A lipophilic copper(II) complex as an optical probe for intracellular detection of NO

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Supplementary information

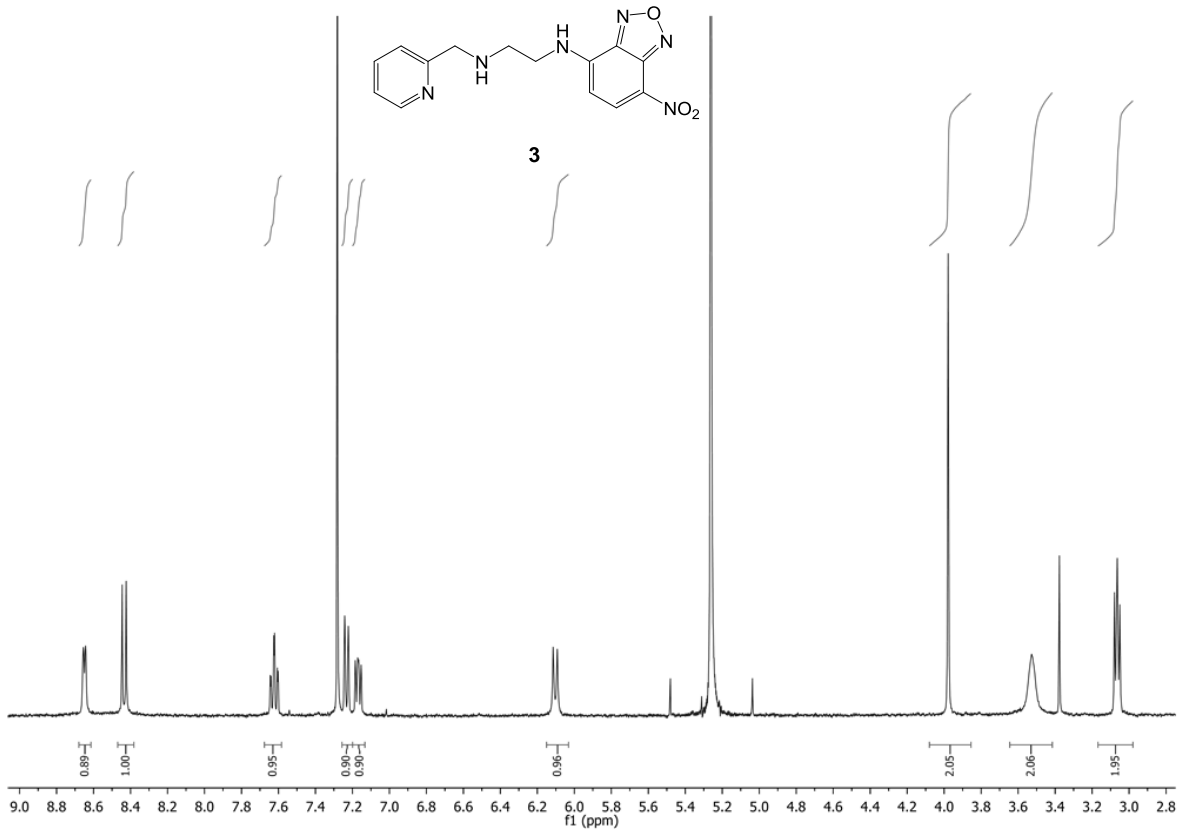

Figure S1. ^{1}H NMR spectrum of ligand **3** in a mixture of $CDCl_3$ and CD_2Cl_2 (peaks at 7.2 and 5.2 ppm correspond to residual solvents)

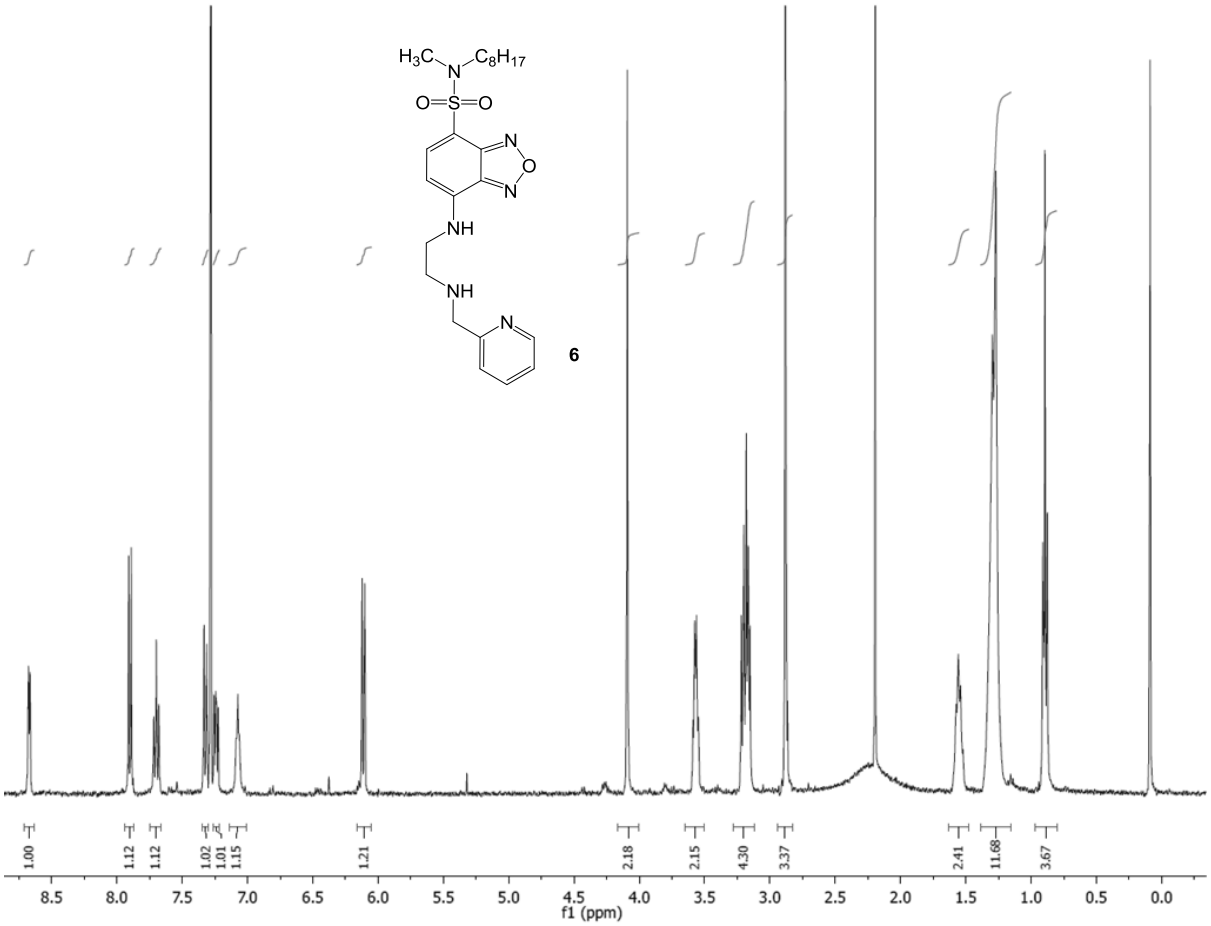

Figure S2. ^{1}H NMR spectrum of ligand **6** in $CDCl_3$

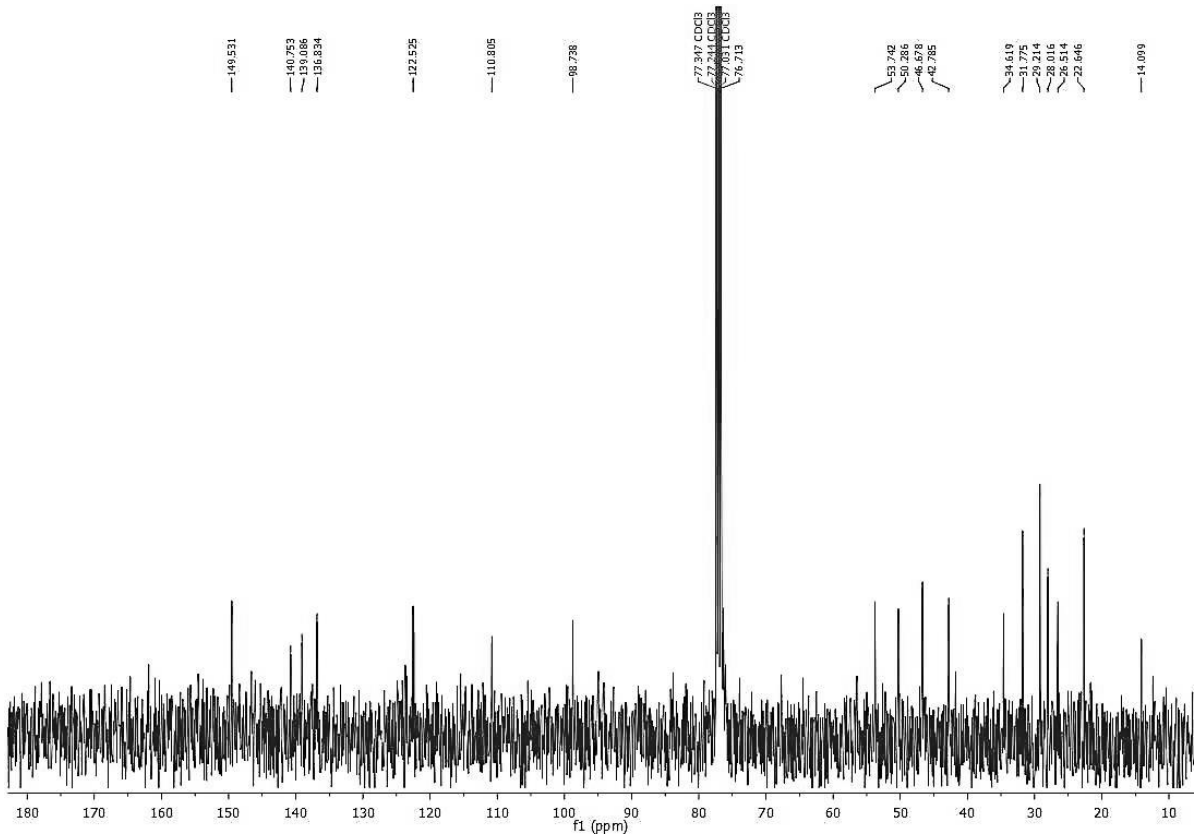

Figure S3. ^{13}C NMR spectrum of ligand **6** in $CDCl_3$.

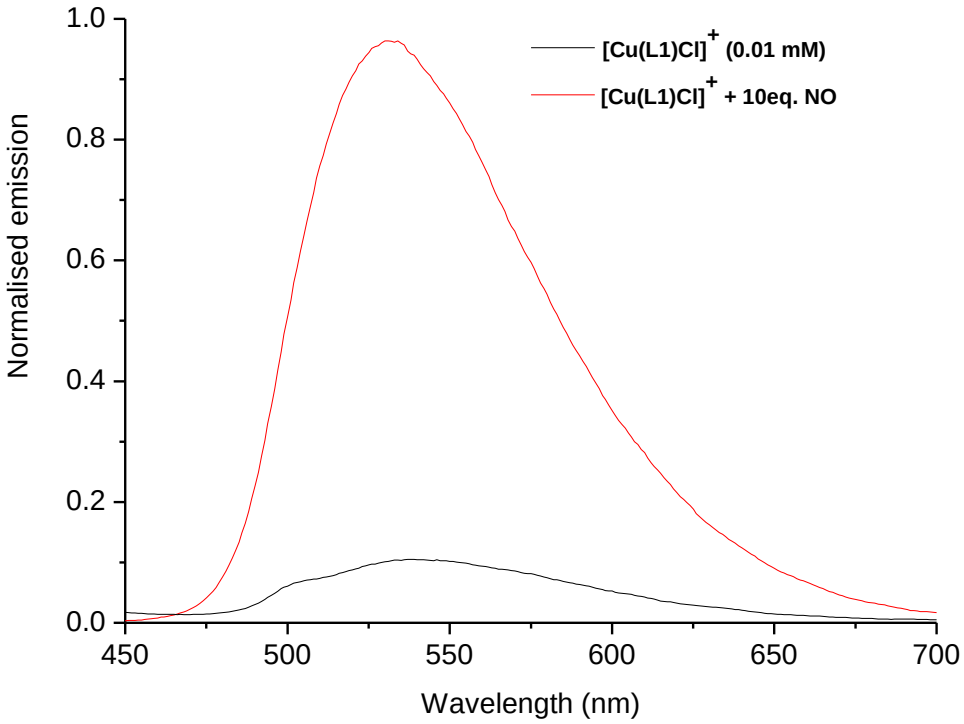

Figure S4. Fluorescent enhancement of complex **4** (0.01 mM) in presence of 10 eq. of NO (red trace) generated from DEA-NO (50 mM PIPES, pH 7.0, 100 mM KCl).

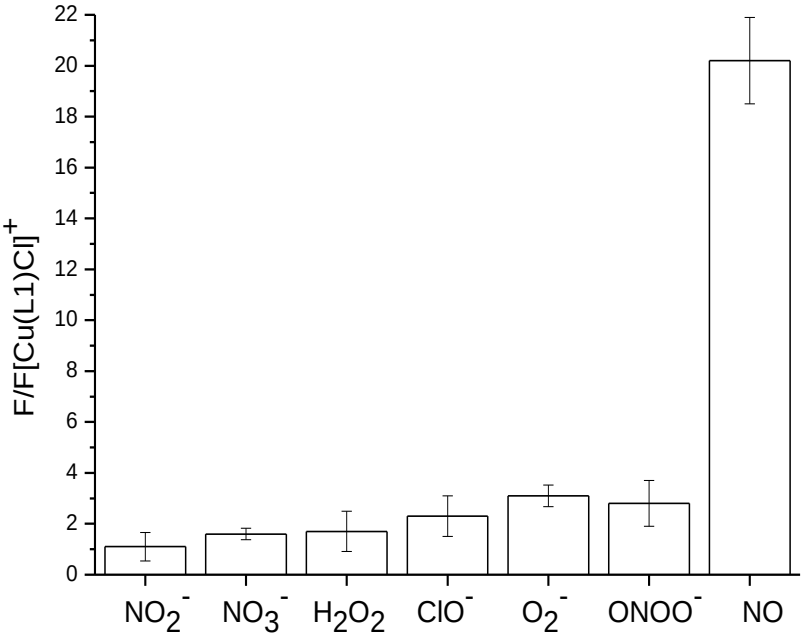

Figure S5. The fluorescent response of complex **4** (540 nm) after addition of 100 eq. of NO_2^-, NO_3^-, H_2O_2, ClO^-, $O_2^{-\cdot}$ and NO for 1 hour in buffered PBS (pH 7). All data (emission intensity, F) are normalised with respect to the emission of a 10 µM solution of **4** (50 mM PIPES, pH 7.0, 100 mM KCl).

Cytotoxicity studies using MTT assay.

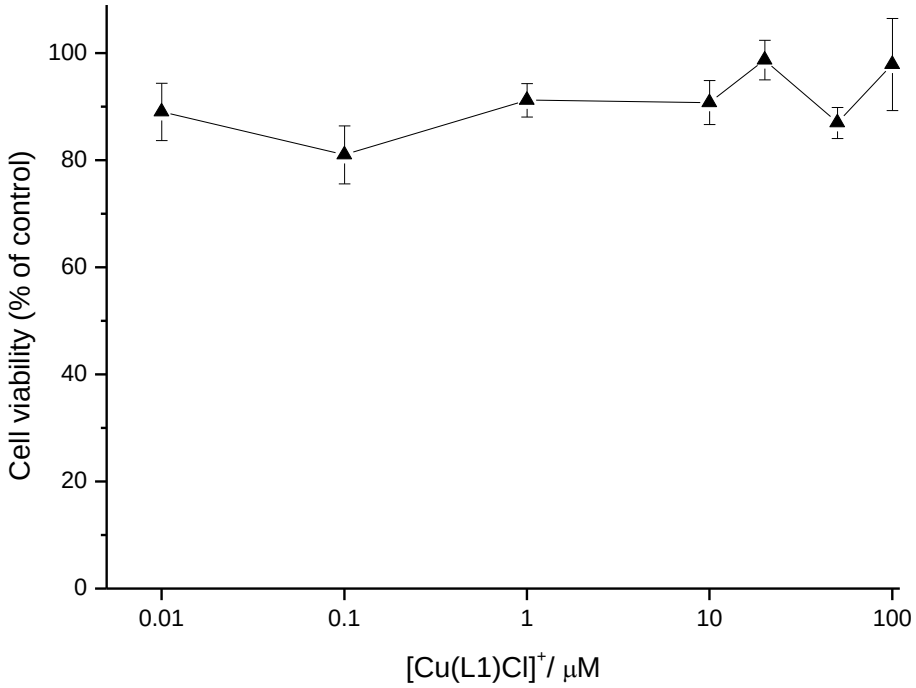

Figure S6. MTT cytotoxicity assay of **4** against NIH 3T3 mouse fibroblasts cells. NIH 3T3 cells were transferred to a 96 well plate in 90 µl of phenol red free media and 10 µl of **4** (0.01 – 100 mM) was added and incubated for 1 hour (37ºC). To each well was then added 20 µL of 5 mg/ml of thiazolyl blue tetrazolium bromide (yellow) and incubated for 3.5 hours (37ºC). The media was removed carefully and the precipitated formazon (purple) was dissolved in 150 µL of MTT solvent (4 mM HCl, 0.1% Nondet P-40). The plate was analysed by UV-vis spectroscopy at 590 nm and compared against the control to determine cell viability in the presence of **4**.

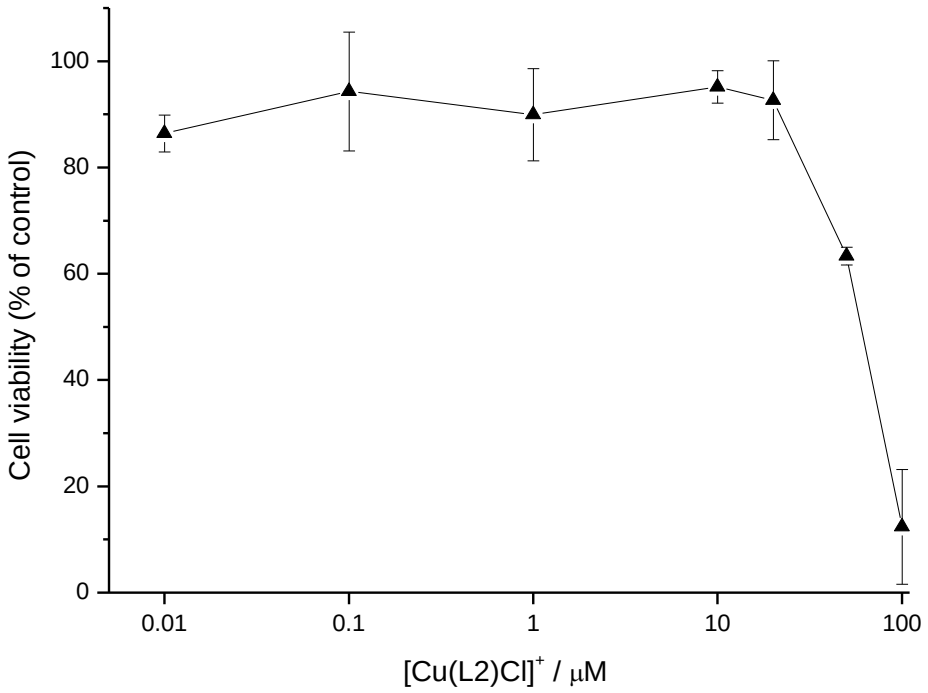

Figure S7. MTT cytotoxicity assay of **7** against NIH 3T3 mouse fibroblasts cells. NIH 3T3 cells were transferred to a 96 well plate in 90 µl of phenol red free media and 10 µL of **7** (0.01 – 100 mM) was added and incubated for 1 hour (37^{o}C). To each well was then added 20 µL of 5 mg/ml of thiazolyl blue tetrazolium bromide (yellow) and incubated for 3.5 hours (37^{o}C). The media was removed carefully and the precipitated formazon (purple) was dissolved in 150 µL of MTT solvent (4 mM HCl, 0.1% Nondet P-40). The plate was analysed by UV-Vis spectroscopy at 590 nm and compared against the control to determine cell viability in the presence of **7**.

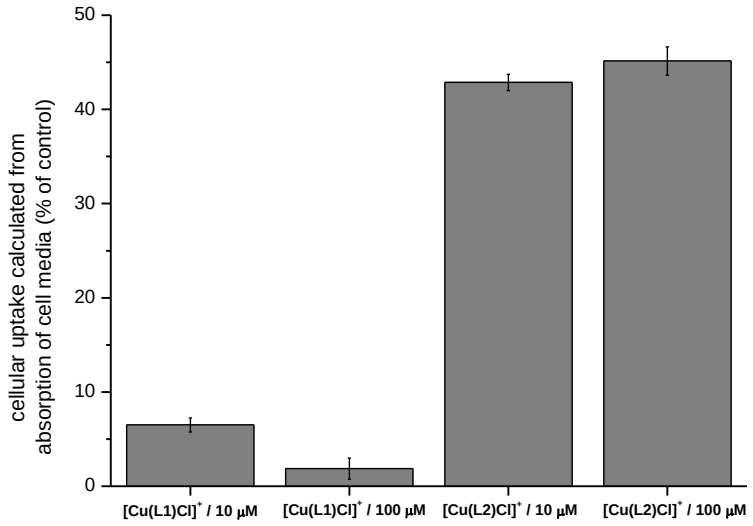

Figure S8. Uptake determination in cell media of **4** (i.e. [Cu(**L1**)Cl]Cl) vs. **7** (i.e. [Cu(**L2**)Cl]Cl) by NIH 3T3 mouse fibroblast cells measured via UV/Vis spectroscopy. NIH 3T3 cells were incubated for 1 hour at 37^{o}C with 500 µL of 10 and 100 µM solutions of **4** or **7** in phenol red free media. After this time, the cells were spun down and 200 µl aliquots of cell free media were transferred to a 96 well plate and the absorbance was measured at 480 nm for **4** and 440 nm for **7.** The values were compared with the controls (known concentration of compound in cell free, phenol red free media) in order to determine the percentage of cellular uptake (triplicate +/- SD) of each compound.