Supplementary materials

Cellular Confocal Fluorescence Studies and Cytotoxic Activity of New Zn(II) Bisthiosemicarbazonato Complexes

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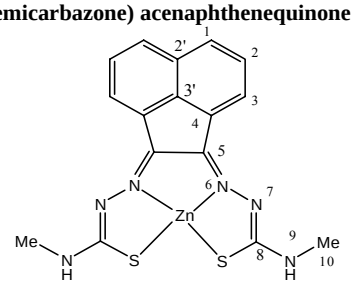
General Information

NMR spectra were run on a Varian Mercury 300 MHz spectrometer at 298K in d^6–DMSO solution and referenced to residual solvent peak. UV spectra measurements were run in DMSO using a Perkin Elmer UV/Vis/NIR spectrometer Lambda 19. Fluorescence spectra were measured in DMSO using a Hitachi F-4500 Fluorescence Spectrophotometer. ES-MS was carried out by the Inorganic Chemistry Spectrometry Service using a MicroMass LC time of flight electrospray mass spectrometer. HPLC spectra were recorded on a Gilson Unipoint instrument using a reverse phase column with a CH_3CN/H_2O mobile phase. LC MS studies were carried out on a Waters 2695 instrument using a Waters Atlantis dC18 column with a CH_3CN/H_2O mobile phase also containing 0.1 % v/v TFA. Elemental analyses were performed by the Inorganic Chemistry Laboratory Analytical Department. Crystallographic data of the extremely small crystals obtained were collected using the synchrotron radiation source at Station 9.8, Daresbury SRS, UK. Confocal Fluorescence Microscopy images were recorded using a Leica Confocal Fluorescence Microscope.

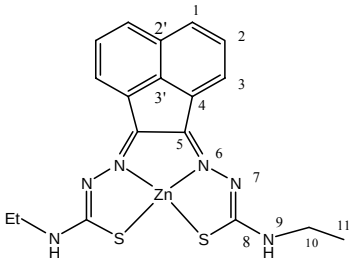
Preparation of zinc bis(4-methyl-3-thiosemicarbazone) acenaphthenequinone (1)



Acenaphthenequinone (0.5 g, 2.74 mmol) and zinc acetate bishydrate (1.807 g, 8.23 mmol) were suspended in glacial acetic acid (10 ml) and heated to 60^0C. 4-Methyl-3-thiosemicarbazide (1.20 g, 11.4 mmol) was then added to the suspension and the mixture heated under reflux for 2 hours. The orange-red solid was isolated by filtration whilst hot, then re-suspended in warm acetic acid (10 ml) and stirred for 15 minutes. The suspension was filtered and washed with diethyl ether (100 ml). The solid was recrystalised from a THF/pentane solvent mix, filtered and then dried under reduced pressure. Yield = 0.78 g, 1.87 mmol, 68%.

^{1}H NMR (300 MHz, d_6 –DMSO, 25^{o}C): δ 11.97 (s, 1H, CH_3COOH), 8.18 (d, 2H, H-3), 7.98 (d, 2H, H-1), 7.88 (m, 2H, NH), 7.75 (t, 2H, H-2), 3.02 (d, 6H, CH_3), 1.89 (s, 3H, CH_3 (acetic acid). **^{13}C NMR** (300 MHz, d_6 –DMSO, 25^{o}C): δ 179.6 (C-8), 172.5 (acetic acid C=O) 140.4 (C-5), 138.5 (C-3'), 131.0 (C-2'), 129.0 (C-2 or 4), 128.6 (C-2 or 4), 127.3 (C-1), 123.5 (C-3), 29.8 (C-10), 21.8 (CH_3 acetic acid). **ES MS**: m/z = 419.0 $[M + H]^+$**. IR**: 3200 w υ(NH), 3050 wυ(NH), 1585 m υ(C=N), 1574 m υ(C=N), 1455 m υ(ring), 1174 s υ(C-S), 1120 mw υ(N-N). **HPLC**: R_t = 16.9 mins. **Elemental:** Calculated (+CH_3COOH): N(17.57 %), C(45.19 %), H(3.79 %), Zn(13.37 %). Found: N(16.73 %), C(44.57 %), H(3.58 %), Zn(13.47 %).

Preparation of zinc bis(4-ethyl-3-thiosemicarbazone) acenaphthenequinone (2)



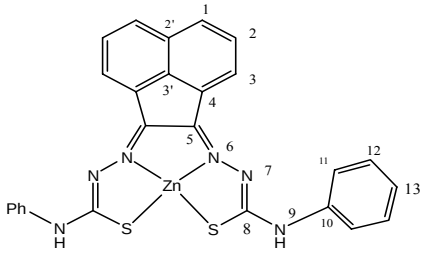
Acenaphthenequinone (0.5 g, 2.74 mmol) and zinc acetate bishydrate (1.807 g, 8.23 mmol) were suspended in glacial acetic acid (10 ml) and heated to 60^0C. 4-Ethyl-3-thiosemicarbazide (3.00 g, 25.16 mmol) was then added to the suspension and the mixture heated under reflux for 30 hours under an atmosphere of nitrogen. The red solid was isolated by filtration whilst hot, then re-suspended in warm acetic acid (10 ml) and stirred for 15 minutes. The suspension was filtered and washed with diethyl ether (100 ml). The solid was then recrystalised from a THF/pentane solvent mix, filtered and dried under reduced pressure. Yield = 0.877 g, 1.97 mmol, 72 %.

^{1}H NMR (300 MHz, d_6 –DMSO, 25^{o}C): 8.15 (d, 2H, H-3), 7.97 (d, 2H, H-1), 7.91 (m, 2H, N(H)Et), 7.70 (d, 2H, H-2), 3.35 (m, 4H, CH_2(Et)), 1.21 (t, 6H, CH_3(Et)), **^{13}C NMR** (300 MHz, d_6 –DMSO, 25^{o}C): δ 178.9(C-8), , 140.1(C-5), 138.4(C-3'), 131.0(C-2'), 129.0(C-2/4), 128.7(C-2/4), 127.3(C-1), 123.2(C-3), 37.7(C-9), 14.9(C-10).^{1}H resonances corresponding to 1 equivalent of acetic acid at δ: 11.97 (s, 1H, H acetic acid), 1.87 (s, 3H, CH_3 acetic acid) as well as ^{13}C resonances at 172.3 (acetic acid C=O) and 21.9 (CH_3 acetic acid) were also observed.
IR: 3254 wbr υ(NH), 1591 mbr υ(CN), 1567 mbr υ(CN), 1384 m υ(ring), 1176 υ(CS), 1142m υ(NN). **ES MS**: m/z = 447.1 $[M + H]^+$. **HPLC**: R_t = 19.3 mins. **Elemental Analysis:** Calculated (+CH_3COOH) N(16.60%), C(47.42%), H(4.38%), Zn(12.63%), Found; N(16.57%), C(46.51%), H(4.27%), Zn(12.74%).

Co-crystallisation of zinc[bis(4-ethyl-3-thiosemicarbazone) acenaphthenequinone] with 4,4'-bipyridyl (bipy) and 1,4-diazabicyclo[2.2.2]octane (DABCO)

Crystals of complexes **2**·DABCO and **2**·Bipy were grown from 1:2 mixtures of the lewis base (DABCO or Bipy) and complex **2**, dissolved in THF and layered with pentane at room temperature. The two complexes were characterized by synchrotron X-ray diffraction.

Preparation of zinc bis(4-phenyl-3-thiosemicarbazone) acenaphthenequinone (3)



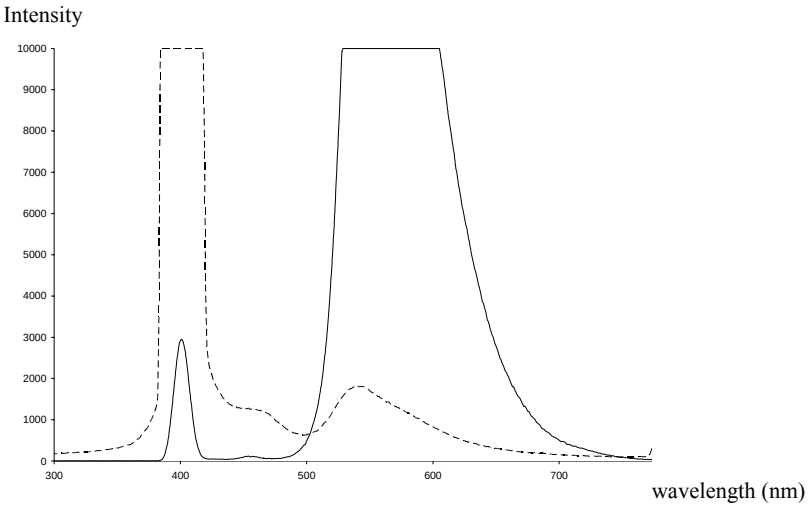
Acenaphthenequinone (0.182 g, 0.997 mmol) and zinc acetate bishydrate (0.656 g, 2.99 mmol) were suspended in glacial acetic acid (10 ml) and heated to 60^{0}C. 4-phenyl-3-thiosemicarbazide (1.50 g, 8.97 mmol) was then added to the suspension and the mixture heated under reflux for 24 hours, under an atmosphere of nitrogen. The dark red solid was isolated by filtration whilst hot, then re-suspended in warm acetic acid (10 ml) and stirred for 15 minutes. The suspension was filtered and washed with diethyl ether (100 ml). The solid was recrystalised from a THF/pentane solvent mix, filtered and then dried under reduced pressure. Yield = 0.397 g, 0.766 mmol, 77 %.
^{1}H NMR (300 MHz, d_6 –DMSO, 25^{o}C): δ 10.05 (s, 2H, N(H)Ph), 8.25 (d, 2H, H-3), 8.15 (d, 2H, H-1), 7.93 (d, 4H, H-11), 7.85 (t, H, H-2), 7.40 (t, 4H, H-12), 7.05 (t, 2H, H-13). **^{13}C NMR** (300 MHz, d_6 –DMSO, 25^{o}C): δ 177.2 (C-8), 142.3 (C-10), 140.4 (C-5), 138.6 (C-3'), 130.7 (C-2'), 128.4 (C-2), 128.3 (C-12), 128.0 (C-4), 127.5 (C-1), 123.5 (C-3), 122.7 (C-13), 120.6 (C11). **IR:** 3402 m υ(NH), 1592 m υ(CN), 1568 m υ(CN), 1479 mbr υ(ring), 1392 ssh υ(ring), 1175 m υ(CS), 1142 m υ(NN). **ES MS**: m/z= 543.1 $[M + H]^+$. **Elemental Analysis:** Calculated N(15.45%), C(57.40%), H(3.34%), Zn(12.02%), Found; N(15.07%), C(56.92%), H (3.54%), Zn(12.24%).

Table S1. Selected UV absorption data, calculated energies ΔE (eV) and molar absorbancies for **1-3**

Compound	λ_1(nm)	E(eV)	ε ($Lmol^{-1}$ cm^{-1})	λ_2(nm)	E(eV)	ε ($Lmol^{-1}$ cm^{-1})
1	359	3.46	28.76	481	2.58	7.68
2	361	3.45	33.74	488	2.54	11.76
3	372	3.34	32.74	510	2.43	16.58

Note. UV visible measurements were carried out in DMSO and in a 5:95 DMSO : water solvent mix (to mimic biological conditions). For all complexes the absorbance was measured at 0.01 mM concentration and λ_1 shows the strongest intensity for all complexes, with 3 to 4 times the absorbance of that observed for λ_2. Although the zinc(II) bis(thiosemicarbazones) absorb strongly at around 350 nm, the excited state does not fluoresce either for symmetry reasons or as a result of the energy being dissipated rapidly via non-radiative processes. Excitation at the weaker absorbing, higher wavelength gives rise to population of a state that fluoresces strongly and on a rapid timescale with respect to non radiative processes.

Figure S1. A comparison of the fluorescence intensities of **2** in (a) DMSO (solid line), and (b) 5:95 DMSO:water solvent mix (dashed line), at concentrations of 0.1 mM (λex = 400 nm)



Note. The fluorescence spectra of **1** and **2** in DMSO:water (5:95) showed emission wavelengths of 533 and 538 nm (λ_{ex} 400 nm, 10 μM conc.). Fluorescence of the acenaphthenequinone starting material was observed only at a concentration of 2.0 mM in 100 % DMSO, with emission observed at 590 nm for excitation at 480 nm.

Quantum yields:

The ratio of photons emitted to photons absorbed was calculated by the following expression:

$$\Phi_s = \Phi_r \cdot \left(\frac{D_s}{D_r}\right) \cdot \left(\frac{A_r}{A_s}\right) \cdot \left(\frac{I_r}{I_s}\right) \cdot \left(\frac{n_s}{n_r}\right)^2$$

Φ = quantum yield	*D* = integrated area under emission peak
s = sample	*A* = absorbance of solution at excitation wavelength
r = reference	*n* = refractive index of (pure) solvent
	I = maximum intensity of emission peak

$[Ru(bipy)_3][PF_6]_2$ in water was used as the reference material, where $\Phi_r = 0.042$

Absorbance was calculated according using the Beer Lambert Law with A=εcl,
where ε= molar absorptivity of sample ($Lmol^{-1}cm^{-1}$), c= concentration($molL^{-1}$) and l= pathlength(cm).

DFT Calculations

To qualitatively appreciate the number and intensities of the absorption bands, the nature of the frontier orbitals was investigated by use of DFT calculations. ADF calculations were performed using Vosko, Wilke and Nusair's local functional [1], with the Becke 88 [2] and the Perdew 86 [3] non local exchange and correlation gradient corrections, on ADF version 2000.02 [4-8]. The basis sets used were uncontracted triple-ξ Slater-type orbitals (STOs). The cores of atoms were frozen, C and N up to the 1s level, S and Zn up to the 2p level.

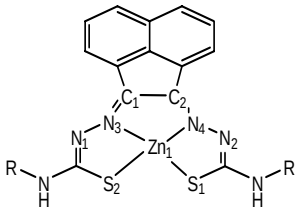
Of course, these calculations best model the gas-phase behavior. However, the results are entirely consistent with the recently reported TD-DFT calculations on Cu(ATSM) and Zn(ATSM) [9] Since the frontier orbital sets of compounds **1**-**3** show strong similarities with those of known Zn(ATSM), similar transitions were assigned to the observed absorption bands in the spectra of **1**. [9] Solution phase DFT calculations would more accurately ascertain what contribution the individual orbital transitions make to the observed absorbance bands. These qualitative calculations strongly indicate that the naphthalene

backbone unit leads to enhanced fluorescence of zinc bis(thiosemicarbazone) units with respect to the known aliphatic backbone compounds.

Table S2. DFT-estimated HOMO-LUMO and HOMO(-1)-LUMO gaps in **1-3.**

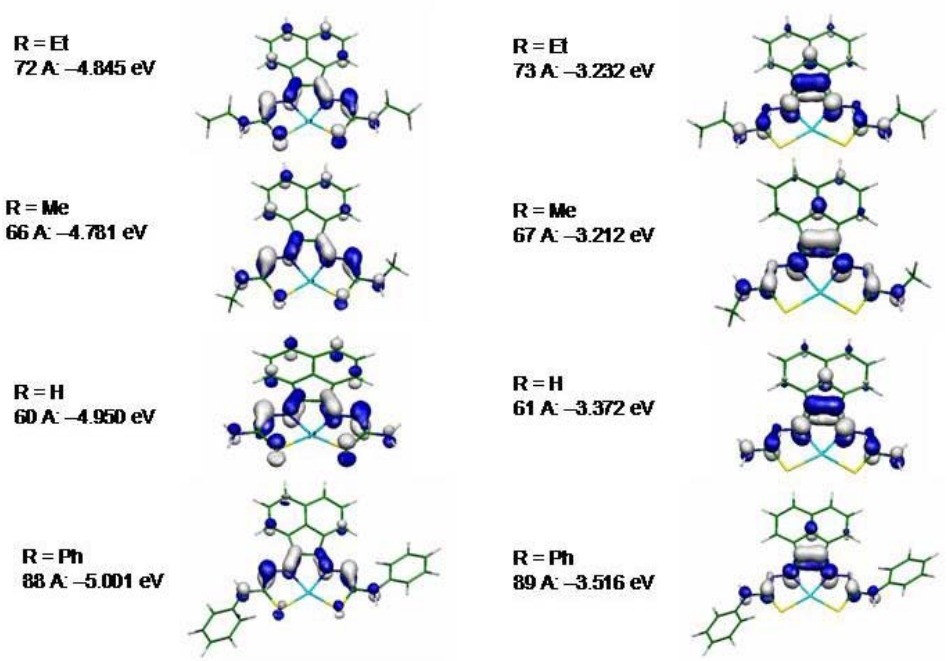
Comp.	HOMO (-1)	HOMO	LUMO	Δ HOMO (-1)-LUMO	Δ HOMO-LUMO
1	-5.332	-4.781	-3.212	2.120	1.569
2	-5.269	-4.845	-3.231	2.038	1.613
3	-5.455	-5.001	-3.516	1.939	1.485

Table S3. DFT-level optimized geometries for **1-3.** (where R = Me (**1**) , Et (**2**), Ph (**3**))



		1	2	3
Bond lengths	Zn1-N1	1.129	2.122	2.096
	Zn1-N2	2.126	2.121	2.137
	Zn1-S1	2.305	2.326	2.289
	Zn1-S2	2.321	2.324	2.298
	C1-C2	1.468	1.472	1.475
	C1-N1	1.290	1.290	1.294
	C2-N2	1.294	1.291	1.292
	N2-N3	1.313	1.317	1.314
	N1-N6	1.320	1.318	1.325
	C3-N3	1.328	1.330	1.340
	C4-N6	1.338	1.331	1.351
Bond angles	N1-Zn1-N2	77.29	77.47	72.88
	N1-Zn1-S1	82.15	81.79	82.87
	N2-Zn1-S2	81.83	81.78	82.02
	S1-Zn1-S2	118.74	118.97	117.29

Figure S2. Schematic representations of gas phase DFT calculated frontier orbitals for Zn(II) complexes with a range of substituents (Me, Et, Ph, H) at the exocyclic nitrogens



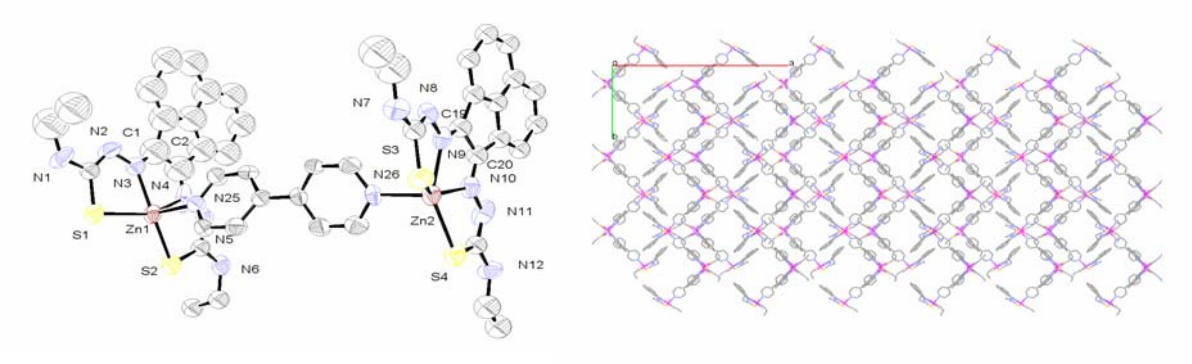
Crystallographic data

Data collection for **2**·DABCO and **2**·Bipy was carried out using the synchrotron radiation source at Station 9.8, Daresbury SRS, UK, on a Bruker SMART CCD diffractometer. The structures were solved by direct methods using the program SIR92 [10]. The refinement (on F) and graphical calculations were performed using the CRYSTALS program suite [11].

Crystal data **2**·DABCO: $C_{42}H_{46}N_{14}S_4Zn_2$ M = 1005.95, Z = 4, monoclinic, space group $C_{2/c}$, a = 11.835(2), b = 21.206(5), c = 23.024(5) β=98.524(2), U = 5714(2) $Å^3$, T = 150 (2) K, μ=1.024 mm^{-1}, synchrotron radiation λ = 0.68920 Å. Of 3495 reflections measured, 3495 were independent (Rint = 0.08). Final R = 0.0761 (1933 reflections with I > 2σ (I)) and wR =0.0802

Crystal data **2**·Bipy: $C_{46}H_{44}N_{14}S_4Zn_2$, C_{46} H_{44} N_{14} S_4 Zn_2, M = 2103.02, Z = 4, monoclinic, space group $P_{21/c}$, a=37.388(2), b=18.0247(12), c=18.1852(11) alpha=90 beta=96.789(1), gamma=90, U = 12169.2(13) $Å^3$, T = 150 (2) K, μ= 0.871 mm^{-1}, synchrotron radiation λ = 0.68920 Å. Of 51183 reflections measured, 20787 were independent (Rint = 0.08). Final R = 0.0893 (5256 reflections with I > 3σ (I)) and wR =0.0929.

Figure S3. Molecular structure (a) and cell packing diagram, view over axis b (b) of the complex **2**·Bipy.



a **b**

Table S4. Selected bond lengths and angles for the X-ray structures of 2 ·DABCO and 2·Bipy

		2 ·DABCO		**2·Bipy**
Bond lengths (Å)	Zn1 . N3 Zn1 . N4 Zn1 . N7 Zn1 . S1 Zn1 . S2 C1 . C2 N2 . N3 N4 . N5 N2 . N3	2.132(9) 2.132(8) 2.168(7) 2.378(3) 2.368(3) 1.508(13) 1.338(11) 1.384(10) 1.338(11) 1.368(15) 1.329(13) 1.384(10) 1.277(12) 1.325(12) 1.405(2) 1.476(2) 1.478(11) 1.476(14) 1.475(12) 1.405(2) 1.476(2) 1.132(16) 1.405(2) 1.019(18) 1.476(2)	Zn1 N3 Zn1 N25 Zn1 N4 Zn1 S1 Zn1 S2 Zn2 N26 Zn2 N10 Zn2 N9 Zn2 S3 Zn2 S4 S1 C13 S2 C16 S3 C31. S4 C34 Zn3 N27 Zn3 N15 Zn3 N16 Zn3 S5 Zn3 S6 Zn4 N28. Zn4 N21 Zn4 N22 Zn4 S7 Zn4 S8. S5 C49 S6 C52. S7 C67 S8 C70 C1 C2 C19 C20 C55 C56 C37 C38. N2 N3 N4 N5 N8 N9 N10 N11 N14 N15 N16 N17 N20 N21 N22 N23	2.119(5) 2.130(5) 2.174(5) 2.3759(16) 2.3949(18) 2.110(5) 2.155(5) 2.157(5) 2.3730(16) 2.3935(18) 1.754(7) 1.741(7) 1.755(7) 1.728(6) .2.122(6) .2.128(6) .2.173(6) 2.356(2) 2.396(2) 2.108(6) 2.150(6) 2.164(7) 2.372(2) 2.404(3) 1.754(8) 1.727(8). 1.731(9) 1.726(9) 1.489(9) 1.491(8) 1.488(11) 1.488(10) 1.375(7) 1.340(7) 1.375(6) 1.349(7) 1.376(8) 1.314(9). 1.382(8) 1.316(9)?.
Bond angles (°)	N3 . Zn1 . N4 N3 . Zn1 . N7 N4 . Zn1 . N7 N3 . Zn1 . S1 N4 . Zn1 . S1 N7 . Zn1 . S1 N3 . Zn1 . S2 N4 . Zn1 . S2 N7 . Zn1 . S2 S1 . Zn1 . S2	75.5(3) 98.0(3) 98.4(3) 79.7(3) 150.7(2) 100.4(2) 150.9(3) 79.6(2) 100.30(19) 118.49(13)	N3 Zn1 N25 N3 Zn1 N4 N25 Zn1 N4 N3 Zn1 S1 N25 Zn1 S1 N4 Zn1 S1 N3 Zn1 S2 N25 Zn1 S2 N4 Zn1 S2 S1 Zn1 S2 N26 Zn2N10 N26 Zn2 N9 N10 Zn2 N9 N26 Zn2 S3 N10 Zn2 S3 N9 Zn2 S3 N26 Zn2 S4 N10 Zn2 S4 N9 Zn2 S4 S3 Zn2 S4	96.1(2) 75.64(19) 97.22(19) 79.82(14) 103.46(13) 149.34(15) 150.59(14) 102.63(15) 79.67(15) 116.70(7) 101.19(18) 97.71(19) 75.06(18) 105.59(13) 144.77(14) 79.00(13) 103.17(14) 79.53(15) 149.72(13) 115.31(6)

Cell culture and fluorescence cell plates preparations

Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 in air and diluted once confluence had been reached. Cells were cultured in DMEM medium with 10% foetal calf serum (FCS) and 100U/ml penicillin. The medium contained no fluorescent indicator dyes such as phenol red and was therefore suitable for use in fluorescent studies. Samples for fluorescence were prepared in the following way: surplus supernatant containing dead cell matter and excess protein was discarded; the live adherent cells were then washed with two 5 ml aliquots of Phosphate Buffer Saline solution to remove any remaining medium containing FCS. FCS inhibits resuspension of the cells as it contains protease inhibitors which inactivate trypsin. To resuspend the cells in solution, they were incubated in 3 ml of trypsin / EDTA (500 mg/L Trypsin, 200 mg/L EDTA) solution for five minutes at 37°C. After trypsinising, fresh DMEM was added to the suspended cells to give a sufficient concentration of cells. The concentration of cells required varies between cell lines and is chosen to be optimal for achieving sufficient coverage and optimal imaging. The cells were plated in a Petri dish containing a glass cover slip and left for 24 hours to adhere before fluorescence imaging measurements were made.

***In vitro* Confocal fluorescence imaging study**

In vitro uptake studies were carried out on a range of adherent human cells to gain an interpretation of the effects different cell lines have on the intracellular distribution. The cell lines used were: IGROV – an epithelial-like ovarian carcinoma, plated at 5,000 cells/ml, MCF-7 – breast carcinoma plated at 5,000 cells/ml, T24 – colon carcinoma, plated at 2,000 cells/ml, SW620 - bladder carcinoma, plated at 6,000 cells/ml and A431 – epidermic carcinoma, plated at 3000 cells/ml.

Figure S4. Uptake profiles determined by fluorescence imaging. (a) Probe **2** in SW620; (b) Probe **3** in SW620; (c) Probe **1** in A431; (d) Probe **2** in A431; (e) Probe **2** in T24 and (f) Probe **3** in T24. (λ_{ex} 488 nm, λ_{em} 515 nm)

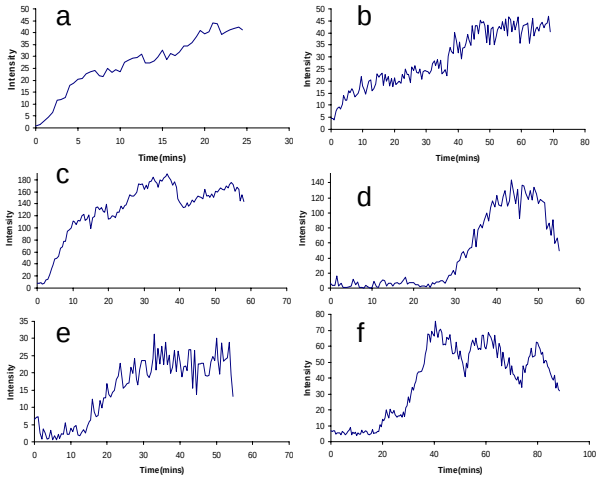
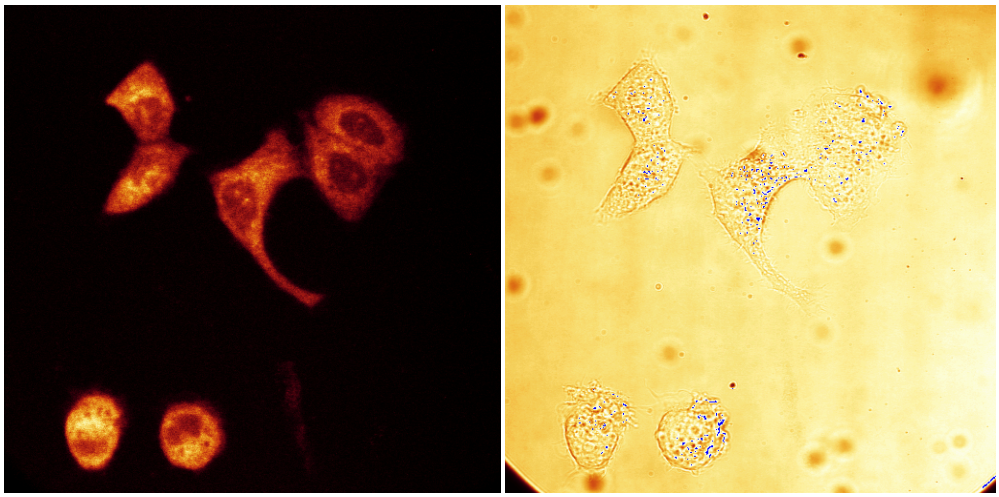
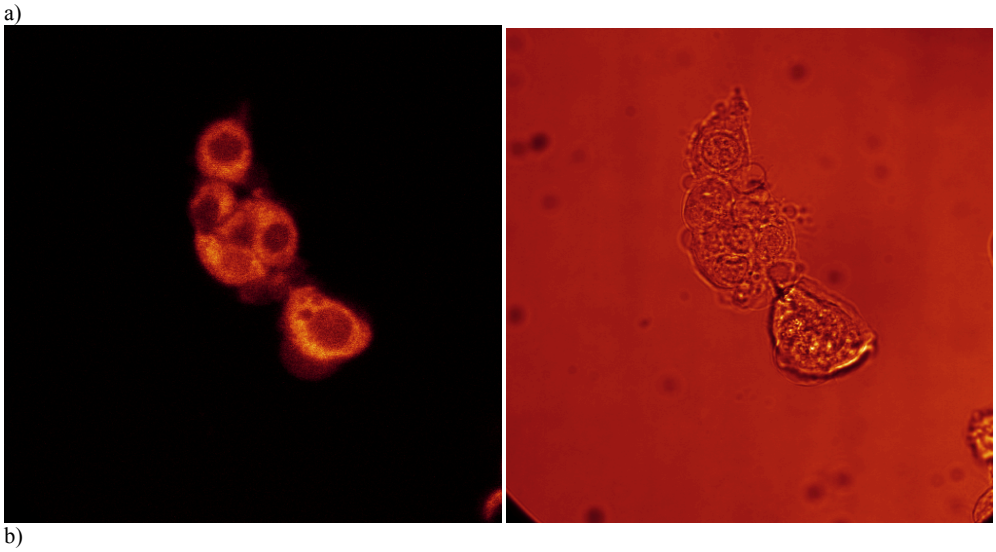


Figure S5. a) MCF-7 cells incubated at 4^{o} C for 3h with Compound **2**: confocal fluorescence and brigthfield images. b) MCF-7 cells incubated at 37^{o} C for 3h with Compound **2**: confocal fluorescence and brigthfield images.
N.B. Only uptake in cytoplasm observed in both cases.

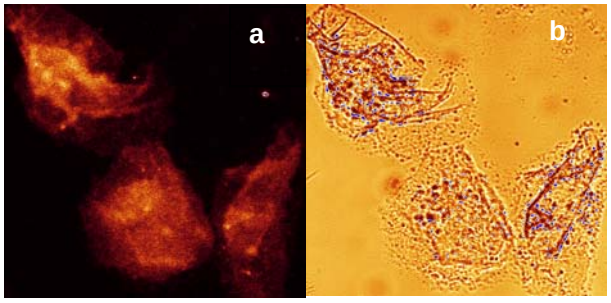


a)



b)

Figure S6. Confocal fluorescence image of **2** in the MCF-7 cell line (λ_{ex} 488 nm, λ_{em} 515 nm, 37^{o} C , after 12 h incubation with **2**) and corresponding brightfield image.



Cytotoxicity studies on U937, IGROV, T24, SW620 and A431 cell lines
The following method details how U937 cells were cultured and the setup of the cytotoxicity experiment with this cell line: Cells were cultured at 37^{0}C in a humidified atmosphere of 5% CO_2 in air. The cells were grown in suspension in RPMI 1640 medium (Invitrogen) with added 2mM glutamine and antibiotics (100 units/ml penicillin and 100μg/ml streptomycin). Cells were diluted when they reached a density of about 5x10^{5}/ml.

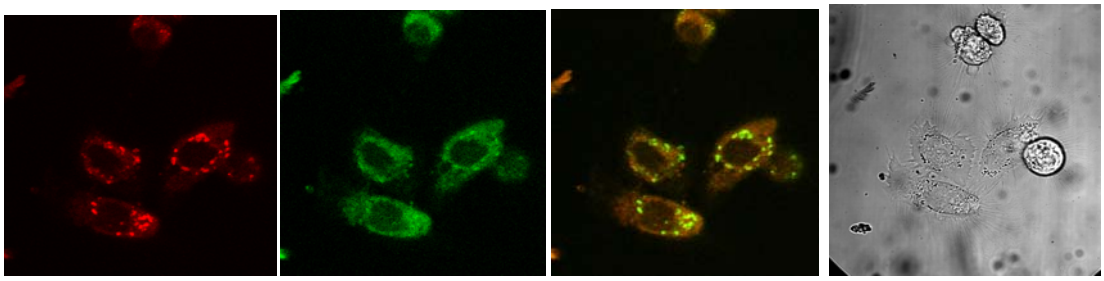
Cells were seeded onto a 96 well plate at cell concentrations of approximately $2x10^5$/ml in 200 µl of cell medium. To this 50 µL of the complex solution was added.

The following methods detail how the adherant cell lines (IGROV, T24, SW620 and A431) were cultured and the setup of the cytotoxicity experiment with these cell lines. As for the U937 cell line the adherent cells were cultured in RPMI 1640 medium as opposed to the DMEM used for fluorescence imaging as the former is the preferred medium for optimal growth.

In testing the cytotoxicity it was of interest to find the LC_{50} of a compound, which is the concentration of the compound that gives rise to a 50% cell death over an incubation period with respect to what is observed in medium alone. Preliminary work involved looking for the best technique by which to measure the numbers of live and dead cells. A selection of methods was attempted before deciding to use a trypan blue stain and haemocytometer grid to count a sample of the cells. The trypan blue stain is taken up by dead cells but cannot permeate the cell membrane of live cells. Initial experiments were run with cell counts taken at 0, 6, 12, 24, 48 and 96 hours, using an arbitrarily set final concentration of compound of 100 µM in a 1:99 DMSO:cell medium solution. Details of experimental setup are as follows: SW620 cells were seeded in 6-well plates at a suspended cell concentration of approximately $5x10^5$ cells/ml in RPMI 1640 medium (4ml per well). The cells were left to adhere for 6 hours. The supernatant was then discarded and replaced with 4 ml fresh medium containing a solution of the compound of interest (100, 50, 25 and 12.5 µM) in DMSO to give a final overall DMSO concentration of 1%. After 48 hours, the supernatant was collected and the adhered cells washed carefully with 2x1 ml PBS and trypsinised with 100 µL of trypsin/EDTA solution for 5 minutes at room temperature. The cell suspension was then made up to 1 ml with fresh medium and cell counts performed. Counts were done on both the supernatant collected and for the newly resuspended cells i.e. those still adhered after 48 hours.

A similar setup was followed for the T24 and A431 cell lines. The T24 cells were seeded at a suspended concentration of $2x10^5$ cells/ml (for compound concentrations of 50, 25, 12.5 and 6.25µM). The A431 cells were seeded at a suspended concentration of $4x10^5$ cells/ml (for compound concentrations of 100, 50, 25 and 12.5 µM).

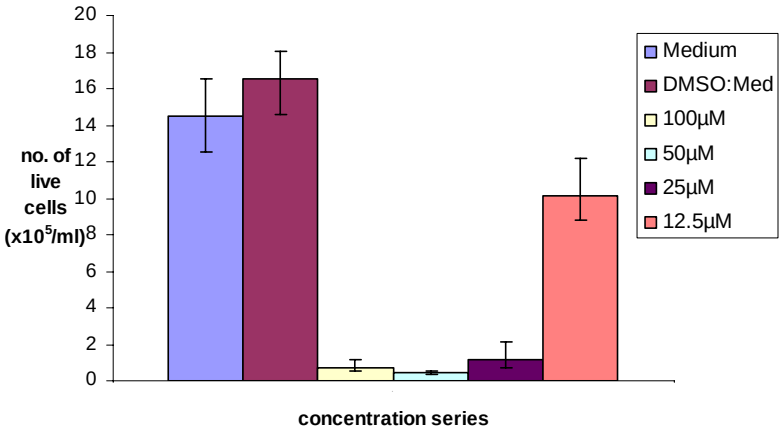
Figure S7. Confocal fluorescence imaging of IGROV cell line: (a) Lysotracker dye (λ_{ex} 568 nm λ_{em} 605 nm); (b) Compound **2** (λ_{ex} 488 nm, λ_{em} 515 nm); (c) Overlay of (a) and (b) indicating the colocalisation of Lysotracker dye and Compound **2** (d) Brightfield image of cells.

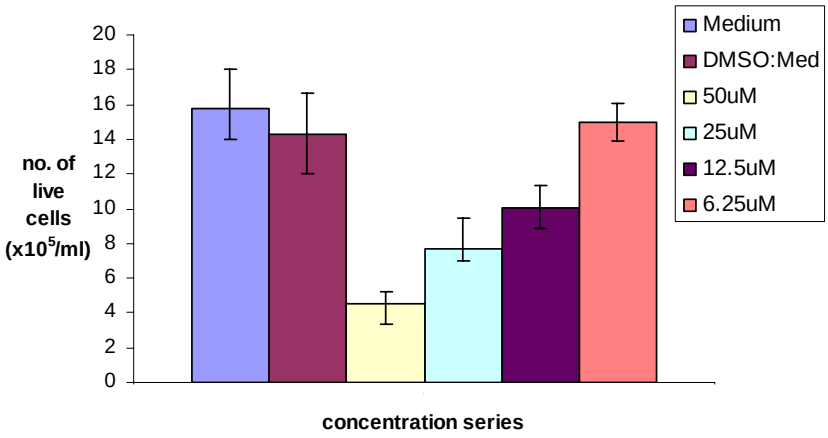


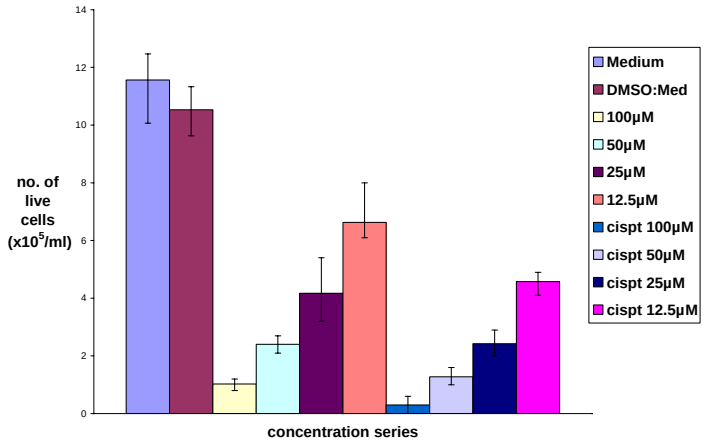
Cytotoxicity studies on MCF-7 cancer cell line

MCF-7 cells; an adherent cell line, were cultured at 37^0C in a humidified atmosphere of 5% CO_2 in air. The cells were cultured in DMEM (Sigma) with added 10% Foetal Calf serum, 2mM glutamine and antibiotics (100 units/ml penicillin and 100µg/ml streptomycin). Cells were diluted when they reached a density of about $1x10^6$/ml.
Cells were seeded in 6-well plates at a suspended cell concentration of approximately $2x10^5$ cells/ml in DMEM (4 mL per well). The cells were left to adhere for 6 hours. The supernatant was then discarded and replaced with 4 ml fresh medium containing a solution of the compound of interest (100, 50, 25 and 12.5 µM) in DMSO to give a final overall DMSO concentration of 1%. After 48 hours, the supernatant was collected and the adhered cells washed carefully with 2x1ml PBS and trypsinised with 100µL of trypsin/EDTA solution for 5 minutes at room temperature.
The cell suspension was then made up to 1 ml with fresh medium and cell counts performed using a trypan blue stain and haemocytometer grid to count a sample of the cells (The trypan blue stain is taken up by dead cells but cannot permeate the cell membrane of live cells). Repeat counts of live and dead cells for each experiment were made on four separate wells. The average number of viable cells is recorded with error bars indicating the range in counts made.

Figure S8. Cytotoxicity studies. Comparison of number of live cells with concentration of **2** for: a) SW620 cell line, a) T24 cell line, and c) MCF-7 cell line with direct comparison with cis-platin.
N.B. Results are averages from four separate counts with error bars indicating the range in numbers recorded.







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