Electronic Supporting Information

for

Nitric oxide-releasing Platinum(IV) Prodrug Efficiently Inhibits Proliferation and Metastasis of Cancer Cells

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Experimental Procedures

Reagents and instruments

Cisplatin was purchased from Shandong Boyuan pharmaceutical Co., Ltd. (Jinan, China). Fetal bovine serum (FBS) were obtained from Lonsera Lonsa science SRL (Uruguay). The high glucose modified eagles medium (DMEM) was purchased from HyClone (Logan, UT). Gelatin (from porcine skin, Type A) was purchased from Sigma-Aldrich (St. Louis, MO). Trypsin-EDTA and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT), JC-1 kit, AnnexinV-FITC apoptosis detection kit, cell cycle and apoptosis analysis kit ,and crystal violet were purchased from Beyotime Biotechnology (Nanjing, China). Ultra-purified water was prepared using a Milli-Q Synthesis System (Millipore, Bedford, MA). Other reagents used in this experiment were analytical grade and used without further purification. NMR spectra were recorded on Bruker ACF-300 MHz spectrometer (Bruker Instruments Ltd)). Fluorescent images were observed on Olympus IX71 fluorescent microscope (Olympus Corporation).

Cell culture

The human hepatocellular carcinoma HepG2, cervical carcinoma HeLa, lung carcinoma A549 cells, ovarian carcinoma A2780 and breast carcinoma MCF-7 were obtained from the American Type Culture Collection (ATCC). 4T1 cell line was purchased from Cell Bank of Shanghai, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM containing 10% FBS at 37 °C in a humidified and 5% CO_2 incubator.

Animals

Balb/c mice (5 weeks old) were purchased from the Shanghai slack laboratory animal Co., LTD (Shanghai, China). All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The procedures were approved by the University of Science and Technology of China Animal Care and Use Committee.

Synthesis of benzofuroxan

Benzofuroxan was prepared according to literature.[1] To a solution of cinnamic acid (5.37 g, 40 mmol) in 8ml glacial acetic acid, 120 mmol saturated water solution of $NaNO_2$ was dropped slowly at less than 70°C. After the dropping, the reaction solution continued to stir for 1 h at room temperature. Then the reaction was diluted using 100 ml of water, extracted with diethyl ether for 3 times. The combination of diethyl ether layer was washed with saturated salt water for 3 times, dried with Na_2SO_4, filtered and removed in vacuum to obtain crude benzofuroxan, followed by further purification using silica gel column chromatography (elution with petroleum ether: ethyl acetate = 4:1). Benzofuroxan, a white solid with a yield of 76%.^{1}H NMR (300 MHz, Chloroform-*d*) δ 2.58 – 2.73 (s, 1H), 4.66 – 4.89 (s, 2H), 7.47 – 7.71 (m, J = 1.8 Hz, 3H), 7.75 – 7.94 (dd, J = 7.5, 2.2 Hz, 2H).

Synthesis of compound 2

To a solution of benzofuroxan (384 mg, 2 mmol) in 20 ml DCM, succinic anhydride (300 mg, 3 mmol) and DMAP (22.9 mg, 0.2 mmol) were added to react overnight at room temperature. After the reaction was finished, the reaction solution was washed with 1% hydrochloric acid for 3 times, dried with anhydrous sodium sulfate, and removed under reduced pressure to obtain the compound **2**, a white solid with a yield of 87%.^{1}H NMR (300 MHz, Chloroform-*d*) δ2.60 – 2.77 (m, 4H), 5.15 – 5.22 (s, 2H), 7.47 – 7.67 (m, 3H), 7.66 – 7.79 (m, 2H).

Synthesis of *c, c, t*-[$Pt(NH_3)_2Cl_2(OCCH_2CH_2COOH)_2$]

Firstly, oxoplatin was synthesized as described in the literature.[2] The reaction mixture of cisplatin (0.3 g, 1.0 mmol) and hydrogen peroxide (30 wt%, 20 mL) was stirred for 5 h at 75 °C. After that, the reaction solution was removed

under reduced pressure to obtain a yellow solid. Then the resulted solid was washed with cold water and dried in vacuum to obtain 0.32 g of oxoplatin. Yield 95.8%. Secondly, to a solution of oxoplatin (0.3 g, 0.9 mmol) in 6 mL anhydrous DMF, succinic anhydride (0.36 g, 3.6 mmol) was added to react for 24 h at 70°C, avoiding light. After that, the reaction solution was diluted with a large amount of diethyl ether to produce a white precipitate. Through further separation with centrifuge, *c, c, t*-[$Pt(NH_3)_2Cl_2(OCCH_2CH_2COOH)_2$] was obtained with a yield of 71%.

Synthesis of Pt-furoxan (1)

A mixture of *c, c, t*-[$Pt(NH_3)_2Cl_2(OCCH_2CH_2COOH)_2$] (160 mg, 0.3 mmol), DCC (129.8 mg, 0.6 mmol), DMAP(7.7 mg, 0.06 mol) and benzofuroxan (115.3 mg, 0.6 mmol) in anhydrous DMF (3 mL) was stirred at room temperature for 24 h in dark. After that, the reaction solution was directly separated using silica gel column chromatography (gradient elution with petroleum ether: ethyl acetate = 2:1 ~ ethyl acetate) to obtain Pt-furoxan, a white solid with yield of 43%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.33 – 2.59 (m, 8H), 5.05 – 5.27 (s, 4H), 6.25 – 6.69 (s, 6H), 7.53 – 7.71 (t, J = 2.7 Hz, 6H), 7.73 – 7.88 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 29.78 , 30.55, 54.59, 112.74, 126.16, 128.19, 129.90, 131.92, 157.53, 172.20, 179.41; ^{195}Pt NMR (400 MHz, DMSO-d_6) δ.1234.53; ESI-MS: m/z=882.92 (calculated 882.52).

The thiol-dependent NO release from Pt-furoxan

Pt-furoxan (50 μM) was dissolved in PBS (50 mM, pH7.4) containing GSH or ascorbic acid (5 mM). The resulted mixture incubated at 37 °C for 24 h, avoiding light. Aliquots (taken at 1, 4, 8, 16 and 24 h) were incubated with Griess reagent A (1.0% sulfanilamine, 5.0% H_3PO_4 in dH_2O) and Griess reagent B (0.1% (N-1-naphthyl)ethylenediamine dihydrochloride in dH_2O) for 10 minutes. Then the results were recorded with images.

Griess assay

Compound **2** or Pt-furoxan (with equivalent concentration of furoxan, 500 μM) was dissolved in PBS (50 mM, pH7.4), then L-cysteine (50 mM) was added. The resulted mixture incubated at 37 °C for 72 h, avoiding light. In addition, sample of compound **2** in absence of L-cysteine was prepared for control. Aliquots (taken at 1, 2, 4, 8, 12, 18, 24, 36, 48 and 72 h) were incubated with Griess reagent A (1.0% sulfanilamine, 5.0% H_3PO_4 in dH_2O) and Griess reagent B (0.1% (N-1-naphthyl)ethylenediamine dihydrochloride in dH_2O) for 10 minutes. The absorbance of the incubation solution was measured at 540 nm using microplate spectrophotometer; the rates of NO release for these compounds were calculated using a standard curve constructed with $NaNO_2$ to yield nitrite concentration.[1]

The NO release from Pt-furoxan tested by fluorescence microscope

HepG2 cells were seeded in a 6-well plate with 5×10^4 cells per well in 2 mL of DMEM containing 10% FBS overnight. Next day, the culture medium was replaced with 2 mL fresh medium containing Pt-furoxan of 5 μM, while the negative control group was directly replaced by fresh culture medium. After further incubation for 24 h, the medium was replaced with 5 μM DAF-FM DA probe or serum-free medium and further incubation at 37 °C spanned 20 min. After that, cells were washed to remove DAF-FM DA probe with pH 7.4 PBS for three times and observed using fluorescence microscope.[3]

The NO release from Pt-furoxan tested by flow cytometry

HepG2 cells were seeded in a 6-well plate with 2×10^5 cells per well in 2 mL of DMEM containing 10% FBS overnight. Next day, the culture medium was replaced with 2 mL fresh medium containing different platinum complexes (equivalent of 5 μM Pt), while the negative control group was directly replaced by fresh culture medium. After further incubation for 24 h, cells were collected, washed with pH 7.4 PBS for 3 times and co-incubated with 5 μM DAF-FM DA probe for 20 min at 37 °C. After that, cells were washed to remove DAF-FM DA probe with pH 7.4 PBS for 3 times and measured using flow cytometry.

Reactions of Pt-furoxan with DNA

The 0.2 mg/mL concentration of herring sperm DNA was prepared with herring sperm DNA (2 mg) and $NaClO_4$ (12.2 mg) in 10 mM phosphate buffer, pH 7.4. The DNA concentration was determined by UV-vis spectra at 260 nm with extinction coefficient 6600 M^{-1}. The mixture of DNA (0.1 mg/mL) with Pt-furoxan (50 μM) in presence of GSH (5 mM) or ascorbic acid (5 mM) was incubated at 37°C in the dark. Aliquots (taken at 0, 1, 4, 8, 16, and 24 h) were incubated with EtBr (0.025 mg/mL) and NaCl (0.1 M) in PBS. Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrometer.

***In vitro* cytotoxicity assays**

Cancer cells were seeded in 96-well plates at the density of 4000 cells/well in 100 μL of DMEM containing 10% FBS, and incubated overnight at 37 °C in 5% CO_2. Then cells was treated with 100 μL fresh medium containing varying concentrations of drugs for 48 h. The 100 μM concentration of Pt-furoxan was dissolved with aid of 0.1% DMSO, while the concentration of compound **2** can reach 4 mM in medium. 0.05% DMSO was used for dissolving all compounds in cellular assays and also used in the control. After the medium containing drugs was replaced with fresh culture medium containing 1 mg/mL MTT, the cells were incubated for 4 h. Finally, 150 μL of DMSO was added to every well when the medium containing MTT was removed. The absorbance was measured at 490 nm using a Bio-Rad 680 microplate reader. The IC_{50} values were calculated using GraphPad Prism software (version 5.01) based on data from three parallel experiments.

The cellular accumulation of Pt-furoxan

HepG2 cells were seeded in 6-well plates overnight and treated with 2 mL fresh medium containing 100 μM of these platinum complexes at 37 °C for 4 h. The cells were washed with PBS (pH 7.4) for 3 times and collected. After the cell numbers were counted by a hemacytometer, the harvested cells were digested by HNO_3 for ICP-MS assay.

***In vitro* antiproliferative assay with PTIO**

HepG2 cells were seeded in 96-well plates at the density of 5000 cells/well in 100 μL of DMEM containing 10% FBS, and incubated overnight at 37 °C in 5% CO_2. After pretreated with 300 μM of PTIO for 1 h, the cells were treated with 100 μL fresh medium containing these platinum complexes. Except that the concentration of Pt-furoxan was 2.5 μM, the concentration of other platinum complexes were all 25 μM. The cells were incubated for another 24 h at 37 °C in a 5% CO_2 humidified incubator. After that, the medium containing drugs was replaced with fresh culture medium containing 1 mg/mL MTT. The cells were co-incubated with MTT for 4 h. Finally, the medium containing MTT was removed, followed by addition of 150 μL of DMSO to every well. The absorbance was measured at 490 nm using a Bio-Rad 680 microplate reader. The cell survival rate were calculated based on data from three parallel experiments.[4]

Mitochondrial membrane potentials assay

Mitochondrial membrane potentials assay was carried out as described in the instructions of JC-1 kit. Briefly, HepG2 cells were seeded in 6-well plates and incubated overnight at 37 °C in 5%CO_2. Next day, the medium was replaced with fresh medium containing 2 μM of these platinum complexes and the cells continued to be cultured for 24 h. After that, the medium containing drugs was removed and the cells was rinsed three times with PBS and treated with 1mL JC-1 staining solution for further incubation of 20 min. After incubation, the cells were washed with buffers provided in JC-1 kit two times and treated with 1 mL serum-free medium. Mitochondrial membrane potentials were monitored by determining fluorescent emissions from mitochondrial JC-1 monomers or aggregates using an Olympus fluorescence microscope.

***In vitro* cell apoptosis assay**

HepG2 cells seeded in 12-well plates were incubated overnight at 37 °C in 5% CO_2 and treated with 5 μM of these platinum complexes. After 24 h and 48 h of incubation, the medium and the cells were all harvested and processed as described in the AnnexinV-FITC apoptosis detection kit (Beyotime Biotechnology, Nanjing, China). The samples were detected with CytoFLEX flow cytometer (Beckman Coulter).

***In vitro* cell cycle assay**

HepG2 cells were seeded in 6-well plates at a density of 2×10^5 and incubated overnight at 37 °C in 5% CO_2. The medium was replaced with fresh medium containing 5 μM of platinum complexes. After 24 h and 48 h of further incubation, the cells were harvested and rinsed twice with PBS. After the cells were fixed with 70% ethanol in phosphate buffer at 4 °C for 24 h and 48 h, the cells were separated by centrifugation and washed with cold PBS. Finally, the cells were processed as described in cell cycle and apoptosis analysis kit (Beyotime Biotechnology, Nanjing, China). The samples were detected with CytoFLEX flow cytometer (Beckman Coulter) and analyzed by MODFit LT for Mac, v5.0, software.[5]

Scratch assay

4T1 cells were incubated in 6-well plates at 37 °C in 5%CO_2. After 95-100% of confluency, the cells were scratched to create a linear wound. The wound was washed with PBS to remove cell debris. After that, the cells were treated with fresh serum-free DMEM medium containing 2 μM of these platinum complexes and cultured further. The cells were observed by microscope at 0, 24 and 48 h. the healed areas in individual group of cells were calculated.

Scratch assay

4T1 cells were incubated in 6-well plates at 37 °C in 5% CO_2. After 95-100% of confluency, the cells were scratched to create a linear wound. The wound was washed with PBS to remove cell debris. After pretreated with 300 μM of PTIO for 1 h, the cells were treated with fresh serum-free DMEM medium containing 2 μM of Pt-furoxan and cultured further. The cells were observed by microscope at 0 and 24 h. the healed areas in individual group of cells were calculated.

Adhesion assay

HUVECs were seeded in 6-well culture plates. After 90% of confluency, HUVECs were pretreated with IL-1β (1 ng/ml) for 4 h. Rhodamine 123-labeled 4T1 cells were co-incubated with the HUVECs monolays in each well, followed by treatment with 5 μM of these platinum complexes for 2 h. After incubation, non-adhered 4T1 cells were washed away (drop-to-drop) with PBS. 20 visual fields selected randomly for each well were taken pictures using fluorescence microscope.[6]

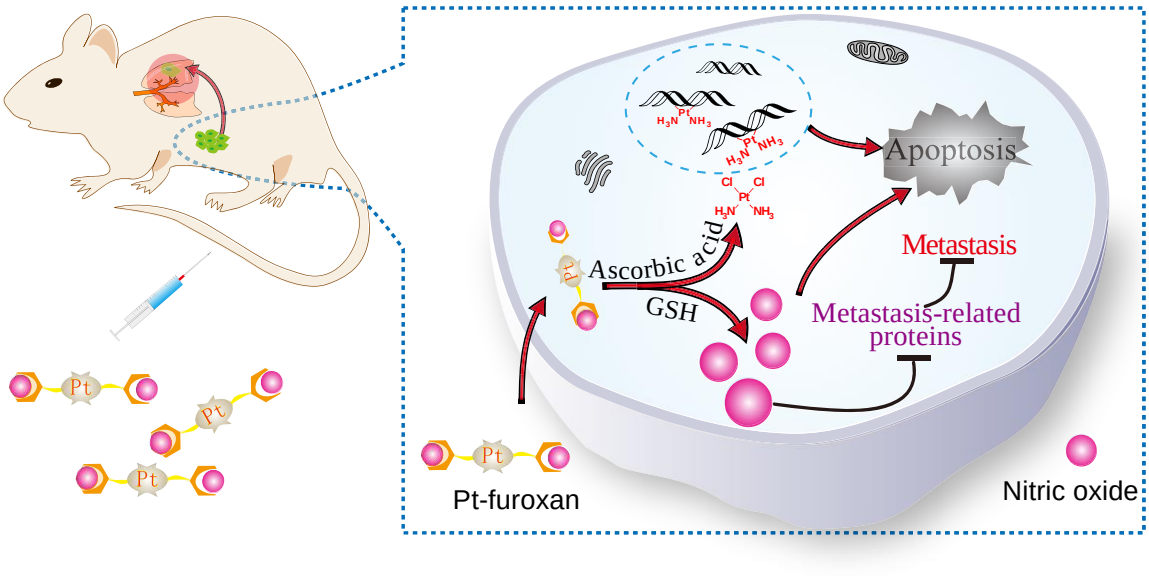
Expression of MMP 2 and MMP 9 tested by gelatin zymography

4T1 cells were seeded in 6-well plates at a density of 5×10^5 and incubated overnight at 37 °C in 5% CO_2. The medium was replaced with fresh serum-free medium containing 2 μM of platinum complexes. After 24 h of incubation, the medium was collected and centrifuged to remove the cell debris at 4 °C. The centrifuged medium was for the gelatin zymography assay. The centrifuged medium of each experimental group (10 μL) was mixed with 6×SDS-PAGE Loading Buffer (10 μL) to load on 10% denaturing SDS polyacrylamide gels containing 1 mg/mL of gelatin. After electrophoresis, the gel was washed 5 times for 20 min in renaturation buffer solution (50 mM Tris-HCl, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 2.5% Triton X-100, pH=7.6). Then it was rinsed 3 times for 20 min in washing buffer solution (50 mM Tris-HCl, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, pH=7.6). Finally, the gel was incubated for 48 h at 37 °C in incubation buffer solution (50

mM Tris-HCl, 5 mM $CaCl_2$, 1μM $ZnCl_2$, 150 mM NaCl, 0.02% Brij-35, pH=7.6). After that, the gel was stained with Coomassie Brilliant Blue R-250 and then destained with water.[7]

Tumor growth inhibition and metastasis suppressor *in vivo*

All live animal experiments were conducted according to the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the Institutional Animal Care and Use Committee of the Animal Experiment Center of University of Science and Technology of China (Hefei, China). For *in vivo* antitumor and anti-metastasis experiment, 3×10^5 4T1 cells were injected to the right mammary gland of female mice. Tumors were allowed to grow to a volume of 100-200 mm^3. The mice bearing metastatic 4T1 breast cancer were randomly assigned to 5 groups (n=5) for the treatment of PBS, oxoplatin+**2**, cisplatin, Pt-furoxan (low dose) or Pt-furoxan (high dose). Drugs were administrated through tail vein injection every other day for 6 times in a dosage of 2.0 mg/kg (or 1.0 mg/kg) platinum. Tumor growth was monitored by measuring the perpendicular diameter of the tumor using calipers every two days. The tumor volume was calculated according to the formula: tumor volume (mm^3) = $0.5\times length\times width^2$. Animals were sacrificed for humane reasons and the heart, liver, spleen, lung, kidney and tumor tissues were immediately excised for image and H&E staining and taken partially for measurement of Pt accumulating in tumor tissues using ICP-MS.



Scheme S1 A schematic illustration of the anti-proliferation and anti-metastasis action of Pt-furoxan.

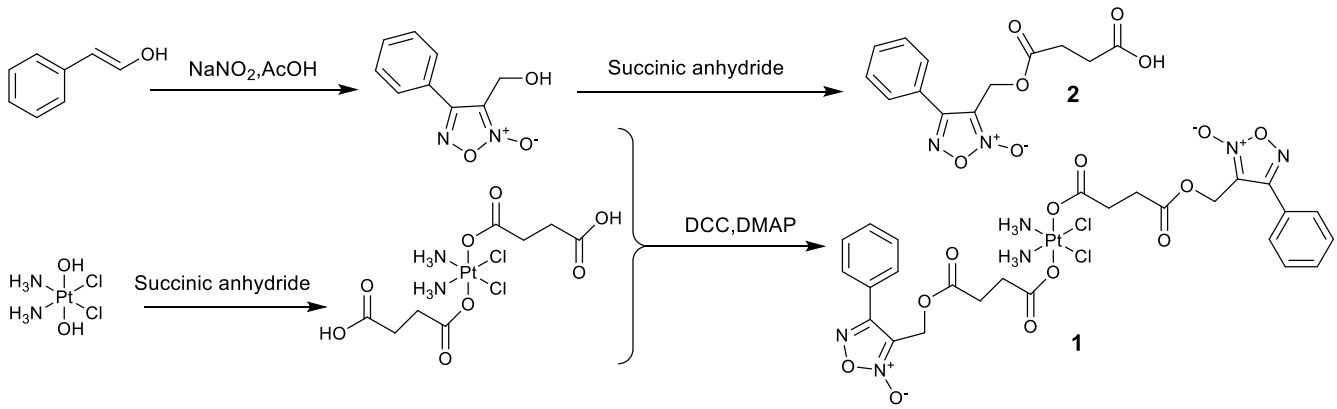


Figure S1. Synthetic route of Pt-furoxan (**1**) and furoxan ligand (**2**).

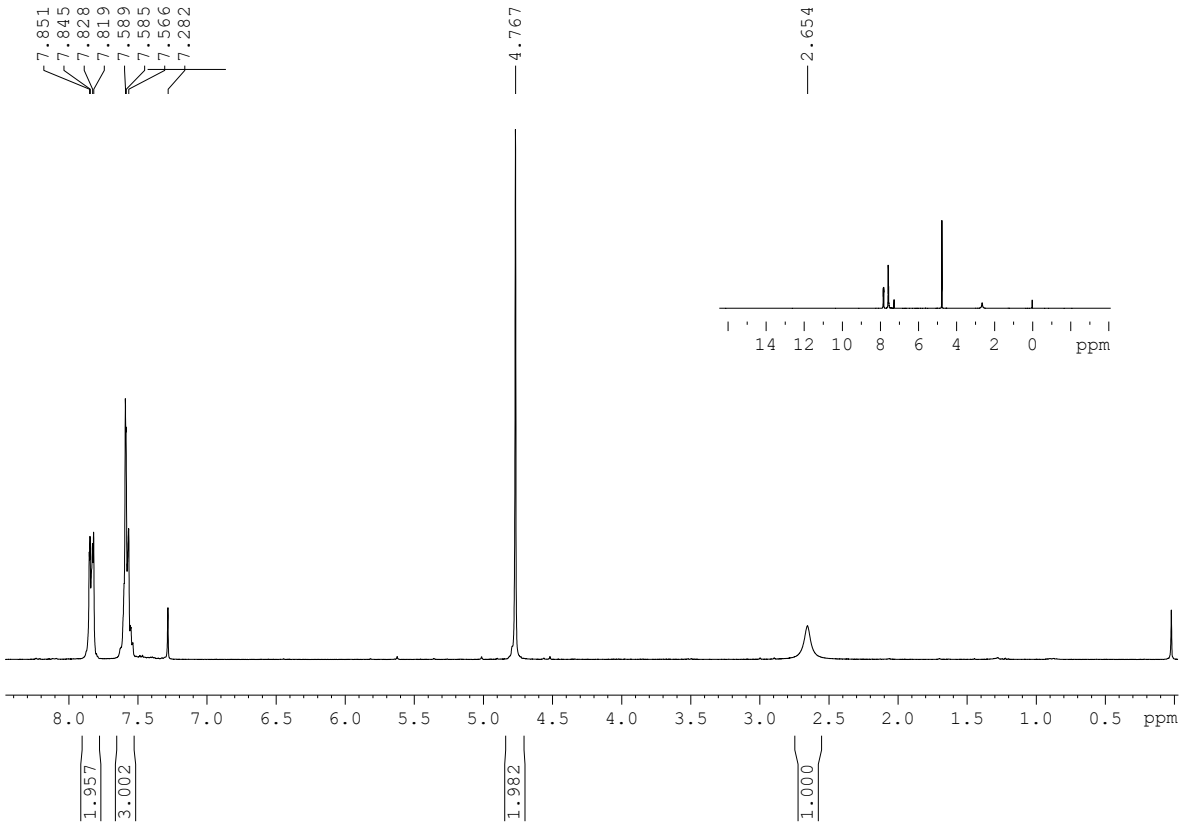


Figure S2. ^{1}H NMR spectrum of benzofuroxan (300 MHz, Chloroform-*d*).

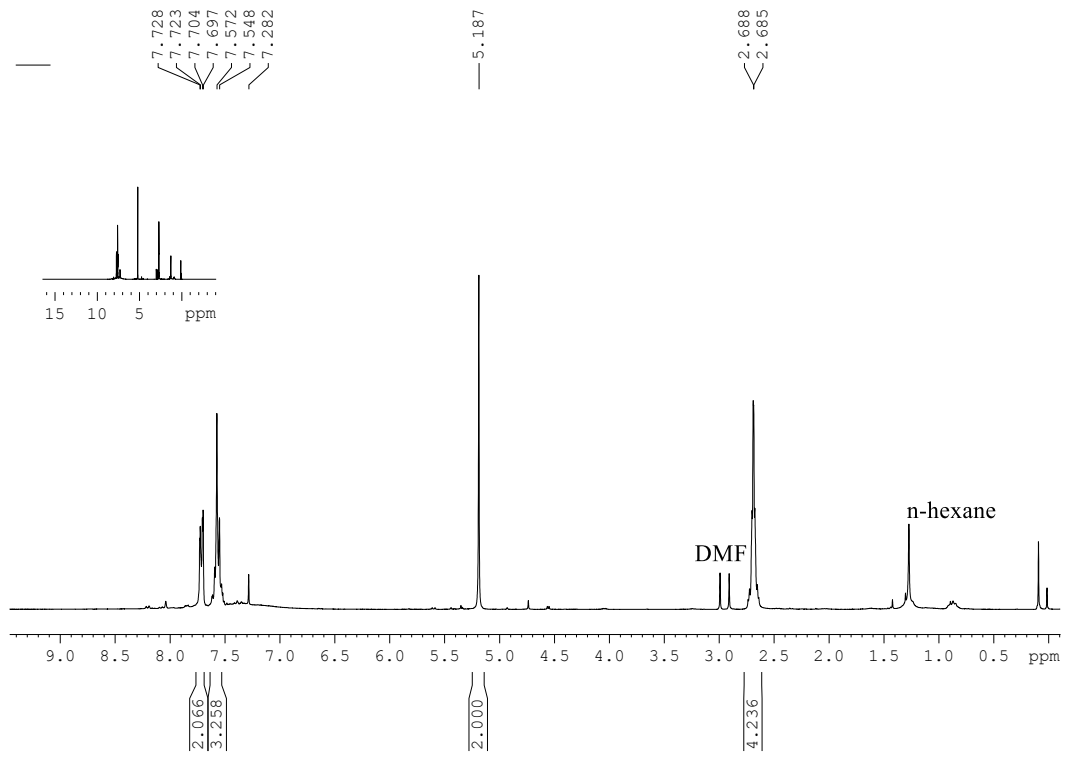


Figure S3. ^{1}H NMR spectrum of furoxan ligand (**2)** (300 MHz, Chloroform-*d*).

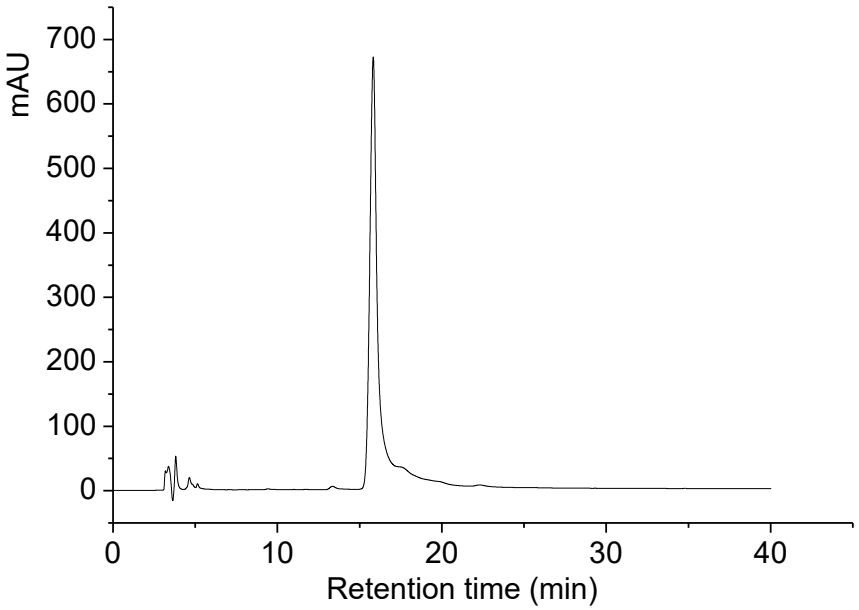


Figure S4 HPLC chromatograms of Pt-furoxan. Mobile phase: 60 % CH3OH in H2O (containing 1% TFA); detection: UV at 220 nm.

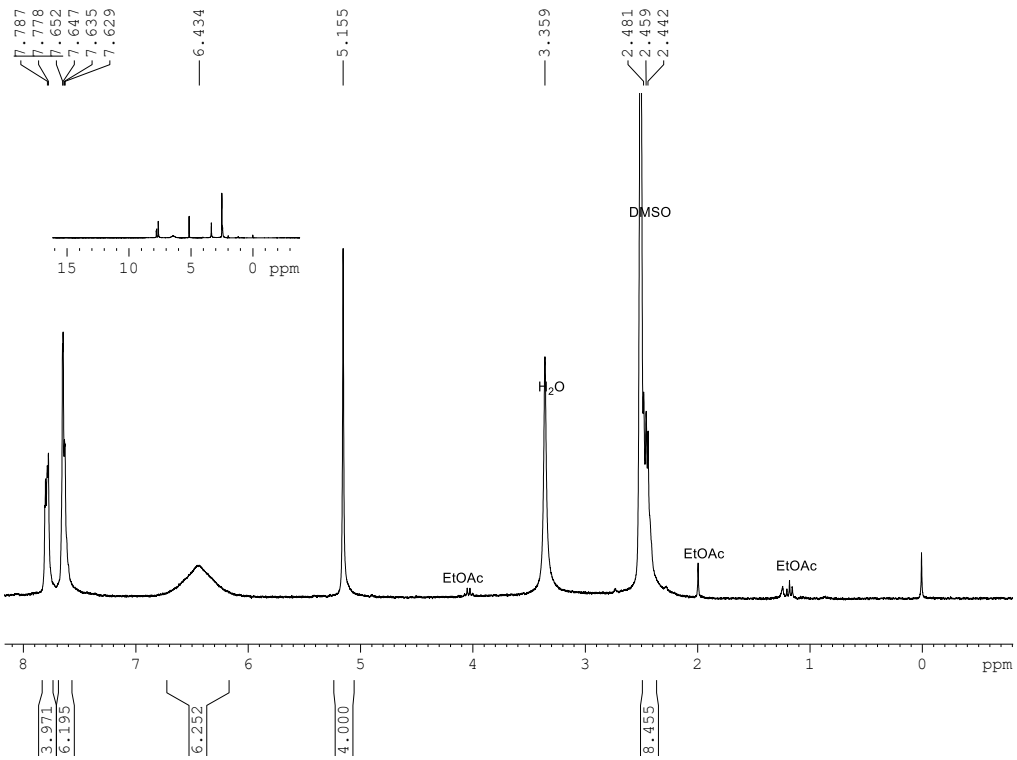


Figure S5. ^{1}H NMR spectrum of Pt-furoxan (**1**) (300 MHz, DMSO-d_6).

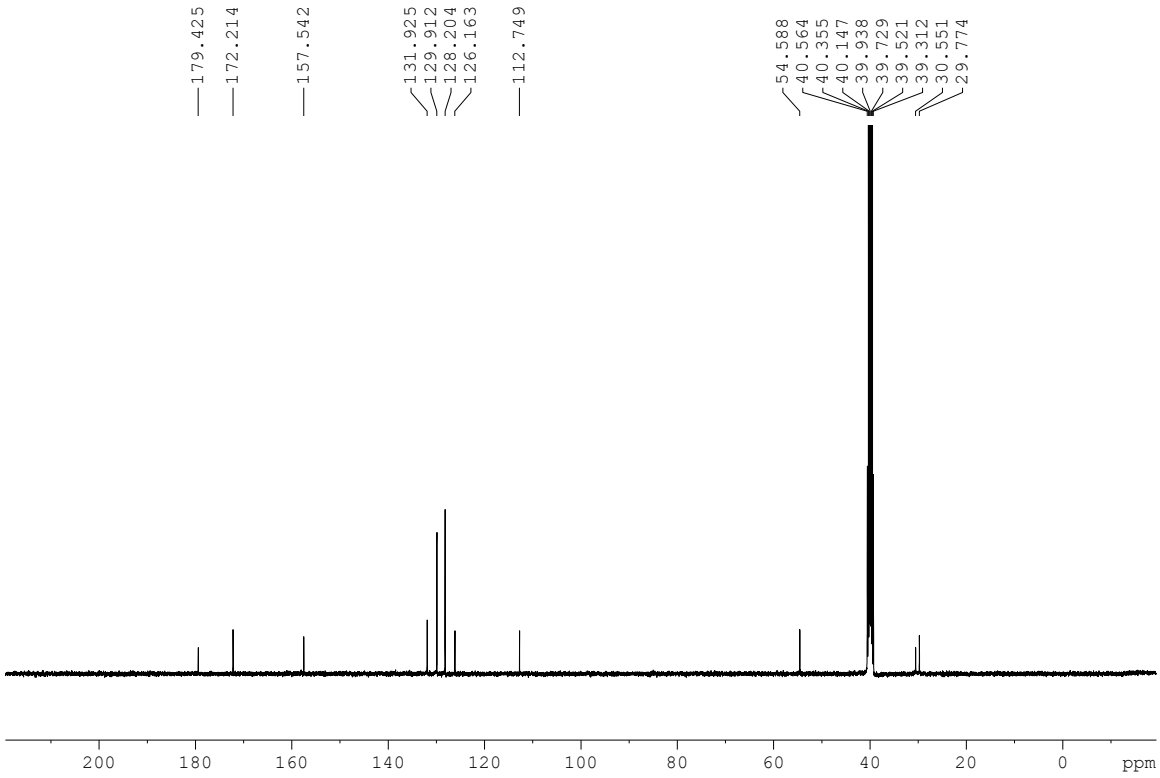


Figure S6. ^{13}C NMR spectrum of Pt-furoxan (**1**) (101 MHz, DMSO-d_6, c=22.6 mM).

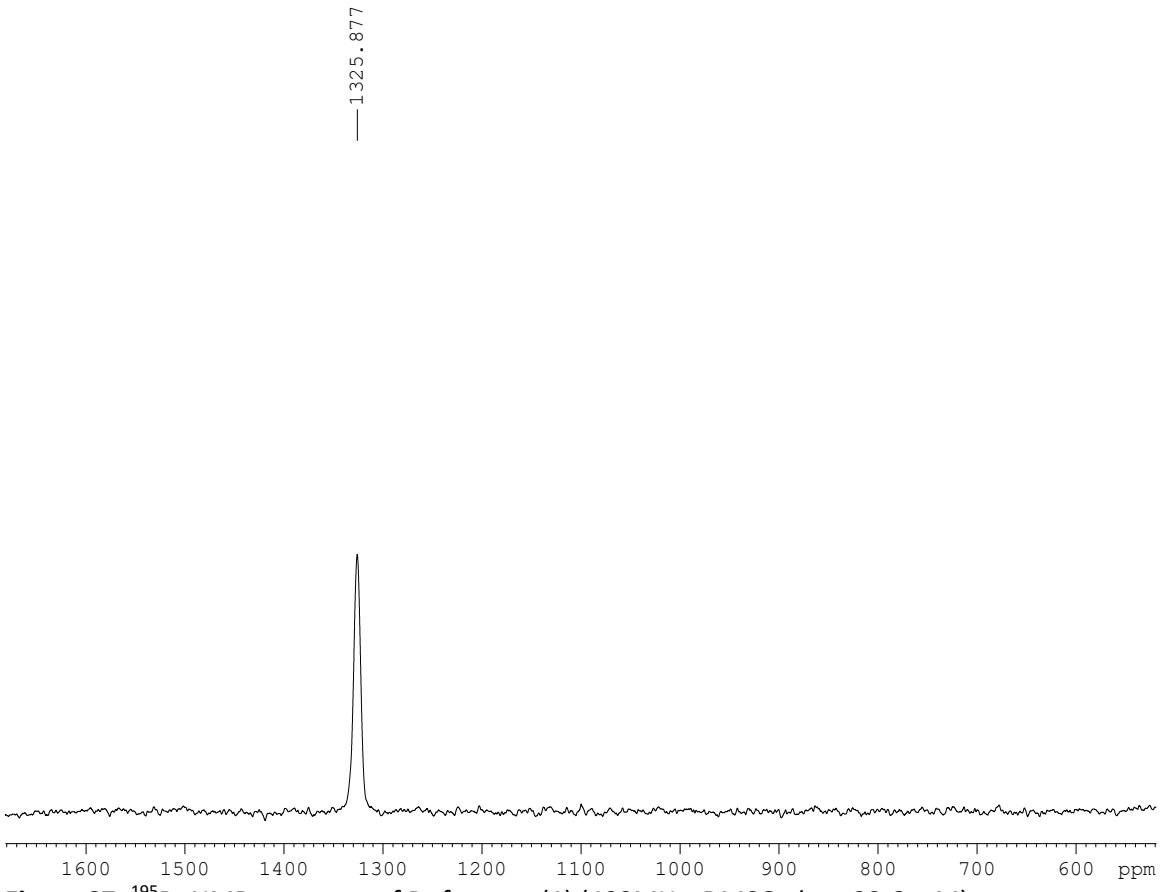


Figure S7. ^{195}Pt NMR spectrum of Pt-furoxan (**1**) (400MHz, DMSO-d_6, c=22.6 mM).

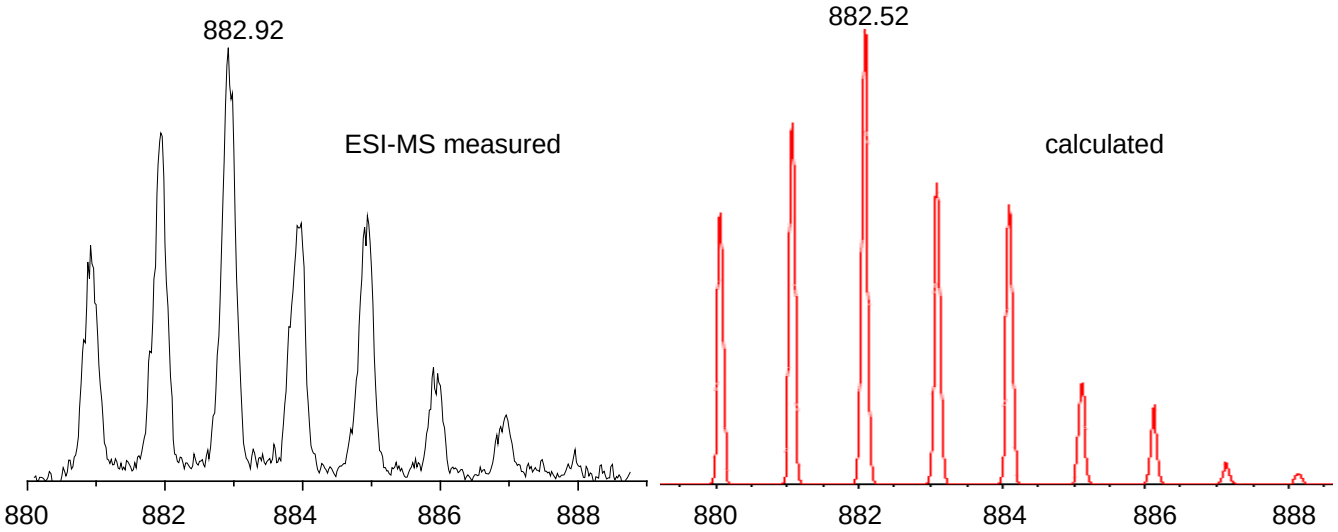


Figure S8. ESI-MS spectrum of Pt-furoxan (**1**).

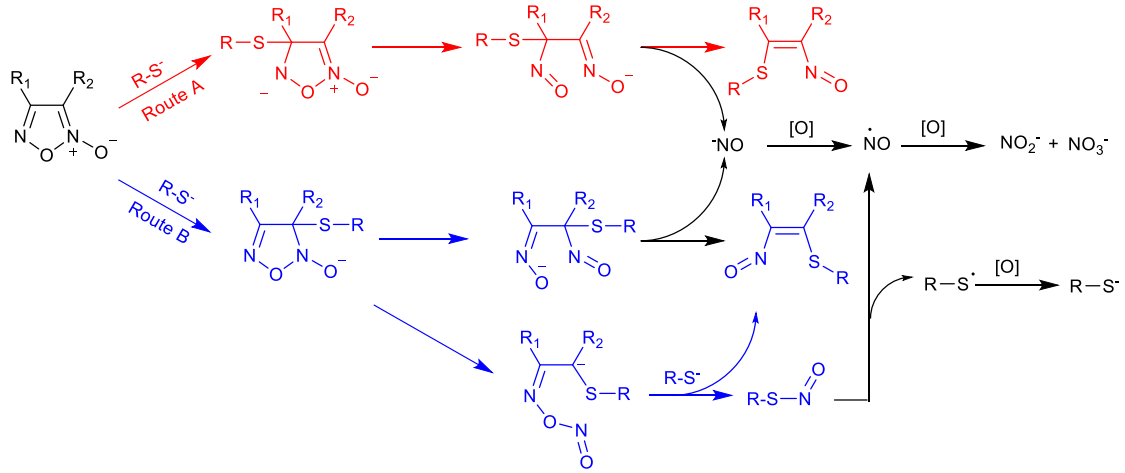


Figure S9. The mechanism of thiol-dependent NO release from furoxans. (From "Molecular Hybridization Tools in the Development of Furoxan-Based NO-Donor Prodrugs" *Chem. Med. Chem*., 2017, 12, 622-638)

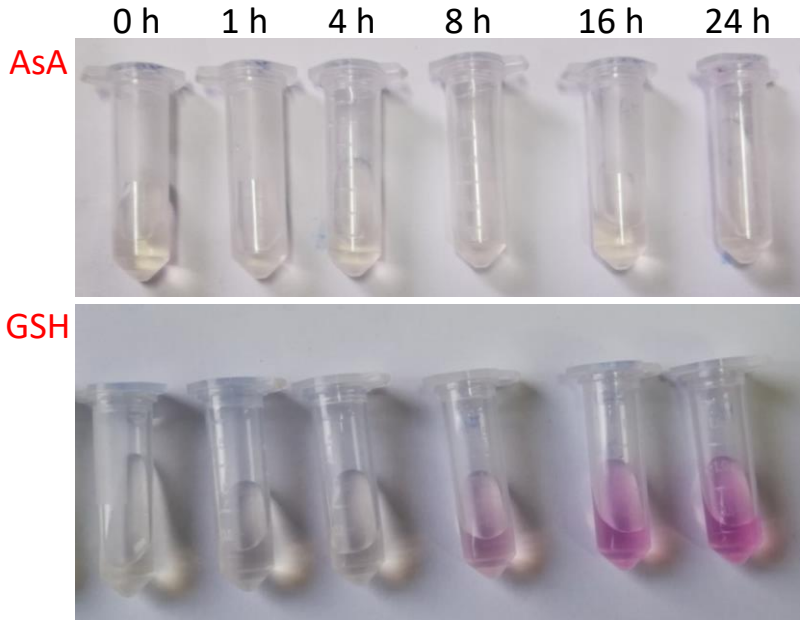


Figure S10. NO release from furoxans in solution based on Griess assay. Pt-furoxan (50 µM) was incubated with GSH (5 mM, lower portion) or ascorbic acid (5 mM, upper portion) for different time at 37°C.

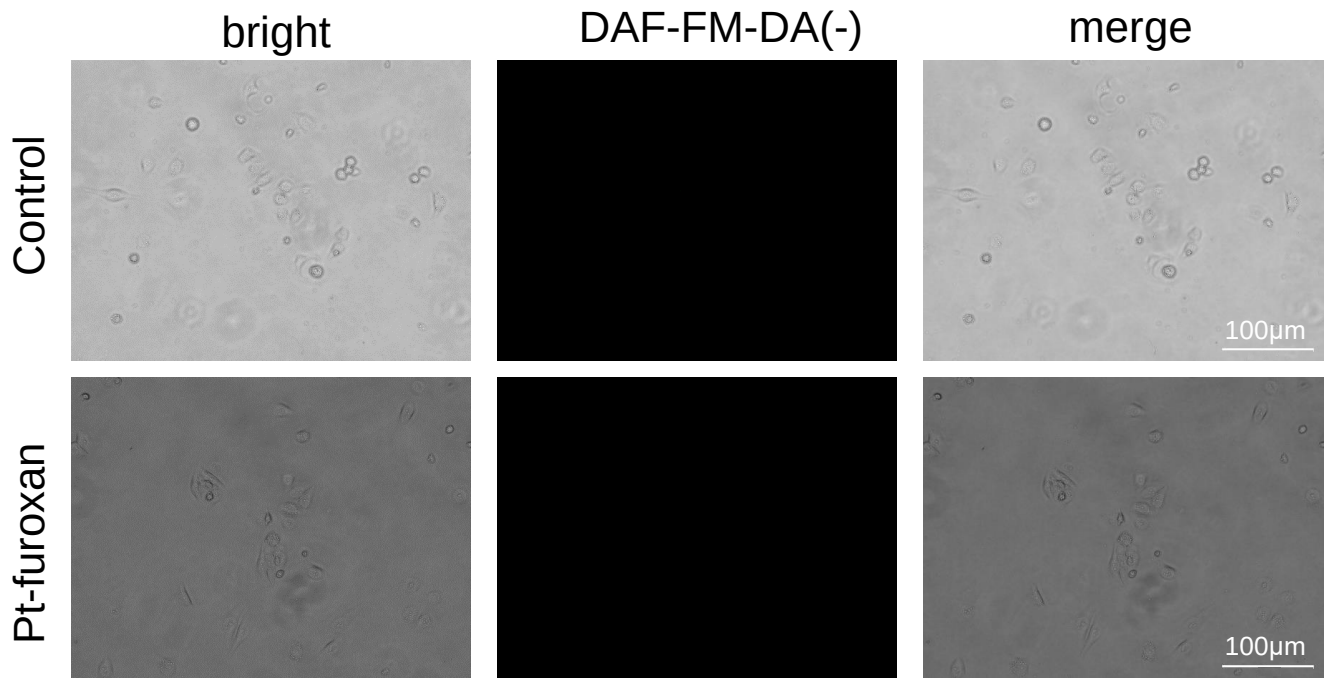


Figure S11. Fluorescence imaging of NO in HepG2 cells. HepG2 cells were co-incubated with 5 µM of Pt-furoxan for 24 h and observed using fluorescence microscope in absence of DAF-FM-DA probe.

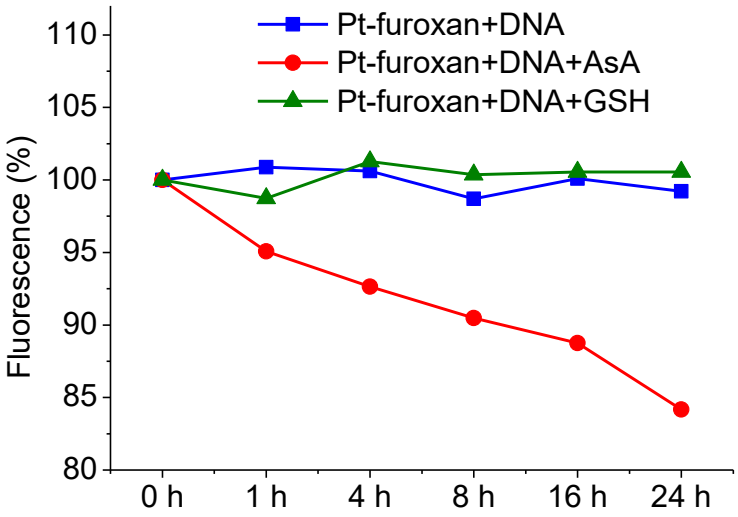


Figure S12 Fluorescence measurement of DNA platination by Pt-furoxan (1) upon reduction. All reactions were performed with 0.1 mg ml^{-1} DNA in 10 mM $NaClO_4$ and 10 mM phosphate buffer (pH = 7.4) and Pt-furoxan (50 μM) at 37 °C for 24 h. 5 mM reducing agent (AsA or GSH) was present for the reduction of Pt-furoxan. The fluorescence probe (EtBr, 0.025 mg mL^{-1}) was added before the fluorescence measurements. The excitation wavelength was 530 nm and the emission was recorded at 592 nm。

Table S1 *In vitro* cytotoxicity (IC50, μM) of Pt-furoxan against cancer cells

	Pt-furoxan	Cisplatin	Oxoplatin	Oxoplatin + **2** [a]	Fold increase[b]
HepG2	0.8 ± 0.2	2.2±0.2	11±2	17±4	2.9
HeLa	0.53±0.06	1.8±0.2	8.9±1.2	7±1	3.3
A549	0.57±0.07	4.8±0.3	15±2	19±3	8.4
A2780	0.6±0.1	3.1±0.6	29±12	33±4	5.2
4T1	0.20±0.03	3.1±0.3	36±10	9.0±2.7	15.6
MCF-7	0.23±0.04	4.5±0.9	12±3	12±3	19.4

[a] The mole ratio of oxoplatin/2 = 1:2;[b] IC50 ratio of cisplatin/Pt-furoxan.

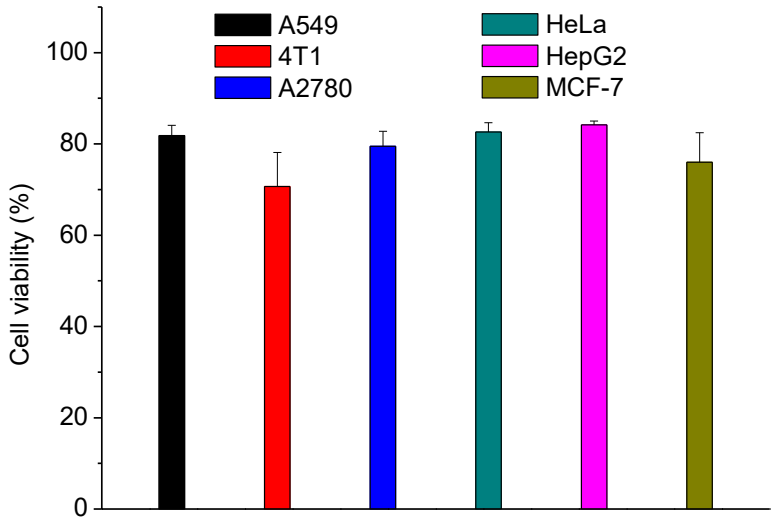


Figure S13. The effect of **2** on growth of cancer cells. Cell viability of cancer cells treated with 200 μM of furoxan ligand (**2**) for 48 h was determined using MTT assay.

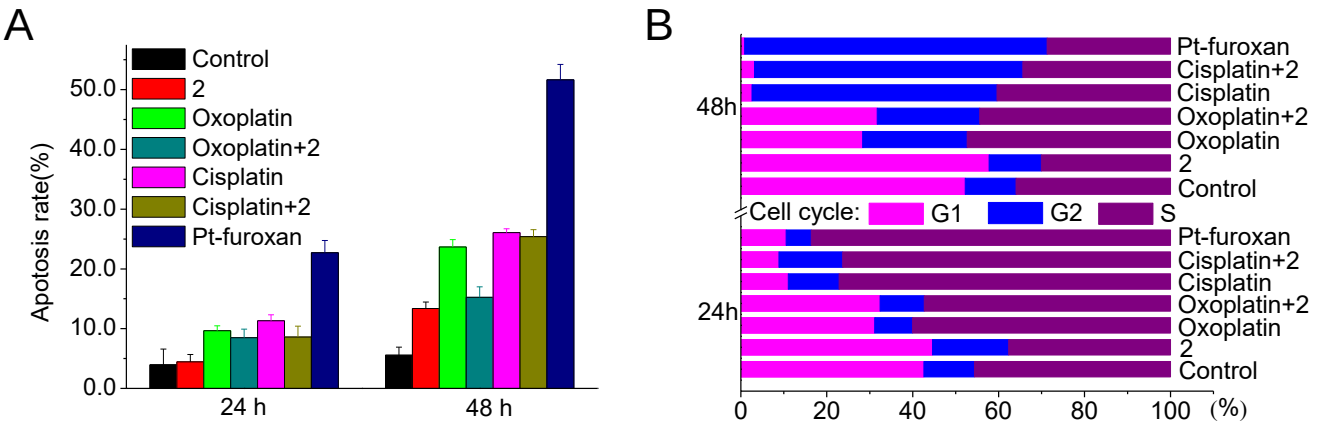


Figure S14. (A) Effects of platinum complexes on apoptosis. HepG2 cells treated with 5 μM of platinum complexes for 24 h or 48 h. Cells were stained with AnnexinV-FITC and PE. (B) Effects of platinum complexes on cell cycle. HepG2 cells treated with 5 μM of platinum complexes for 24 h or 48 h before PE stain. Two molar equivalents of **2** was used alone or in the mixture.

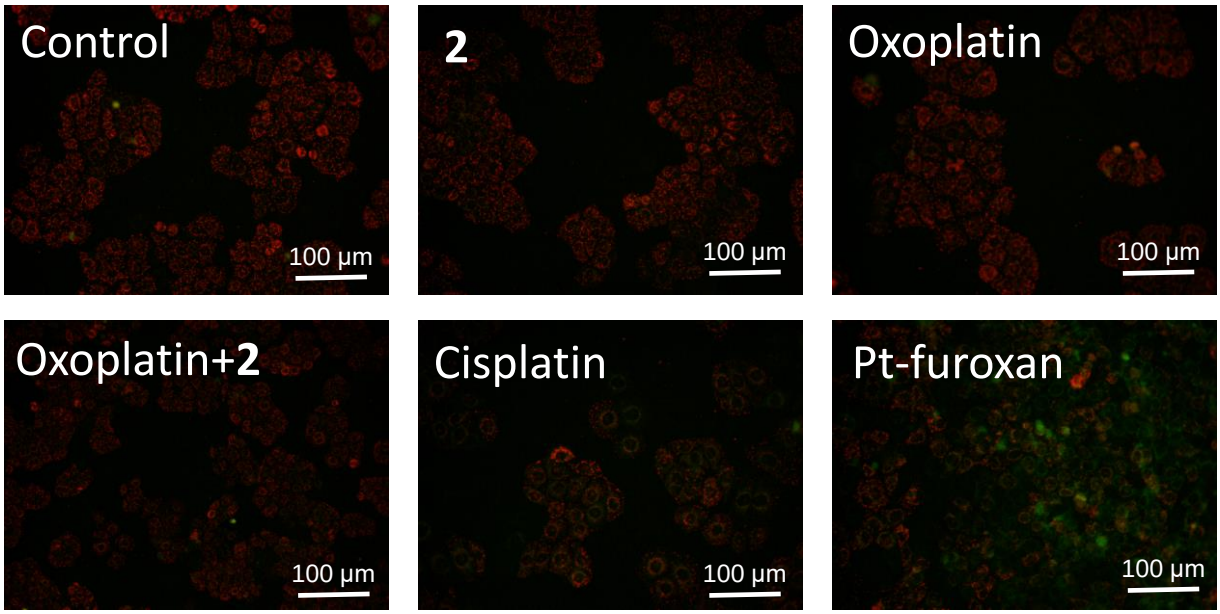


Figure S15. Effects of platinum complexes on mitochondrial membrane potentials. HepG2 cells were treated with 2 μM of platinum complexes for 24 h. Two molar equivalents of **2** was used alone or in the mixture in the assay. Cells were stained with JC-1 probe before taking imaging using fluorescence microscope.

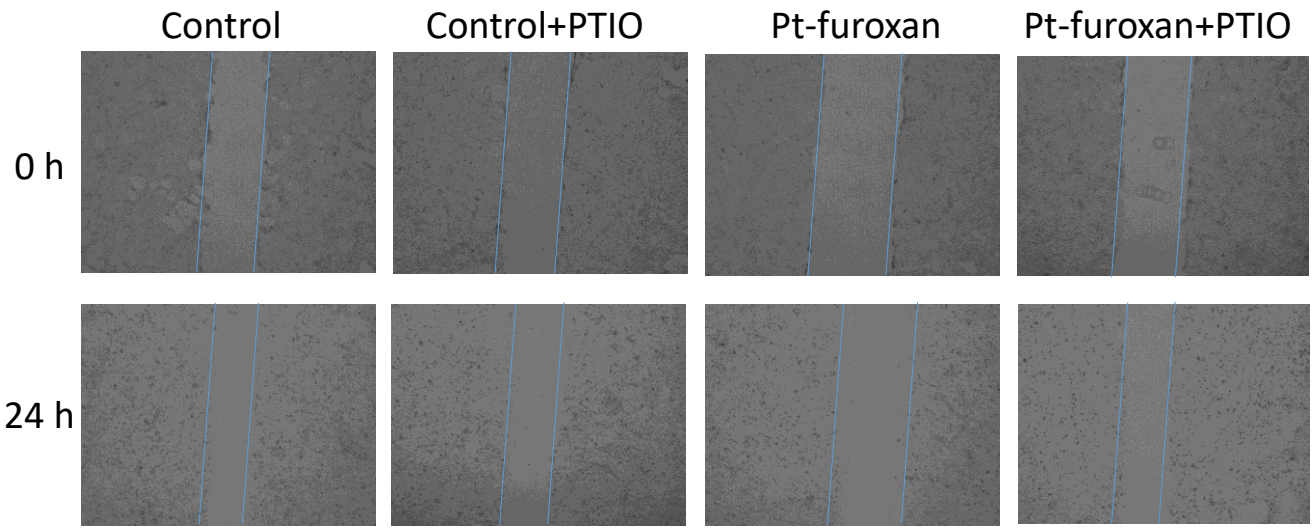


Figure S16 Analysis of the effect of PTIO on migration of 4T1 cells using wound-healing assay. 4T1 cells were pre-treated with 300 μM PTIO for 1 h before the treatment of 2.0 μM Pt-furoxan.

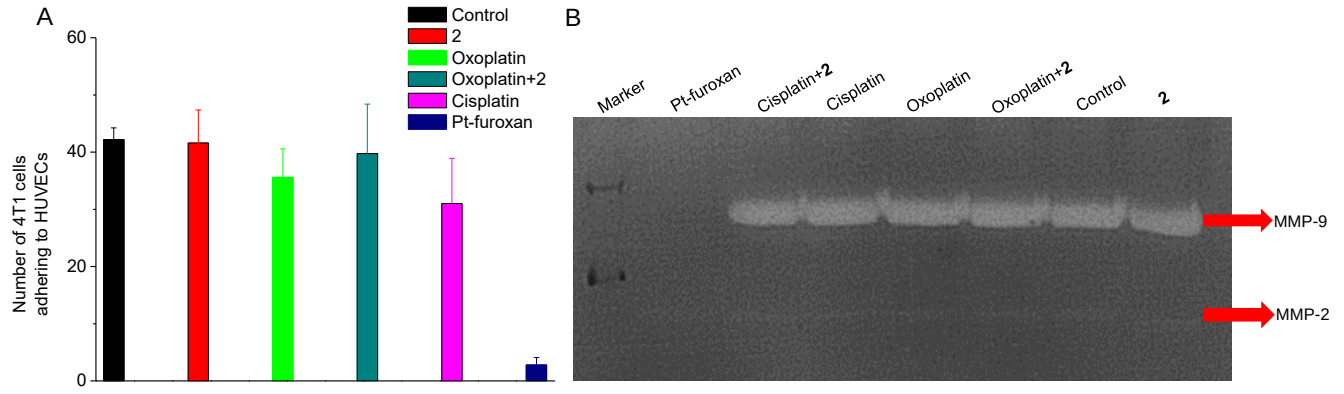


Figure S17. Pt-furoxan inhibited adhesion of 4T1 cells to HUVECs and expression of MMP. (A) The effect of compounds on adhesion of 4T1 cells (green) to HUVECs measured using fluorescence microscope. Co-incubation of Rhodamine 123-labeled 4T1 cells with HUVECs pre-treated with IL-1β (1 ng/ml) in presence of 5 μM of these platinum complexes for 2 h. (B) The activity of MMP-9 and MMP-2 measured by gelatin zymography. 4T1 cells treated with 2 μM of platinum complexes for 24 h. Two molar equivalents of 2 was used alone or in the mixture in all assays.

Figure S18. The representative images of tumor at the end of the experiment. Balb/c mice bearing 4T1 tumors were injected through the tail vein with PBS, the mixture of oxoplatin+**2** (2 mg/kg, Pt/body weight, two molar equivalents of **2** was used in the mixture), cisplatin (2 mg/kg, Pt/body weight) and Pt-furoxan (1 mg/kg or 2 mg/kg, Pt/body weight), (n = 5). PBS was used as a control. Mice were treated every-other-day 6 times.

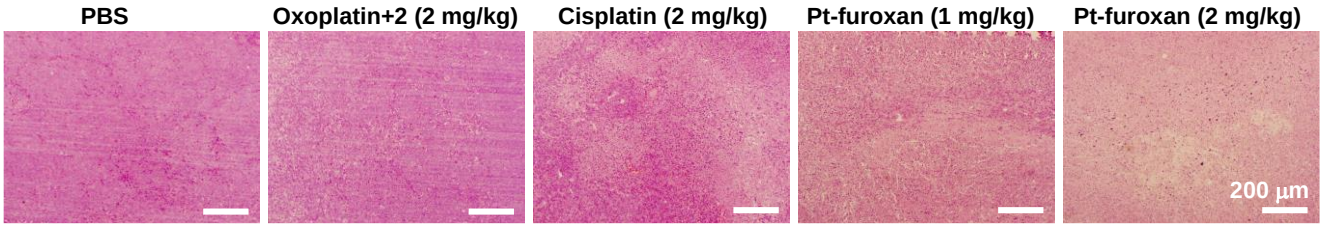


Figure S19 Analyses on cell apoptosis of tumor via HE staining at the end of the experiment. Balb/c mice-bearing 4T1 tumors were injected through the tail vein with PBS, the mixture of oxoplatin+**2** (2 mg Pt/kg body weight, two molar equivalents of **2** was used in the mixture), cisplatin (2 mg/kg, Pt/body weight) and Pt-furoxan (1 mg/kg or 2 mg/kg, Pt/body weight), (n = 5). PBS was used as a control. Mice were treated every-other-day 6 times.

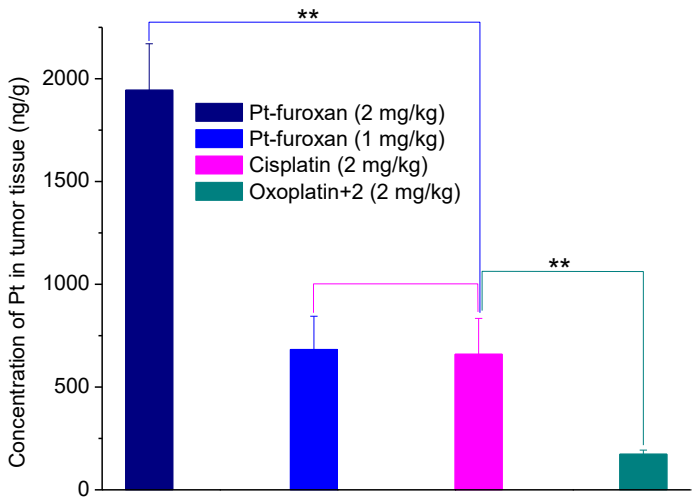


Figure S20. 14-day Pt concentration in tumor of mice from drug experimental group by ICP-MS at the end of the experiment. Balb/c mice bearing 4T1 tumors were injected through the tail vein with PBS, the mixture of oxoplatin+2 (2 mg/kg, Pt/body weight, two molar equivalents of **2** was used in the mixture), cisplatin (2 mg/kg, Pt/body weight) and Pt-furoxan (1 mg/kg or 2 mg/kg, Pt/body weight), (n = 5). PBS was used as a control. Mice were treated every-other-day 6 times. Error bars denote standard deviations. *p<0.05, **p<0.01, ***p<0.001.

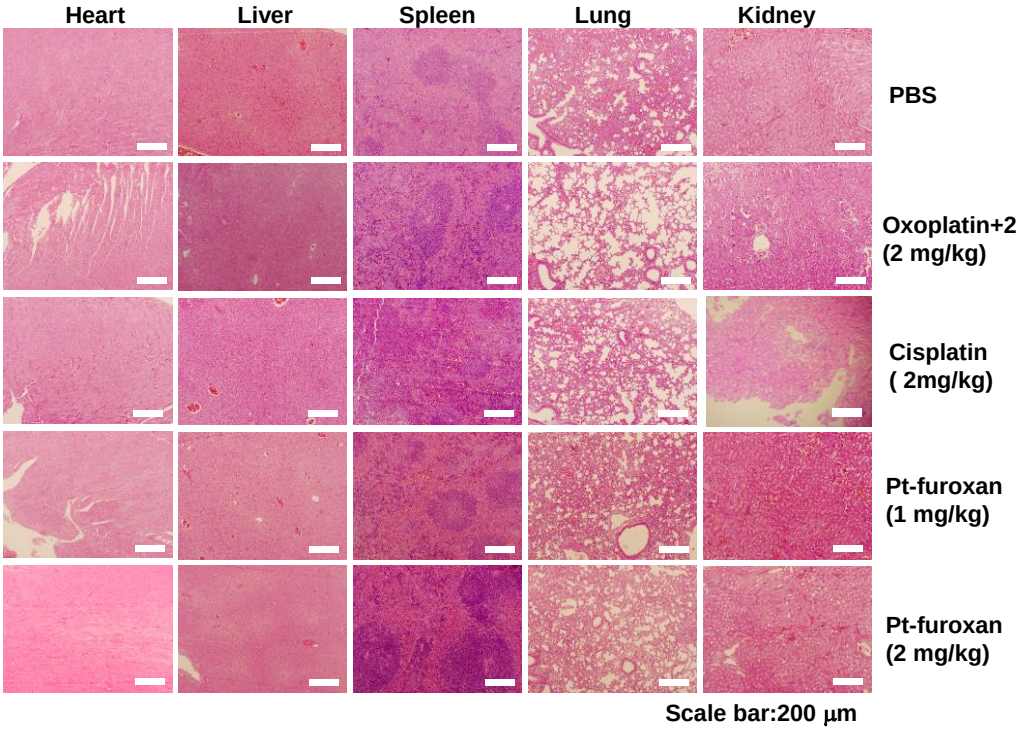


Figure S21. Analyses on the histological tissue damage caused by Pt-furoxan and cisplatin using H&E staining at the end of the experiment. Balb/c mice bearing 4T1 tumors were injected through the tail vein with PBS, the mixture of oxoplatin+**2** (2 mg/kg, Pt/body weight, two molar equivalents of **2** was used in the mixture), cisplatin (2 mg/kg, Pt/body weight) and Pt-furoxan (1 mg/kg or 2 mg/kg, Pt/body weight), (n = 5). PBS was used as a control. Mice were treated every-other-day 6 times.

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