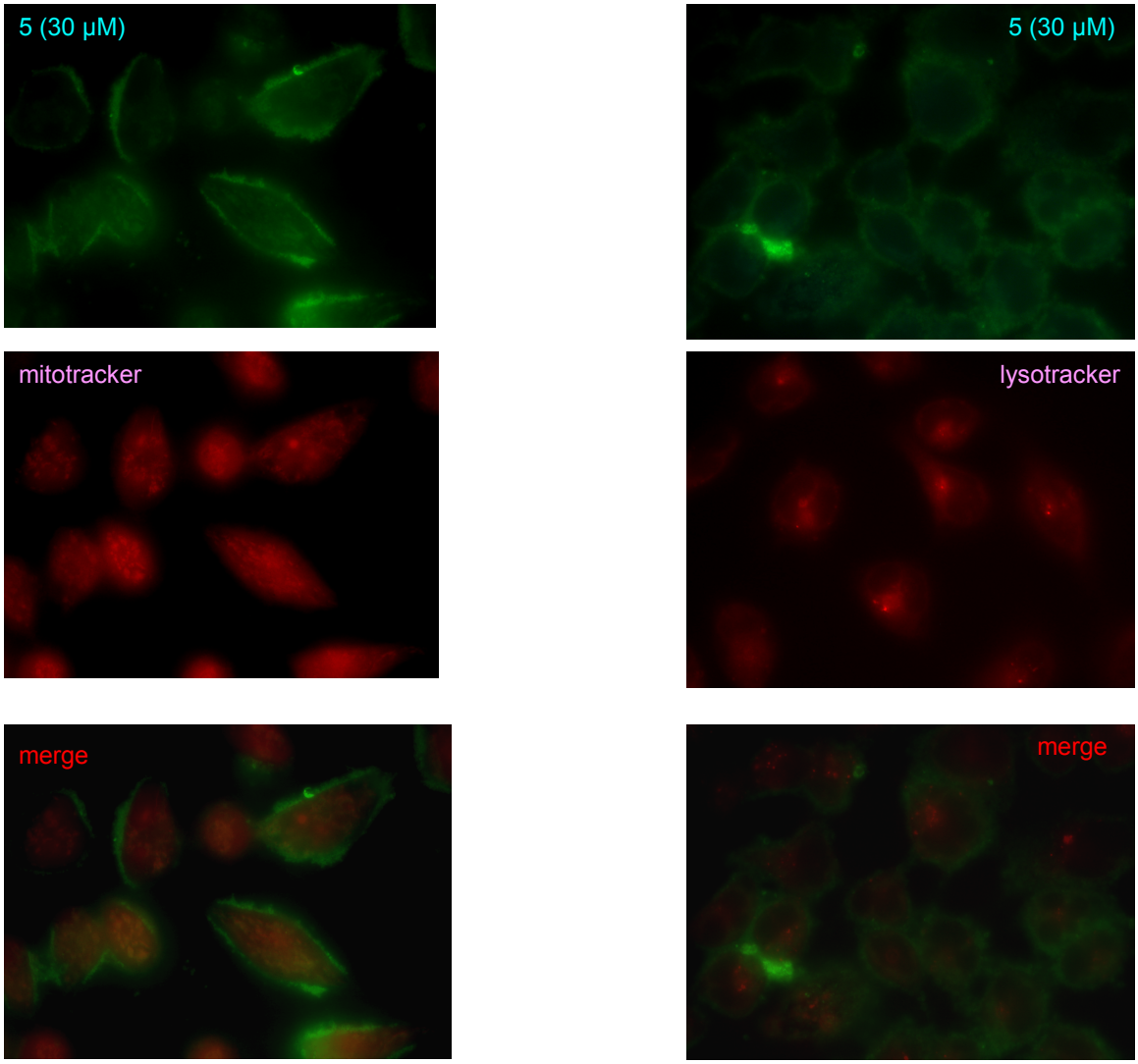
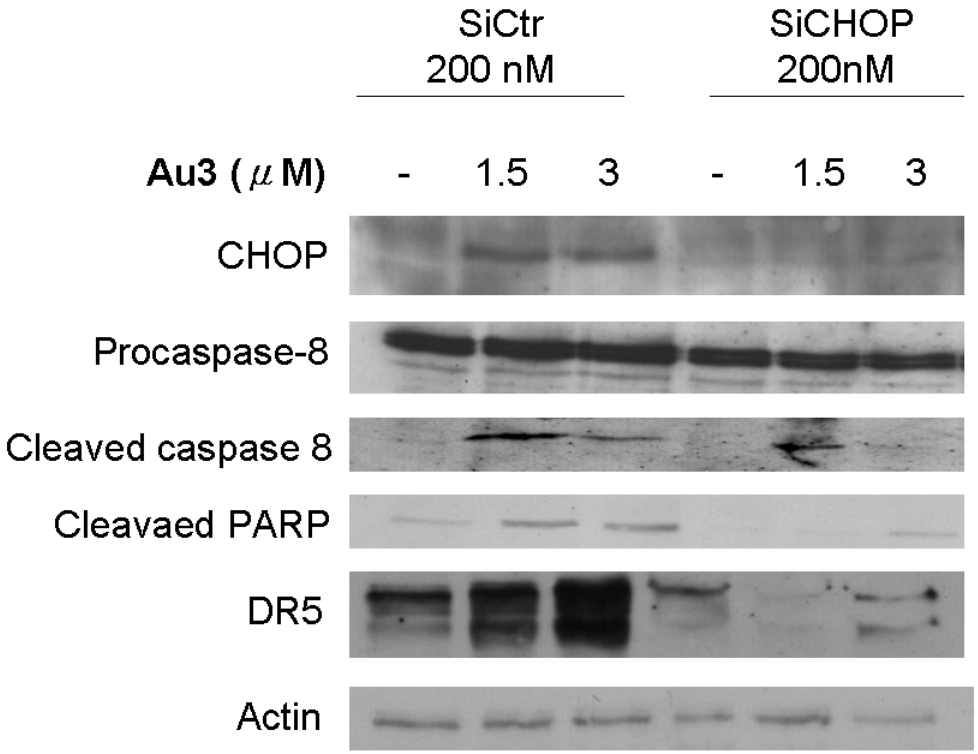


Fig. S4 HepG2 cells were transfected with CHOP siRNA and control siRNA. (Upper) After 24 h, cells were treated with **Au3** (1.5 or 3 μM) for 24 h, and whole-cell extracts were prepared and subjected to immunoblot analysis. Equal protein loading was confirmed by β-actin. Representative of three separate experiments. (Lower) The cytotoxicity profiles (24 h) of **Au3** alone or combined treatment of **Au3** and TRAIL (50 ng/mL) towards HepG2 cells transfected with CHOP siRNA or control siRNA.



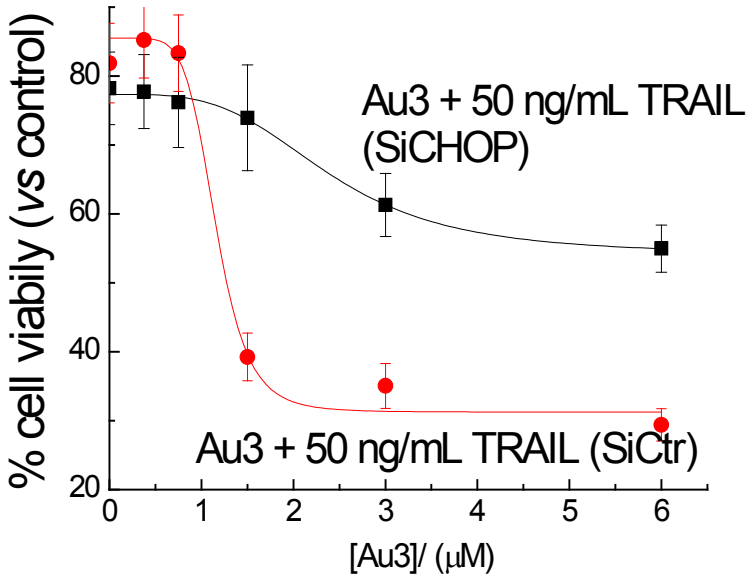


Fig. S5 Western blot analysis showing the expression of apoptosis-related proteins in cancer cells after treatment with **Au3** (5 μM) for 0, 4, 8, 16 or 24 h.

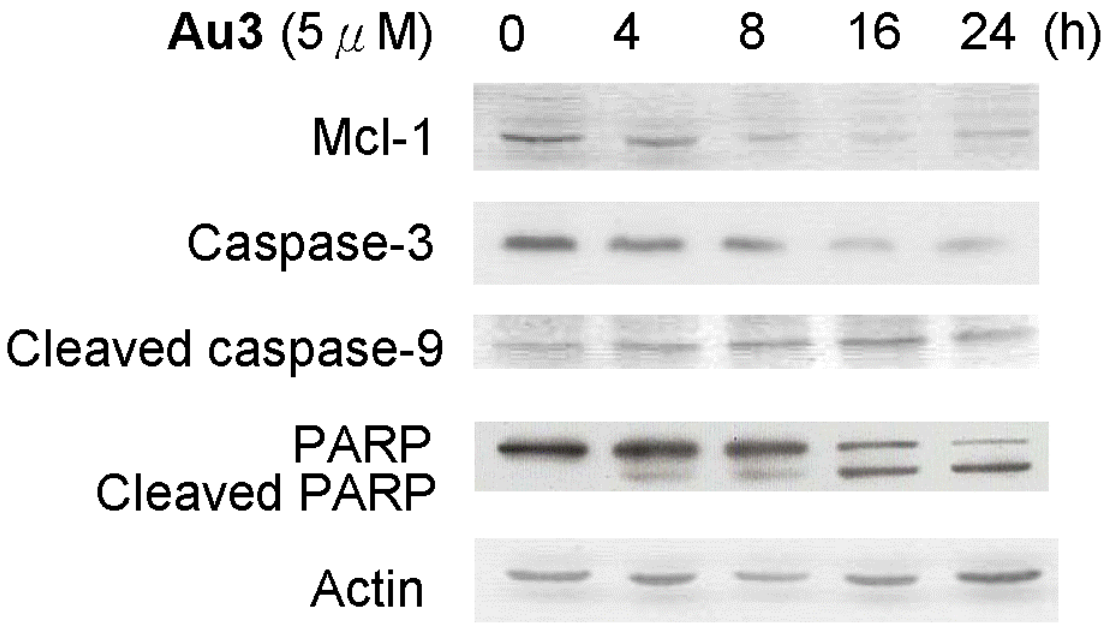


Fig. S6 Cytotoxicity profiles of HepG2 cells treated with **Au3** or **5** for 24 h.

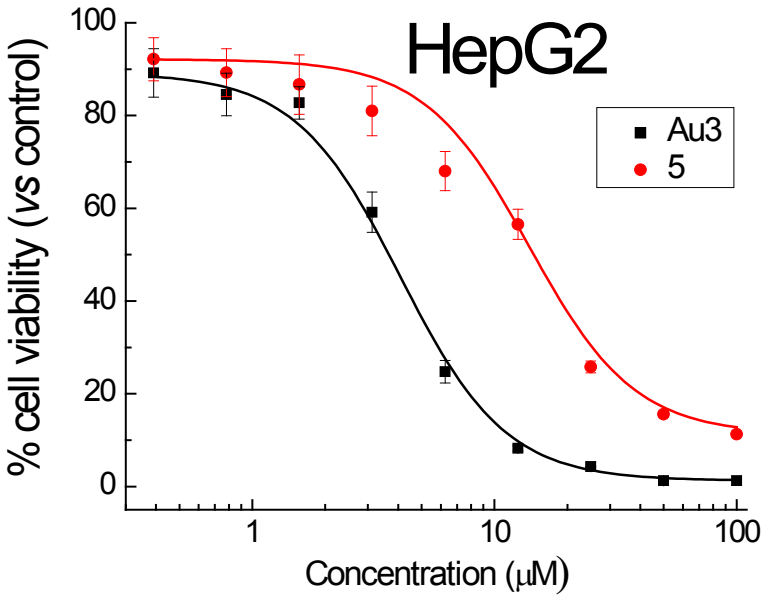


Fig. S7 The isobologram was used to analyze the synergistic cytotoxic effects of **Au3** and TRAIL. The additivity line connects the IC_{50} value of each compound used alone. A and B represents two different dose pairs of each compound. Both A and B are well below the line and is located in the synergy region.

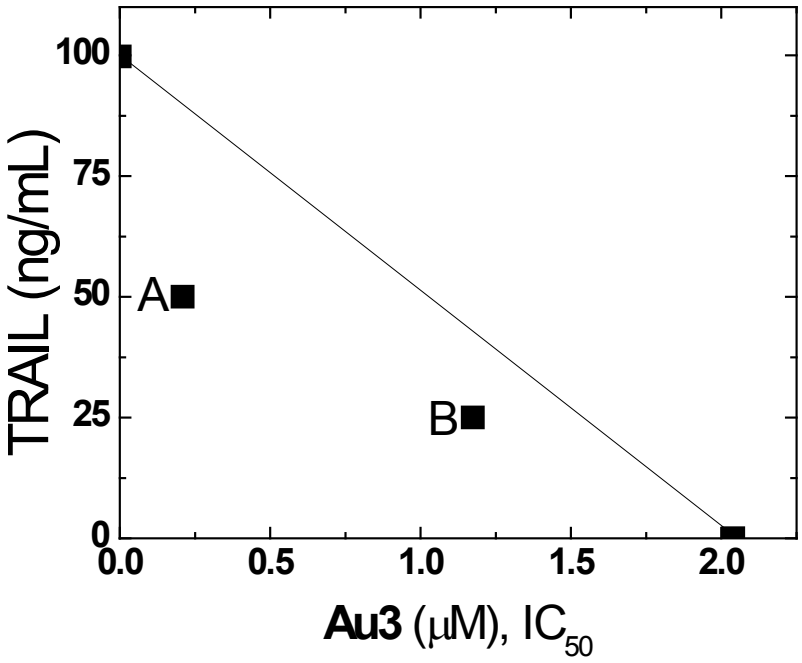


Fig. S8 The cytotoxicity profiles (24 h) of **Au3** alone or combined treatment of **Au3** and TRAIL (50 ng/mL) towards PLC cells.

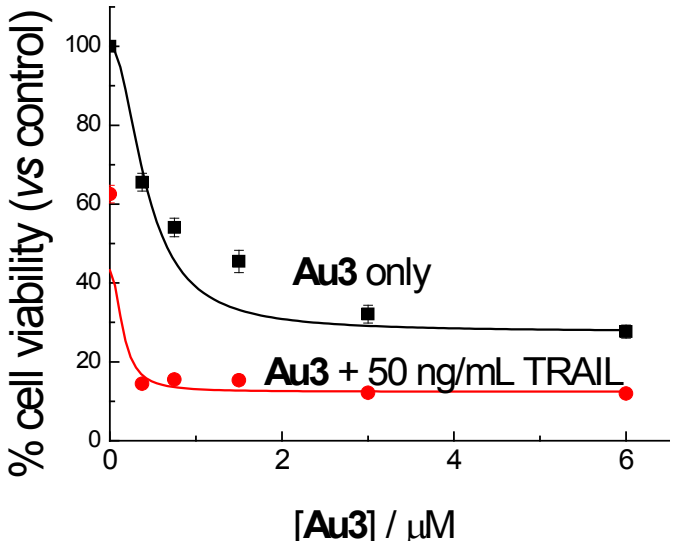
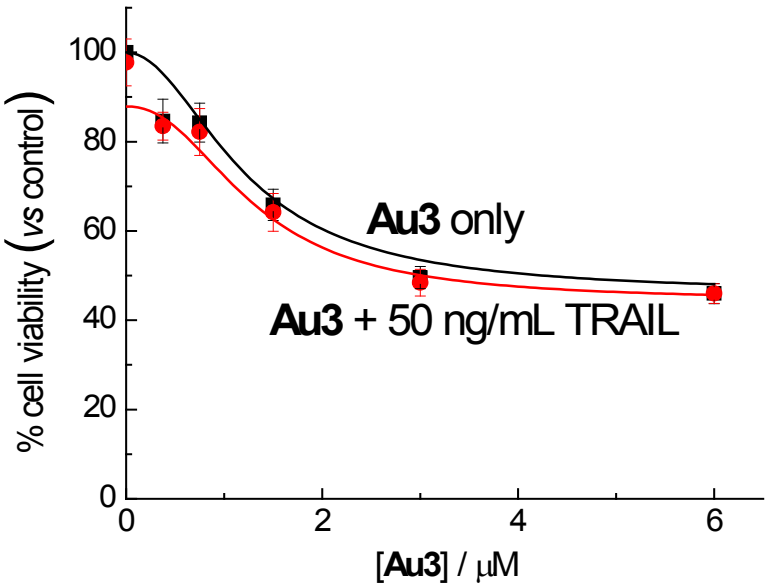


Fig. S9 The cytotoxicity profiles (24 h) of **Au3** alone or combined treatment of **Au3** and TRAIL (50 ng/mL) in CCD-19Lu (upper) and MiHA cells (lower).

CCD-19Lu cells:



MiHA cells:

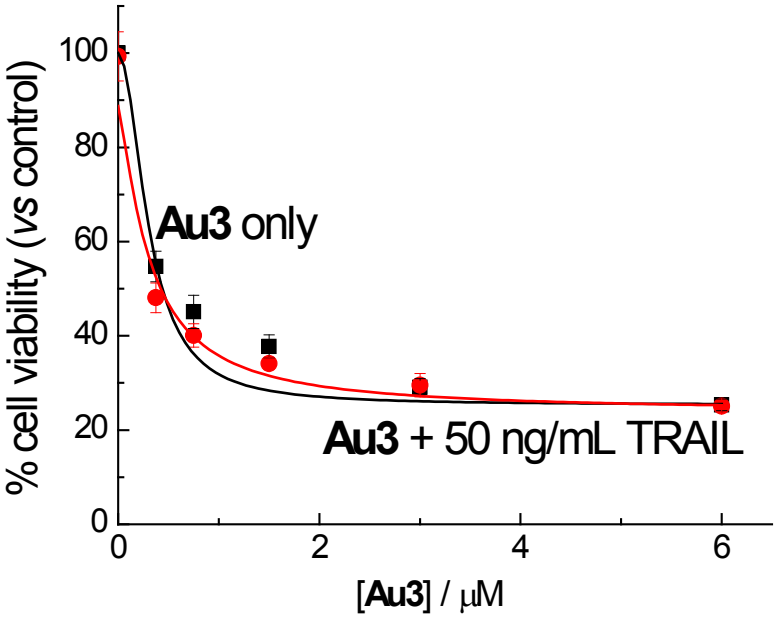


Fig. S10 Western blot analysis showing the DR5 expressions in HepG2 cells transfected with *DR5* siRNA (SiDR5) and control siRNA (SiCtr).

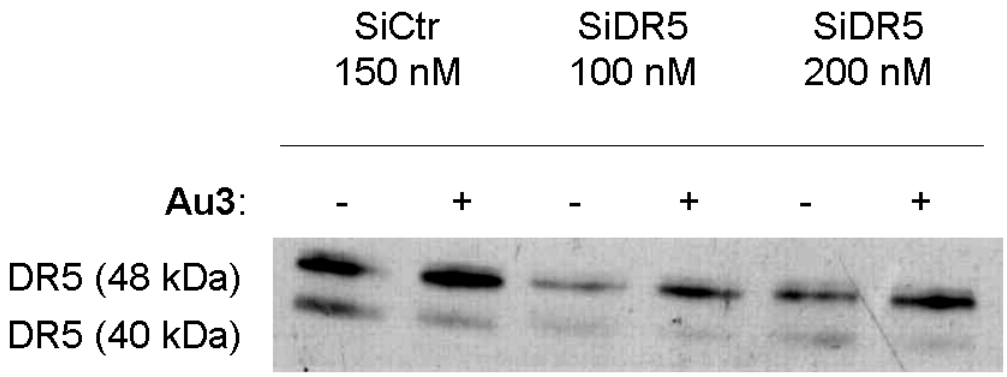


Fig. S11 The cytotoxicity profiles (24 h) of **Au3** alone or combined treatment of **Au3** and TRAIL (25 or 50 ng/mL) in HepG2 cells transfected with *DR5* siRNA.