Supporting Information for

Gallium(III)-polypyridyl complexes as anti-osteosarcoma stem cell agents

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Experimental Details

Materials and Methods. All synthetic procedures were performed under normal atmospheric conditions or under nitrogen. Fourier transform infrared (FTIR) spectra were recorded with an IRAffinity-1S Shimadzu spectrophotometer. High resolution electron spray ionisation mass spectra were recorded on a BrukerDaltronics Esquire 3000 spectrometer by Dr. Lisa Haigh (Imperial College London). UV-Vis absorption spectra were recorded on a Cary 3500 UV-Vis spectrophotometer. ^{1}H NMR spectra were recorded on a BrukerAvance 400 MHz Ultrashield NMR spectrometer. ^{1}H NMR spectra were referenced internally to residual solvent peaks, and chemical shifts are expressed relative to tetramethylsilane, $SiMe_4$ (δ = 0 ppm). Elemental analysis of the compounds prepared was performed commercially by London Metropolitan University. The gallium(II) complex, **1** was prepared according to a previously reported protocol.[1] $GaCl_3$, $Ga(NO_3)_3$, salinomycin, 1,10-phenantroline, 5-methyl-1,10-phenanthroline, and 3,4,7,8-tetramethyl-1,10-phenanthroline were purchased from Sigma Aldrich and used as received. Cisplatin and carboplatin were purchased from TCI Chemicals and used as received. For all biological studies, the concentrations of **1-3** were based on gallium concentration.

Synthesis of [Ga(III)(5-methyl-1,10-phenanthroline)$_2$Cl$_2$][GaCl$_4$], 2. To a solution of $GaCl_3$ (212.0 mg, 1.20 mmol) in 30 mL of dry dichloromethane, 5-methyl-1,10-phenanthroline (243.6 mg, 1.25 mmol) was added. The resulting mixture was stirred at room temperature for 46 hours. The solution was then dried under vacuum, the solid was suspended in diethyl ether (30 mL), filtrated, and washed with diethyl ether (40 mL) to yield the crude product as an orange solid (432.0 mg). The crude product was dissolved in acetonitrile (4 mL) and this solution was added to diethyl ether (40 mL), resulting in a white precipitate. The precipitate was filtrated and washed with diethyl ether (40 mL) to yield pure **2** as a white solid (345.6 mg, 37%); ^{1}H NMR (400 MHz, CD_3CN) δ_H 10.20 (t, 1H, J = 4), 10.10 (t, 1H, J = 4), 9.30 (dt, 1H, J = 8, 2), 9.09 (dt, 1H, J = 8, 2), 8.82-8.79 (m, 1H), 8.61-8.58 (m, 1H), 8.53 (ddd, 1H, J = 8, 4, 2), 8.45 (ddd, 1H, J = 8, 4, 2), 8.23 (s, 1H), 8.10 (s, 1H), 7.68-7.64 (m, 2H), 7.60-7.57 (m, 2H), 2.99 (br s, 3H), 2.87 (d, 3H, J = 2); IR (solid, cm^{-1}): 1653, 1629, 1610, 1590, 1528,1488, 1427, 1385, 1341, 1313, 1266, 1224, 1192, 1151, 1120, 1077, 1034, 100, 905, 875, 819, 733, 724, 663, 648, 620, 552; ESI-MS Calcd. for $C_{26}H_{20}Cl_2GaN_4$ [M-$GaCl_4$]$^+$: 529.0312 a.m.u. Found [M-$GaCl_4$]$^+$: 529.0322 a.m.u., Calcd. for $GaCl_4$ [$GaCl_4$]$^-$: 210.7980 a.m.u. Found [$GaCl_4$]$^-$: 210.7984; Anal. Calcd. For $C_{26}H_{20}Ga_2N_4Cl_6$: C, 42.17; H, 2.72; N, 7.57. Found: C, 42.15; H, 2.57; N, 7.42.

Synthesis of [Ga(III)(3,4,7,8-tetramethyl-1,10-phenanthroline)$_2$Cl$_2$][GaCl$_4$], 3. To a solution of $GaCl_3$ (161.0 mg, 0.91 mmol) in 30 mL of dry dichloromethane, 3,4,7,8-tetramethyl-1,10-phenanthroline (222.8 mg, 0.94 mmol) was added. The resulting mixture was stirred at room temperature for 23 hours. The solution was then dried under vacuum, the solid was suspended in diethyl ether (50 mL), filtrated, and washed with diethyl ether (40 mL) to yield the crude product as a beige solid (376.0 mg). The crude product was dissolved in acetonitrile (4 mL) and this solution was added to diethyl ether (40 mL), resulting in a white precipitate. The precipitate was filtrated and washed with diethyl ether (40 mL) to yield pure **3** as a white solid (276.5 mg, 36%); ^{1}H NMR (400 MHz, CD_3CN) δ_H 9.87 (s, 2H), 8.53 (d, 2H, J = 10), 8.42 (d, 2H, J = 10), 7.41 (s, 2H), 3.03 (s, 6H), 2.85 (s, 6H), 2.71 (s, 6H), 2.18 (s, 6H); IR (solid, cm^{-1}): 1653, 1636, 1530, 1490, 1433, 1387, 1315, 1266, 1199, 1079, 1019, 892, 869, 826, 809, 722, 641, 622, 574, 558, 555, 523, 475, 471, 464, 454; ESI-MS Calcd. for $C_{32}H_{32}Cl_2GaN_4$ [M-$GaCl_4$]$^+$: 613.1230 a.m.u. Found [M-$GaCl_4$]$^+$: 613.1254 a.m.u., Calcd. for

$GaCl_4$ $[GaCl_4]^-$: 210.7980 a.m.u. Found $[GaCl_4]^-$: 210.7989; Anal. Calcd. For $C_{32}H_{32}Ga_2N_4Cl_6$: C, 46.60; H, 3.91; N, 6.79. Found: C, 46.46; H, 3.77; N, 6.62.

X-ray Single Crystal Diffraction Analysis. Standard procedures were used to mount the crystal on a Bruker APEX 2000 diffractometer, with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) at 293 K. The crystal structure was solved using direct methods in SHELXS and refined by full-matrix least-squares routines, based on F^2, using the SHELXL program.[2] All the H atoms were placed in geometrically idealised positions and constrained to ride on their parent atoms. The structure has been deposited with the Cambridge Crystallographic Data Centre (CCDC 1947711). This information can be obtained free of charge from www.ccdc.cam.ac.uk/data_request/cif.

Measurement of water-octanol partition coefficient (LogP). The LogP value for **1-3** was determined using the shake-flask method and UV-Vis spectroscopy. The octanol used in this experiment was pre-saturated with water. An aqueous solution of **1-3** (500 μL, 100 μM) was incubated with octanol (500 μL) in a 1.5 mL tube. The tube was shaken at room temperature for 24 h. The two phases were separated by centrifugation and the **1-3** content in each phase was determined by UV-Vis spectroscopy.

Cell Lines and Cell Culture Conditions. The U2OS bone osteosarcoma, HEK 293T embryonic kidney, and GMO7575 skin fibroblast cell lines were acquired from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% penicillin and 10% fetal bovine serum. The BEAS-2B bronchial epithelium cell line was acquired from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in RPMI 1640 medium with 2 mM L-glutamine supplemented with 1% penicillin and 10% fetal bovine serum. The human epithelial breast MCF710A cell line was acquired from American Type Culture Collection (ATCC, Manassas, VA, USA). MCF10A cells were maintained in Mammary Epithelial Cell Growth Medium (MEGM) with supplements and growth factors (BPE, hydrocortisone, hEGF, insulin, and gentamicin/amphotericin-B). The cells were grown at 310 K in a humidified atmosphere containing 5% CO_2. To gain access to OSC-enriched cells, a full T75 flask of U2OS cells was treated with methotrexate (300 nM) for 4 days. The cells (labelled U2OS-MTX cells) were used immediately.

Flow cytometry. U2OS and U2OS-MTX cells were seeded in 6-well plates at a density of 5 × 10^5 cells/ mL and the cells were allowed to attach overnight. The cells were harvested by trypsinization and suspended in PBS (500 μL). The PE labelled anti-CD117 antibody (5 μL) was then added to the cell suspension, which was subsequently incubated in the dark for 20 min. The cells were analyzed using a FACSCanto II flow cytometer (BD Biosciences) (20,000 events per sample were acquired) at the University of Leicester FACS Facility. The FL2 channel was used to assess CD117 expression. Cell populations were analysed using the FlowJo software (Tree Star).

In order to probe the effect of the gallium complexes, **2**, **3**, and $Ga(NO_3)_3$ on the U2OS cell membrane, flow cytometry studies were carried out. U2OS cells were incubated with and without **2**, **3**, and $Ga(NO_3)_3$ (0.25 μM for 24 h) at 37°C. Cells were harvested from adherent cultures by trypsinization. The FITC Annexin V/Dead Cell Apoptosis Kit was used to probe the effect on the U2OS cell membrane. The manufacture's (Thermo Fisher Scientific) protocol was followed to carry out this experiment. Briefly, untreated and treated cells (1 × 10^6) were suspended in 1× Annexin binding buffer (100 μL) (10 mM HEPES, 140 mM NaCl,

2.5 mM $CaCl_2$, pH 7.4), then 5 μL FITC Annexin V and 1 μL PI (100 μg/ mL) were added to each sample and incubated at room temperature for 15 min. After which more 1× Annexin binding buffer (400 μL) was added while gently mixing. The cells were analyzed using a FACSCanto II flow cytometer (BD Biosciences) (20,000 events per sample were acquired) at the University of Leicester FACS Facility. The FL1 channel was used to assess Annexin V binding and the FL3 channel was used to assess PI uptake. Cell populations were analysed using the FlowJo software (Tree Star).

Cytotoxicity MTT assay. The colourimetric MTT assay was used to determine the toxicity of **1-3**, cisplatin, carboplatin, salinomycin, $Ga(NO_3)_3$, 1,10-phenantroline, 5-methyl-1,10-phenanthroline, and 3,4,7,8-tetramethyl-1,10-phenanthroline. U2OS, U2OS-MTX, and HEK 293T, BEAS-2B, MCF10A, and GMO7575 cells (5×10^3) were seeded in each well of a 96-well plate. After incubating the cells overnight, various concentrations of the compounds (0.2-250 μM), were added and incubated for 72 h (total volume 200 μL). Stock solutions of the compounds were prepared as 5 or 10 mM solutions in DMSO or PBS and diluted using media. The final concentration of DMSO in each well was 0.5% and this amount was present in the untreated control as well. After 72 h, 20 μL of a 4 mg/mL solution of MTT in PBS was added to each well, and the plate was incubated for an additional 4 h. The DMEM/MTT or RPMI/MTT or MEGM/MTT mixture was aspirated and 200 μL of DMSO was added to dissolve the resulting purple formazan crystals. The absorbance of the solutions in each well was read at 550 nm. Absorbance values were normalized to (DMSO-containing) control wells and plotted as concentration of test compound versus % cell viability. IC_{50} values were interpolated from the resulting dose dependent curves. The reported IC_{50} values are the average of three independent experiments, each consisting of six replicates per concentration level (overall n = 18).

Sarcosphere Formation and Viability Assay. U2OS-MTX cells (5×10^3) were plated in ultralow-attachment 96-well plates (Corning) and incubated in DMEM/F12 medium supplemented with N2 (Invitrogen), human EGF (10 ng/mL), and human bFGF (10 ng/mL) for 10 days. Studies were also conducted in the presence of **2**, **3**, cisplatin, carboplatin, salinomycin, $Ga(NO_3)_3$, 5-methyl-1,10-phenanthroline, and 3,4,7,8-tetramethyl-1,10-phenanthroline (0-133 μM). Sarcospheres treated with **2**, **3**, cisplatin, carboplatin, salinomycin, $Ga(NO_3)_3$, 5-methyl-1,10-phenanthroline, and 3,4,7,8-tetramethyl-1,10-phenanthroline (at their respective IC_{20} values, 10 days) were imaged using an inverted microscope. The viability of the sarcospheres was determined by addition of a resazurin-based reagent, TOX8 (Sigma). After incubation for 16 h, the solutions were carefully transferred to a black 96-well plate (Corning), and the fluorescence of the solutions was read at 590 nm (λ_{ex} = 560 nm). Viable sarcospheres reduce the amount of the oxidized TOX8 form (blue) and concurrently increases the amount of the fluorescent TOX8 intermediate (red), indicating the degree of sarcosphere cytotoxicity caused by the test compound. Fluorescence values were normalized to DMSO-containing controls and plotted as concentration of test compound versus % sarcosphere viability. IC_{50} values were interpolated from the resulting dose dependent curves. The reported IC_{50} values are the average of two independent experiments, each consisting of three replicates per concentration level (overall n = 6).

Cellular Uptake. To measure the cellular uptake of **2**, **3**, and $Ga(NO_3)_3$ *ca.* 1 million U2OS cells were treated with **2**, **3**, and $Ga(NO_3)_3$ (0.25 μM) at 37 ºC for 24 h. After incubation, the media was removed and the cells were washed with PBS (2 mL × 3), and harvested. The number of cells was counted at this stage, using a haemocytometer. This mitigates any cell death induced by **2**, **3**, and $Ga(NO_3)_3$ at the administered concentration and experimental

cell loss. The cells were centrifuged to form pellets. The cellular pellets were dissolved in 65% HNO_3 (250 μL) overnight. The cellular pellets were also used to determine the gallium content in the nuclear fraction. The Thermo Scientific NE-PER Nuclear and Cytoplasmic Extraction Kit was used to extract and separate the nuclear fraction. The fractions were dissolved in 65% HNO_3 overnight (250 μL final volume). All samples were diluted 5-fold with water and analysed using inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer NexION 350D). Gallium levels are expressed as Ga (ppb) per million cells. Results are presented as the mean of five determinations for each data point.

Immunoblotting Analysis. U2OS cells (5×10^3 cells) were incubated with **3** (at various concentrations, 50-200 nM) for 72 h at 37 ^oC. Cells were washed with PBS, scraped into SDS-PAGE loading buffer (64 mM Tris-HCl (pH6.8)/ 9.6% glycerol/ 2%SDS/ 5% β-mercaptoethanol/ 0.01% Bromophenol Blue), and incubated at 95 ^oC for 10 min. Whole cell lysates were resolved by 4-20 % sodium dodecylsulphate polyacylamide gel electrophoresis (SDS-PAGE; 200 V for 25 min) followed by electro transfer to polyvinylidene difluoride membrane, PVDF (350 mA for 1 h). Membranes were blocked in 5% (w/v) non-fat milk in PBST (PBS/0.1% Tween 20) and incubated with the appropriate primary antibodies (Cell Signalling Technology). After incubation with horseradish peroxidase-conjugated secondary antibodies (Cell Signalling Technology), immune complexes were detected with the ECL detection reagent (Bio-Rad) and analysed using a chemiluminescence imager (Bio-Rad ChemiDoc Imaging System).

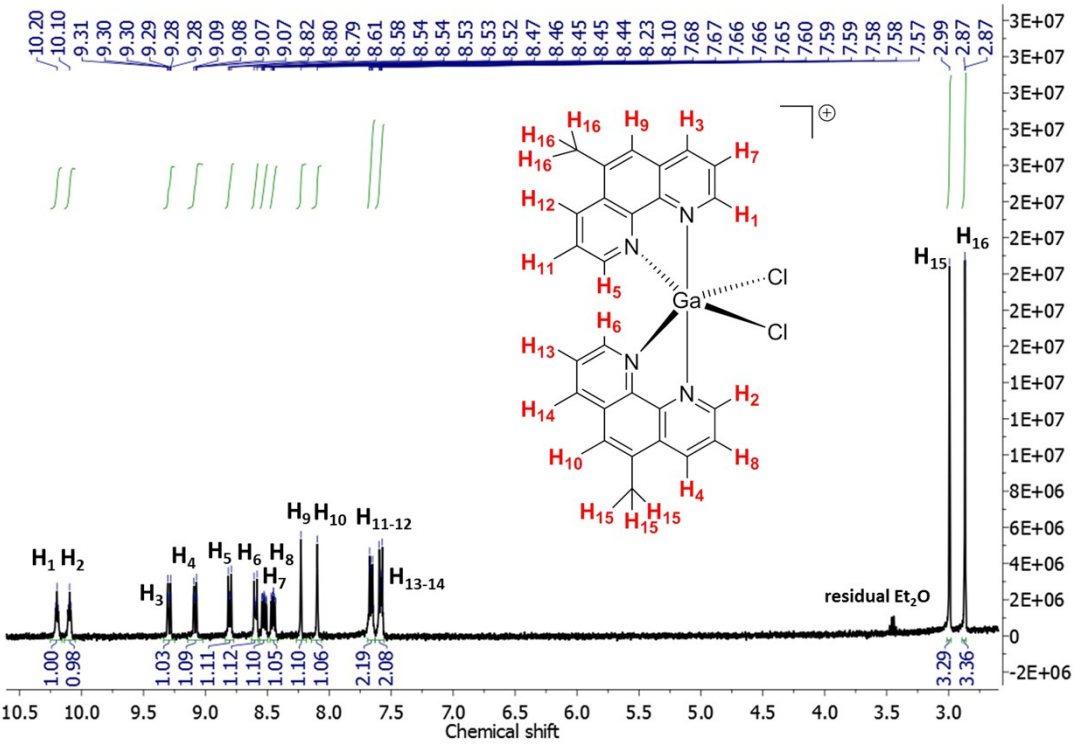

Fig. S1 ^{1}H NMR spectrum of **2** in CD_3CN.

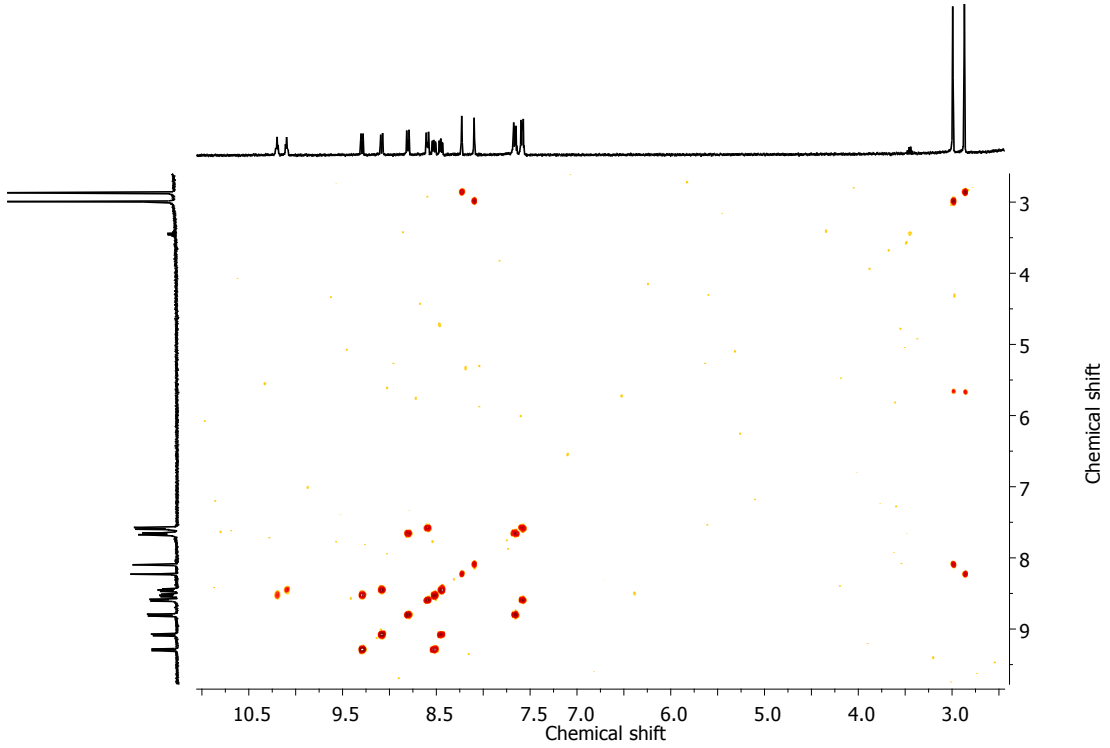

Fig. S2 ^{1}H-^{1}H COSY NMR spectrum of **2** in CD_3CN.

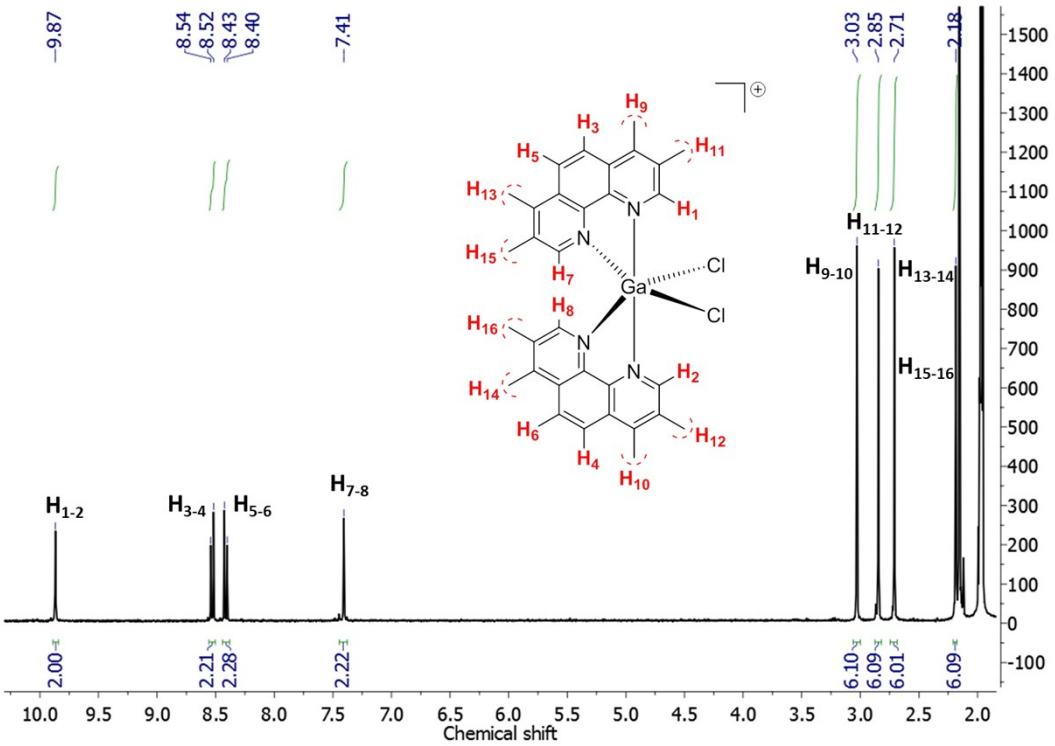

Fig. S3 ^{1}H NMR spectrum of **3** in CD_3CN.

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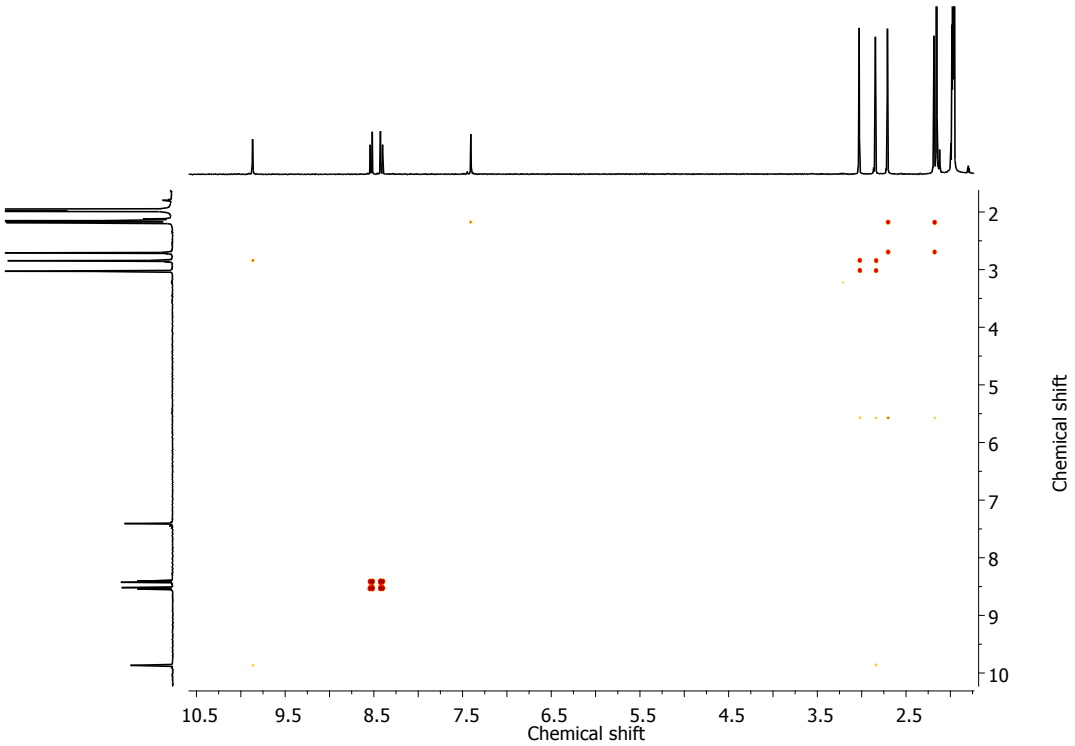


Fig. S4 ^{1}H-^{1}H COSY NMR spectrum of **3** in CD_3CN.

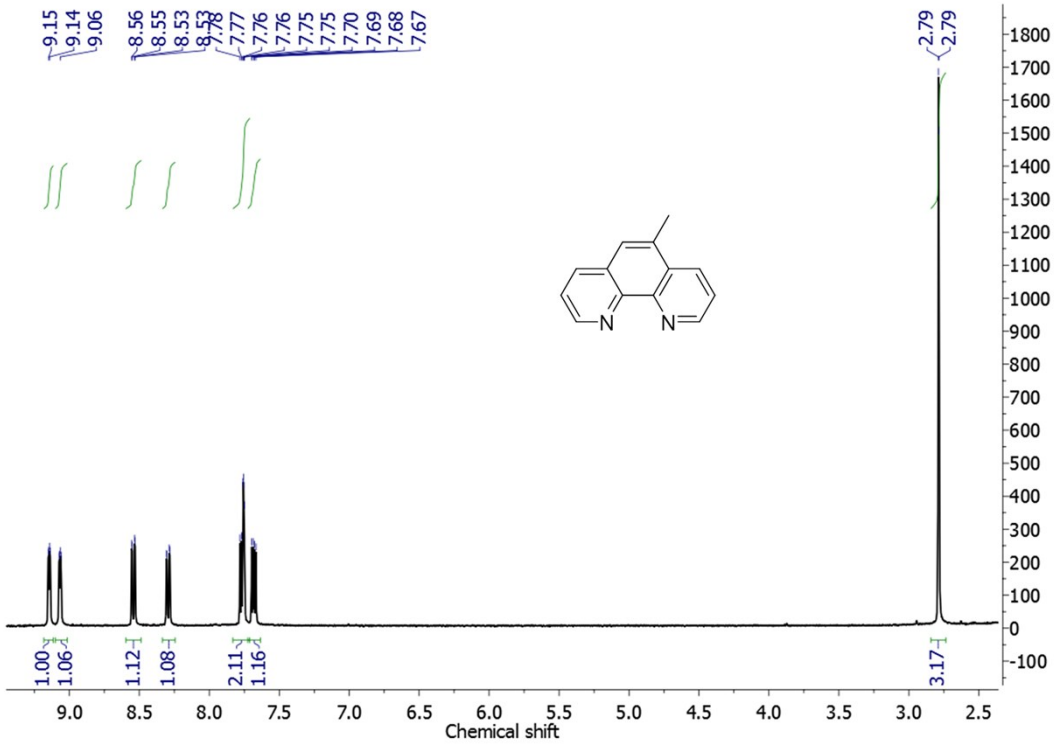


Fig. S5 ^{1}H NMR spectrum of 5-methyl-1,10-phenanthroline in CD_3CN.

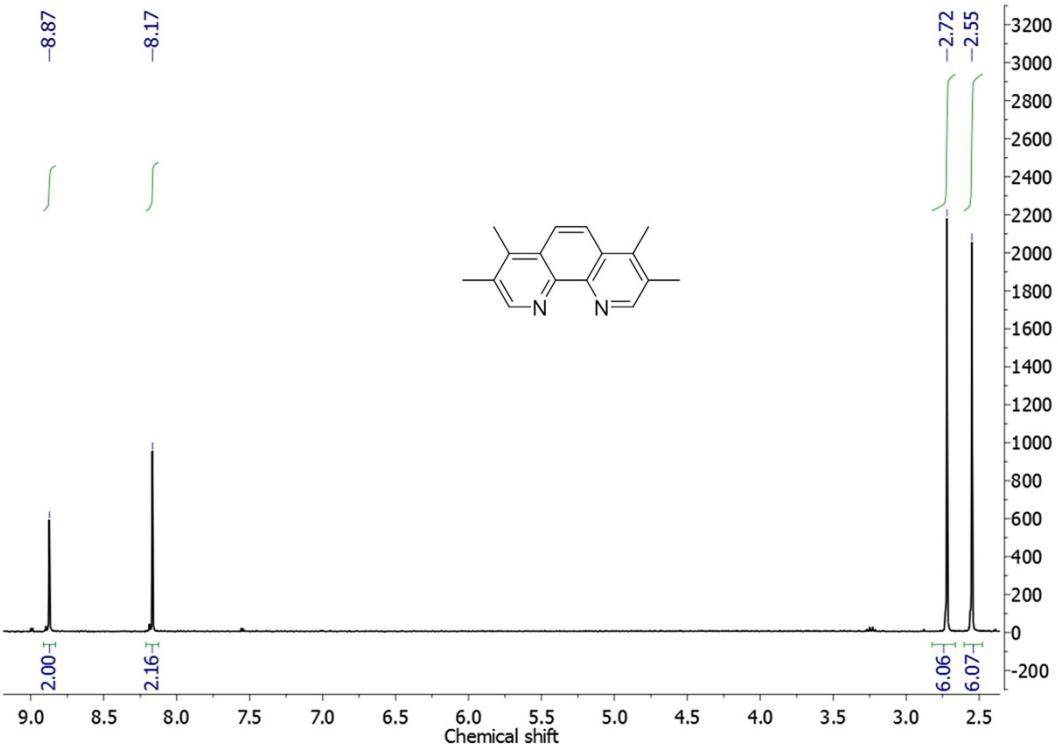


Fig. S6 ^{1}H NMR spectrum of 3,4,7,8-tetramethyl-1,10-phenanthroline in CD_3CN.

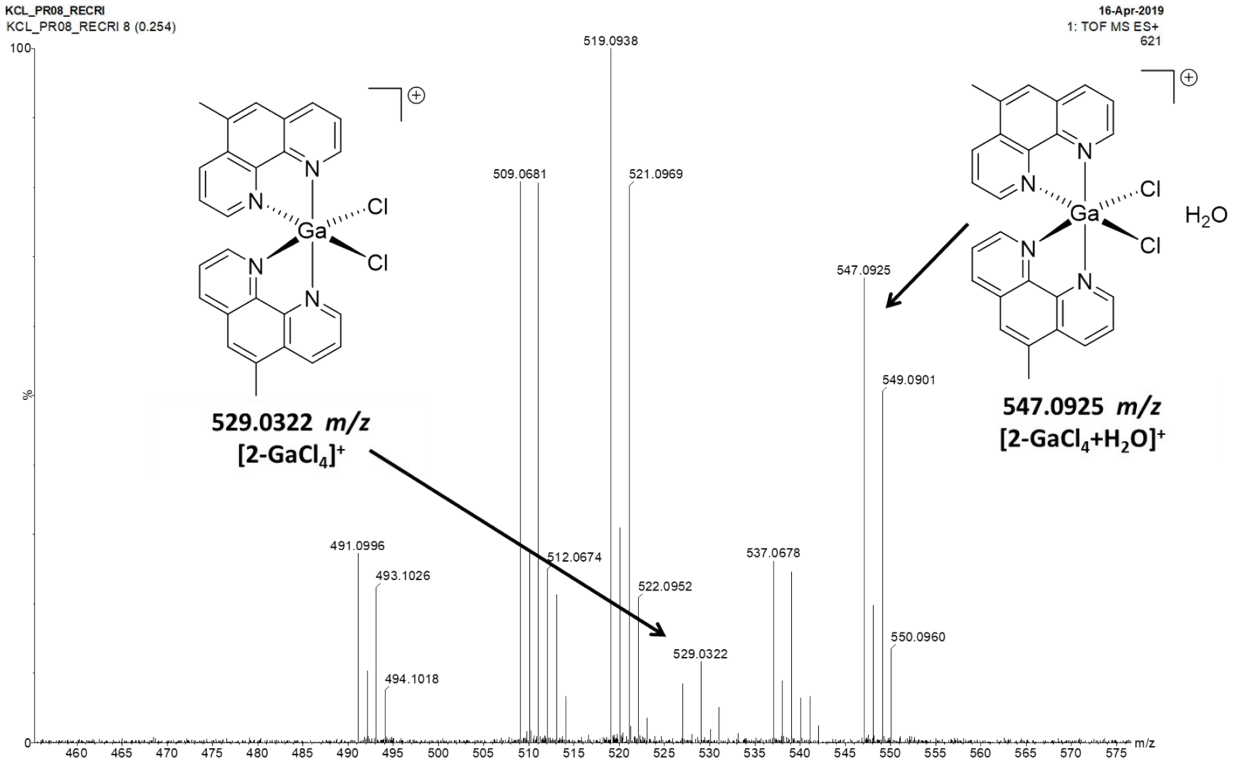


Fig. S7 High resolution ESI mass spectrum (positive mode) of **2**.

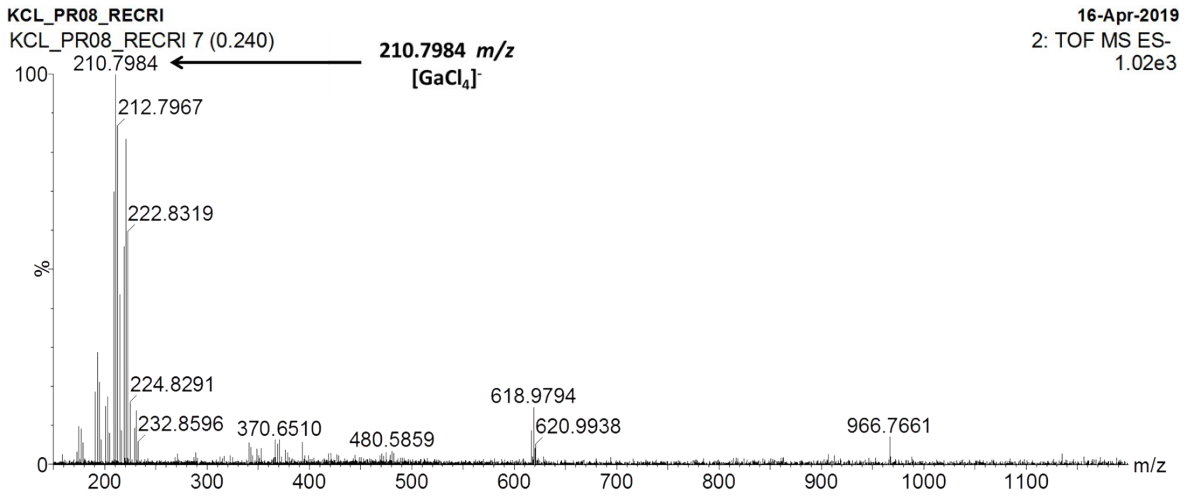


Fig. S8 High resolution ESI mass spectrum (negative mode) of **2**.

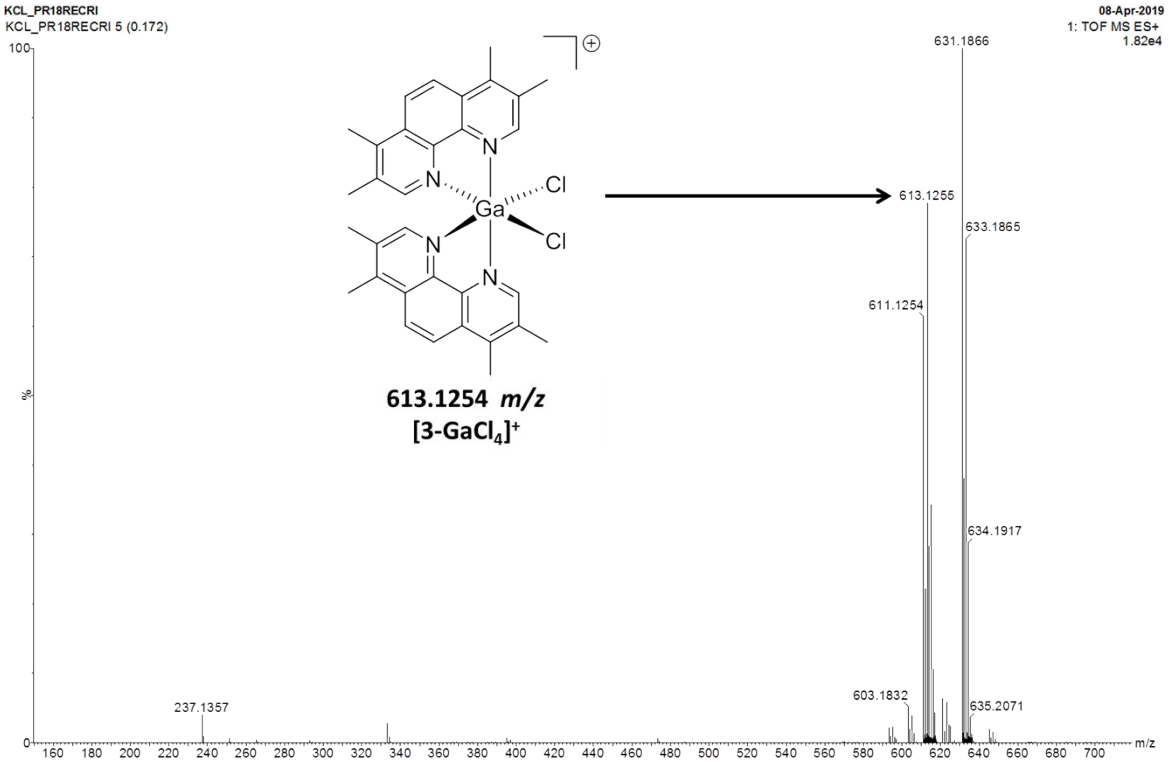


Fig. S9 High resolution ESI mass spectrum (positive mode) of **3**.

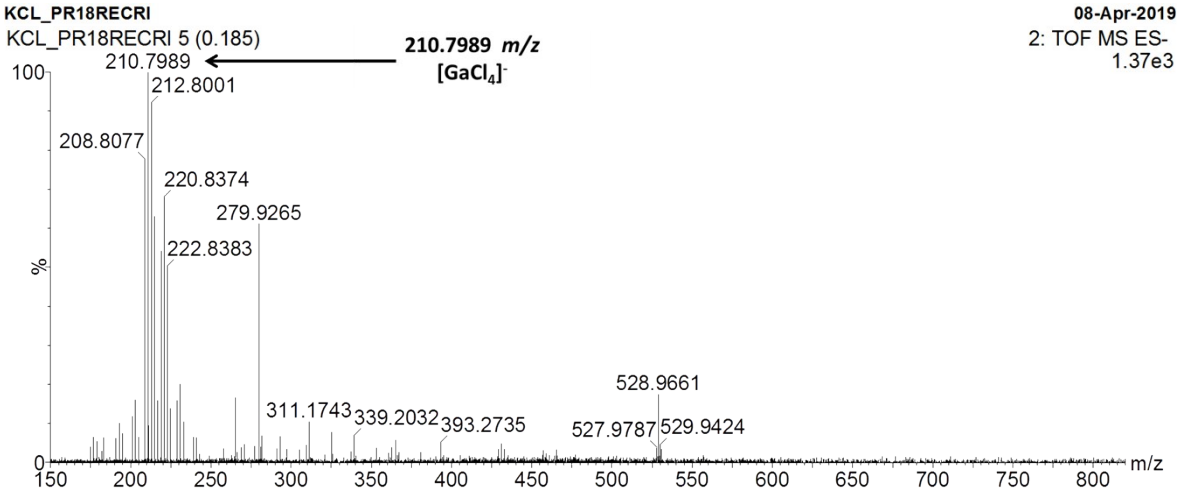


Fig. S10 High resolution ESI mass spectrum (negative mode) of **3**.

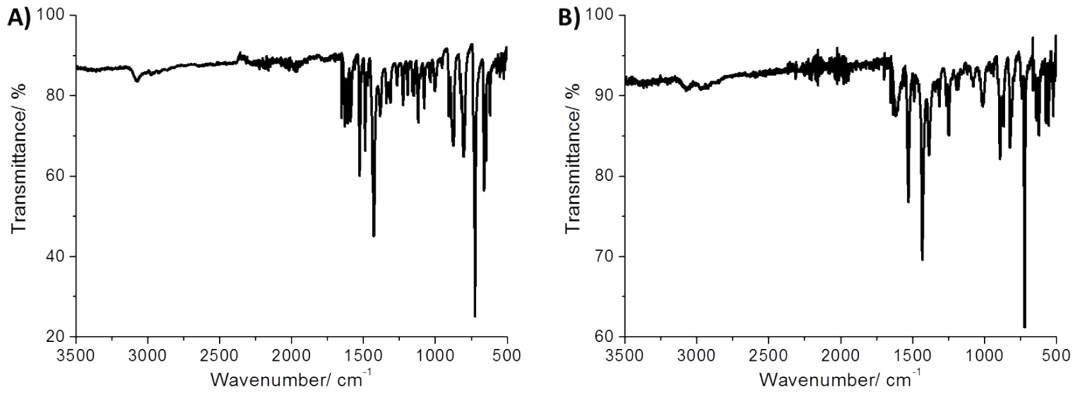


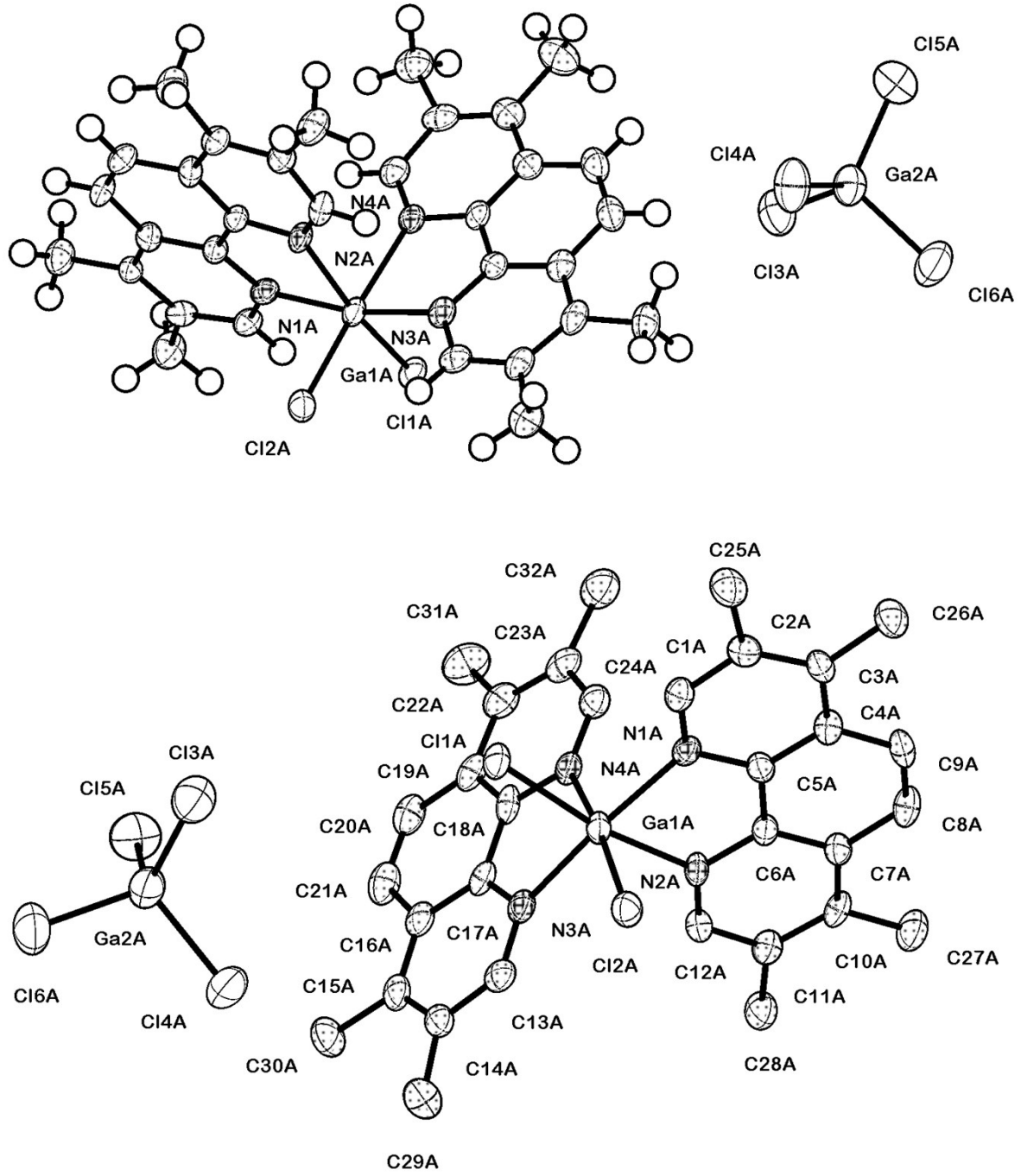
Fig. S11 IR spectrum of (A) **2** and (B) **3** in the solid form.

Table S1. Crystallographic data of **3**.

formula	$C_{32}H_{32}Cl_2GaN_4$, Cl_4Ga, $2CH_3CN$
Fw	906.86
crystal system	triclinic
space group	*P-1*
a, Å	10.884(3)
b, Å	16.710(5)
c, Å	22.133(6)
α, deg.	76.662(5)
β, deg.	86.906(5)
γ, deg.	86.074(5)
V, Å^3	3904.6(19)
Z	4
D_{calcd}, g/cm^3	1.403
Reflections collected	30646
Reflections independent (R_{int})	15153, 0.080
Goodness-of-fit on F^2	0.78
R indices (all data)	R1 = 0.0604, wR2 = 0.1419

$R_1 = [\Sigma \, || F_0| - |F_c||/\Sigma|F_0|]$, $wR_2 = [\Sigma w(F_0^2 - F_c^2)^2/\Sigma w(F_0^2)^2]^{1/2}$

Table S2. Selected bond lengths (Å) and angles (°) for **3**.



Ga(1A)-N(4A) 2.165(4)	Ga(1)-N(1) 2.087(5)
Ga(1A)-N(1A) 2.057(4)	Ga(1)-N(2) 2.164(4)
Ga(1A)-N(2A) 2.125(4)	Ga(1)-N(3) 2.072(5)
Ga(1A)-N(3A) 2.082(4)	Ga(1)-N(4) 2.107(5)
Ga(1A)-Cl(1A) 2.2720(15)	Ga(1)-Cl(1) 2.2765(16)
Ga(1A)-Cl(2A) 2.2762(16)	Ga(1)-Cl(2) 2.2738(15)
Ga(2A)-Cl(3A) 2.172(2)	Ga(2B)-Cl(6B) 2.165(3)
Ga(2A)-Cl(4A) 2.178(2)	Ga(2B)-Cl(5B) 2.156(6)

Ga(2A)-Cl(5A) 2.164(2)	Ga(2B)-Cl(3B) 2.159(4)
Ga(2A)-Cl(6A) 2.1673(19)	Ga(2B)-Cl(4B) 2.159(4)
Ga(2')-Cl(5') 2.131(11)	Ga(2')-Cl(3') 2.109(12)
Ga(2')-Cl(4') 2.156(12)	Ga(2')-Cl(6') 2.16(2)
Cl(1A)-Ga(1A)-N(3A) 92.50(12)	N(1)-Ga(1)-N(4) 93.59(18)
Cl(1A)-Ga(1A)-N(4A) 86.82(13)	Cl(1)-Ga(1)-Cl(2) 96.99(5)
Cl(2A)-Ga(1A)-N(1A) 92.40(11)	N(2)-Ga(1)-N(3) 89.45(17)
Cl(2A)-Ga(1A)-N(2A) 92.39(11)	Cl(2)-Ga(1)-N(4) 171.66(12)
Cl(2A)-Ga(1A)-N(3A) 93.64(12)	Cl(2)-Ga(1)-N(3) 95.11(13)
Cl(2A)-Ga(1A)-N(4A) 170.24(14)	N(1)-Ga(1)-N(3) 165.47(18)
Cl(1A)-Ga(1A)-Cl(2A) 98.35(5)	Cl(2)-Ga(1)-N(2) 88.18(13)
N(2A)-Ga(1A)-N(4A) 83.27(16)	Cl(2)-Ga(1)-N(1) 91.25(13)
N(3A)-Ga(1A)-N(4A) 77.80(17)	Cl(1)-Ga(1)-N3 95.68(12)
Cl(1A)-Ga(1A)-N(1A) 95.89(12)	N(3)-Ga(1)-N(4) 78.67(18)
Cl(1A)-Ga(1A)-N(2A) 168.22(11)	N(1)-Ga(1)-N(2) 77.69(18)
N(1A)-Ga(1A)-N(2A) 78.75(15)	Cl(1)-Ga(1)-N(4) 89.20(12)
N(2A)-Ga(1A)-N(3A) 91.63(15)	Cl(1)-Ga(1)-N2 172.35(14)
N(1A)-Ga(1A)-N(4A) 95.31(16)	Cl(1)-Ga(1)-N(1) 96.50(13)
N(1A)-Ga(1A)-N(3A) 168.85(16)	N(2)-Ga(1)-N(4) 86.24(17)
Cl(5A)-Ga(2A)-Cl(6A) 109.71(7)	Cl(4B)-Ga(2B)-Cl(6B) 109.39(15)
Cl(3A)-Ga(2A)-Cl(6A) 110.91(7)	Cl(5B)-Ga(2B)-Cl(6B) 109.2(2)
Cl(3A)-Ga(2A)-Cl(4A) 109.00(7)	Cl(3B)-Ga(2B)-Cl(5B) 110.7(3)
Cl(3A)-Ga(2A)-Cl(5A) 108.27(7)	Cl(3B)-Ga(2B)-Cl(4B) 110.29(18)
Cl(4A)-Ga(2A)-Cl(5A) 111.89(7)	Cl(4B)-Ga(2B)-Cl(5B) 109.3(2)
Cl(4A)-Ga(2A)-Cl(6A) 107.08(7)	Cl(3B)-Ga(2B)-Cl(6B) 107.93(16)
Cl(3')-Ga(2')-Cl(6') 109.2(7)	Cl(5')-Ga(2')-Cl(6') 106.0(8)
Cl(4')-Ga(2')-Cl(5') 108.5(6)	Cl(3')-Ga(2')-Cl(4') 108.0(5)
Cl(4')-Ga(2')-Cl(6') 112.4(7)	Cl(3')-Ga(2')-Cl(5') 112.8(6)

Table S3. Experimentally determined LogP values for **1-3**.

Gallium complex	LogP
1	-0.47 ± 0.04
2	-0.16 ± 0.05
3	-0.39 ± 0.09

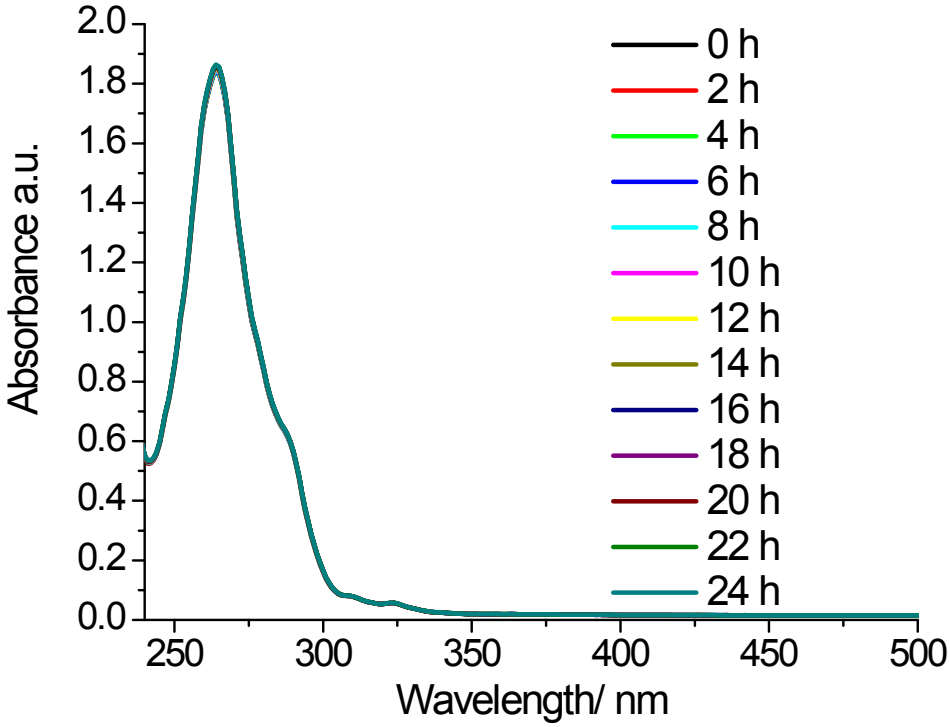


Fig. S12 UV-Vis spectrum of **1** (25 μM) in PBS:DMSO (200:1) over the course of 24 h at 37 ^{o}C.

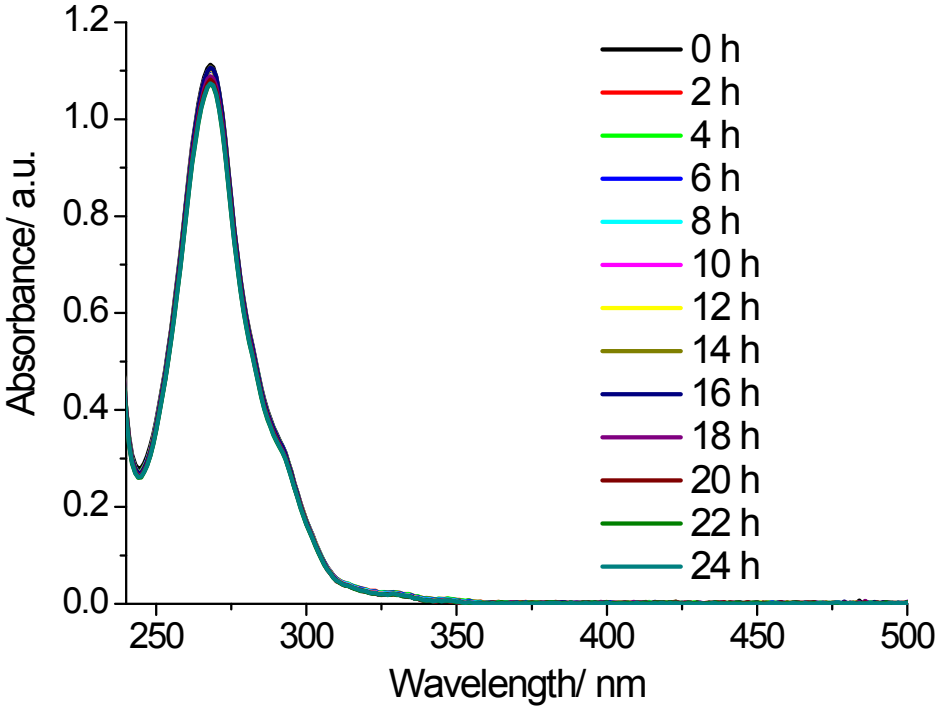


Fig. S13 UV-Vis spectrum of **2** (25 μM) in PBS:DMSO (200:1) over the course of 24 h at 37 ^{o}C.

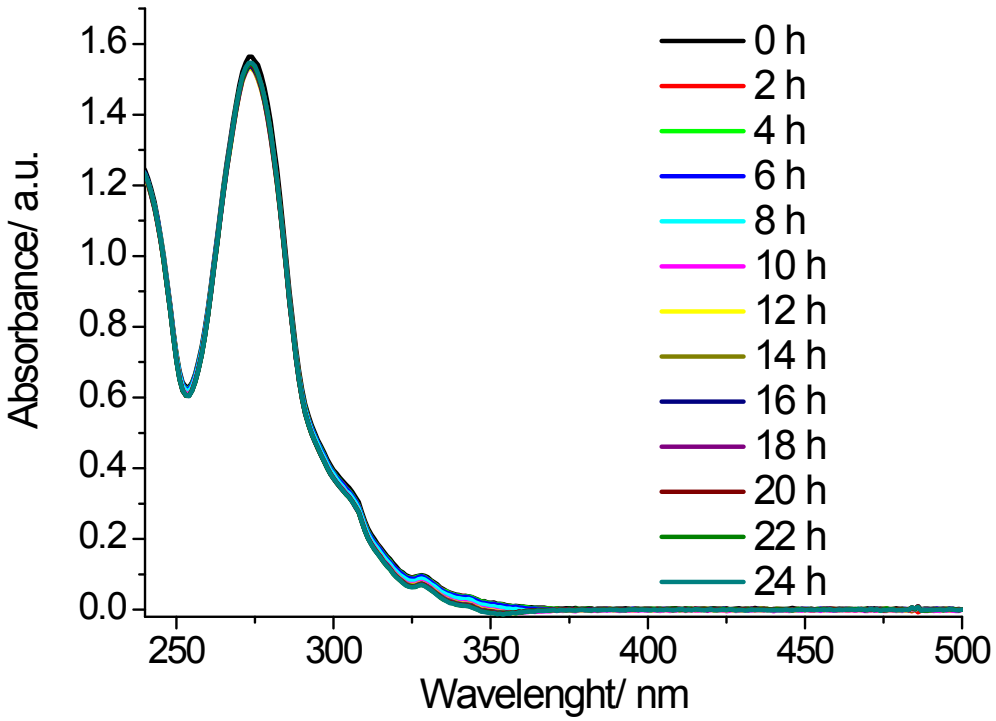


Fig. S14 UV-Vis spectrum of **3** (25 μM) in PBS:DMSO (200:1) over the course of 24 h at 37 ^{o}C.

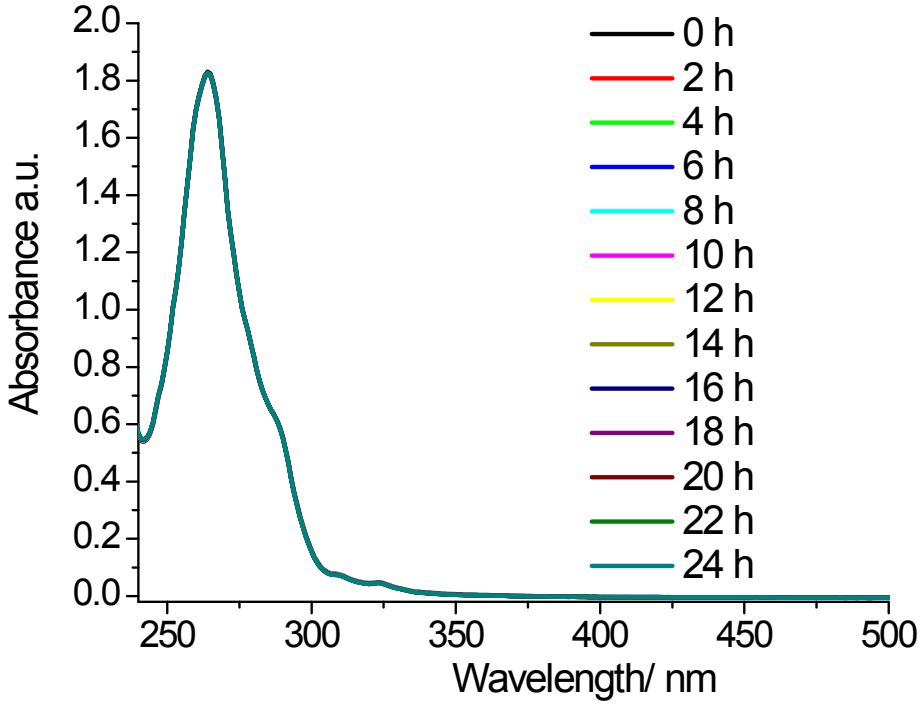


Fig. S15 UV-Vis spectrum of **1** (25 μM) in the presence of ascorbic acid (250 μM) in PBS:DMSO (200:1) over the course of 24 h at 37 ^{o}C.

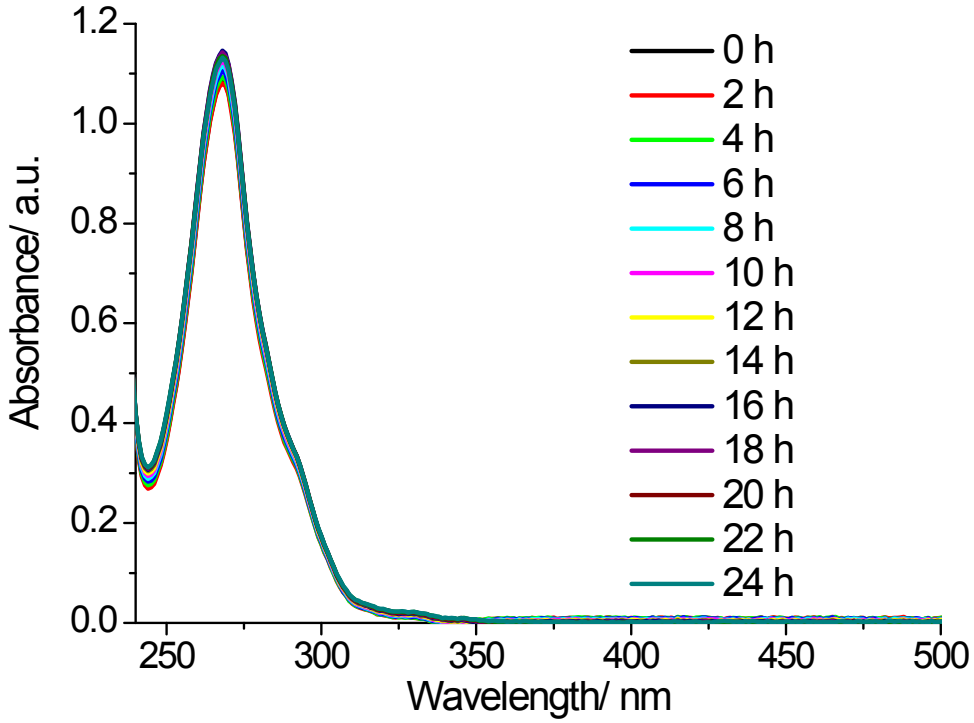


Fig. S16 UV-Vis spectrum of **2** (25 μM) in the presence of ascorbic acid (250 μM) in PBS:DMSO (200:1) over the course of 24 h at 37 °C.

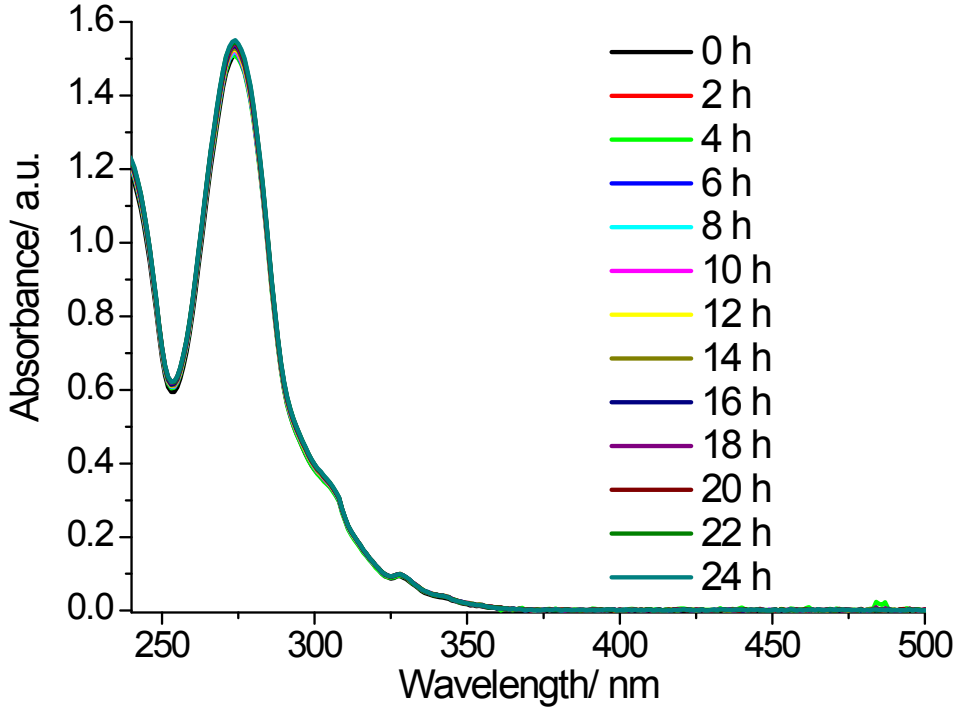


Fig. S17 UV-Vis spectrum of **3** (25 μM) in the presence of ascorbic acid (250 μM) in PBS:DMSO (200:1) over the course of 24 h at 37 °C.

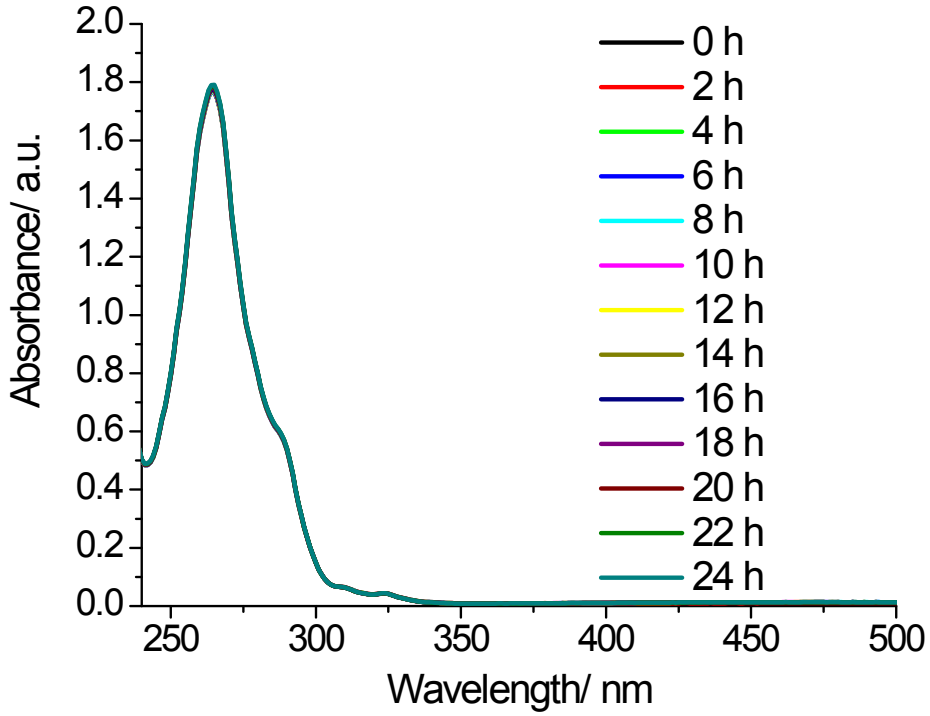


Fig. S18 UV-Vis spectrum of **1** (25 μM) in cell media:DMSO (200:1) over the course of 24 h at 37 °C.

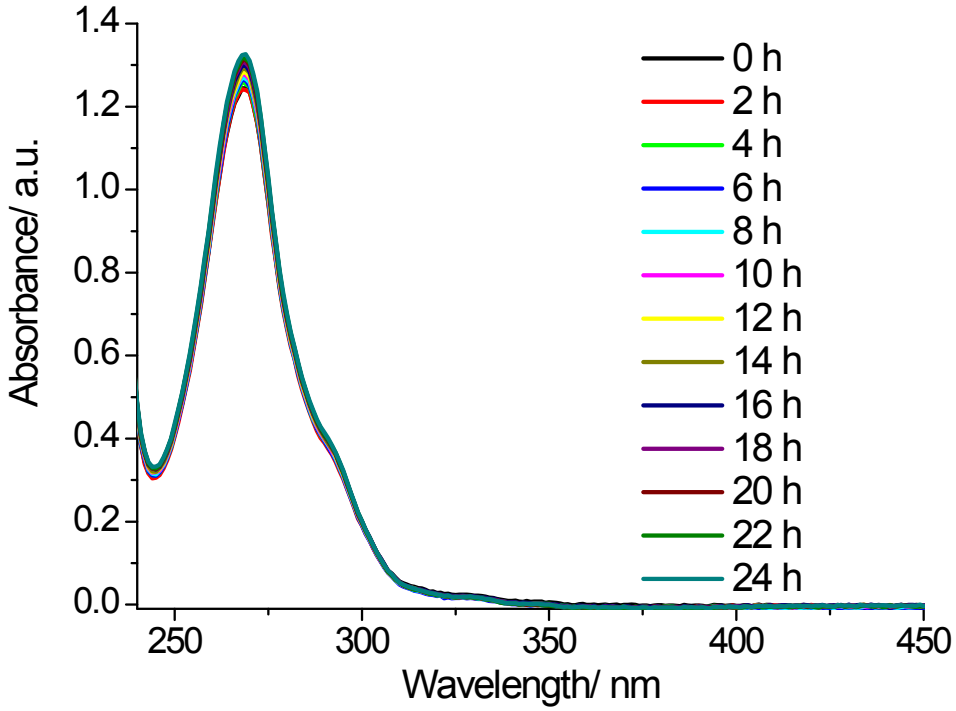


Fig. S19 UV-Vis spectrum of **2** (25 μM) in cell media:DMSO (200:1) over the course of 24 h at 37 °C.

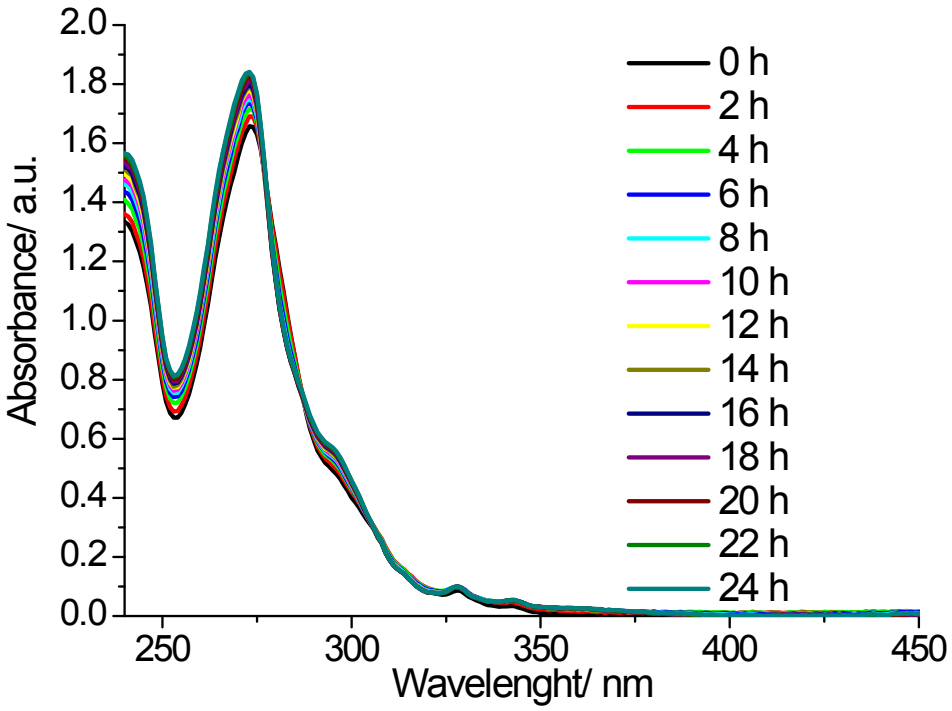


Fig. S20 UV-Vis spectrum of **3** (25 μM) in cell media:DMSO (200:1) over the course of 24 h at 37 ºC.

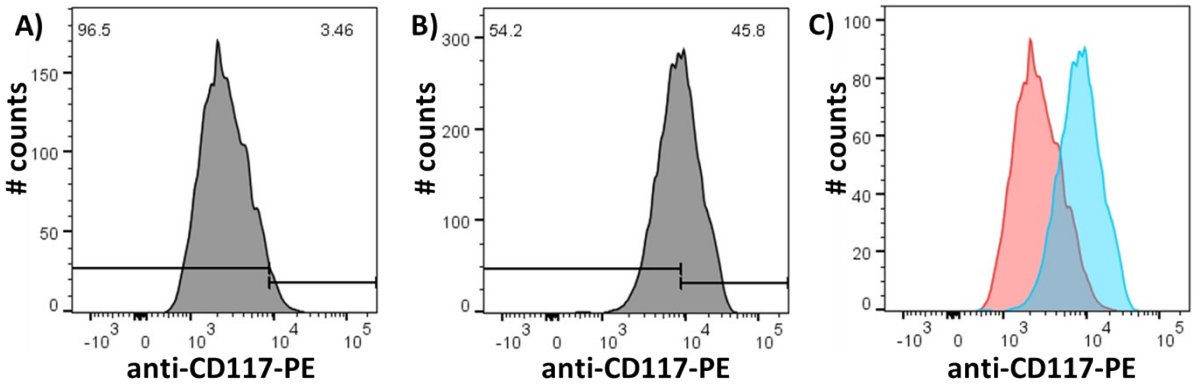


Fig. S21 Representative histograms displaying the PE fluorescence emitted by anti-CD117-PE antibody (10 μg/mL) stained U2OS (A, and red histogram in C) and U2OS-MTX (B, and blue histogram in C) cells. In this example, U2OS cells contain ~3.5% $CD117^{high}$ cells and U2OS-MTX contain ~45.8% $CD117^{high}$ cells.

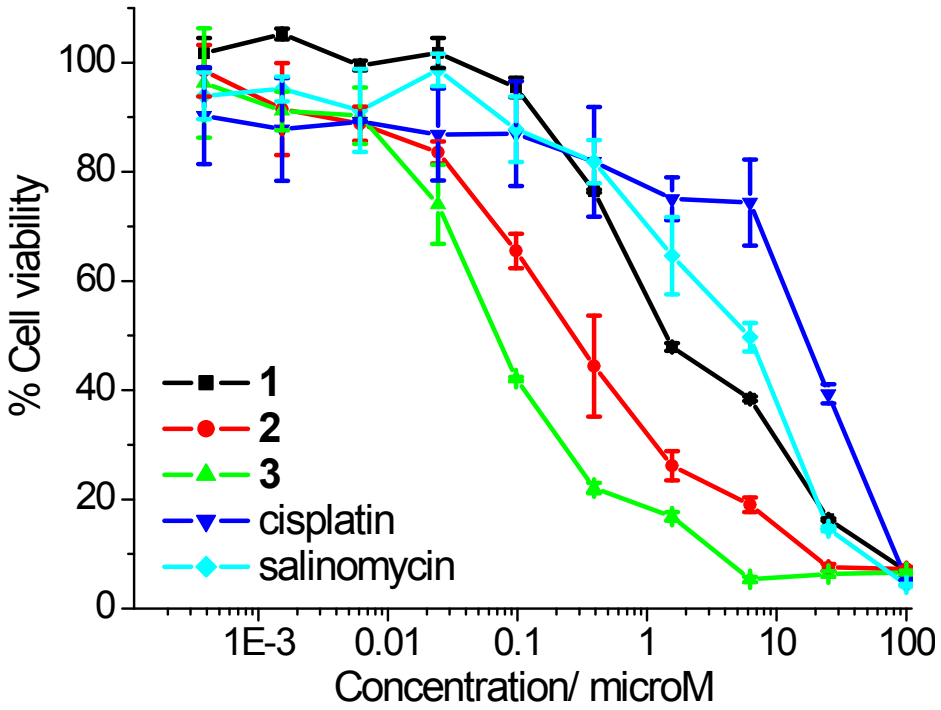


Fig. S22 Representative dose-response curves for the treatment of U2OS cells with **1**-**3**, cisplatin, or salinomycin after 72 h incubation.

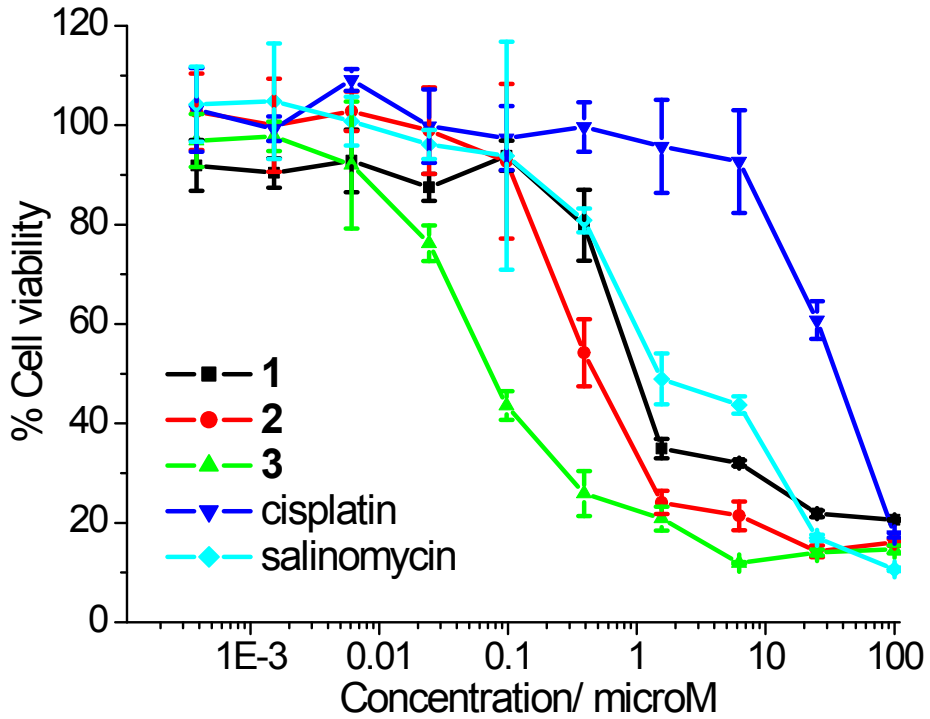


Fig. S23 Representative dose-response curves for the treatment of U2OS-MTX cells with **1**-**3**, cisplatin, or salinomycin after 72 h incubation.

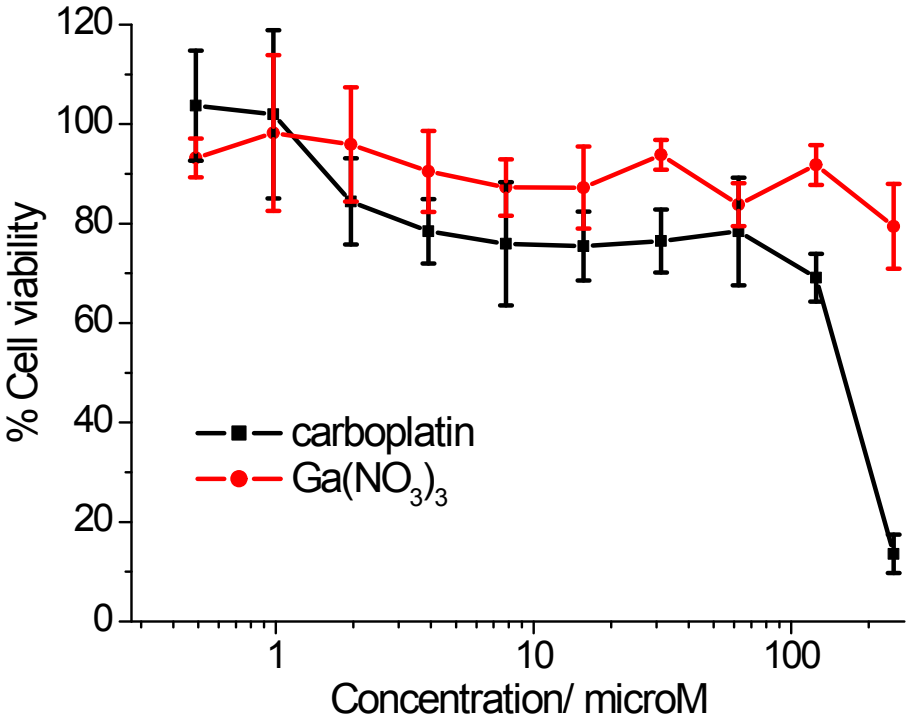


Fig. S24 Representative dose-response curves for the treatment of U2OS cells with carboplatin or $Ga(NO_3)_3$ after 72 h incubation.

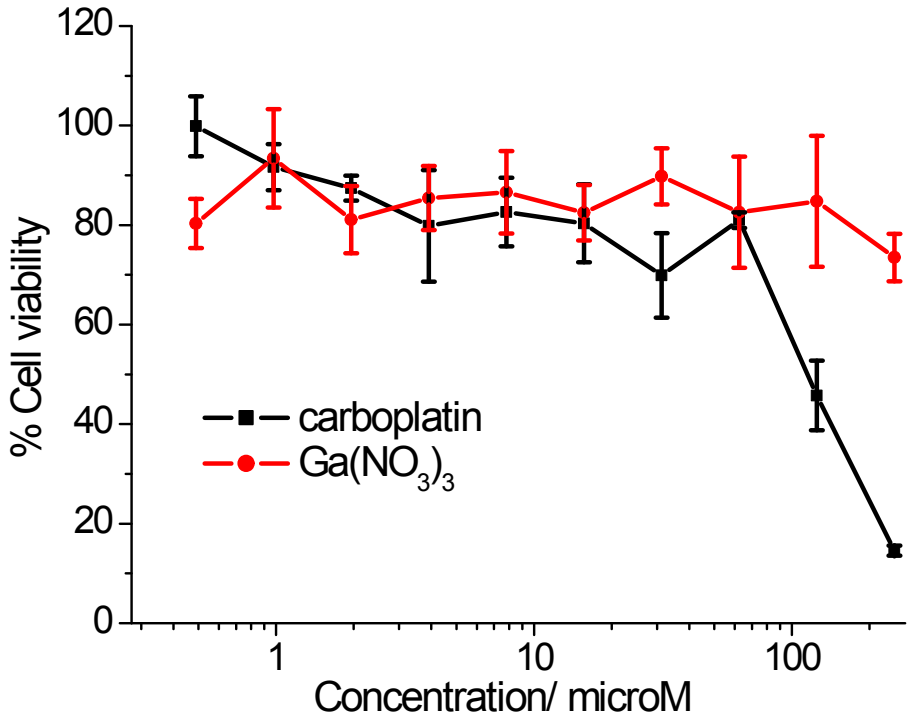


Fig. S25 Representative dose-response curves for the treatment of U2OS-MTX cells with carboplatin or $Ga(NO_3)_3$ after 72 h incubation.

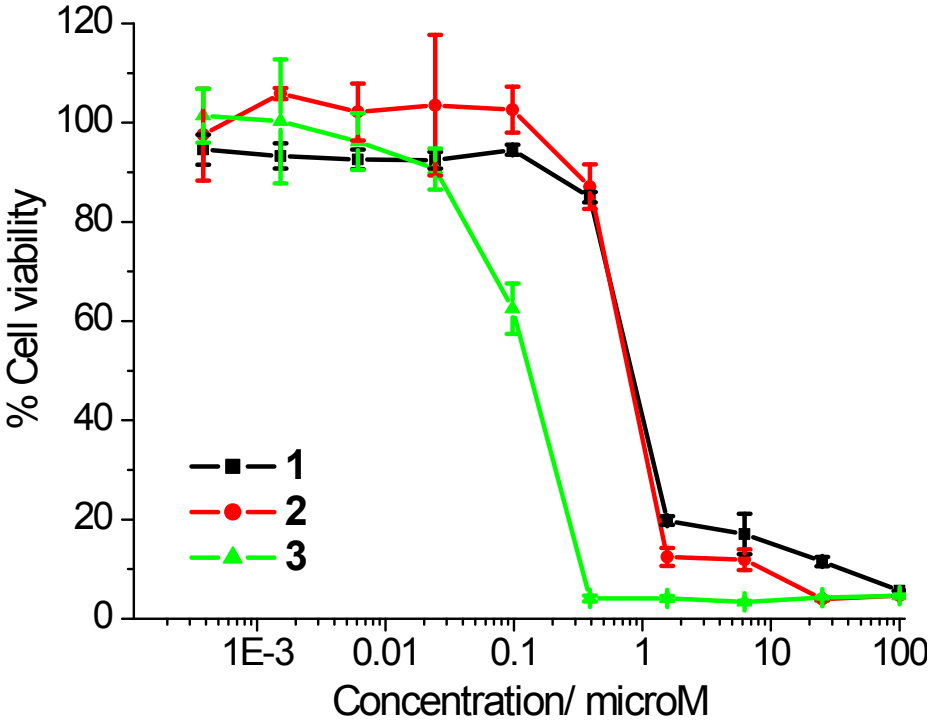


Fig. S26 Representative dose-response curves for the treatment of HEK 293T cells with **1**-**3** after 72 h incubation.

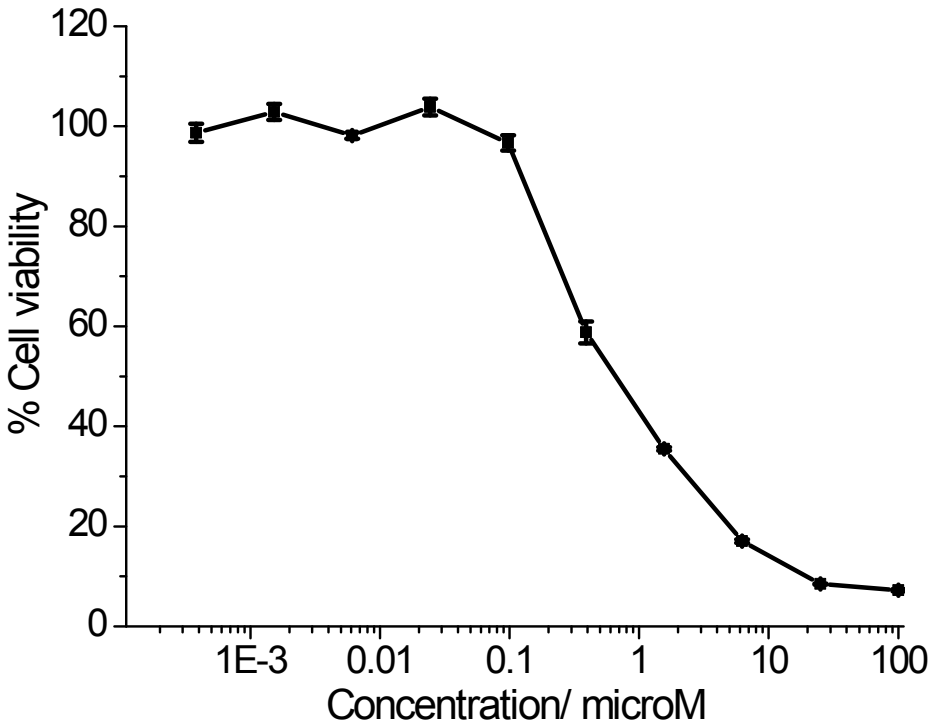


Fig. S27 Representative dose-response curves for the treatment of MCF10A cells with **3** after 72 h incubation.

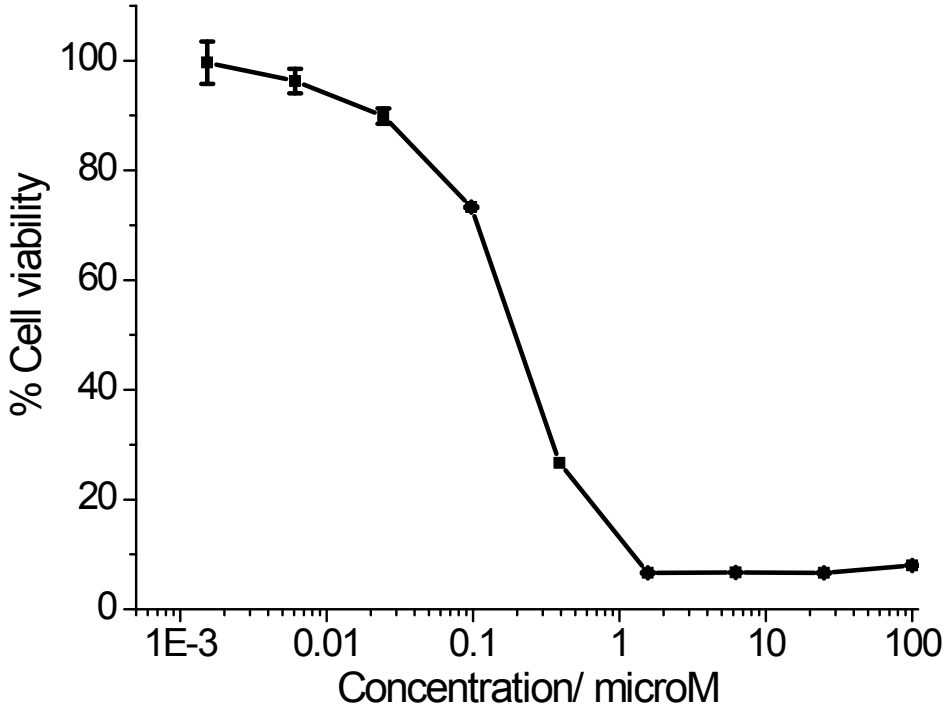


Fig. S28 Representative dose-response curves for the treatment of BEAS-2B cells with **3** after 72 h incubation.

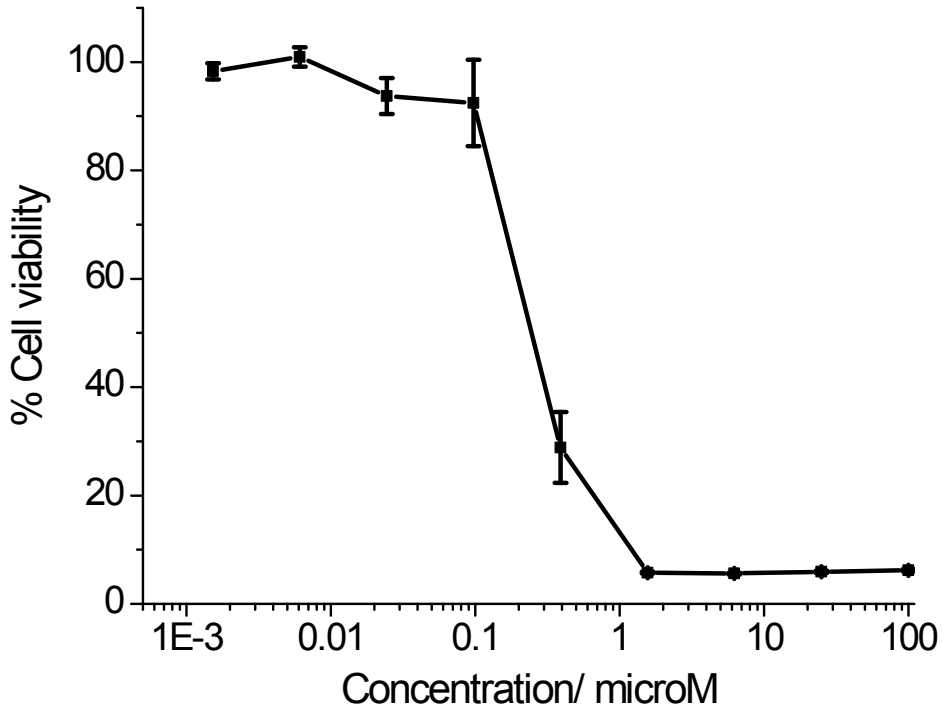


Fig. S29 Representative dose-response curves for the treatment of GMO7575 cells with **3** after 72 h incubation.

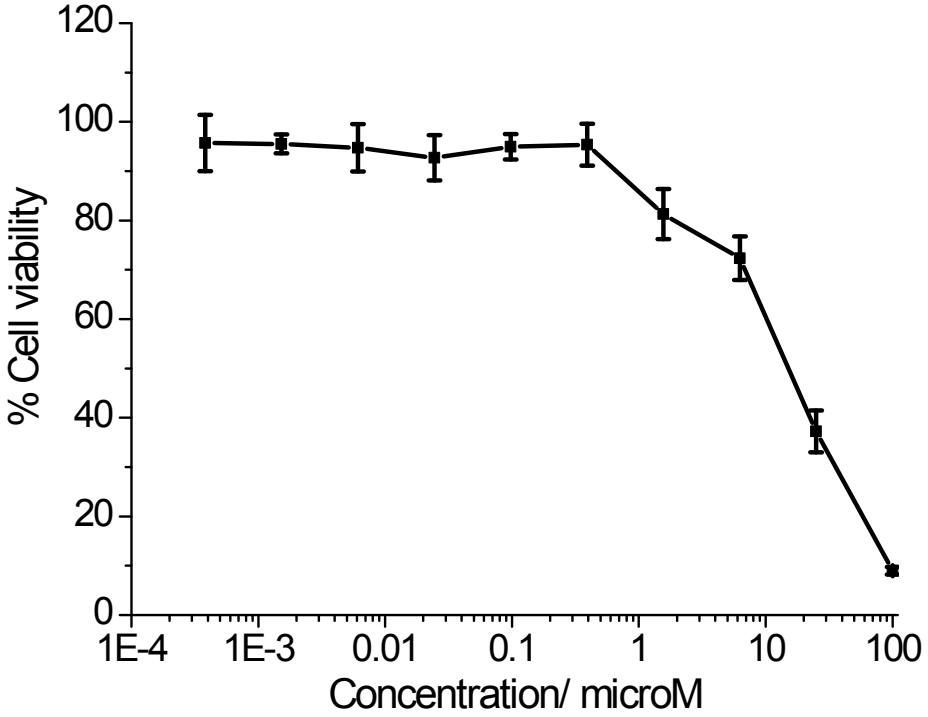


Fig. S30 Representative dose-response curves for the treatment of HEK 293T cells with cisplatin after 72 h incubation.

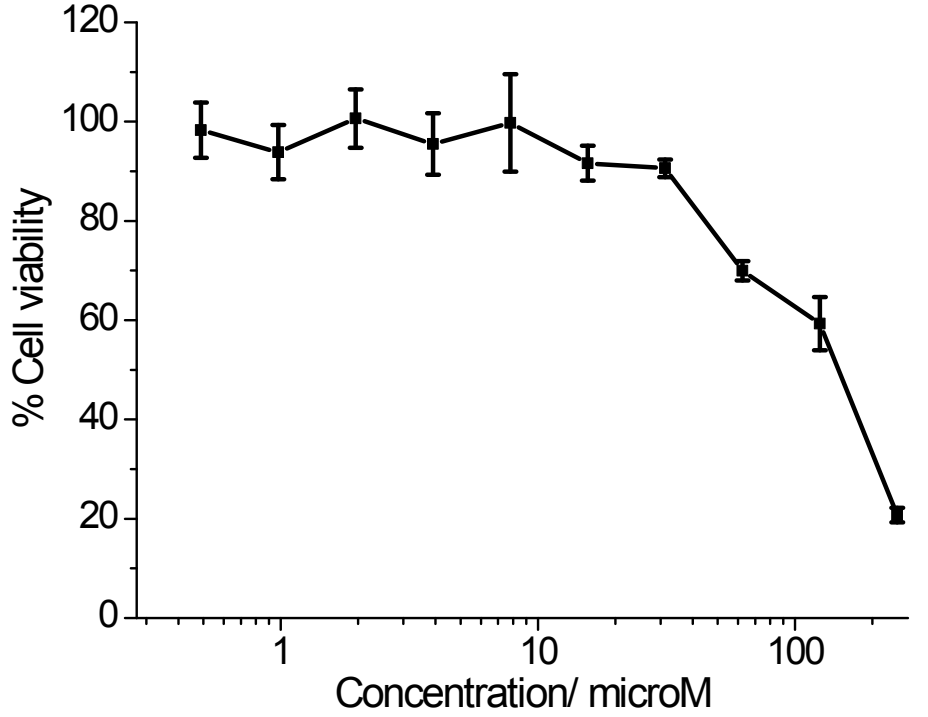


Fig. S31 Representative dose-response curves for the treatment of HEK 293T cells with carboplatin after 72 h incubation.

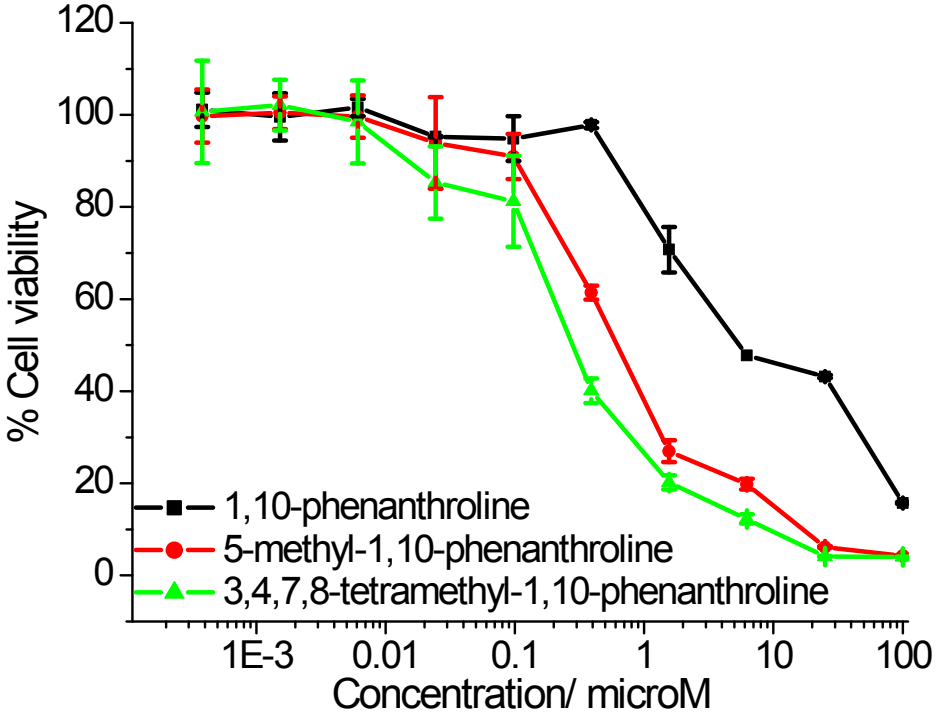


Fig. S32 Representative dose-response curves for the treatment of U2OS cells with 1,10-phenantroline, 5-methyl-1,10-phenanthroline, or 3,4,7,8-tetramethyl-1,10-phenanthroline after 72 h incubation.

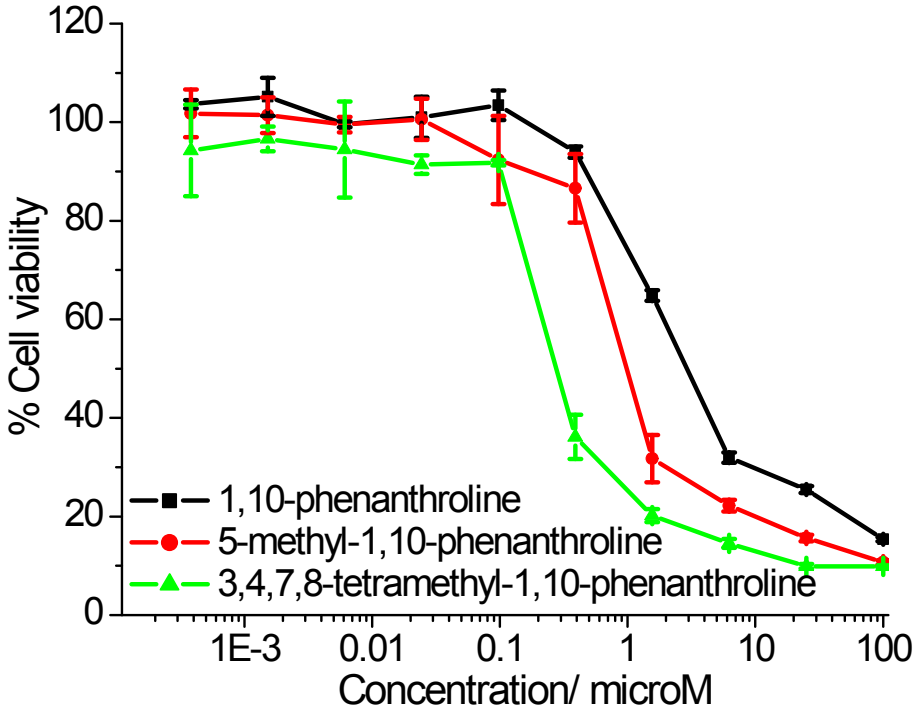


Fig. S33 Representative dose-response curves for the treatment of U2OS-MTX cells with 1,10-phenantroline, 5-methyl-1,10-phenanthroline, or 3,4,7,8-tetramethyl-1,10-phenanthroline after 72 h incubation.

Table S4. IC_{50} values of 1,10-phenanthroline, 5-methyl-1,10-phenanthroline, and 3,4,7,8-tetramethyl-1,10-phenanthroline against U2OS and U2OS-MTX cells, and U2OS-MTX sarcospheres after 3 or 10 days incubation (mean of three independent experiments ± SD). n.d. not determined.

Polypyridyl ligand	IC_{50} value/ μM against U2OS cells	IC_{50} value/ μM against U2OS-MTX cells	IC_{50} value/ μM against U2OS-MTX sarcospheres
1,10-phenanthroline	5.49 ± 0.31	2.92 ± 0.08	n.d.
5-methyl-1,10-phenanthroline	0.62 ± 0.01	0.98 ± 0.02	5.59 ± 0.03
3,4,7,8-tetramethyl-1,10-phenanthroline	0.28 ± 0.01	0.28 ± 0.03	5.37 ± 0.02

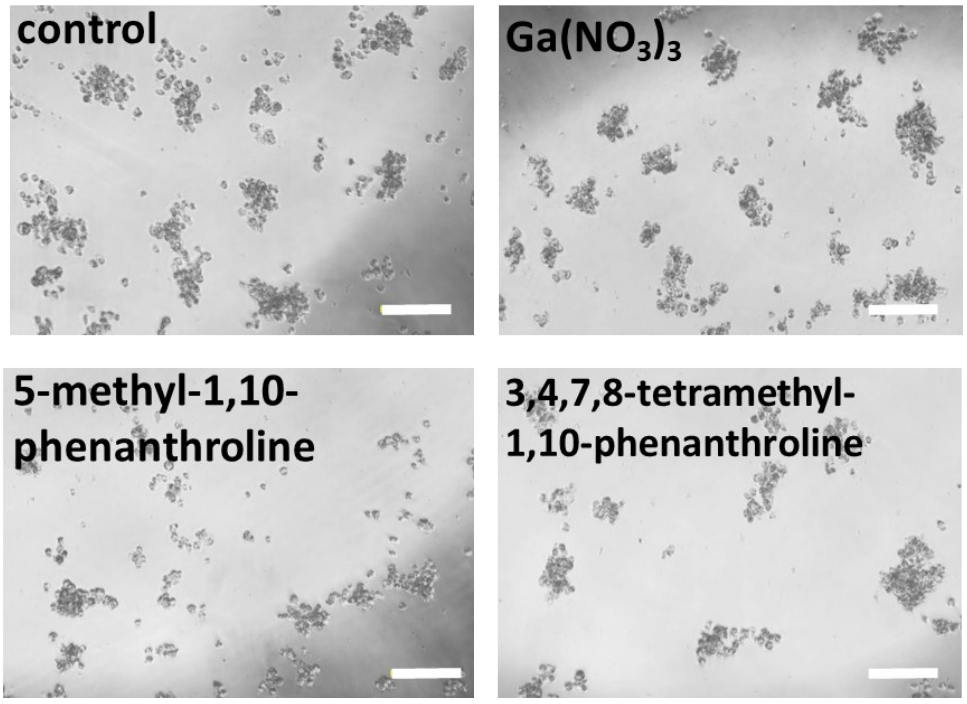


Fig. S34 Representative bright-field images (× 10) of U2OS-MTX sarcospheres in the absence and presence of $Ga(NO_3)_3$, 5-methyl-1,10-phenanthroline, or 3,4,7,8-tetramethyl-1,10-phenanthroline at its IC_{20} value (10 days incubation). Scale bar = 100 μm.

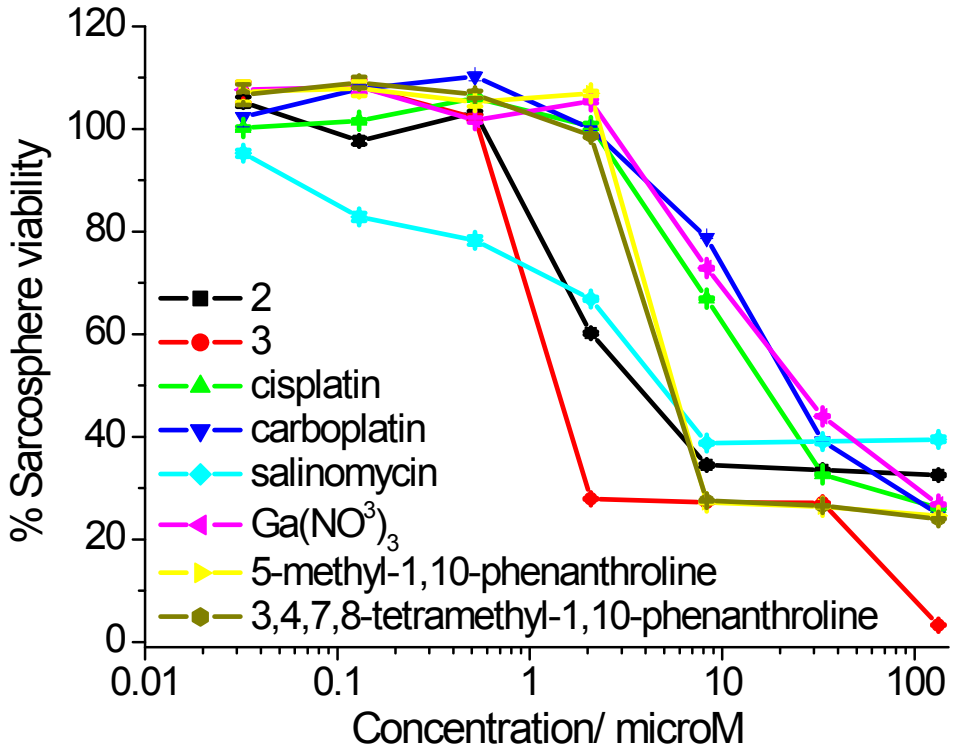


Fig. S35 Representative dose-response curves for the treatment of U2OS-MTX sarcospheres with **2**, **3**, cisplatin, carboplatin, salinomycin, $Ga(NO_3)_3$, 5-methyl-1,10-phenanthroline, or 3,4,7,8-tetramethyl-1,10-phenanthroline after 10 days incubation.

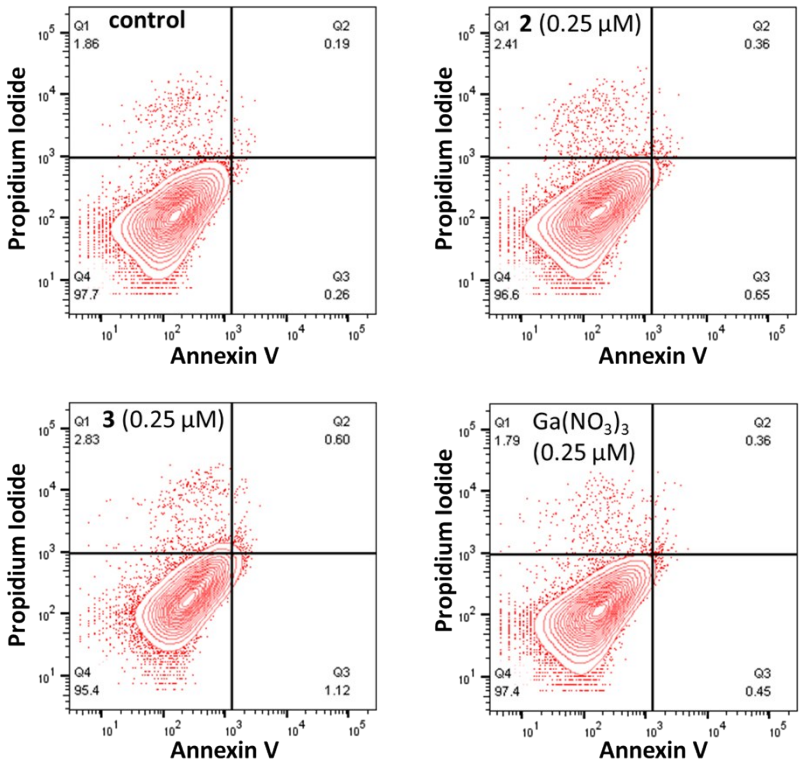


Fig. S36 FITC Annexin V/PI binding assay of untreated U2OS cells and U2OS cells treated with **2**, **3**, or $Ga(NO_3)_3$ (0.25 µM for 24 h).

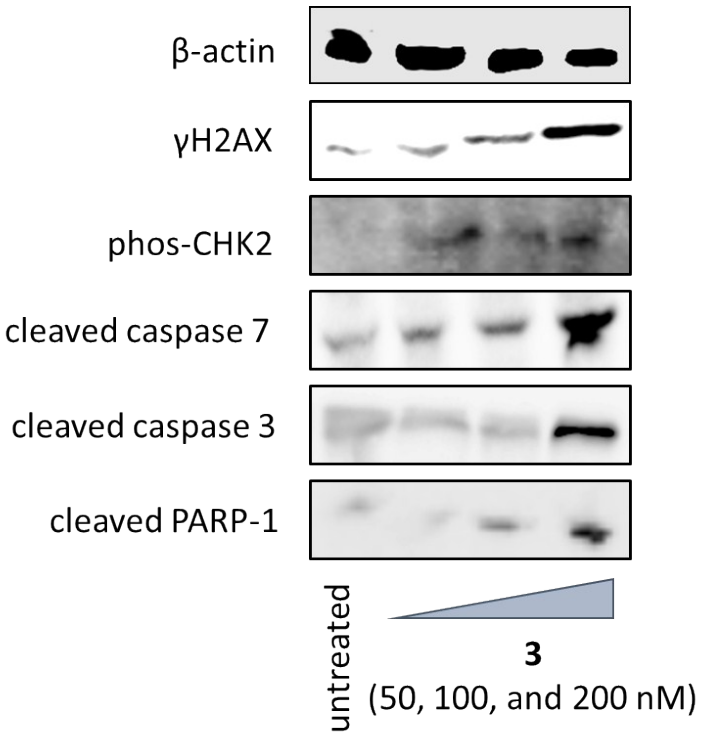


Fig. S37 Immunoblotting analysis of proteins related to the DNA damage and apoptosis pathways. Protein expression in U2OS cells following treatment with **3** (50, 100 and 200 nM for 72 h).

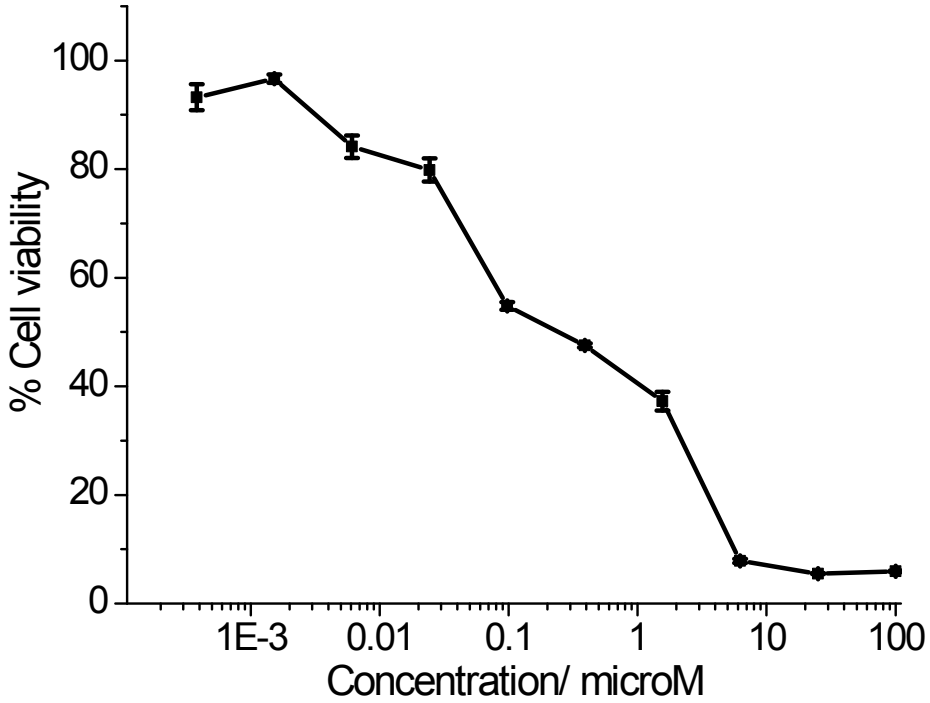


Fig. S38 Representative dose-response curves of **3** against U2OS cells in the presence of z-VAD-FMK (5 μM) after 72 h incubation.

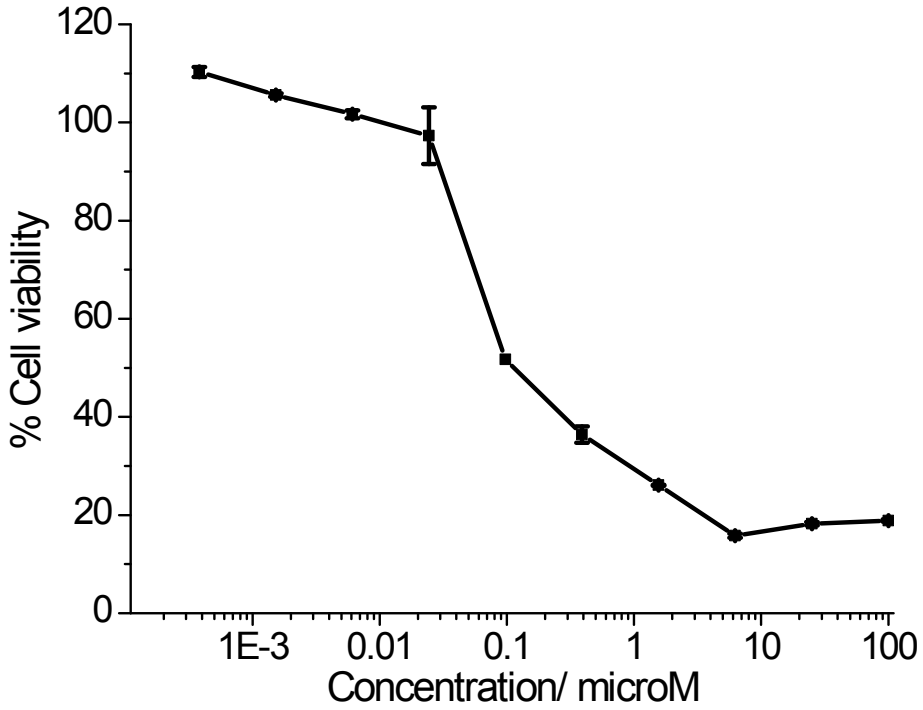


Fig. S39 Representative dose-response curves of **3** against U2OS-MTX cells in the presence of z-VAD-FMK (5 μM) after 72 h incubation.

References

1. A. J. Carty, K. R. Dymock and P. M. Boorman, *Can. J. Chem.*, 1970, **48**, 3524-3529.
2. G. Sheldrick, *Acta Cryst.* , 2008, **A64**, 112-122.