Supporting Information for

Diflunisal-adjoined cobalt(III)-polypyridyl complexes as anti-cancer stem cell agents

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Experimental Details

Materials and Methods. All synthetic procedures were performed under normal atmospheric conditions. ^{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). High resolution electron spray ionisation mass spectra were recorded on a BrukerDaltronics Esquire 3000 spectrometer by Dr. Lisa Haigh (Imperial College London). Fourier transform infrared (FTIR) spectra were recorded with an IR Affinity-1S Shimadzu spectrophotometer. Liquid chromatography mass spectrometry was recorded on an Advion expression compact mass spectrometer with Aligent Technologies 1260 Infinity II HPLC using a Poroshell 120 EC-C18 2.7µm column (4.6×50mm); methodology reported in Table S1. Elemental analysis was performed commercially by London Metropolitan University. 2,2'-bipyridine, 1,10-phenanthroline, 4,7-diphenyl-1,10-phenanthroline, and diflunisal were purchased from Sigma Aldrich and used as received. The *cis*-dichlorobis-(ammine)cobalt(III) chloride complexes, **1-3** was prepared according to a previously reported protocol.[1] For all biophysical and cellular studies, a 10 mM stock solution in DMSO was initially prepared. The stock solution was diluted in the appropriate biological solution to the working concentration(s).

Synthesis of [Co(2,2'-bipyridine)$_2$(diflunisal)][PF$_6$] (4). Diflunisal (100 mg, 0.40 mmol), [Co(2,2'-bipyridine)$_2$Cl$_2$]Cl (120 mg, 0.25 mmol), and Ag_2O (165 mg, 0.71 mmol) were stirred in MeOH (5 ml) pre-dried with Na_2SO_4 for 4 days at room temperature. The resulting silver salts were removed yielding a dark green solution which was reduced to *ca.* 5ml. Diethyl ether (100 ml) was added resulting in a pale green precipitate which was isolated and washed with diethyl ether (3 x 10ml). The solid was dissolved in acetone (10 ml) giving a dark green solution, which was dispersed in a solution of $NaPF_6$ (200 mg, 1.19 mmol) in water (10 ml). The resulting green solid was filtered and washed with water (3 x 10ml). The crude solid was then purified using alumina column chromatography [(95:5) DCM:MeOH] to yield **4** as a dark green solid (27.2 mg, 0.044 mmol, 24%); IR (solid state, cm^{-1}): 1613.12, 1582.66, 1467.89, 1448.60, 1437.03, 1404.29, 1320.33, 1275.00, 1278.00, 1139.01, 1107.19, 964.45, 834.25, 764.81, 728.16, 651.97, 593.14, 553.59; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.04 (d, 1H), 8.99 (d, 1H), 8.94 (d, 1H), 8.89 (d, 1H), 8.74 (d, 1H), 8.66 - 8.56 (m, 3H), 8.40 (ddd, 2H), 8.08 (t, 1H), 8.02 (t, 1H), 7.83 (s, 1H), 7.66 (d, 1H), 7.62 - 7.54 (m, 3H), 7.43 (ddd, 1H), 7.26 (td, 1H), 7.16 (dt, 1H), 7.10 (td, 1H), 6.80 (d, 1H); ^{19}F-(^{1}H) NMR (400 MHz, DMSO-d$_6$); δ_F -70.11 (d, J = 756 Hz), -112.79 (d, J = 8), -114.03 (d, J = 8); ESI-MS Calcd. For $C_{33}H_{22}CoF_2N_4O_3$ [M-PF$_6$]$^+$: 619.0987 a.m.u., Found [M-PF$_6$]$^+$: 619.0995; Anal. Calcd. For $C_{33}H_{22}CoF_2N_4O_3PF_6 \cdot 0.25H_2O$: C, 51.55; H, 2.95; N, 7.29. Found: C, 51.36; H, 2.97; N, 6.93.

Synthesis of [Co(1,10-phenanthroline)$_2$(diflunisal)][PF$_6$] (5). Diflunisal (100 mg, 0.40 mmol), [Co(1,10-phenanthroline)$_2$Cl$_2$]Cl (94 mg, 0.18 mmol), and Ag_2O (165 mg, 0.71 mmol) were stirred in MeOH (5 ml) pre-dried with Na_2SO_4 for 4 days at room temperature. The resulting silver salts were removed yielding a dark green solution which was reduced to *ca.* 5ml. Diethyl ether (100 ml) was added resulting in a pale green precipitate which was isolated and washed with diethyl ether (3 x 10ml). The solid was dissolved in acetone (10 ml) giving a dark green solution, which was dispersed in a solution of $NaPF_6$ (200 mg, 1.19 mmol) in water (10 ml). The resulting green solid was filtered and washed with water (3 x 10 ml). The crude solid was then purified using alumina column chromatography [(95:5) DCM:MeOH] to yield **5** as a dark green solid (26.9 mg, 0.040 mmol, 22%); IR (solid state, cm^{-1}): 1615.45, 1581.70, 1520.94, 1467.89, 1428.35, 1406.17, 1318.40, 1276.9, 1226.78,

1139.01, 1099.47, 965.41, 904.65, 830.39, 716.59, 699.23, 653.90, 555.59; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 9.28 (d, 1H), 9.23 (d, 1H), 9.07 (d, 1H), 8.99 - 8.92 (m, 3H), 8.58 - 8.38 (m, 6H), 7.88 (t, 1H), 7.83 - 7.74 (m, 4H), 7.44 (ddd, 1H), 7.26 (td, 1H), 7.15 (dt, 1H), 7.10 (td, 1H), 6.76 (d, 1H); ^{19}F-(^{1}H) NMR (400 MHz, DMSO-d_6) δ_F -70.13 (d, J = 756 Hz), -112.79 (d, J = 8), -114.02 (d, J = 8); ESI-MS Calcd. For $C_{37}H_{22}CoF_2N_4O_3$ $[M\text{-}PF_6]^+$: 667.0988 a.m.u., Found $[M\text{-}PF_6]^+$: 667.0998; Anal. Calcd. For $C_{37}H_{22}CoF_2N_4O_3PF_6$: C, 54.70; H, 2.73; N, 6.90. Found: C, 54.61; H, 2.76; N, 6.79.

Synthesis of [Co(4,7-diphenyl-1,10-phenanthroline)$_2$(diflunisal)][PF$_6$] (6). Diflunisal (80 mg, 0.32 mmol), [Co(4,7-diphenyl-1,10-phenanthroline)$_2$Cl$_2$]Cl (100 mg, 0.12 mmol), and Ag_2O (110 mg, 0.47 mmol) were stirred in MeOH (5 ml) pre-dried with Na_2SO_4 for 4 days at room temperature. The resulting silver salts were removed yielding a dark green solution which was reduced to *ca.* 5ml. Diethyl ether (100 ml) was added resulting in a pale green precipitate which was isolated and washed with diethyl ether (3 x 10ml). The solid was dissolved in acetone (10 ml) giving a dark green solution, which was dispersed in a solution of $NaPF_6$ (200 mg, 1.19 mmol) in water (10 ml). The resulting green solid was filtered and washed with water (3 x 10 ml). The crude solid was then purified using alumina column chromatography [(95:5) DCM:MeOH] to yield **6** as a dark green solid (22.3 mg, 0.001 mmol, 8%).); IR (solid state, cm^{-1}): 1615.75, 1604.84, 1560.48, 1519.97, 1469.82, 1428.35, 1419.67, 1405.20, 1318.40, 1274.03, 1228.71, 1138.05, 1100.44, 1020.39, 964.45, 831.36, 761.91, 734.91, 700.19, 636.53, 552.63; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 9.21 (d, 1H), 9.13 (d, 1H), 8.49 (t, 2H), 8.40 (t, 2H), 8.30 (dd, 2H), 8.04 (t, 2H), 7.96 (d, 1H), 7.90 (t, 4H), 7.81 - 7.66 (m, 18H), 7.46 (ddd, 1H), 7.30 – 7.22 (m, 2H), 7.11 (td, 1H), 6.94 (d, 1H); ^{19}F-(^{1}H) NMR (400 MHz, DMSO-d_6) δ_F -70.13 (d, J = 756 Hz), -112.72 (d, J = 8), -114.01 (d, J = 8); ESI-MS Calcd. For $C_{61}H_{38}CoF_2N_4O_3$ $[M\text{-}PF_6]^+$: 971.2237 a.m.u. Found $[M\text{-}PF_6]^+$: 971.2253; Anal. Calcd. For $C_{61}H_{38}CoF_2N_4O_3PF_6$: C, 65.60; H, 3.43; N, 5.02. Found: C, 65.32; H, 3.73; N, 4.94.

Measurement of water-octanol partition coefficient (LogP). The LogP value for **4-6** 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 **4-6** (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 **4-6** content in each phase was determined by UV/Vis spectroscopy.

Cell Lines and Cell Culture Conditions. The human mammary epithelial cell line, HMLER and HMLER-shEcad were kindly donated by Prof. R. A. Weinberg (Whitehead Institute, MIT). HMLER and HMLER-shEcad cells were maintained in Mammary Epithelial Cell Growth Medium (MEGM) with supplements and growth factors (BPE, hydrocortisone, hEGF, insulin, and gentamicin/amphotericin-B). GMO7575 normal fibroblast skin cells and MDA-MB-231 human breast adenocarcinoma cells were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with foetal bovine serum (10 %) and penicillin/streptomycin (1 %). The cells were grown at 310 K in a humidified atmosphere containing 5% CO_2.

Cytotoxicity MTT assay. The colorimetric MTT assay was used to determine the toxicity of **1-6**, 2,2'-bipyridine, 1,10-phenanthroline, 4,7-diphenyl-1,10-phenanthroline, diflunisal, and carboplatin. HMLER, HMLER-shEcad, or 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, as determined by UV-vis spectroscopy (0.0004-100 μM), were added and incubated for 72 h (total volume 200 μL). Stock solutions of the compounds were prepared as 10 mM solutions in DMSO and diluted using MEGM or DMEM. 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 MEGM/MTT or DMEM/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).

Tumorsphere Formation and Viability Assay. HMLER-shEcad cells (5×10^3) were plated in ultralow-attachment 96-well plates (Corning) and incubated in MEGM supplemented with B27 (Invitrogen), 20 ng/mL EGF, and 4 μg/mL heparin (Sigma) for 5 days. Studies were also conducted in the presence of **2**, **4**-**6**, diflunisal, and salinomycin (0-133 μM). Mammospheres treated with **2**, **4**-**6**, diflunisal, and salinomycin (at their respective IC_{20} values, 5 days) were counted and imaged using an inverted microscope. The viability of the mammospheres 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 mammospheres reduce the amount of the oxidized TOX8 form (blue) and concurrently increases the amount of the fluorescent TOX8 intermediate (red), indicating the degree of mammosphere cytotoxicity caused by the test compound. Fluorescence values were normalized to DMSO-containing controls and plotted as concentration of test compound versus % mammospheres 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 **4**-**6** *ca.* 1 million HMLER-shEcad cells were treated with **4**-**6** (10 μM) at 37 ºC for 24 h. After incubation, the media was removed, 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 **4**-**6** 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. For **5**, cellular pellets were also used to determine the cobalt content in the nuclear, cytoplasmic, and membrane fractions. The Thermo Scientific NE-PER Nuclear and Cytoplasmic Extraction Kit was used to extract and separate the nuclear, cytoplasmic, and membrane fractions. 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). Cobalt levels are expressed as Co (ppb) per million cells. Results are presented as the mean of five determinations for each data point.

DNA Cleavage Studies. Plasmid DNA (pUC19) was purchased from Invitrogen. The DNA cleavage activity of **2** and **5** was determined by monitoring the conversion of supercoiled plasmid DNA (form I) to nicked circular DNA (form II) in Tris-HCl buffer (5mM, pH 7.4), using agarose gel electrophoresis. To probe the effect of **2** and **5** concentration on cleavage, solutions containing DNA (100 ng) and **2** and **5** (0-100 μM) with and without ascorbic acid (10-fold excess), with a total reaction volume of 20 μL, were incubated at 37 ^{o}C for 16 h. To

determine the oxidative cleavage mechanism, solutions containing DNA (100 ng), **5** (10 μM), ascorbic acid (100 μM), and various radical scavenges (10 mM or 40 mM of KI, DMSO, ^{t}BuOH, and NaN_3), with a total reaction volume of 20 μL, were incubated at 37 °C for 16 h. Reactions were also conducted in the presence of methyl green (50 μM), DAPI (50 μM), and TO (10 μM). After incubation, loading buffer (5 μL, containing 0.25% bromophenol blue, 0.25% xylene cyanol and 60% glycerol) was added and reaction mixtures were immediately loaded onto a 1% agarose gel containing SYBR safe. The DNA fragments were separated by applying 60 V for 2 h in Tris-acetate EDTA (TAE) buffer. The DNA bands were analysed under UV light using a Fujifilm Image Reader LAS- 3000.

Immunoblotting Analysis. HMLER-shEcad cells (5 × 10^3 cells) were incubated with **5** (at various concentrations, 1, 2 and 4 μM) or diflunisal (50 μM) for 72 h at 37 °C. 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 °C 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 (BioRad) and analysed using a chemiluminescence imager (Amersham Imager 600).

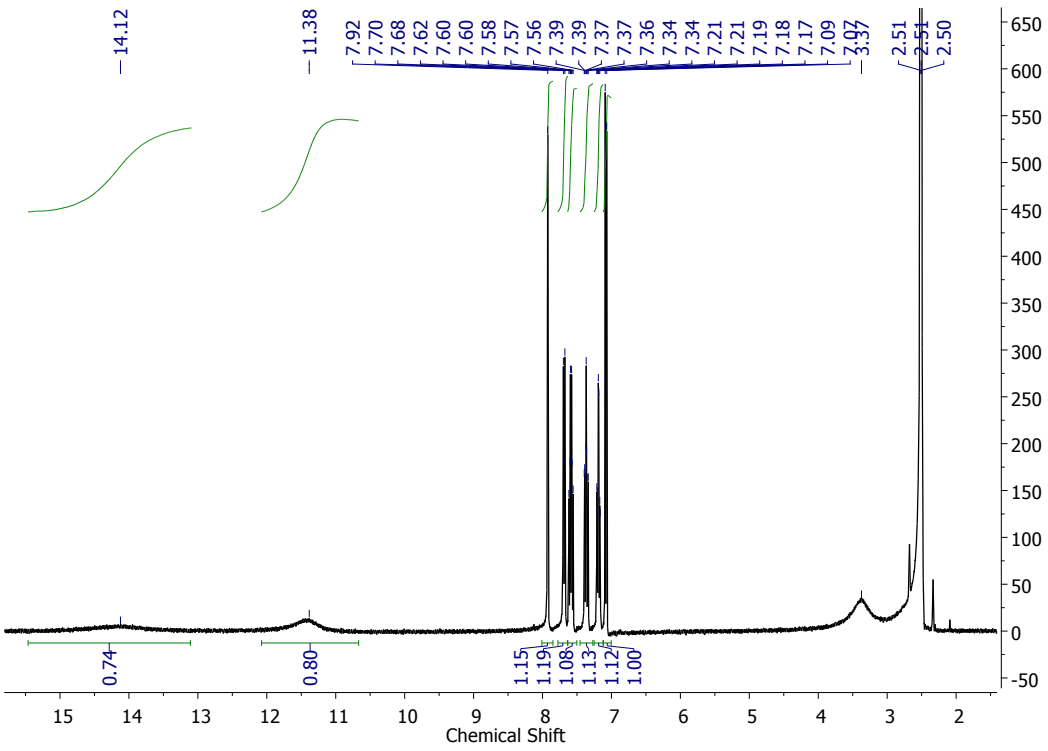

Fig. S1 ^{1}H NMR spectrum of diflunisal in DMSO-d_6.

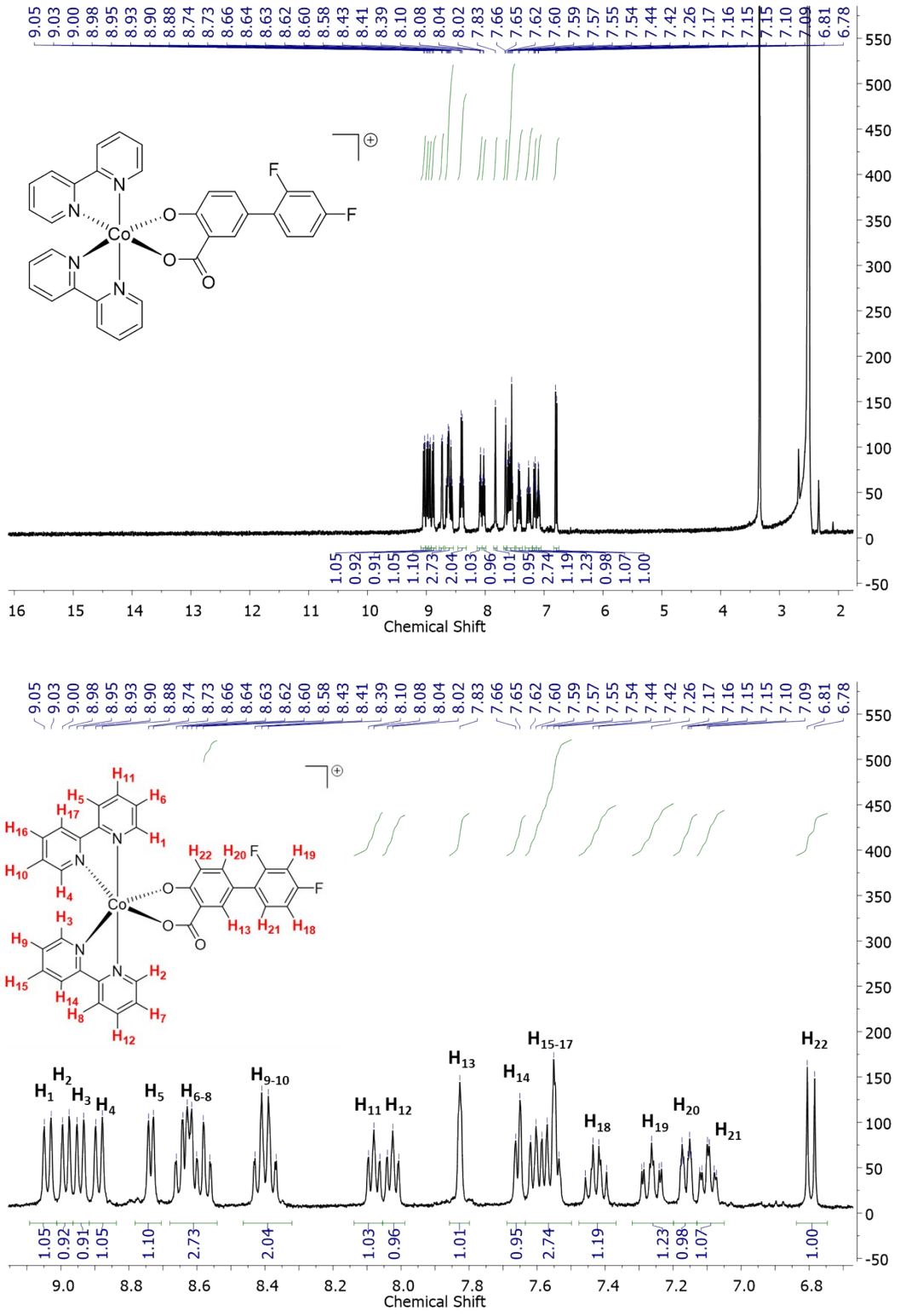

Fig. S2 ^{1}H NMR spectrum of **4** in DMSO-d_6.

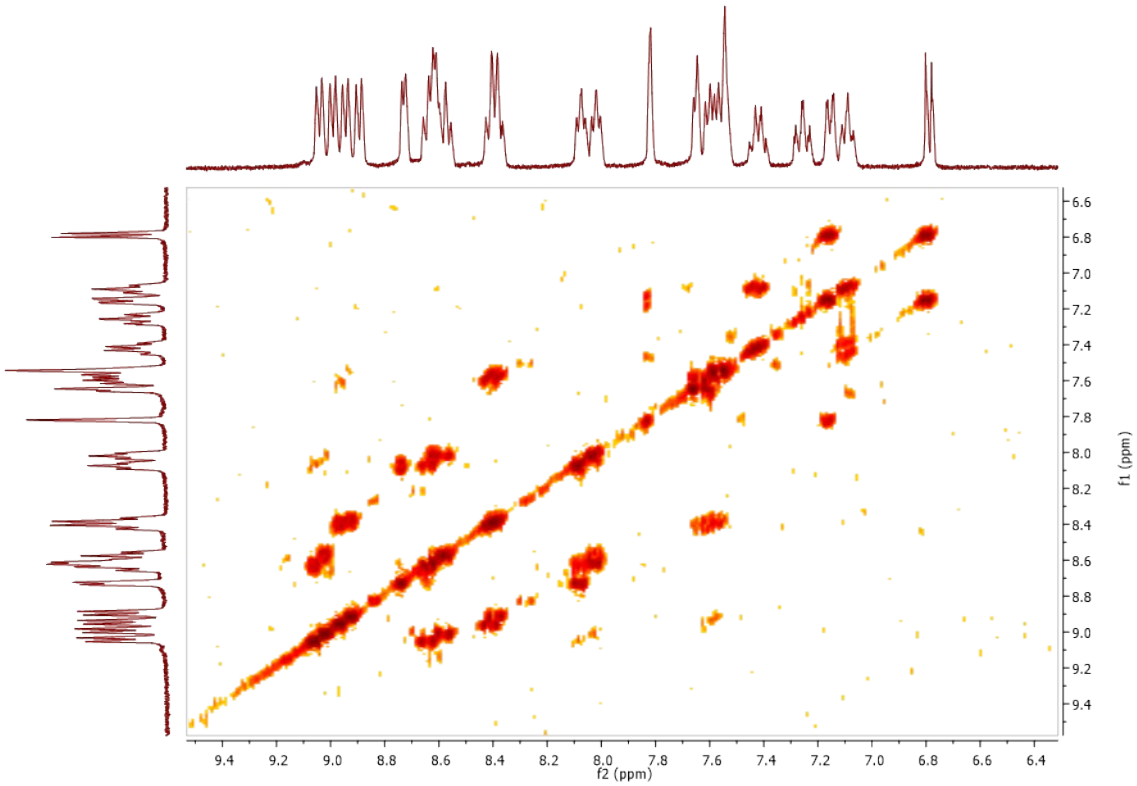

Fig. S3 ^{1}H COSY NMR spectrum of **4** in DMSO-d_6.

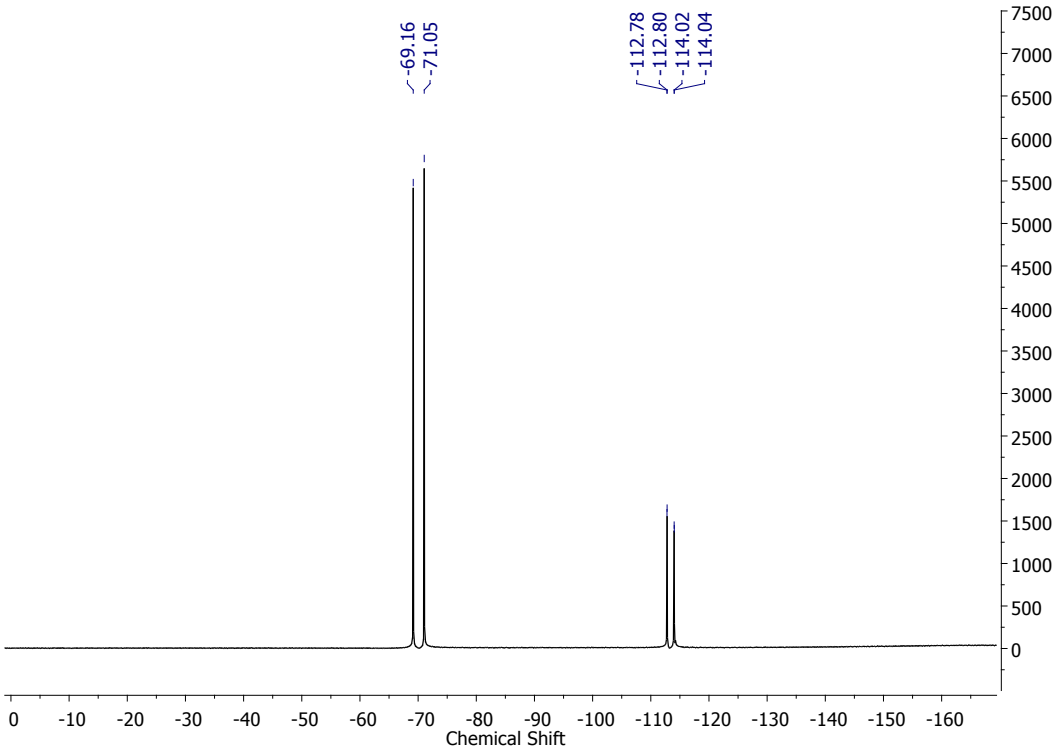

Fig. S4 ^{19}F-(^{1}H) NMR spectrum of **4** in DMSO-d_6.

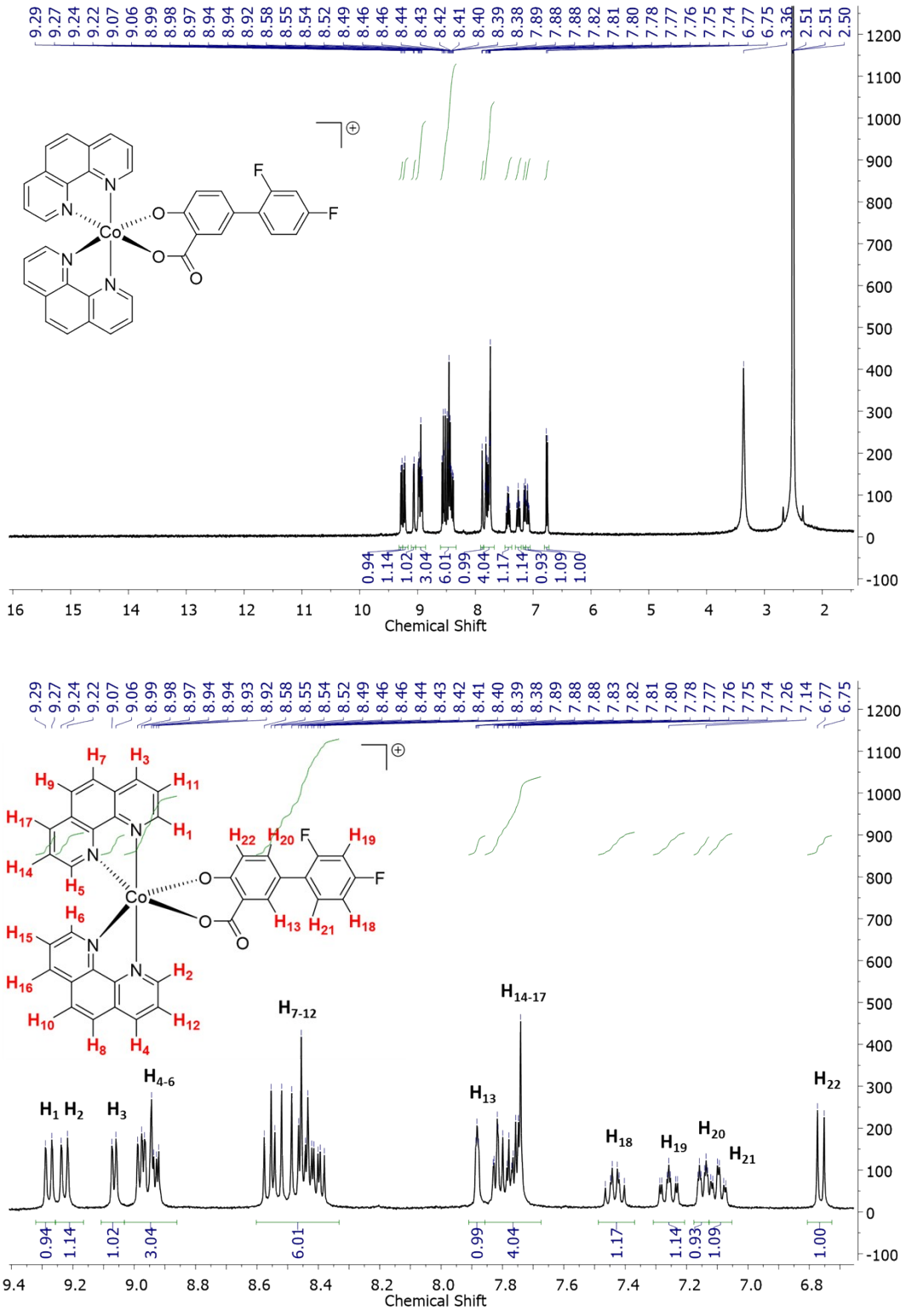

Fig. S5 ^{1}H NMR spectrum of **5** in DMSO-d_6.

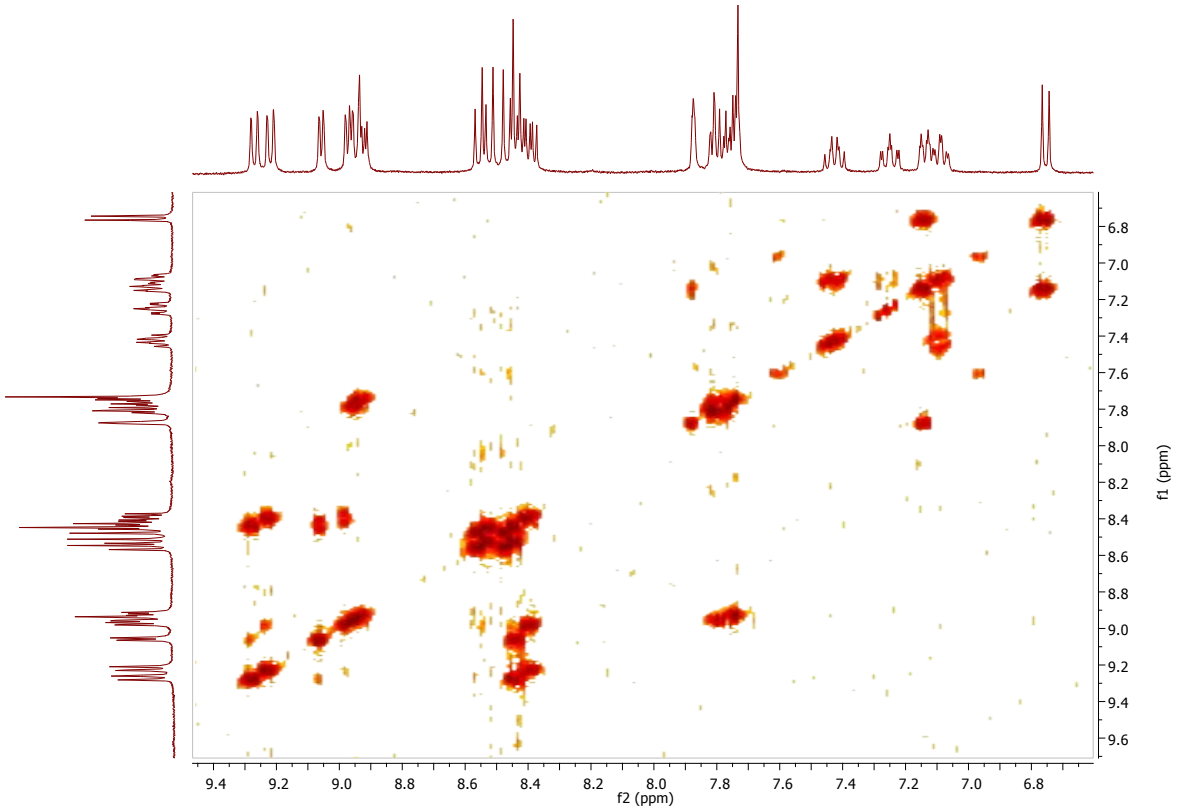

Fig. S6 ^{1}H COSY NMR spectrum of **5** in DMSO-d_6.

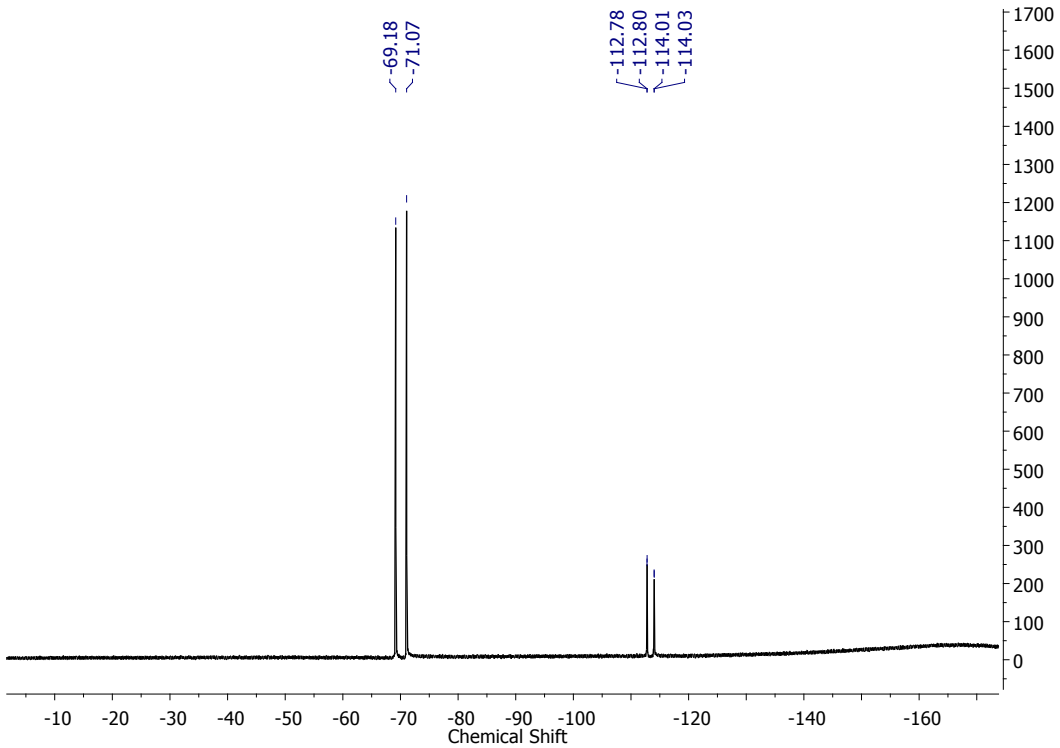

Fig. S7 ^{19}F-(^{1}H) NMR spectrum of **5** in DMSO-d_6.

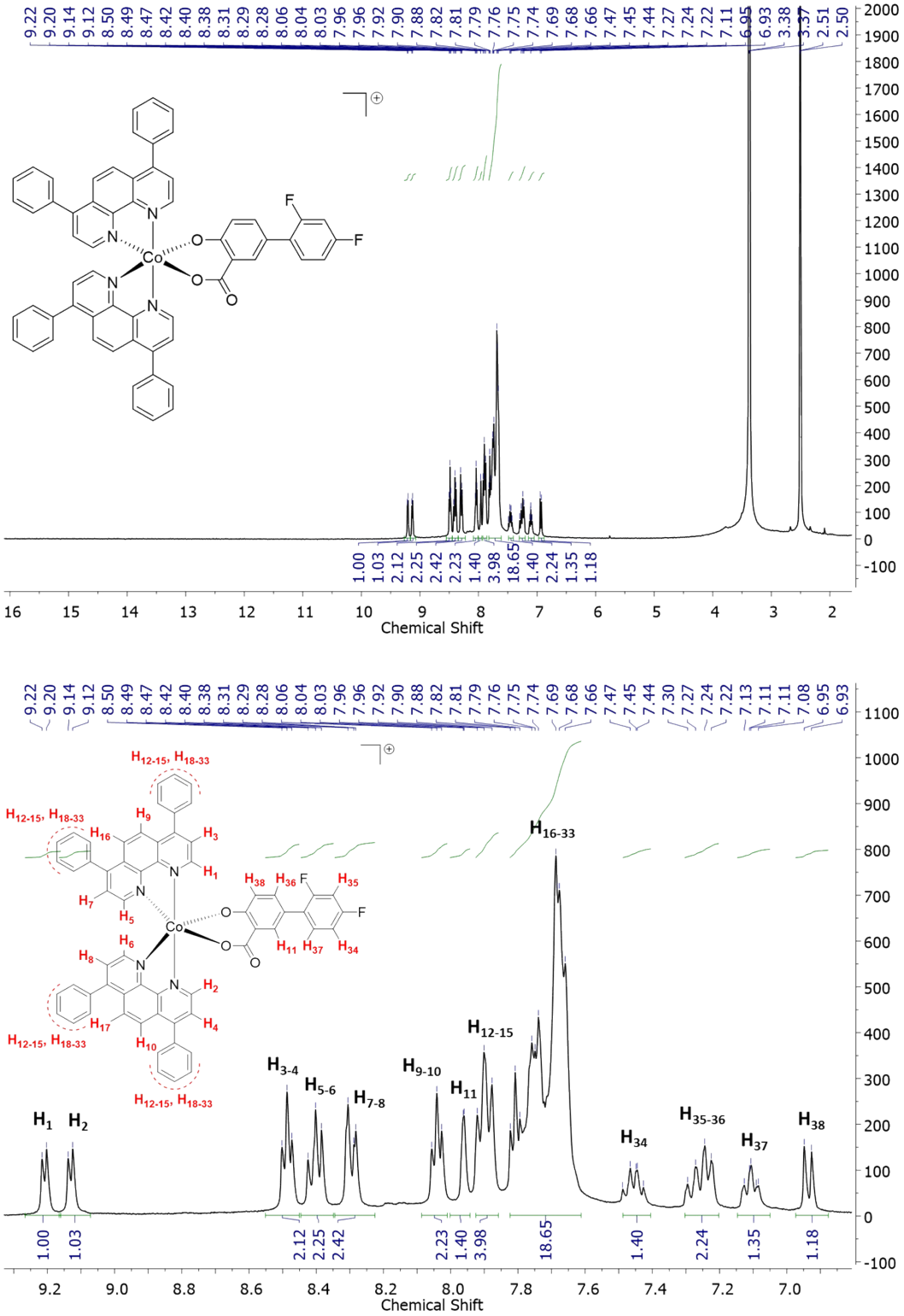

Fig. S8 ^{1}H NMR spectrum of **6** in DMSO-d_6.

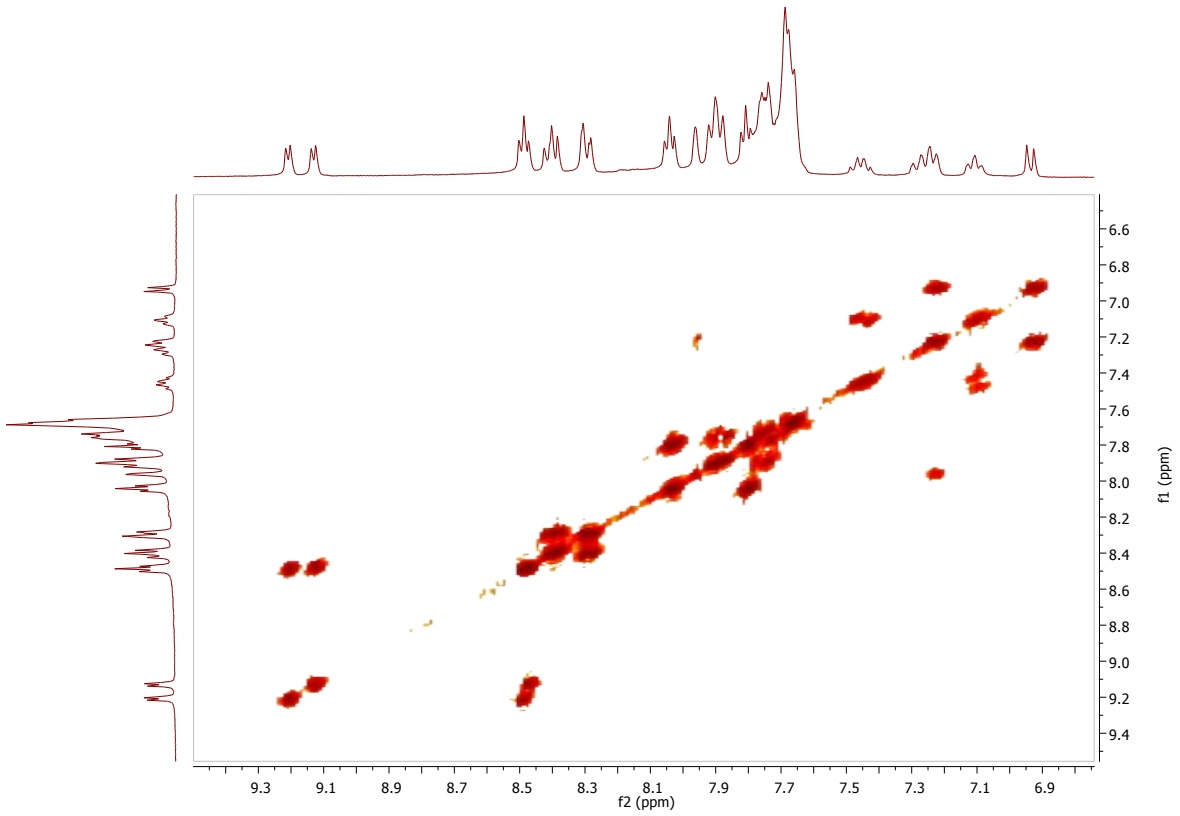

Fig. S9 ^{1}H COSY NMR spectrum of **6** in DMSO-d_6.

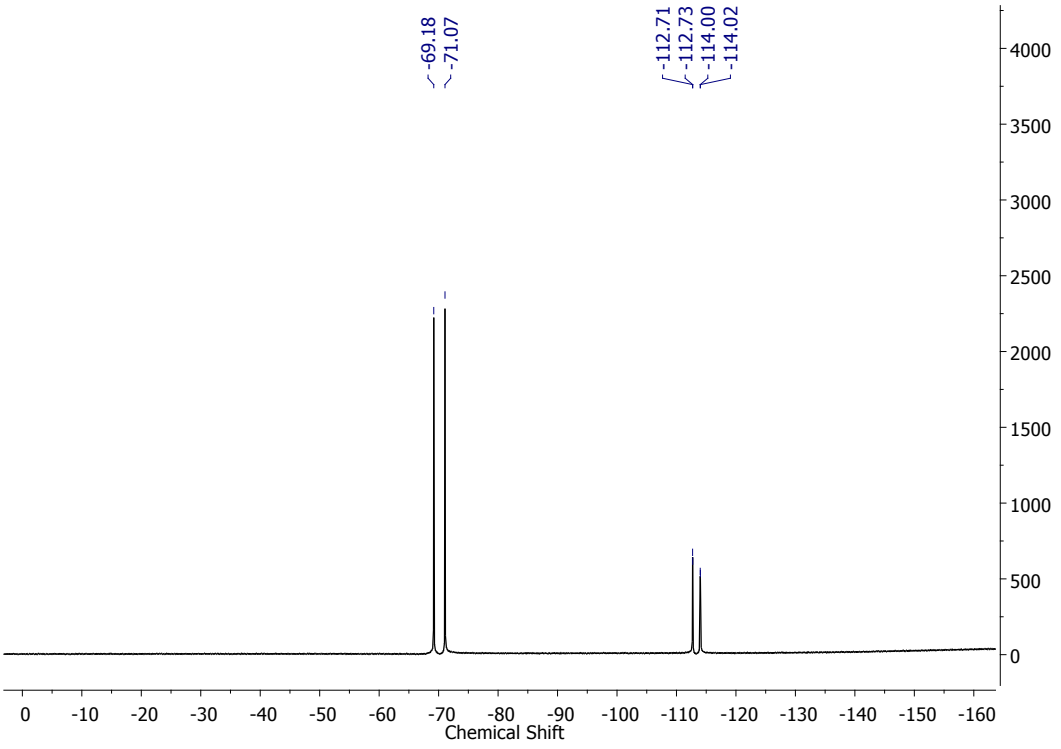

Fig. S10 ^{19}F-(^{1}H) NMR spectrum of **6** in DMSO-d_6.

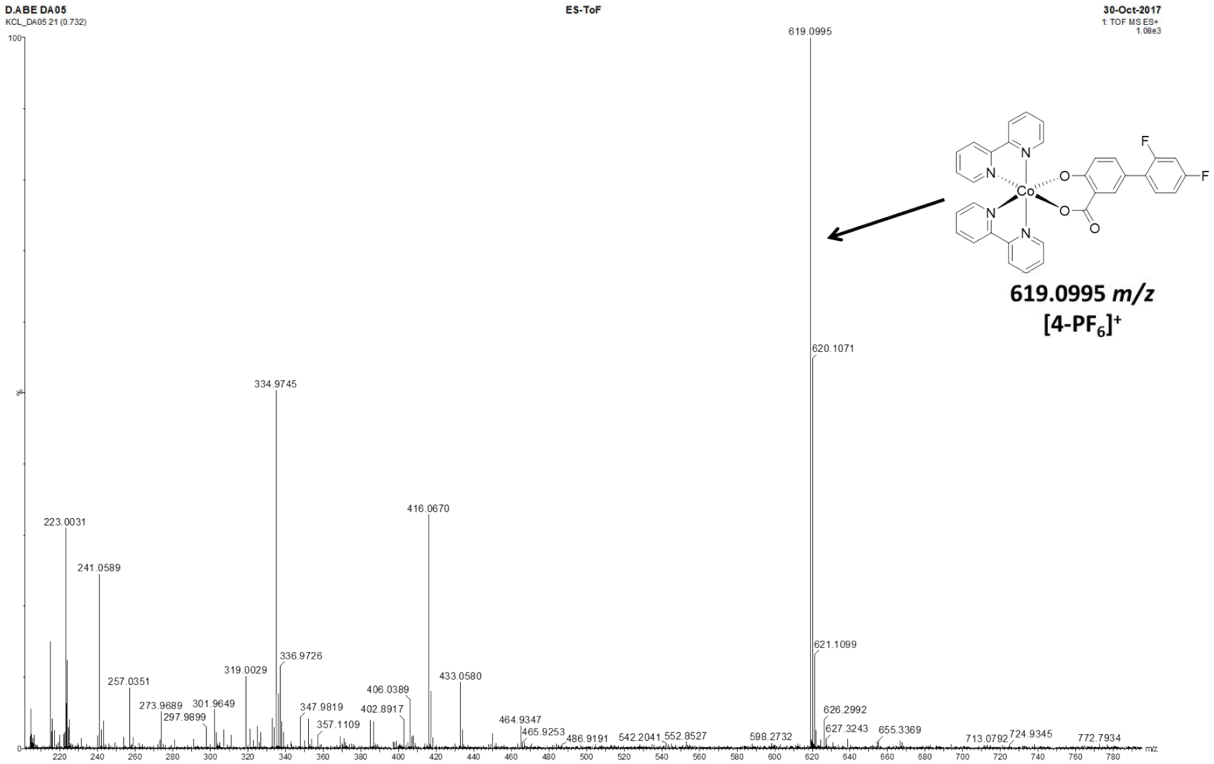

Fig. S11 High resolution ESI mass spectrum (positive mode) of **4**.

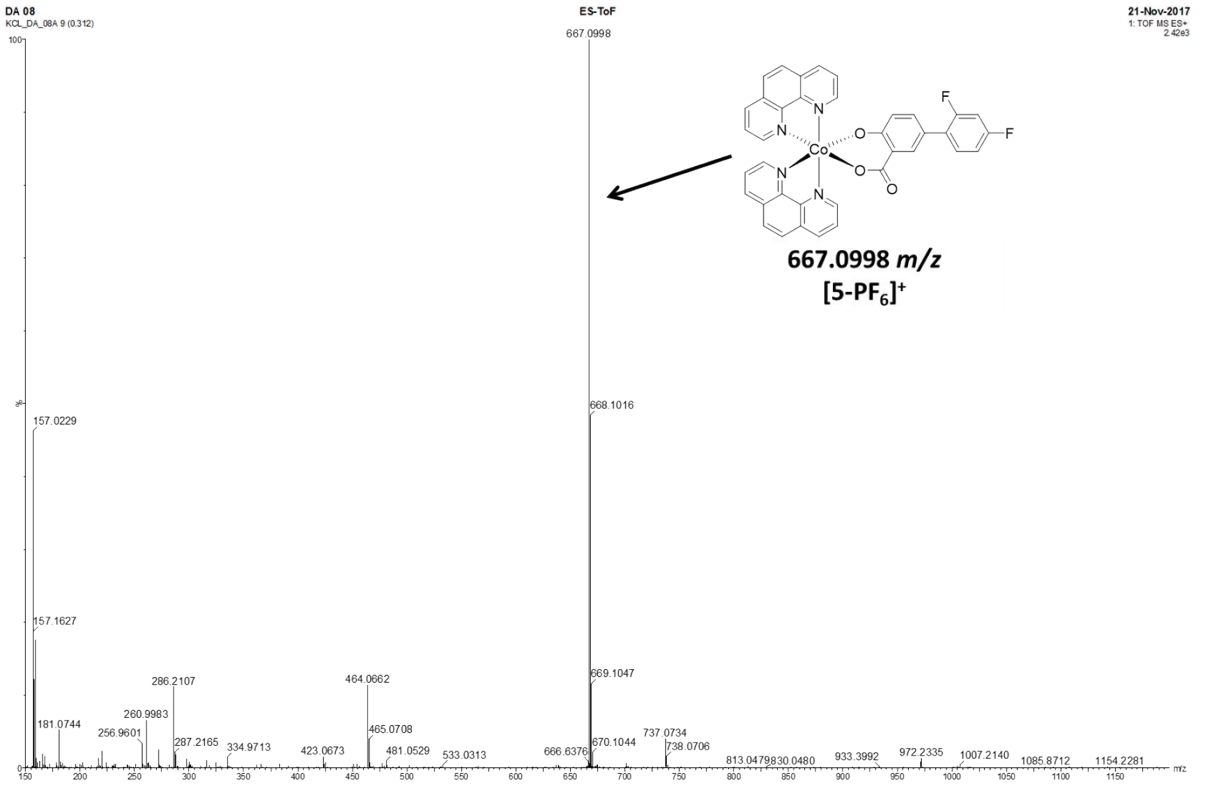

Fig. S12 High resolution ESI mass spectrum (positive mode) of **5**.

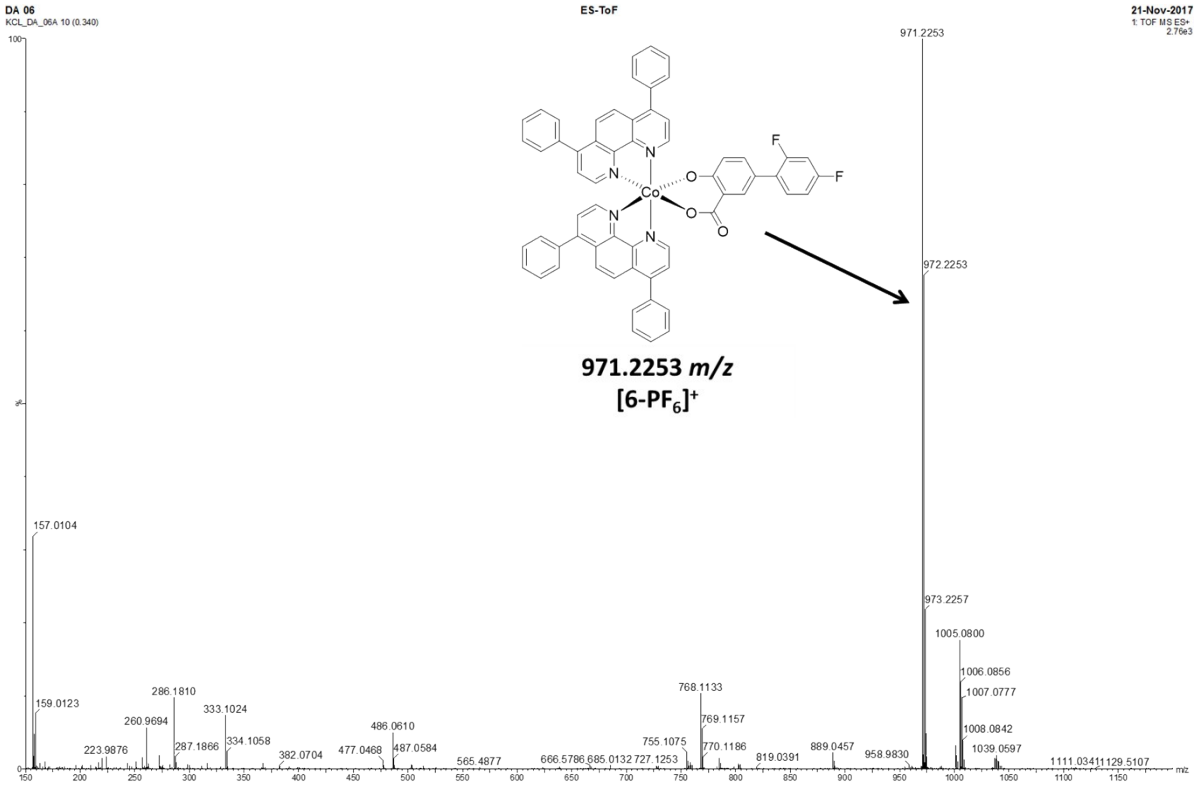

Fig. S13 High resolution ESI mass spectrum (positive mode) of **6**.

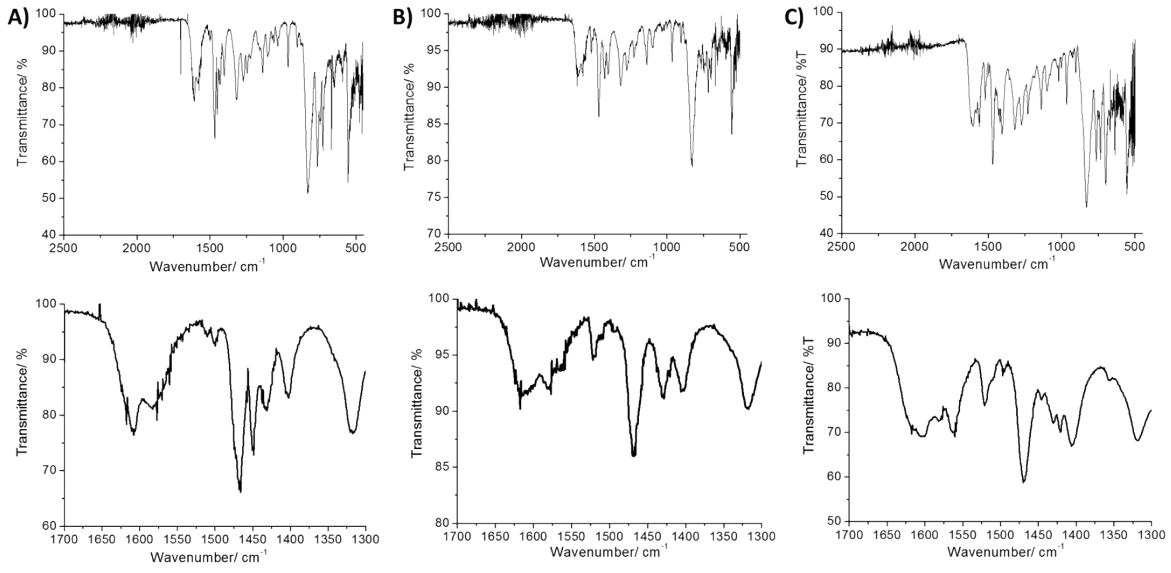

Fig. S14 IR spectrum of (A) **4**, (B) **5**, and (C) **6** in the solid form. The 1700-1300 cm^{-1} region has been magnified and shown below each full IR spectrum.

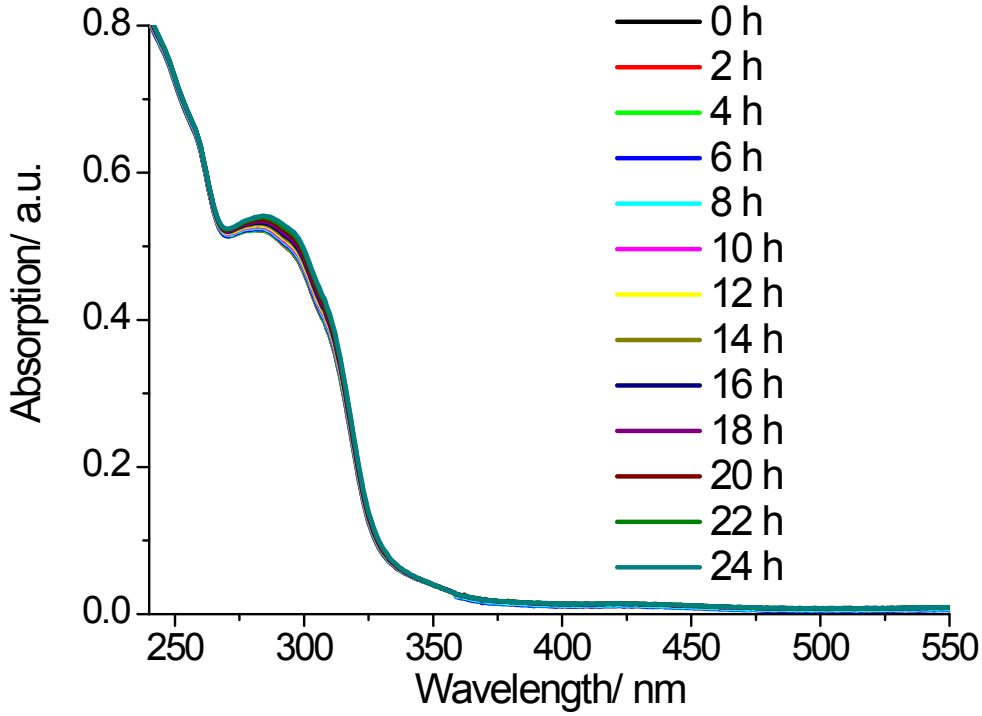

Fig. S15 UV-Vis spectrum of **4** (12.5 μM) in PBS over the course of 24 h at 37 °C.

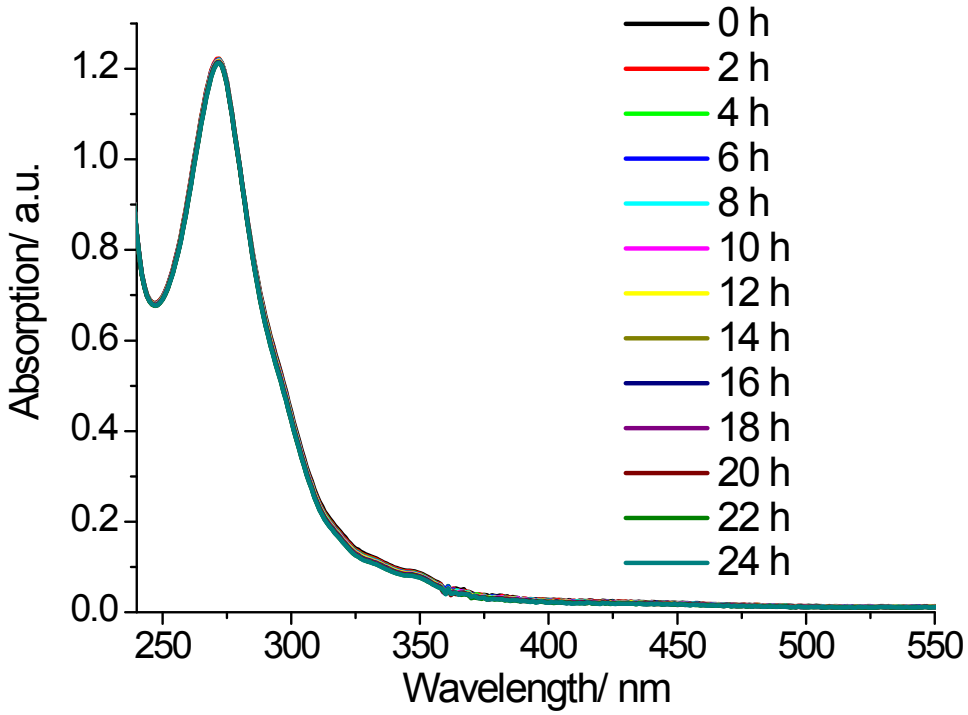

Fig. S16 UV-Vis spectrum of **5** (12.5 μM) in PBS over the course of 24 h at 37 °C.

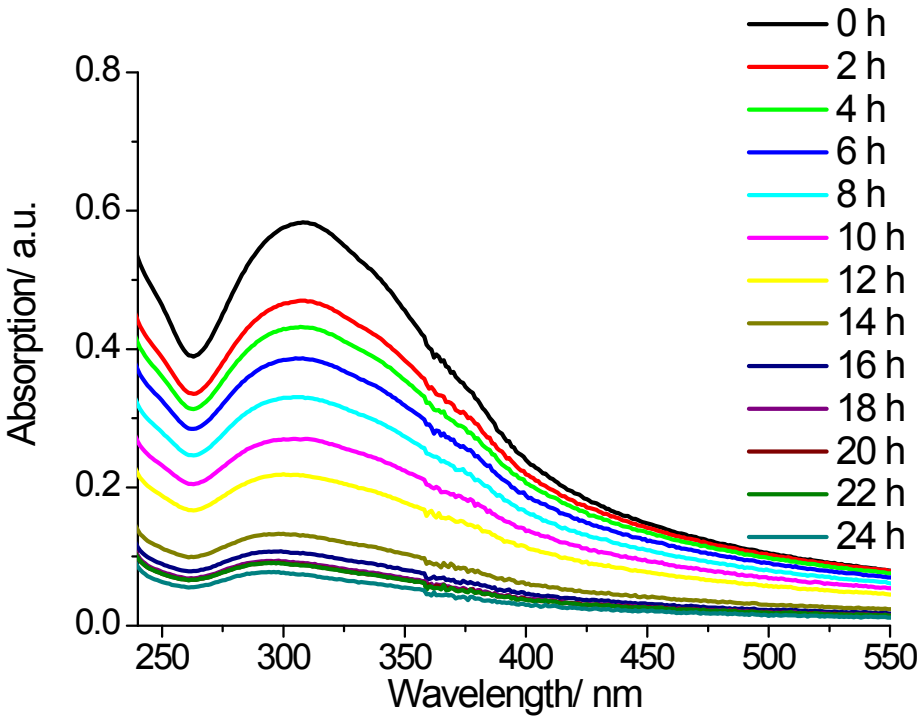

Fig. S17 UV-Vis spectrum of **6** (12.5 μM) in PBS over the course of 24 h at 37 °C.

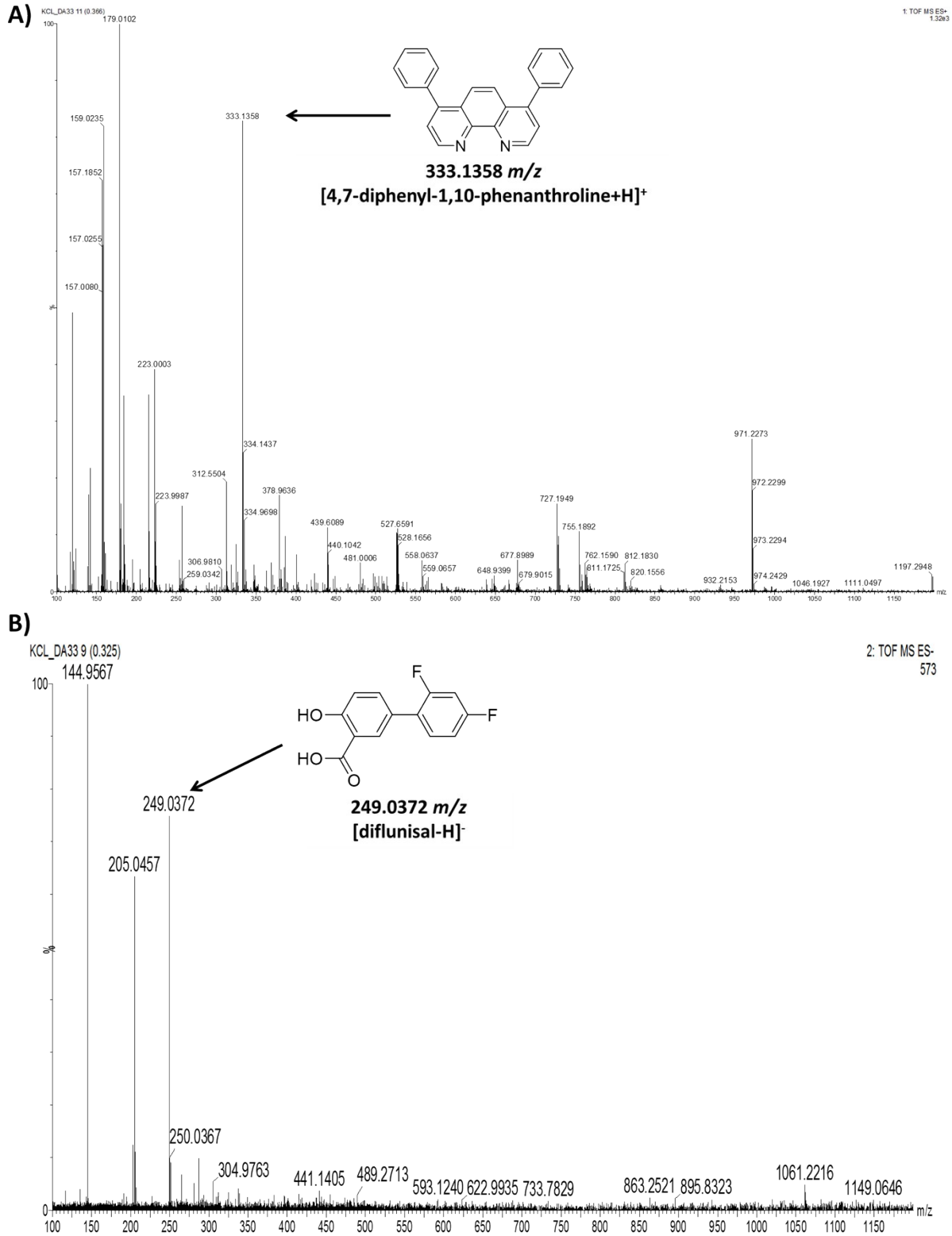

Fig. S18 ESI-TOF mass spectra of **6** (50 μM) in PBS after 72 h incubation at 37 ºC. (A) Positive mode and (B) negative mode.

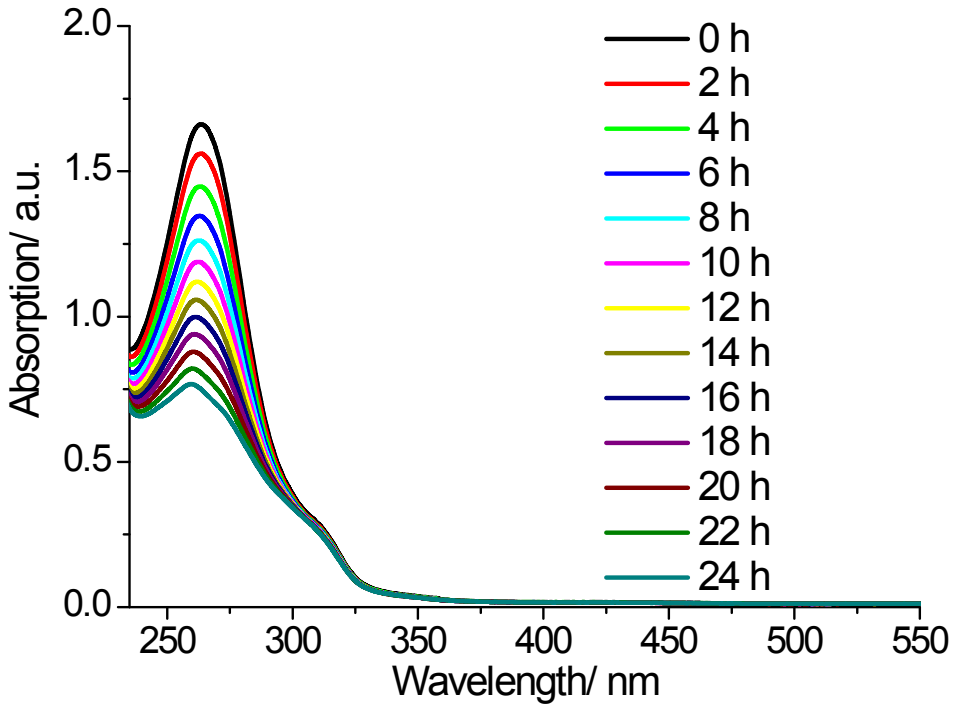

Fig. S19 UV-Vis spectrum of **4** (12.5 μM) in the presence of ascorbic acid (125 μM) in PBS over the course of 24 h at 37 °C.

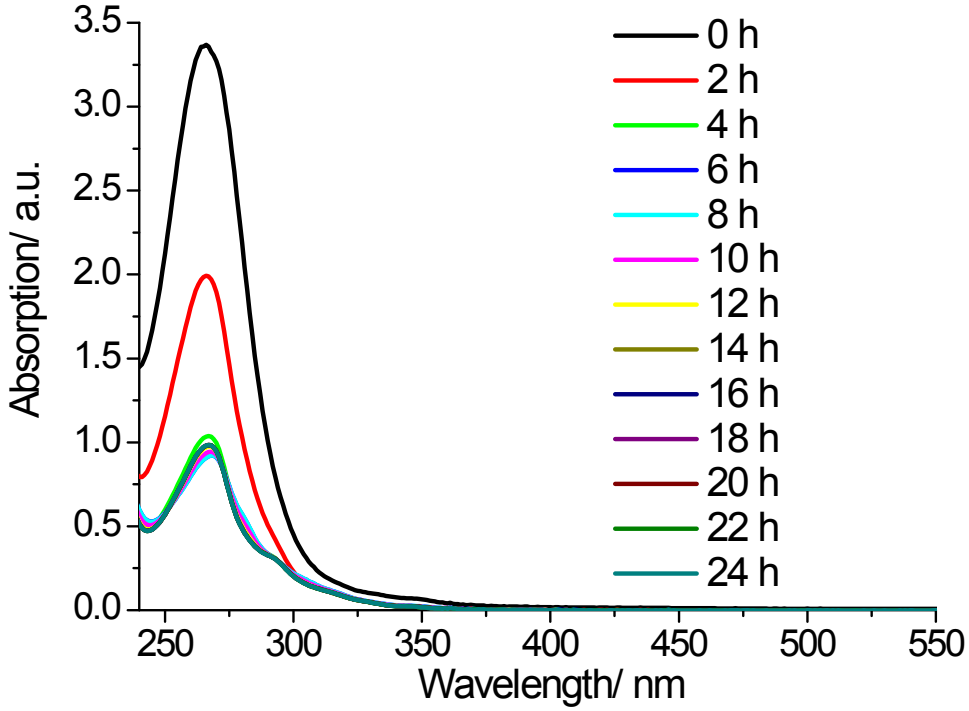

Fig. S20 UV-Vis spectrum of **5** (12.5 μM) in the presence of ascorbic acid (125 μM) in PBS over the course of 24 h at 37 °C.

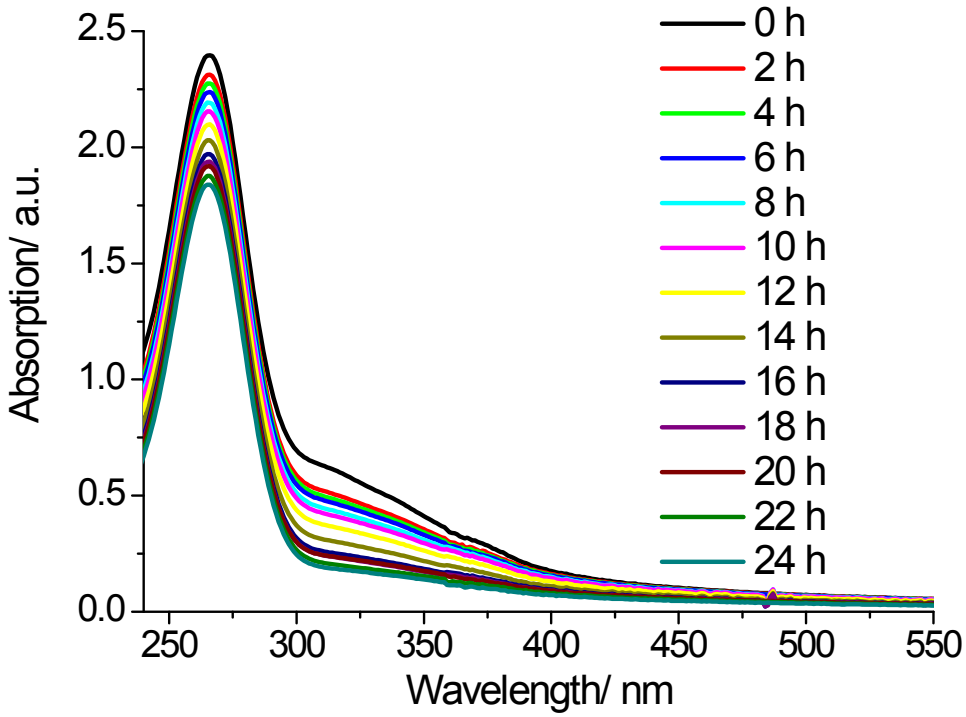

Fig. S21 UV-Vis spectrum of **6** (12.5 μM) in the presence of ascorbic acid (125 μM) in PBS over the course of 24 h at 37 °C.

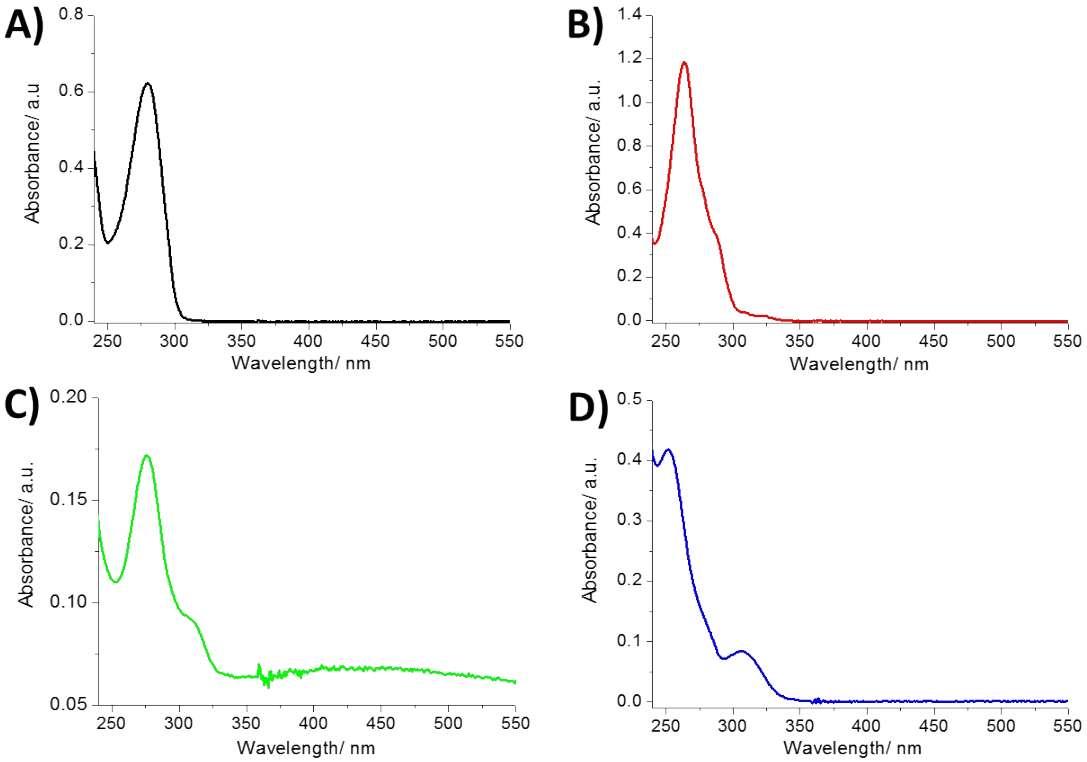

Fig. S22 UV-Vis spectrum of (A) 2,2'-bipyridine, (B) 1,10-phenanthroline, (C) 4,7-diphenyl-1,10-phenanthroline, and (D) diflunisal (all at 12.5 μM) in PBS at 37 °C.

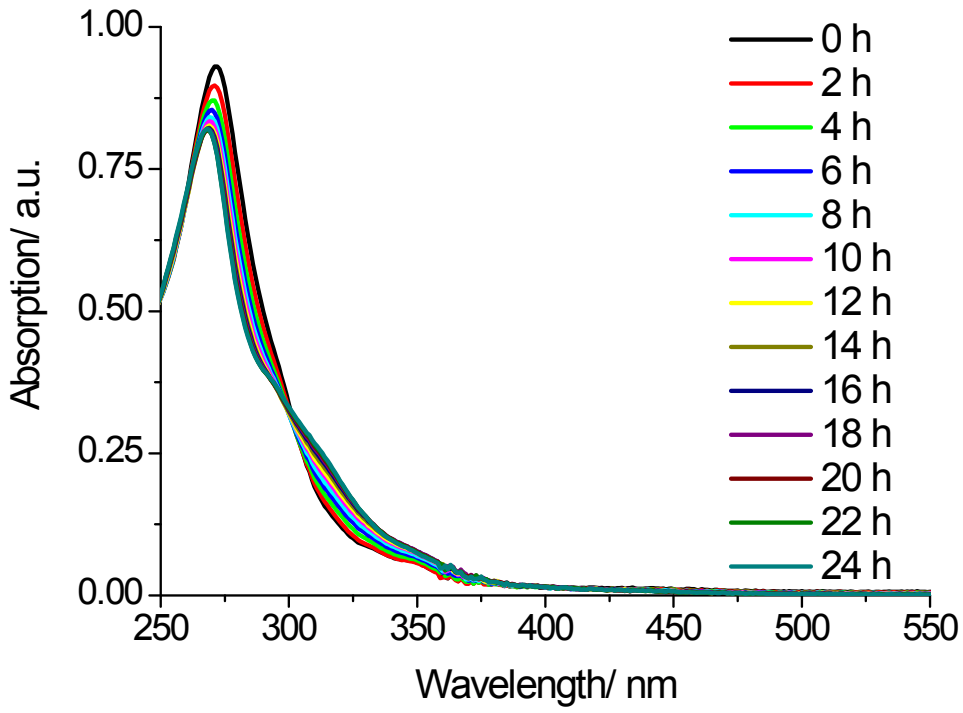

Fig. S23 UV-Vis spectrum of **5** (12.5 μM) in the presence of glutathione (125 μM) in PBS over the course of 24 h at 37 °C.

Table S1. Liquid chromatography mass spectrometry (LCMS) methodology table; volumes and solvents used over the time frame. Flow rate:1 ml/min; max pressure 600 bar; injection volume: 40μL.

Time (min)	Water [%]	Methanol [%]
0.00	90.0	10.0
2.00	90.0	10.0
22.00	5.0	95.0
25.00	5.0	95.0
28.00	90.0	10.0
35.00	90.0	10.0

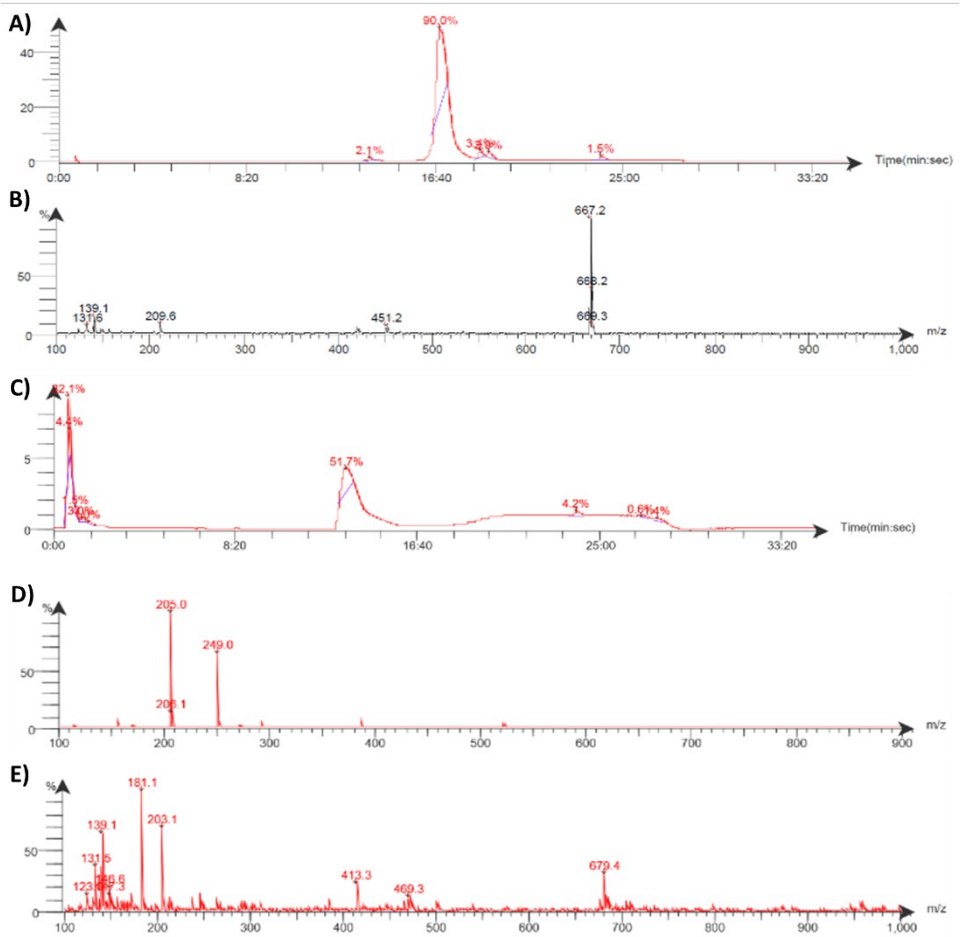

Fig S24 (A) LCMS UV-Vis chromatograph of **5** in H_2O:DMSO (100:1) after 72 h incubation at 37 ^oC. (B) ESI mass spectrum (positive mode) of UV-Vis signal detected at a RT: 16:30-17:14 min. (C) LCMS UV-Vis chromatograph of **5** in H_2O:DMSO (100:1) in the presence of ascorbic acid (500 µM, 10 equivalence) after 72 h incubation at 37 ^oC. (D) ESI mass spectrum (negative mode) of UV-Vis signal detected at a RT: 12:50-15:41 min. (E) ESI mass spectrum (positive mode) of UV-Vis signal detected at a RT: 23:38-24:18 min.

Table S2. Experimentally determined LogP values for **4-6**.

Co(III) complex	LogP
4	-0.30
5	0.04
6	0.56

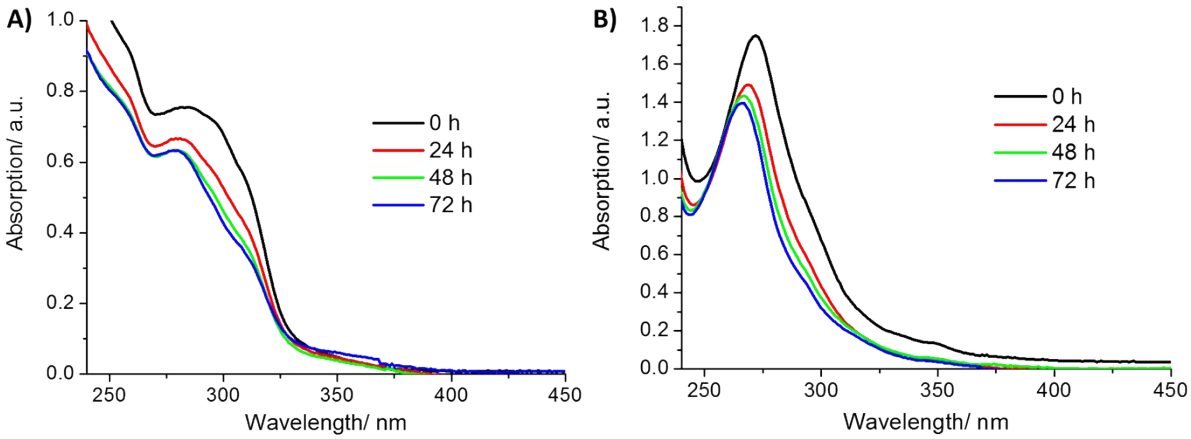

Fig. S25 UV-Vis spectrum of (A) **4** and (B) **5** (25 μM) in mammary epithelial cell growth medium (MEGM):DMSO (200:1) over the course of 72 h at 37 °C.

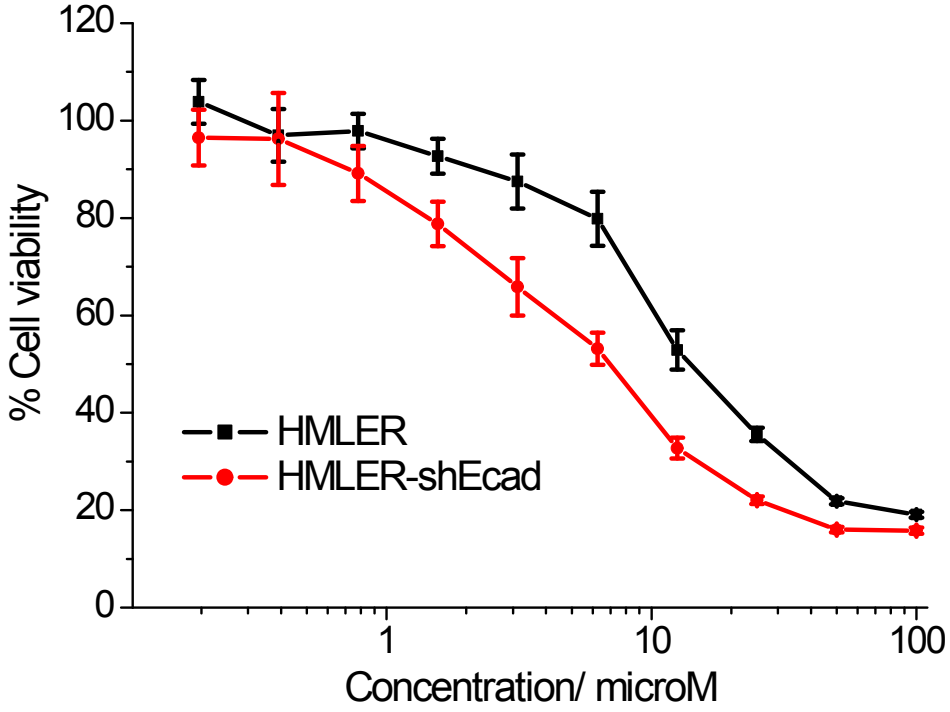

Fig. S26 Representative dose response curves of **4** against HMLER and HMLER-shEcad cells after 72 h incubation.

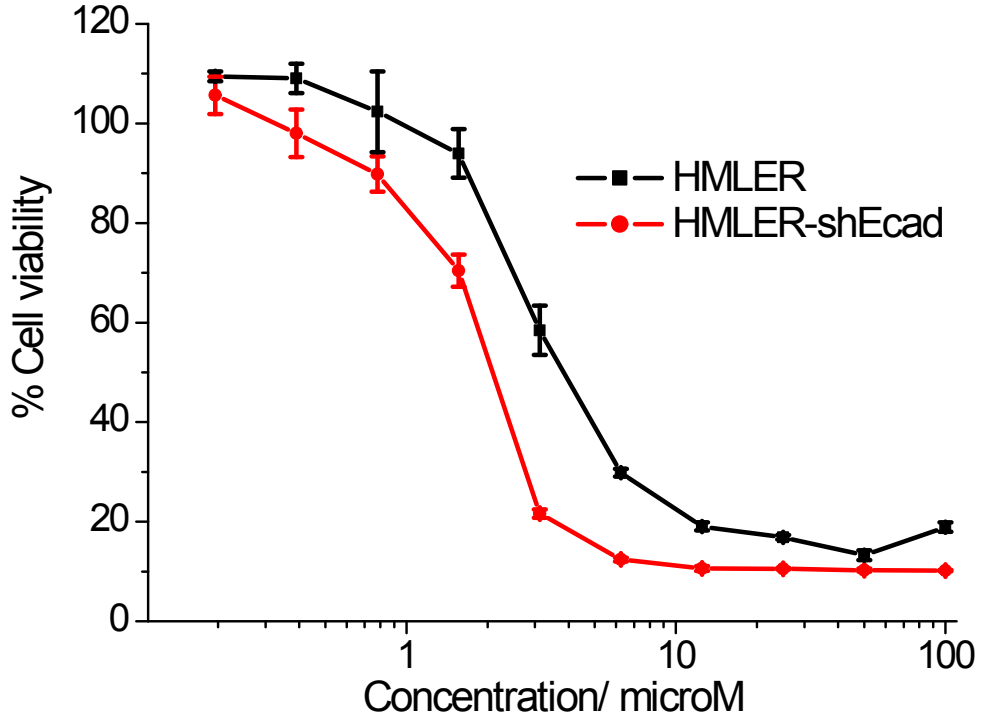

Fig. S27 Representative dose response curves of **5** against HMLER and HMLER-shEcad cells after 72 h incubation.

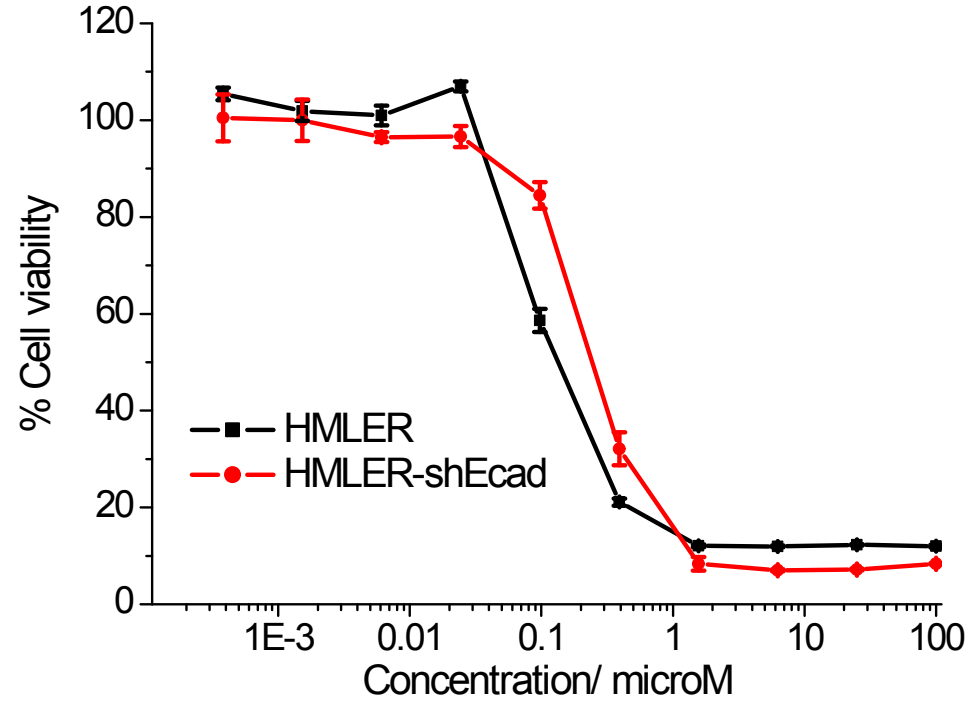

Fig. S28 Representative dose response curves of **6** against HMLER and HMLER-shEcad cells after 72 h incubation.

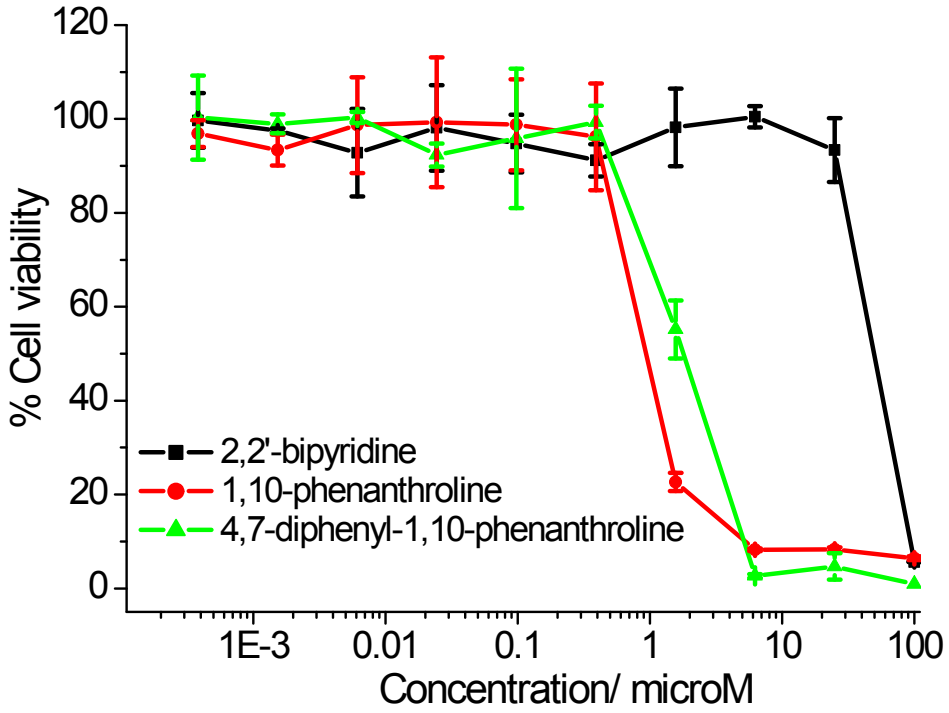

Fig. S29 Representative dose-response curves for the treatment of HMLER-shEcad cells with 2,2'-bipyridine, 1,10-phenanthroline, and 4,7-diphenyl-1,10-phenanthroline, after 72 h incubation.[2]

Table S3. IC_{50} values of **1-3**, 2,2'-bipyridine, 1,10-phenanthroline, and 4,7-diphenyl-1,10-phenanthroline against HMLER-shEcad cells after 72 h incubation (mean of three independent experiments ± SD). [a] taken from reference 2.

Compound	IC_{50} value against HMLER-shEcad cells
1	8.8 ± 0.2
2	2.3 ± 0.1
3	0.2 ± 0.04
2,2'-bipyridine[a]	47.2 ± 0.04
1,10-phenanthroline[a]	1.0 ± 0.04
4,7-diphenyl-1,10-phenanthroline[a]	1.9 ± 0.08

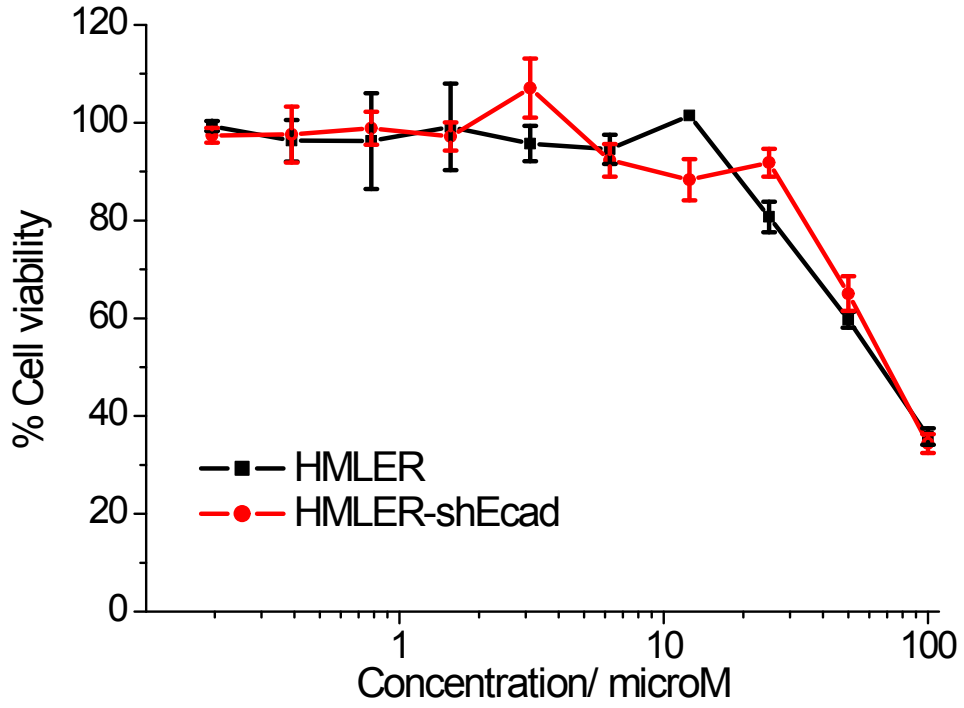

Fig. S30 Representative dose response curves of carboplatin against HMLER and HMLER-shEcad cells after 72 h incubation.

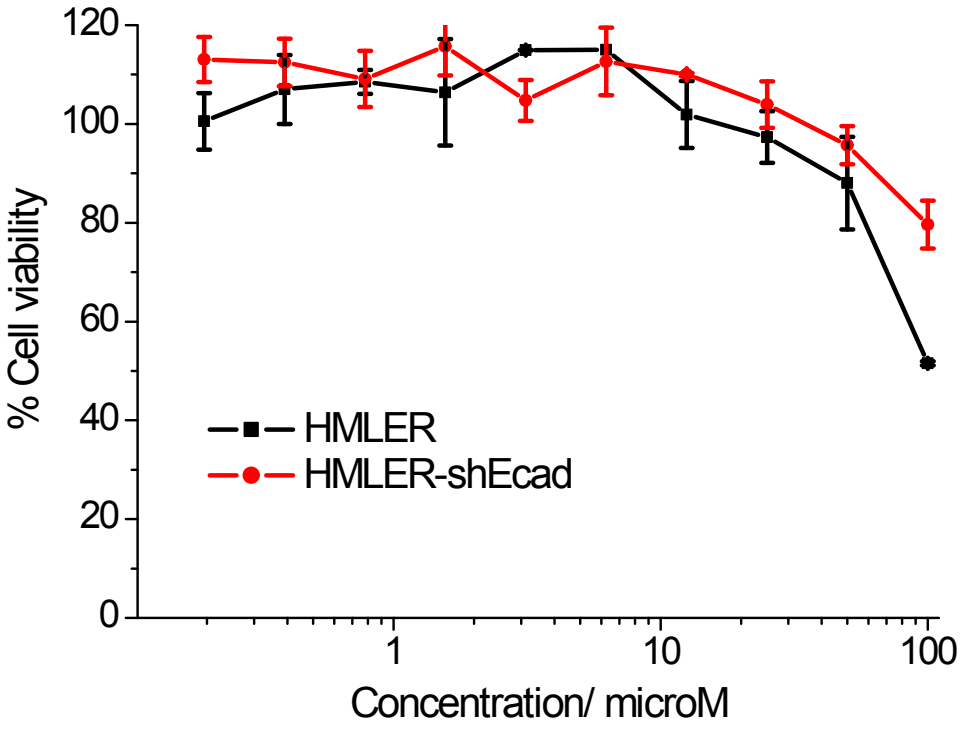

Fig. S31 Representative dose response curves of diflunisal against HMLER and HMLER-shEcad cells after 72 h incubation.

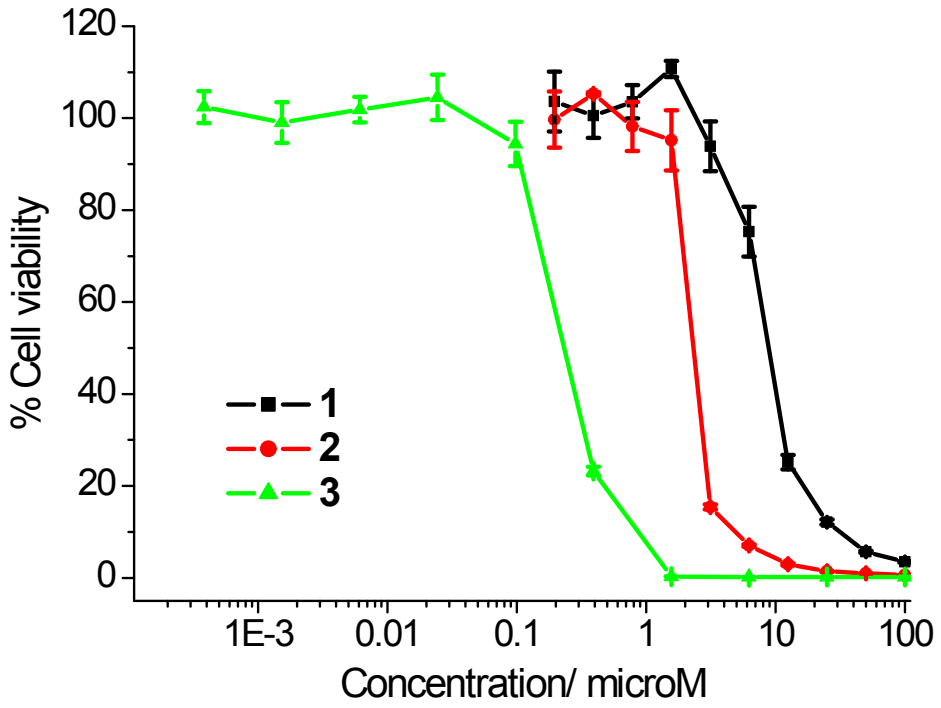

Fig. S32 Representative dose response curves of **1-3** against HMLER-shEcad cells after 72 h incubation.

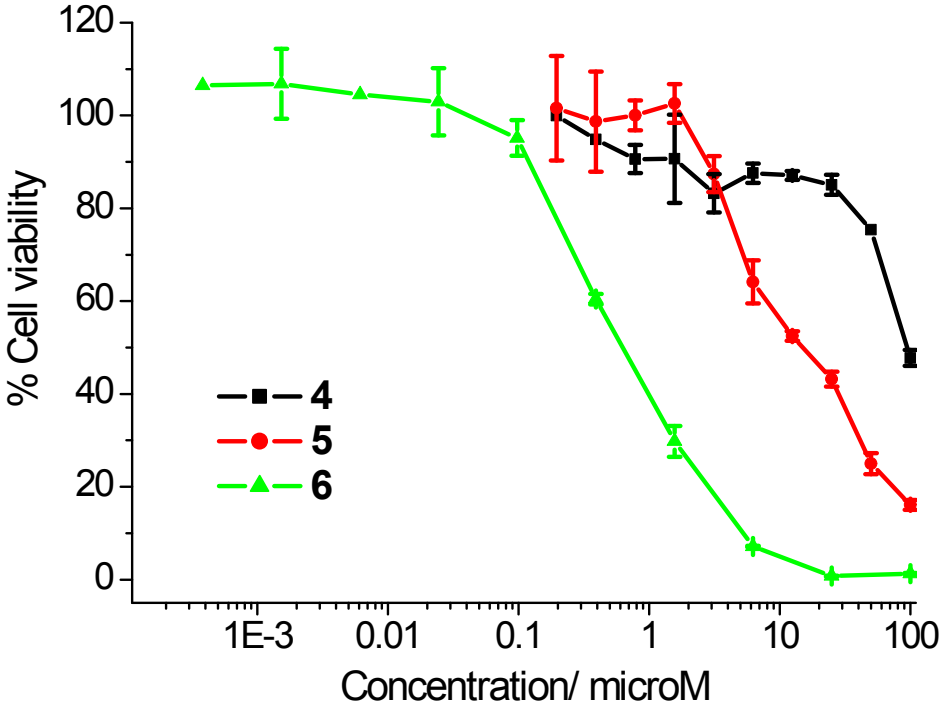

Fig. S33 Representative dose response curves of **4-6** against MDA-MB-231 cells after 72 h incubation.

Table S4. IC_{50} values of **4-6** against MDA-MB-231 and GMO7575 cells after 72 h incubation (mean of three independent experiments ± SD).

Co(III) complex	IC_{50} value against MDA-MB-231 cells	IC_{50} value against GMO7575 cells
4	96.5 ± 2.7	73.2 ± 2.0
5	15.2 ± 1.9	21.2 ± 1.3
6	0.7 ± 0.1	2.3 ± 0.1

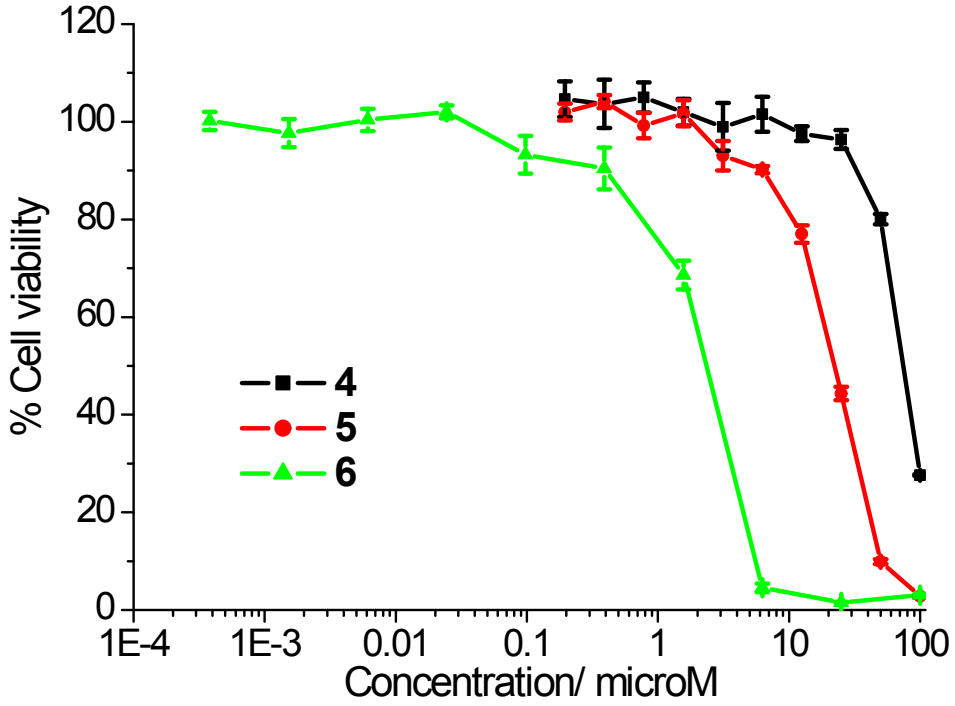

Fig. S34 Representative dose response curves of **4-6** against GMO7575 cells after 72 h incubation.

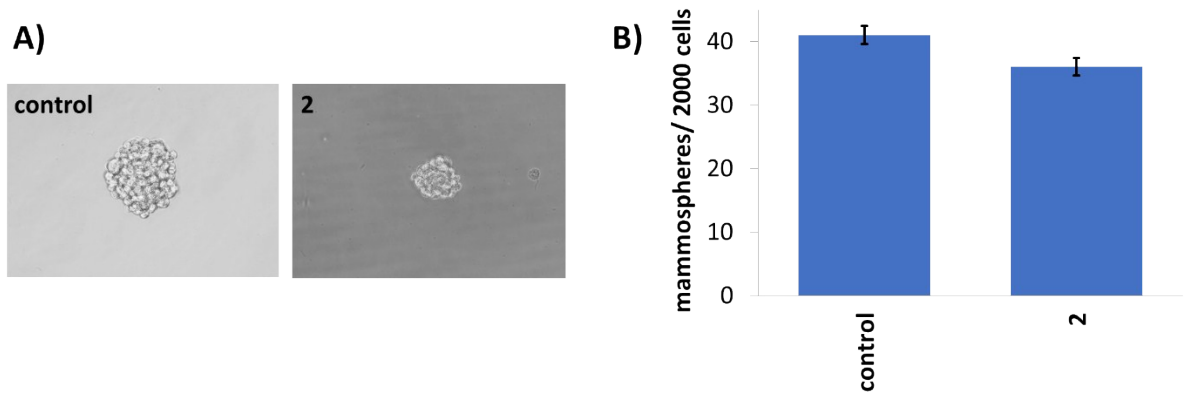

Fig. S35 (A) Representative images (×20) of HMLER-shEcad mammospheres in the absence (control) and presence of **2** at its respective IC_{20} value for 5 days. (B) Quantification of mammosphere formation with HMLER-shEcad cells untreated and treated with **2** at its IC_{20} values for 5 days.

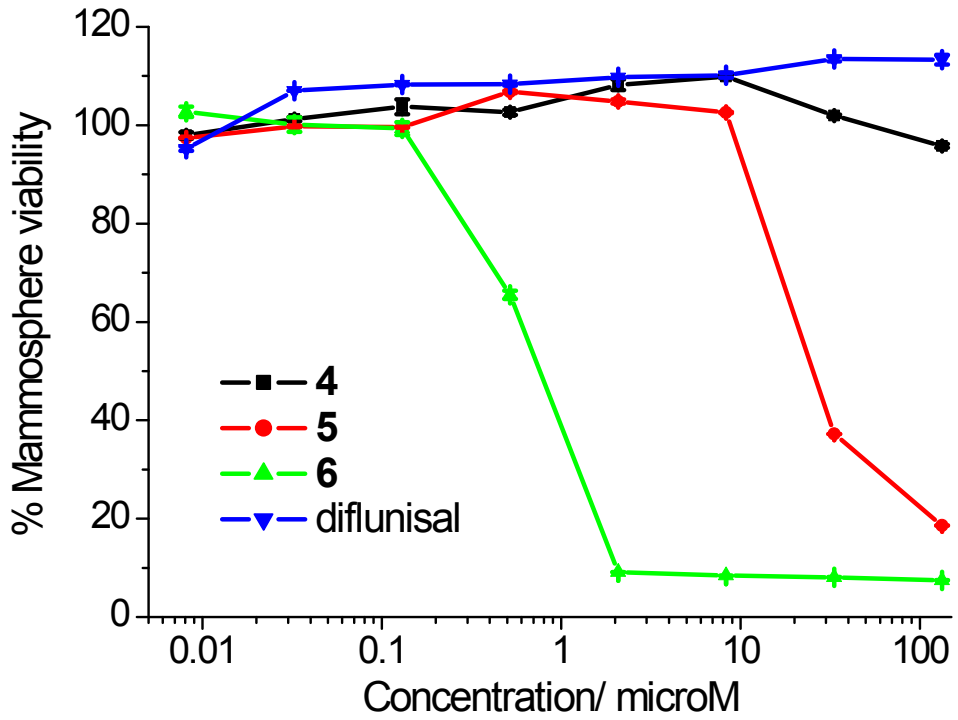

Fig. S36 Representative dose-response curves for the treatment of HMLER-shEcad mammospheres with **4**-**6** and diflunisal after 5 days incubation.

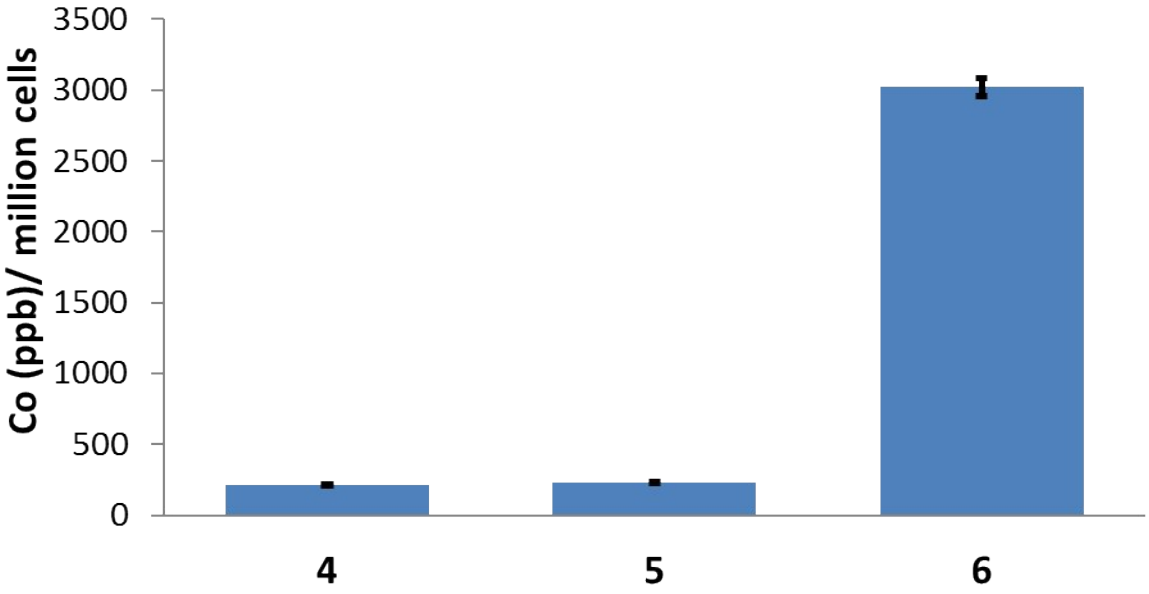

Fig. S37 Cobalt content in whole-cell isolated from HMLER-shEcad cells treated with **4-6** (10 μM for 24 h).

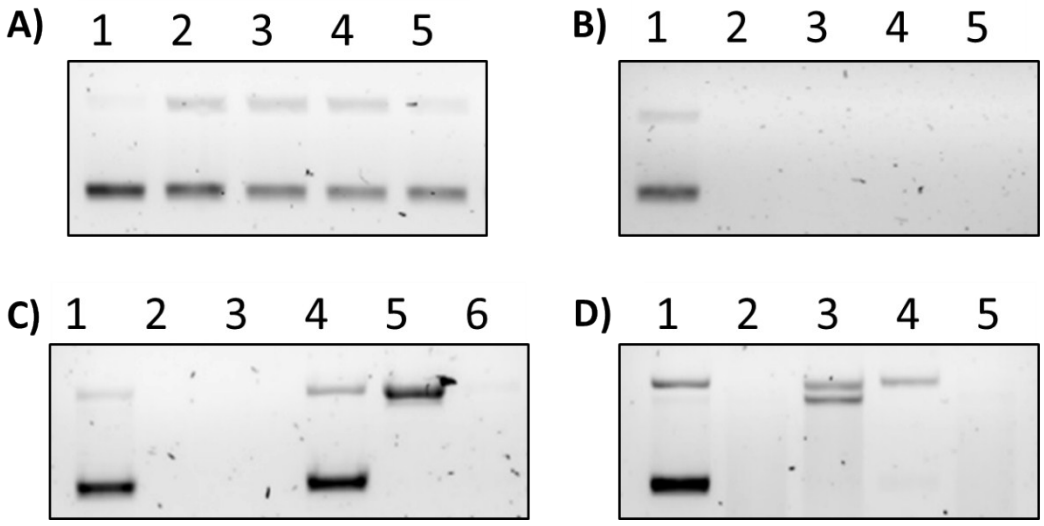

Fig. S38 DNA cleavage by **5** after a 16 h incubation period under various conditions. (A) Lane 1: DNA only; Lane 2-5: DNA + 10, 25, 50, and 100 μM of **5**. (B) Lane 1: DNA only, Lane 2-4: DNA + 10, 25, 50, and 100 μM of **5** with 10 equivalents of ascorbic acid. (C) Lane 1: DNA only, Lane 2: DNA + **5** (10 μM) with 10 equivalents of ascorbic acid, Lane 3-6: DNA + **5** (10 μM) with 10 equivalents of ascorbic acid + ^{t}BuOH (10 mM), KI (40 mM), NaN_3 (40 mM), DMSO (10 mM). (D) Lane 1: DNA only, Lane 2: DNA + **5** (10 μM) with 10 equivalents of ascorbic acid, Lane 3: DNA + **5** (10 μM) with 10 equivalents of ascorbic acid + methyl green (50 μM), Lane 4: DNA + **5** (10 μM) with 10 equivalents of ascorbic acid + DAPI (50 μM), Lane 5: DNA + **5** (10 μM) with 10 equivalents of ascorbic acid + thiazole orange (10 μM).

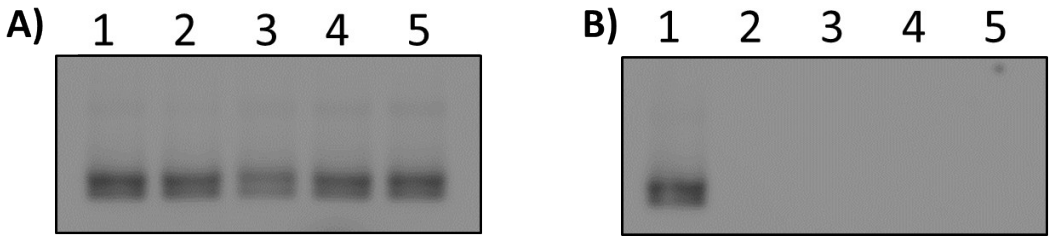

Fig. S39 DNA cleavage by **2** after a 16 h incubation period under various conditions. (A) Lane 1: DNA only; Lane 2-5: DNA + 10, 25, 50, and 100 μM of **2**. (B) Lane 1: DNA only, Lane 2-4: DNA + 10, 25, 50, and 100 μM of **2** with 10 equivalents of ascorbic acid.

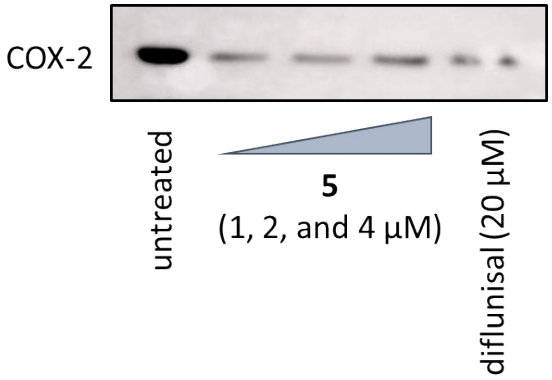

Fig. S40 COX-2 expression in HMLER-shEcad cells pre-treated with lipopolysaccharide (LPS) (2.5 μM for 24 h), followed by **5** (1-4 μM for 48 h) or diflunisal (50 μM for 48 h).

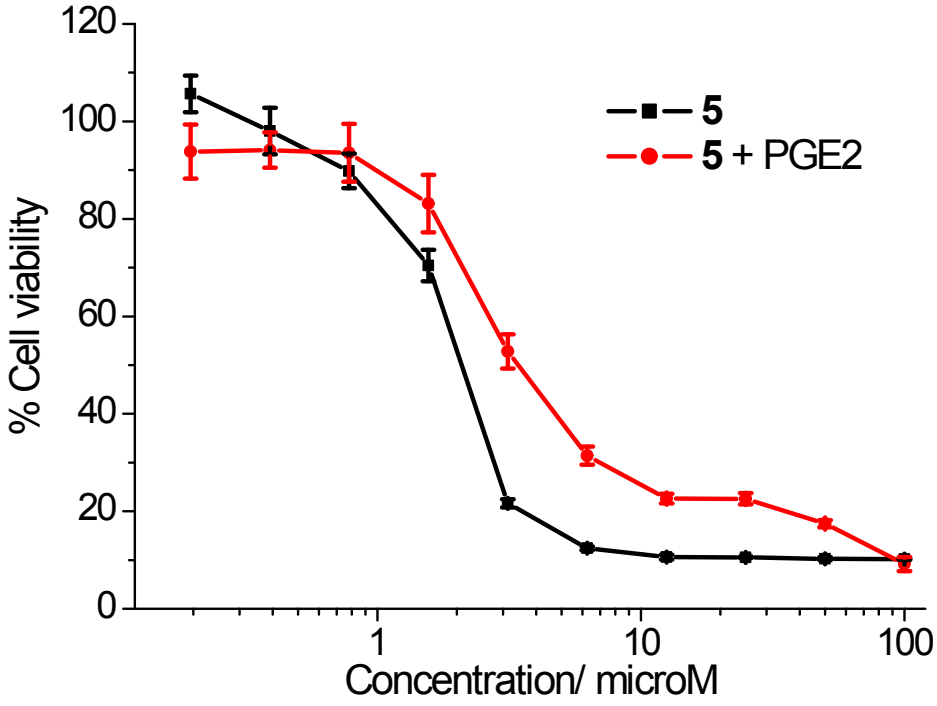

Fig. S41 Representative dose response curves of **5** against HMLER-shEcad cells in the absence and presence of PGE2 (20 μM) after 72 h incubation.

Reference

1. S. Ghosh, A. C. Barve, A. A. Kumbhar, A. S. Kumbhar, V. G. Puranik, P. A. Datar, U. B. Sonawane and R. R. Joshi, *J. Inorg. Biochem.*, 2006, **100**, 331-343.

2. K. Laws, A. Eskandari, C. Lu and K. Suntharalingam, *Chemistry*, 2018, DOI: 10.1002/chem.201803521.