Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

2-nitroimidazole based fluorescent probes for nitroreductase; monitoring reductive stress *in cellulo*

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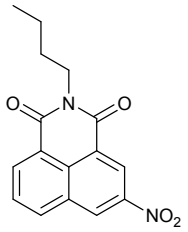
General remarks

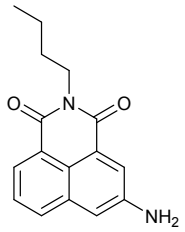
^{1}H NMR spectra were recorded using a Bruker Avance III 500 at a frequency of 500.13 MHz, and are reported as parts per million (ppm) with either DMSO-*d*$_6$ (δ_H 2.50 ppm) or $CDCl_3$ (δ_H 7.26 ppm) as an internal reference. The data are reported as chemical shift (δ), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (*J* Hz) and relative integral. ^{13}C NMR spectra were recorded using a Bruker Avance III 500 at a frequency of 125.76 MHz and are reported as parts per million (ppm) with either DMSO-*d*$_6$ (δ_H 39.5 ppm) or $CDCl_3$ (δ_H 77.2 ppm) as an internal reference. High resolution ESI spectra were recorded on an Agilent 6310 LCMS TOF. Analytical TLC was performed using pre-coated silica gel plates (Merck Kieselgel 60 F254). Microwave irradiation of reaction mixtures was performed using a CEM Discover SP microwave controlled by Synergy^{TM} software. Synthesis of compound **3** was carried out as described by Conway and co-workers.[1]

3-nitro-*N*-butyl-1,8-naphthalimide (7)



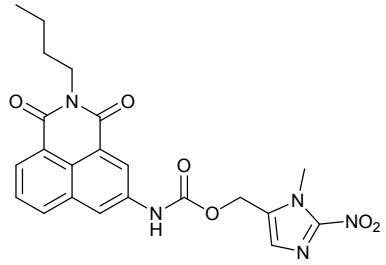
3-nitro-1,8-naphthalic anhydride (1 g, 4.1 mmol, 1 eq.) and butyl amine (0.31 g, 0.406 ml, 4.1 mmol, 1 eq.) were dissolved in EtOH (17 mL) before being heated at 110 ^{o}C for 1 hr under microwave irradiation. The mixture was allowed to cool to room temperature before the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography eluting with DCM. The purified product was isolated as a cream solid (1.2 g, 97% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) σ 9.41 (d, 1H, J = 2.3 Hz), 8.89 (d, 1H, J = 2.3Hz), 8.72 (dd, 1H, J = 8.4/0.82 Hz), 8.62 (dd, 1H, J = 7.4/1.2 Hz), 8.01 (dt, 1H, J = 7.4/0.75 Hz), 4.03 (t, 2H, J = 7.49 Hz), 1.62 (quin, 2H, J = 7.48 Hz), 1.36 (sex, 2H, J = 7.47 Hz), 0.93 (t, 3H, J = 7.47 Hz); **^{13}C NMR** (125 MHz, DMSO-d_6): 163.24, 162.73, 146.29, 136.74, 134.38, 131.31, 130.12, 129.97, 129.71, 124.48, 123.32, 123.04, 29.99, 20.25, 14.19; **HRMS** (ESI): Calculated for $C_{16}H_{15}N_2O_4$ $[M+H]^+$, expected: 299.1035, observed: 299.1026, PPM: 3.03; **ν_{max} (film)/cm^{-1}:** 3089.47, 2965.46, 2937.14, 2876.14, 1993.10, 1837.2, 1707.12, 1598.98, 1547.54, 1508.57, 1462.63, 1437.21, 1418.73, 1352.05, 1327.24, 1267.73, 1241.87, 1203.19. **MP:** 130~136 ^{o}C.

3-amino-*N*-butyl-1,8-naphthalimide (5)



3-nitro-*N*-butyl-1,8-naphthalimide (1.2g, 4 mmol, 1 eq.) was dissolved in MeOH (70 ml) before Pd/C (0.25 g) was added and the reaction was placed under an atmosphere of H_2. The reaction was allowed to proceed for 2 hrs before being filtered through a pad of celite. The solvent was removed under reduced pressure to yield a bright yellow solid. (1.05 g, 98% yield) **^{1}H NMR** (500 MHz, DMSO-d_6) σ 8.13 (dd, 1H, J = 7.12/1.13Hz), 8.08 (dd, 1H, J = 8.4/1.0Hz), 8.02 (d, 1H, J = 2.32Hz), 7.67 (dt, 1H, J = 7.3/1.03Hz), 7.34 (d, 1H, J = 2.32Hz), 6.04 (s, 1H), 4.08 (t, 2H, J = 7.4Hz), 1.65 (quin, 2H, J = 7.32Hz), 1.41 (sex, 2H, J = 7.48Hz), 0.98 (t, 3H, J = 7.48Hz). **^{13}C NMR** (125 MHz, DMSO-d_6): 164.24, 164.05, 148.34, 134.02, 131.92, 127.42, 125.88, 123.04, 122.24, 122.18, 121.04, 112.18, 79.42, 30.17, 20.27, 14.19; **HRMS** (ESI): Calculated for $C_{16}H_{17}N_2O_2$ $[M+H]^+$, expected: 269.1298, observed: 269.1285, PPM: 4.86; **ν_{max} (film)/cm^{-1}:** 3467.96, 3364.16, 3065.44, 2959.11, 2863.58, 1691.26, 1652.47, 1629.40, 1580.68, 1516.78, 1449.69, 1388.59, 1341.53, 1306.06, 1218.14. **MP:** 150~162 °C.

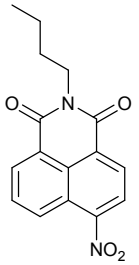
(2-Butyl-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)-carbamic acid 3-methyl-2-nitro-3H-imidazol-4-ylmethyl ester (2)



3-amino-*N*-butyl-1,8-naphthalimide (48 mg, 0.18 mmol, 1 eq.) and DMAP (66 mg, 0.54 mmol, 3 eq.) were dissolved in anhydrous DCM (20 mL) and placed under an argon atmosphere. The reaction was cooled to -10°C using an ice and salt bath before phosgene solution (15 wt. % in toluene; 0.7 ml, 1.07 mmol, 6 eq.) was added dropwise and the reaction mixture was allowed warm to room temperature and stirred for 4 hrs. The reaction mixture was reduced to dryness by bubbling with argon before the residue was redissolved in anhydrous DCM and cooled to 0 °C (20 mL). (3-Methyl-2-nitro-3H-imidazol-4-yl)-methanol **3** (84 mg, 0.53 mmol, 3 eq) was added and the reaction was allowed to return to room temp before being stirred overnight under an argon atmosphere. The reaction was quenched with water (10 mL) and extracted with DCM (3 x 10 ml). The resulting organic layer was dried over $MgSO_4$ before the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography eluting with EtOAc/Pet Ether (6:4 to

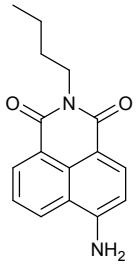
8:2) to yield a beige solid. (51 mg, 63% yield) **^{1}H NMR** (500 MHz, $CDCl_3$) σ 8.56 (brs, 1H, NH), 8.5 (d, 1H, J = 7.4Hz), 8.3 (d, 1H, J = 2.3Hz), 8.16 (d, 1H, J = 8.3Hz), 7.75 (t, 1H, J = 7.5Hz), 7.32 (s, 1H), 7.08 (s, 1H), 5.31 (s, 2H), 4.16 (t,2H, J = 7.6Hz), 4.11 (s, 3H), 1.7 (quin, 2H, J = 7.4), 1.44 (sex, 2H, J = 7.5), 0.97 (t, 3H, J = 7.4Hz). **^{13}C NMR** (125 MHz, $CDCl_3$): 163.86, 163.60, 153.36, 146.62, 138.24, 134.01, 133.55, 132.60, 129.46, 129.38, 128.18, 124.24, 123.63, 123.36, 122.36, 120.12, 56.06, 34.79, 30.10, 20.27, 14.20; **HRMS** (ESI): Calculated for $C_{22}H_{22}O_6N_5$ $[M+H]^+$, expected: 452.1543, observed: 452.1562, PPM: -4.76; **ν_{max} (film)/cm^{-1}:** 3414.37, 2960.13, 1738.57, 1699.25, 1655.81, 1626.98, 1562.83, 1490.27, 1467.33, 1432.17, 1345.74, 1272.53, 1226.28, 1176.74, 1135.72, 1067.69, 1037.12, 837.81, 783.86. **MP:** 212 °C.

4-nitro-*N*-butyl-1,8-naphthalimide (8)



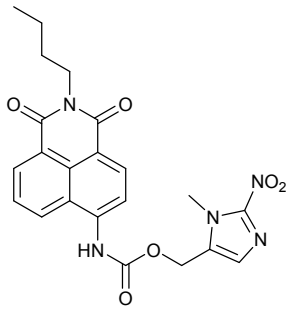
4-nitro-1,8-naphthalic anhydride (1 g, 4.1 mmol, 1 eq.) and butyl amine (0.31 g, 0.406 ml, 4.1 mmol 1 eq.) were dissolved in EtOH (17 mL) before being heated at 110 °C for 1 hr under microwave irradiation. The mixture was allowed to cool to room temperature before the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography eluting with DCM. The purified product was isolated as a cream solid (1.18 g, 95% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) σ 8.58 (dd, 1H, J = 8.65/0.84Hz), 8.51 (dd, 1H, J = 7.34/0.8Hz), 8.48 (d, 1H, J = 8.1Hz), 8.46 (t, 1H, J = 7.8Hz), 7.99 (dd, 1H, J = 7.62/0.86Hz), 3.98 (t, 2H, J = 7.58Hz), 1.59 (quin, 2H, J = 7.45Hz), 1.35 (sex, 2H, J = 7.42Hz), 0.92 (t, 3H, J = 7.42Hz). **^{13}C NMR** (500 MHz, DMSO-d_6): 163.24, 162.44, 149.39, 132.06, 130.50, 129.99, 129.09, 128.60, 126.89, 124.68, 123.05, 122.99, 29.91, 20.25, 14.15; **HRMS** (ESI): Calculated for $C_{16}H_{15}N_2O_4$ $[M+H]^+$, expected: 299.1025, observed: 299.1026, PPM: -0.4; **ν_{max} (film)/cm^{-1}:** 3106.27, 3069.79, 3040.62, 2961.54, 2873.57, 1949.06, 1708.53, 1654.57, 1623.75, 1583.15, 1529.35, 1461.93, 1439.21, 1408.28, 1346.72, 1269.49, 1231.81, 1188.64, 1082.40. **MP:** 104~108 °C.

4-amino-*N*-butyl-1,8-naphthalimide (4)



4-nitro-*N*-butyl-1,8-naphthalimide (1.1g, 3.7 mmol, 1 eq.) was dissolved in MeOH (70 ml) before Pd/C (0.25 g) was added and the reaction was placed under an atmosphere of H_2. The reaction was allowed to proceed for 2 hrs before being filtered through a pad of celite. The solvent was removed under reduced pressure to yield a bright yellow solid. (1.02 g, 95% yield) **^{1}H NMR** (500 MHz, DMSO-d_6) σ 8.59 (dd, 1H, J = 8.45/0.89Hz), 8.41 (dd, 1H, J = 7.33/0.85Hz), 8.18 (d, 1H, J = 8.4Hz), 7.63 (dd, 1H, J = 7.48/0.83Hz), 7.43 (s, 2H), 6.82 (d, 1H, J = 8.4Hz), 3.99 (t, 2H, J = 748Hz), 1.56 (quin, 2H, J = .48Hz), 1.31 (sex, 2H, J = 7.48Hz), 0.9 (t, 3H, J = 7.48Hz); **^{13}C NMR** (125 MHz, DMSO-d_6): 163.94, 163.40, 153.86, 140.79, 133.47, 132.13, 131.46, 129.74, 129.51, 128.80, 126.99, 124.48, 122.76, 117.91, 56.48, 34.82, 30.14, 20.28, 14.21; **HRMS** (ESI): Calculated for $C_{16}H_{17}N_2O_2$ $[M+H]^+$, expected: 269.1273, observed: 269.1285, PPM: -4.33; **ν_{max} (film)/cm^{-1}:** 3410.38, 3356.55, 3254.13, 2957.81, 2930.08, 2861.62, 1676.07, 1636.78, 1613.97, 1575.31, 1528.50, 1480.40, 1431.25, 1378.83, 1362.04, 1304.33, 1244.87, 1115.97, 1077.63. **MP:** 178~186 °C.

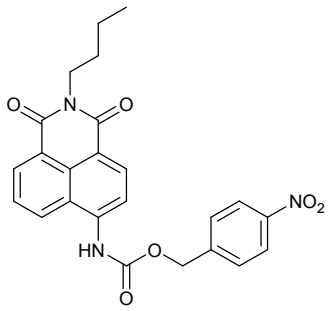
(2-Butyl-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-carbamic acid 3-methyl-2-nitro-3H-imidazol-4-ylmethyl ester (1)



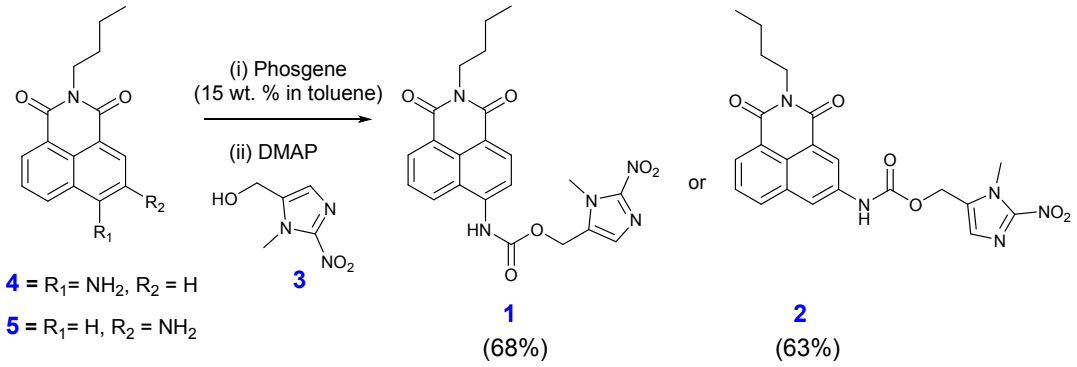
4-amino-*N*-butyl-1,8-naphthalimide (48 mg, 0.18 mmol, 1 eq.) and DMAP (66 mg, 0.54 mmol, 3 eq.) were dissolved in anhydrous DCM (20 mL) and placed under an argon atmosphere. The reaction was cooled to -10°C using an ice and salt bath before phosgene solution (15 wt. % in toluene; 0.7 ml, 1.07 mmol, 6 eq.) was added dropwise and the reaction mixture was allowed warm to room temperature and stirred for 4 hrs. The reaction mixture was reduced to dryness by bubbling with argon before the residue was redissolved in anhydrous DCM and cooled to 0 °C (20 mL). (3-Methyl-2-nitro-3H-imidazol-4-yl)-methanol **3** (84 mg, 0.53 mmol, 3 eq) was added and the reaction was allowed to return to room temp before being stirred overnight under an argon atmosphere. The reaction was quenched with water (10 mL) and extracted with DCM (3 x 10 ml). The resulting organic layer was dried over $MgSO_4$ before the solvent was removed under reduced

pressure. The resulting residue was purified by column chromatography eluting with EtOAc/Pet Ether (6:4 to 8:2) to yield a beige solid (55 mg, 68% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) σ 10.45 (Bs, NH), 8.67 (dd, 1H, J = 8.59/1.0Hz), 8.5 (dd, 1H, J = 7.26/0.9Hz), 8.48 (d, 1H, J = 8.24Hz), 8.17 (d, 1H, J = 8.27Hz), 7.84 (dt, 1H, J = 7.38/1.2Hz), 7.36 (s,1H), 5.39 (s, 2H), 4.02 (t,2H, J = 7.74Hz), 4.01 (s, 3H), 1.6 (quin, 2H, J = 7.5Hz), 1.34 (sex, 2H, J = 7.48Hz), 0.92 (t, 3H, J = 7.47Hz); **^{13}C NMR** (500 MHz, DMSO-d_6): 163.94, 163.40, 153.86, 140.79, 133.47, 132.13, 131.46, 129.74, 129.51, 128.80, 126.99, 124.48, 122.76, 117.91, 56.48, 34.82, 30.14, 20.28, 14.21; **HRMS** (ESI): Calculated for $C_{22}H_{22}O_6N_5$ $[M+H]^+$, expected: 452.1536, observed: 452.1565, PPM: -6.26; **ν_{max} (film)/cm^{-1}:** 3444.50, 3234.96, 2928.75, 2870.81, 1698.32, 1654.59, 1592.32, 1542.07, 1486.61, 1390.09, 1358.23, 1228.60, 1181.95. **MP:** 230~242 °C.

(2-Butyl-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-carbamic acid 4-nitro-benzyl ester (NB)



4-amino-*N*-butyl-1,8-naphthalimide (60 mg, 0.22 mmol, 1 eq.) and DMAP (82 mg, 0.67 mmol, 3 eq.) were dissolved in anhydrous DCM (20 mL) and placed under an argon atmosphere. The reaction was cooled to -10°C using an ice and salt bath before phosgene solution (15 wt. % in toluene; 1.0 ml, 1.33 mmol, 6 eq.) was added dropwise and the reaction mixture was allowed warm to room temperature and stirred for 4 hrs. The reaction mixture was reduced to dryness by bubbling with argon before the residue was redissolved in anhydrous DCM and cooled to 0 °C (20 mL). 4-nitrobenzyl alcohol (103 mg, 0.67 mmol, 3 eq.) was added and the reaction was allowed to return to room temp before being stirred overnight under an argon atmosphere. The reaction was quenched with water (10 mL) and was extracted with DCM (3 x 10 ml). The resulting organic layer was dried over $MgSO_4$ before the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography eluting with MeOH/DCM (0:100 to 4:96) to yield a beige solid (60 mg, 60% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) σ 10.50 (s, NH), 8.74 (d, 1H, J = 8.68Hz), 8.54 (d, 1H, J = 7.28Hz), 8.5 (d, 1H, J = 8.29Hz), 8.3 (d, 2H, J = 8.46Hz), 8.2 (d, 1H, J = 8.1Hz), 7.87 (t, 1H, J = 7.83Hz), 7.78 (d, 2H, 8.47Hz), 4.08 (dd, 2H, 5.24Hz), 4.05 (t, 2H, 7.42Hz), 1.63 (quin, 2H, J = 7.51Hz), 1.36 (sex, 2H, J = 7.77Hz), 0.94 (t, 3H, J = 7.4Hz). **^{13}C NMR** (125 MHz, DMSO-d_6): 163.96, 163.41, 147.66, 144.66, 132.15, 131.43, 129.07, 126.94, 124.13, 65.76, 55.38, 49.07, 40.61, 30.16, 20.28, 14.29. **HRMS** (ESI): Calculated for $C_{24}H_{21}N_3O_6$ $[M+H]^+$, expected: 447.1435, observed: 447.143, PPM: 0.95; **ν_{max} (film)/cm^{-1}:** 3309.52, 2956.91, 2872.62, 1707.63, 1694.55, 1646.75, 1621.35, 1593.24, 1544.41, 1518.43, 1447.13, 1393.43, 1365.32, 1346.34, 1224.021065.63. **MP:** 240~244 °C.



Scheme S1: Synthesis of target 2-nitroimidazole based probes 1 and 2.

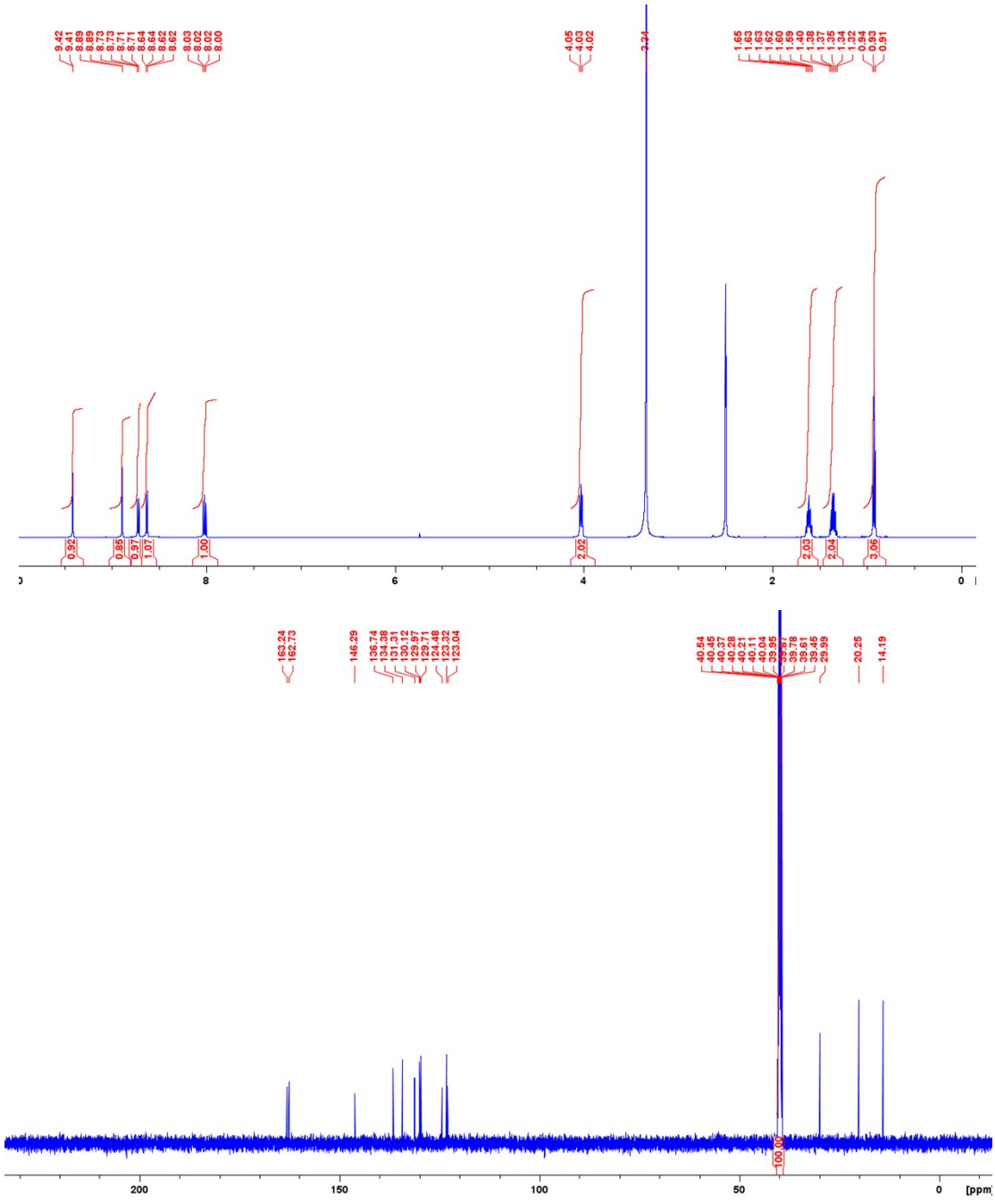


Figure S1: ^{1}H NMR (DMSO-d$_6$, 500 MHz) and ^{13}C NMR (DMSO-d$_6$, 125 MHz) spectra of 7.

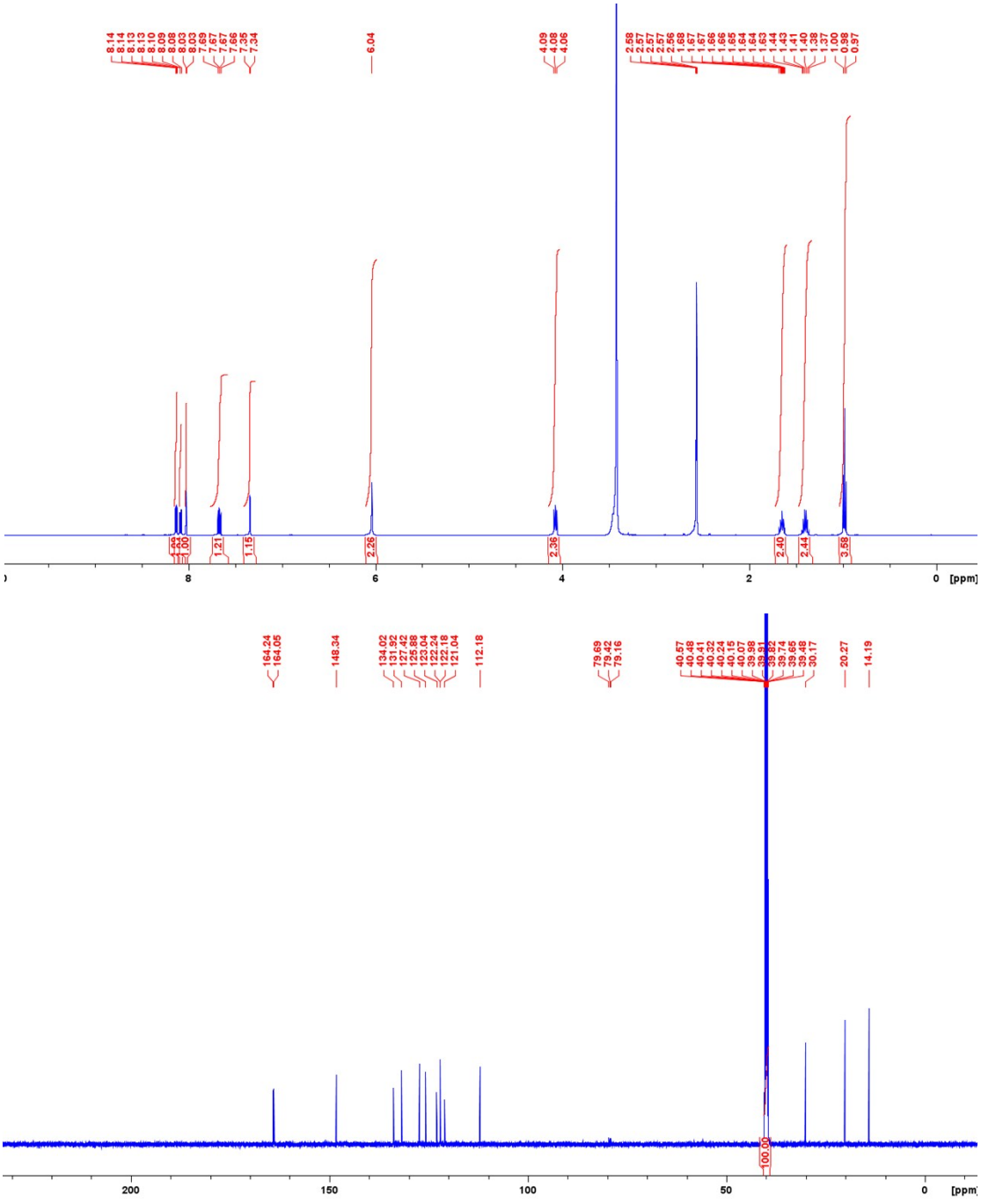


Figure S2: ^{1}H NMR (DMSO-d$_6$, 500 MHz) and ^{13}C NMR (DMSO-d$_6$, 125 MHz) spectra of 5.

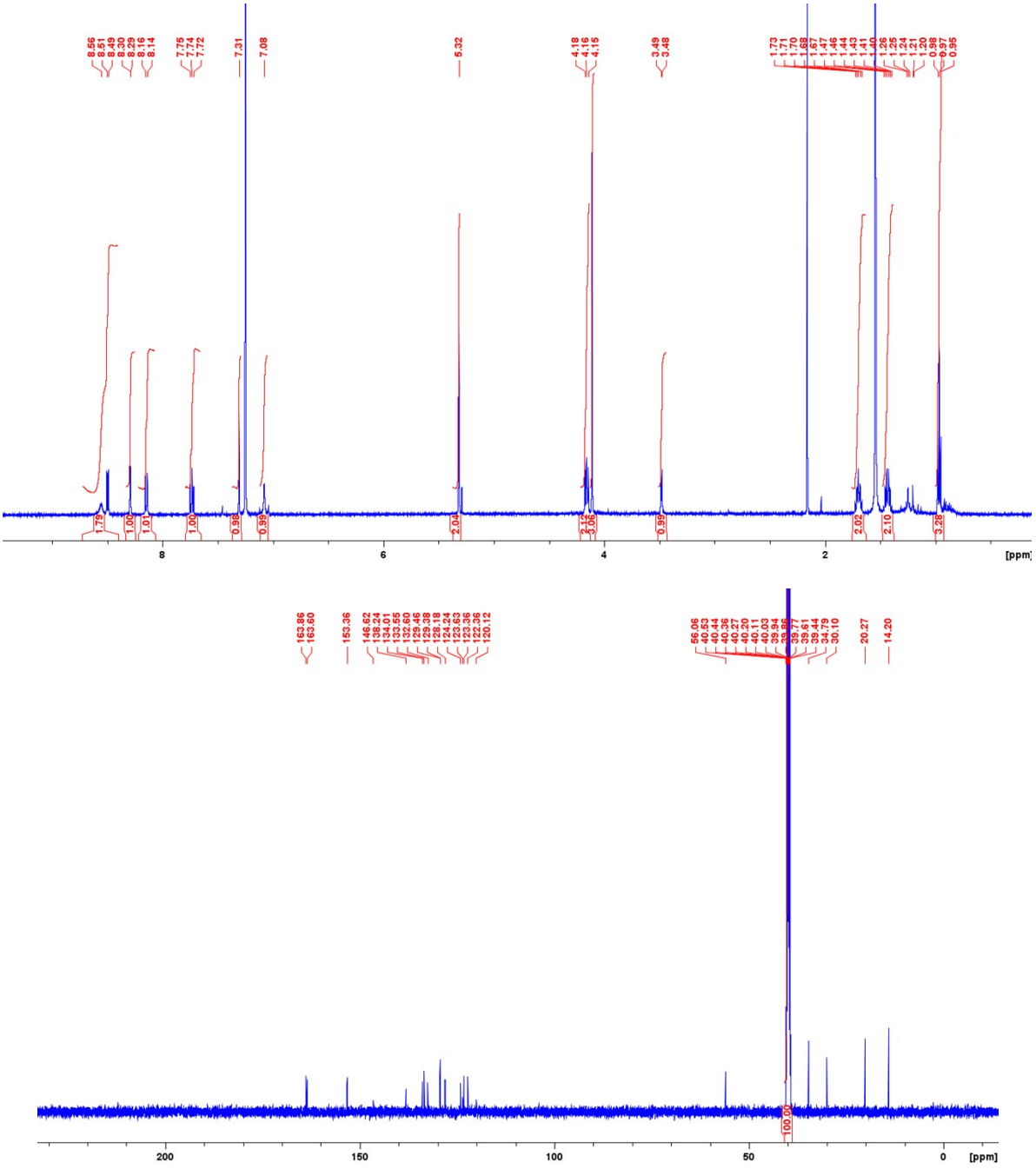


Figure S3: ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) spectra of 2.

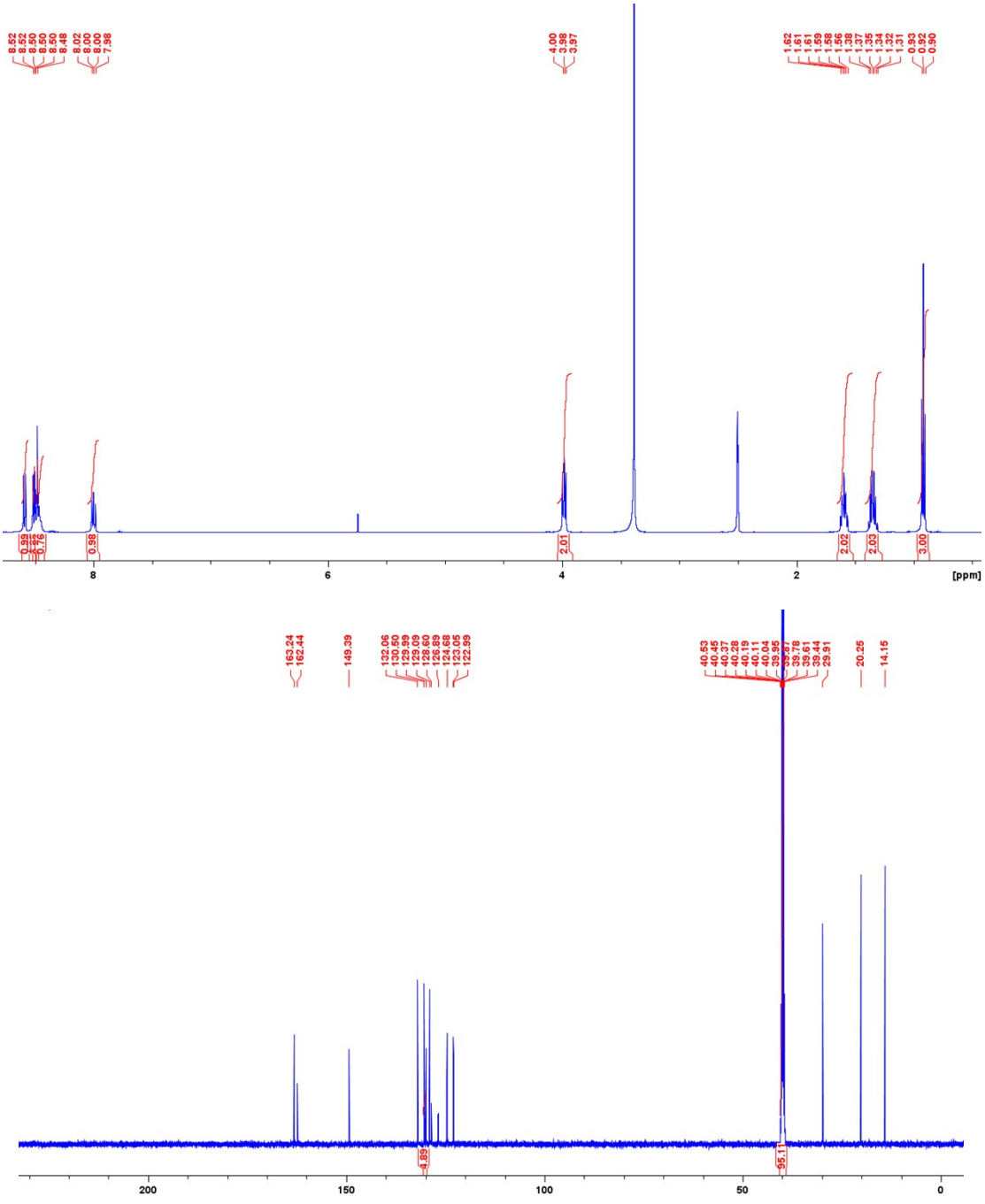


Figure S4: ^{1}H NMR (DMSO-d$_6$, 500 MHz) and ^{13}C NMR (DMSO-d$_6$, 125 MHz) spectra of 8.

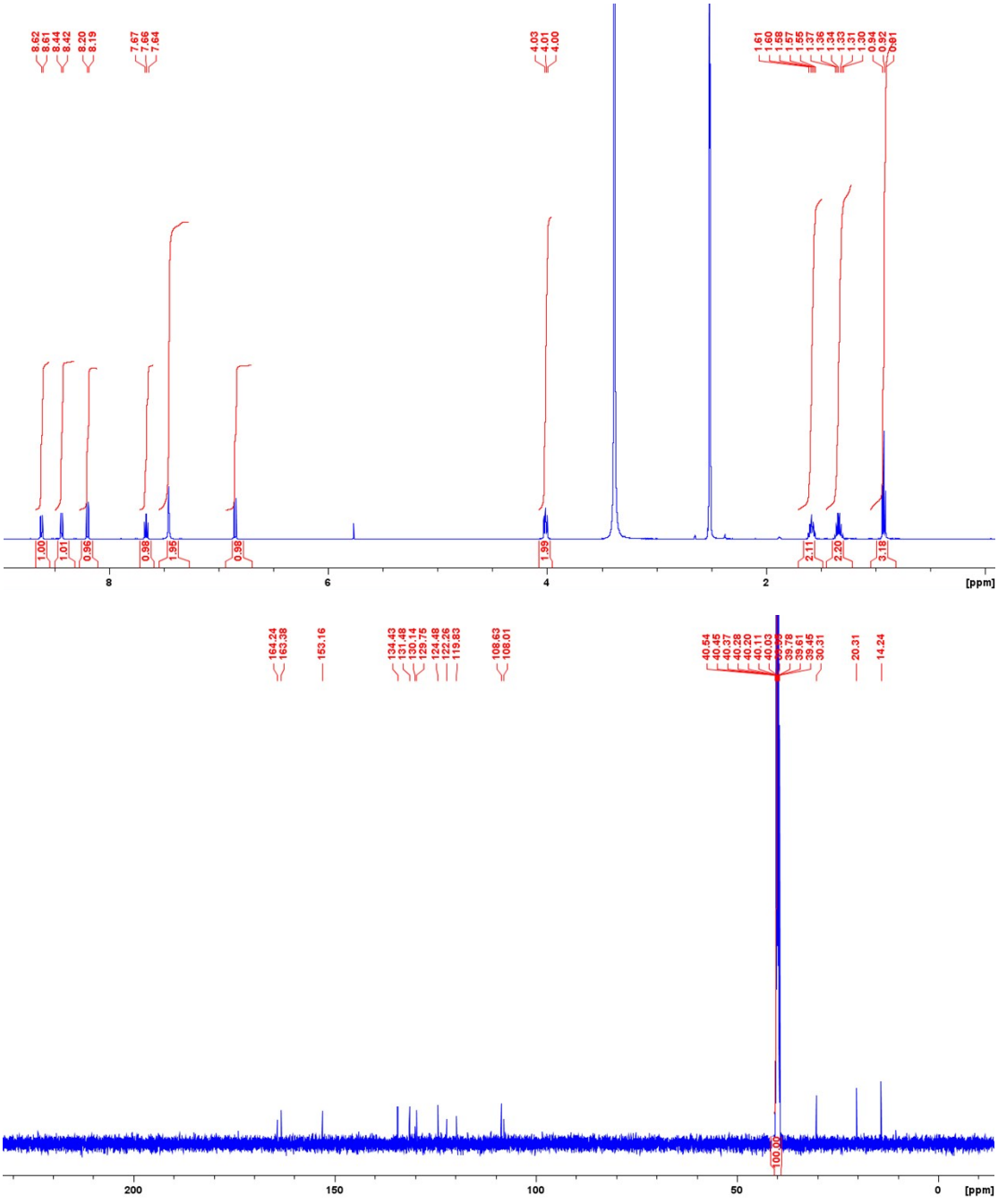


Figure S5: ^{1}H NMR (DMSO-d$_6$, 500 MHz) and ^{13}C NMR (DMSO-d$_6$, 125 MHz) spectra of 4.

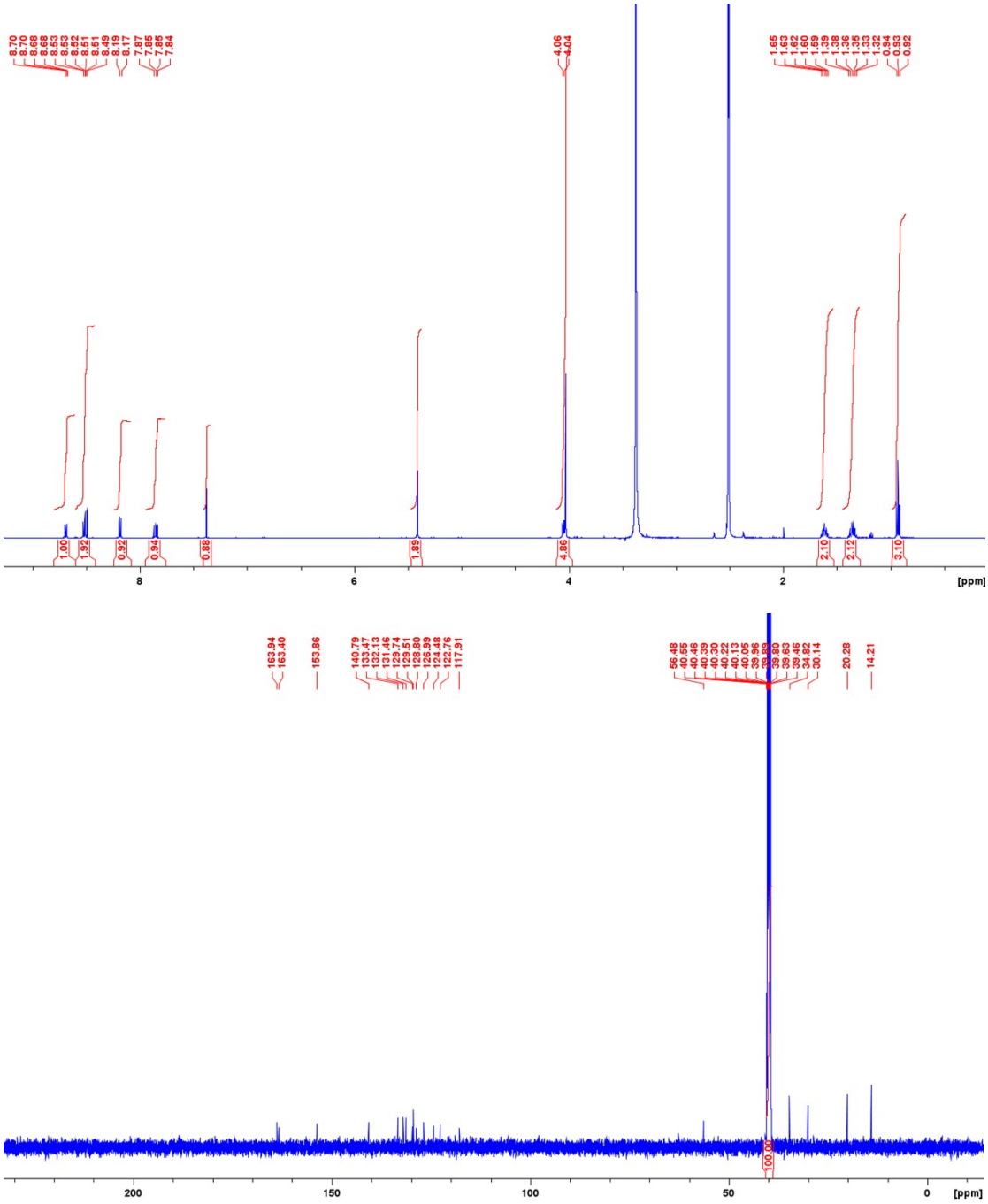


Figure S6: ^{1}H NMR (DMSO-d$_6$, 500 MHz) and ^{13}C NMR (DMSO-d$_6$, 125 MHz) spectra of 1.

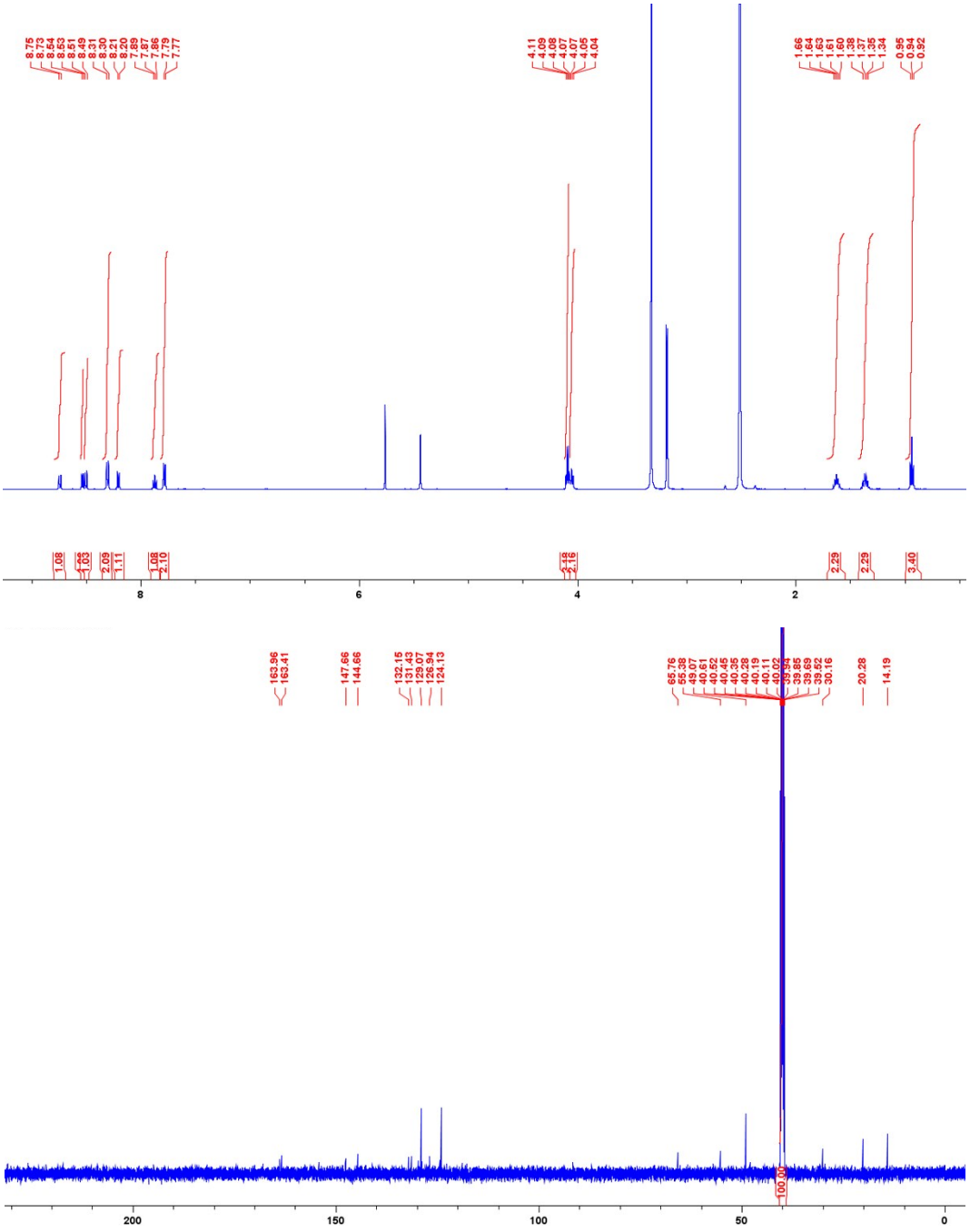


Figure S7: ^{1}H NMR (DMSO-d$_6$, 500 MHz) and ^{13}C NMR (DMSO-d$_6$, 125 MHz) spectra of NB.

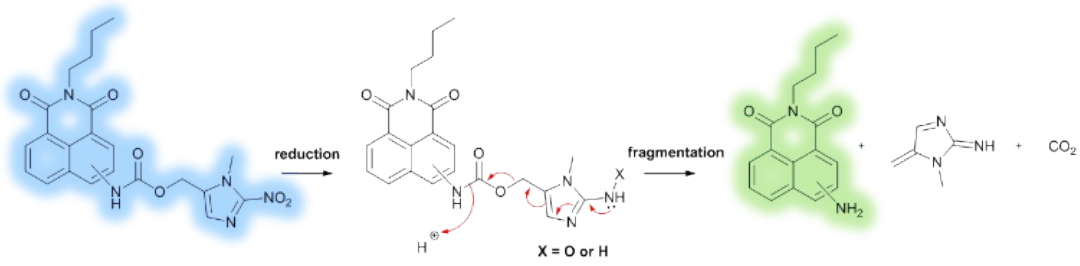


Figure S8: Proposed release of amino-1,8-naphthalimide fluorophores from probes 1 and 2 under reductive stress.

Materials and Methods for Biological Experiments:

Nitroreductase Studies

Fluorescence titrations were carried out using a Cary Eclipse Fluorescence Spectrometer or a Jasco FP6300 spectrofluorimeter. A stock solution of the fluorescent probe under study was prepared in DMSO before being diluted in a 3 mL quartz fluorescence cell to a final concentration of 1×10^{-5} M with NADH (50 μM) in 10 mM phosphate buffer solution (PBS) where the total amount of DMSO present was < 0.1%. Spectroscopic titrations were performed by additions of aliquots of a 1 μg/μL Nitroreductase (from Escherichia coli, ≥90% (SDS-PAGE), recombinant, expressed in E. coli), followed by either continuous scanning of a single wavelength or by intermittent fluorescence spectra recorded from 400 nm to 750 nm. Typically, 0 up to 24 μg/mL of NTR was added to the solution. ESI-LCMS studies were conducted on an Agilent 6310 LCMS TOF with samples prepared to a final concentration of 1×10^{-3} M with NADH (50 μM) in 10 mM phosphate buffer solution (PBS) where the total amount of DMSO present was < 0.1%. Due to the hydrophobic nature of the probes under study it is feasible to suggest that some aggregation of probes **1**, **2** and **NB** may occur. In this instance we observed no evidence to suggest that this was the case and all of the spectroscopic measurements carried out showed no precipitation of the probes at a concentration of 1×10^{-5} M.[2]

Cell culture

HeLa cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 50 μg/ml penicillin/streptomycin at 37˚C in a humidified atmosphere of 5% CO_2. For reducing conditions, compounds were pre-treated for 3 hrs with nitroreductase and its cofactor NADH before being added to cells. For endocytosis studies, cells were pre-treated for 3hrs with 100 μM dynasore.

Viability assay

$5x10^3$ cells/well were seeded in a 96-well plate and treated with the respective compound for 24 h. Alamar Blue (20 μl) (BioSource) was then added to each well and incubated for 4 h. Fluorescence was read using at 590 nm (excitation 544 nm). The control untreated cells represented 100% cell viability. All data points (expressed as means ± S.E.M.) were analysed using GRAPHPAD Prism (version 4) software (Graphpad software Inc., San Diego, CA).

Confocal microscopy

HeLa cells were seeded at a density of 1×10^5 cells /2 mL and left for 24 h before the required treatment. Cells were then washed twice, stained with the red nuclear stain DRAQ5 and analysed by live confocal microscopy using an Olympus FV1000 point scanning microscope with a 60x oil immersion lens with an NA (numerical aperture) of 1.42. The software used to collect images was FluoView Version 7.1 software. Compounds were excited by a 405 nm argon laser, emission 425-475 and 525-575 nm. DRAQ5 was excited by a 633 nm laser, emission >650 nm. For spectra, emission from 410 to 770 nm was quantified using a Leica SP8 confocal microscope (60X oil immersion lens), excitation 405 nm.

Flow cytometry

Following the required treatment, cells were trypsinised, washed twice with PBS, resuspended in 400 μL of ice cold PBS and assayed for flow cytometry (FACS CyAn, Bectin Dickson). Analysis was performed using appropriate gates, counting 10,000 cells and the FlowJo software package. The compounds were excited by a 405 nm laser, emission filters 425 nm-475nm and 510 nm-550nm.

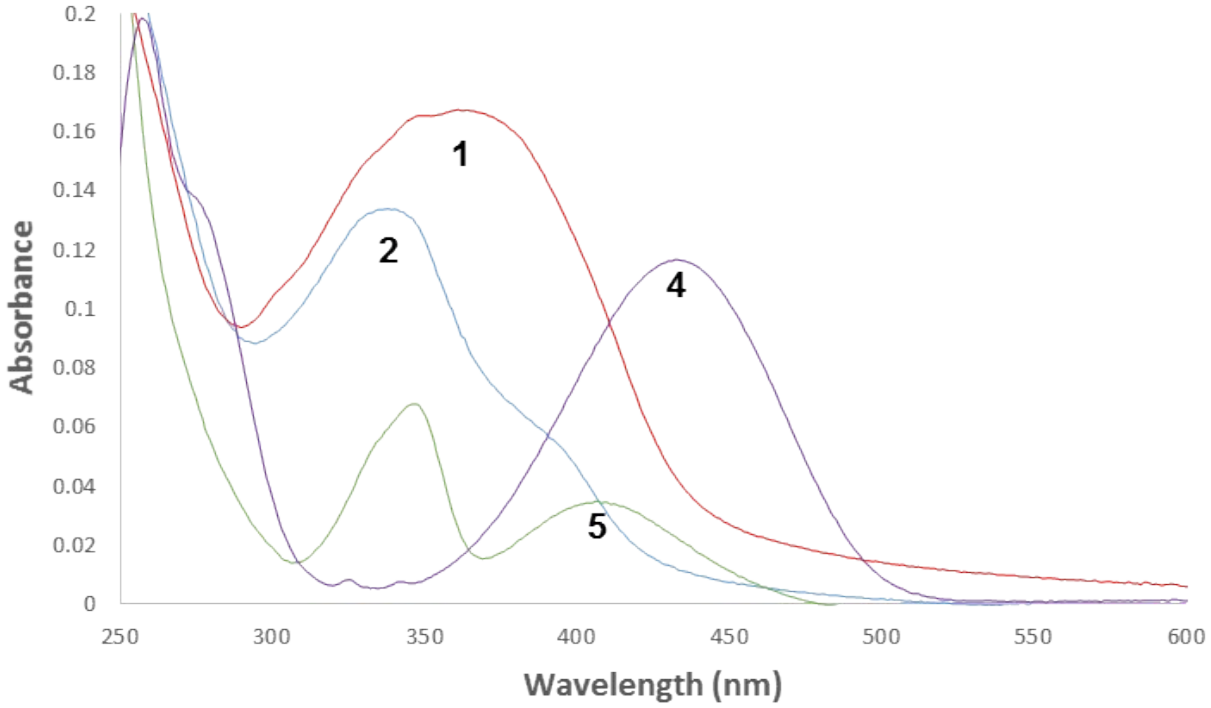


Figure S9: UV absorption spectra of **1** (red), **2** (blue), **4** (purple) and **5** (green) (10 μM) in 10 mM phosphate buffered solution at pH 7.4 (<0.1% DMSO).

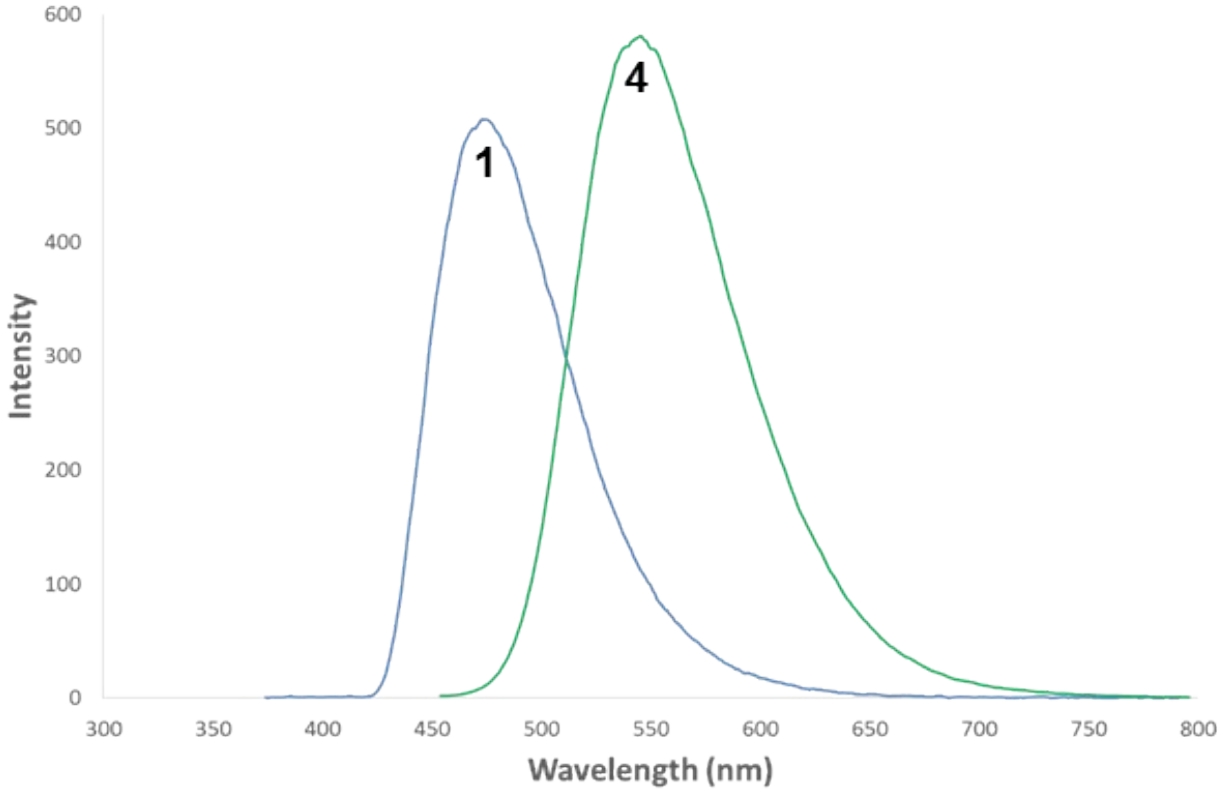


Figure S10: Emission spectrum of **1** (blue) and **4** (green) (10 μM) (λ_{ex} 345 nm) in 10 mM phosphate buffered solution at pH 7.4 (<0.1% DMSO).

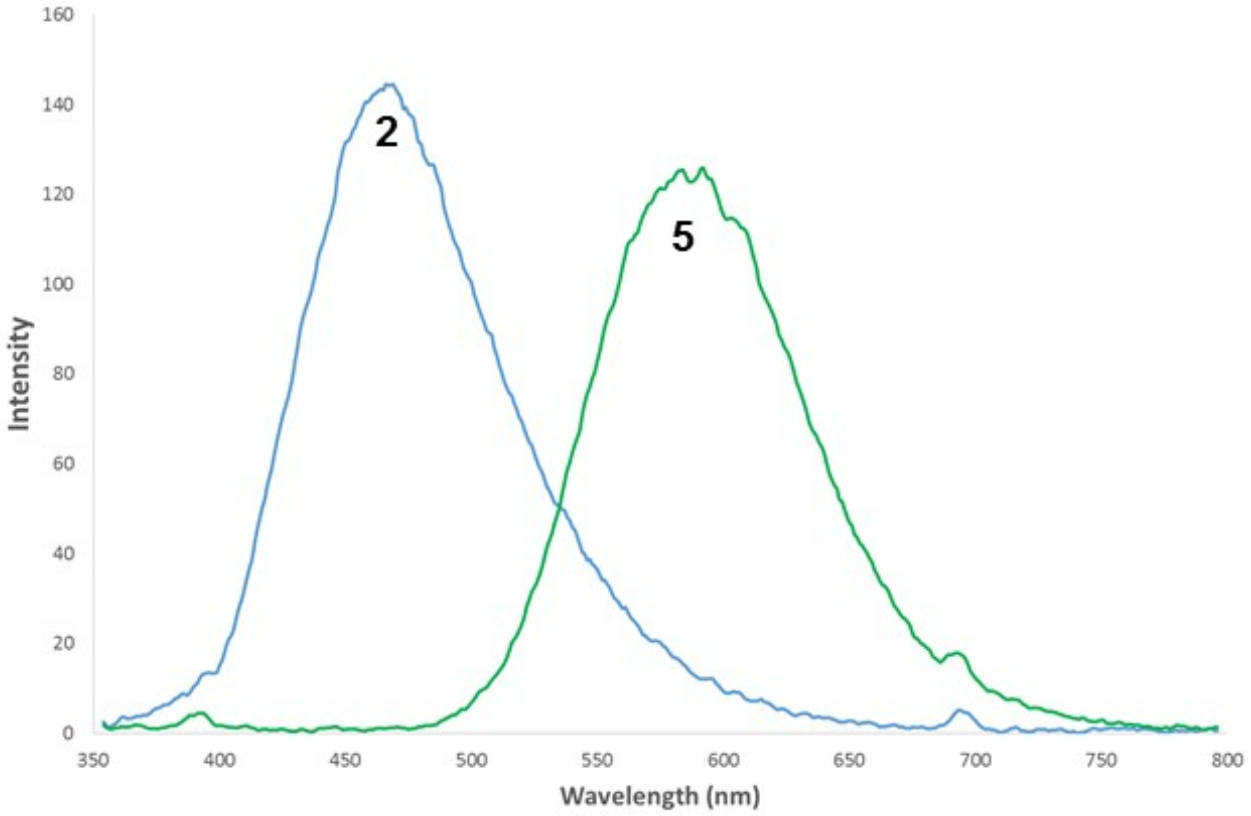


Figure S11: Emission spectrum of **2** (blue) and **5** (green) (10 μM) (λ_{ex} 345 nm) in 10 mM phosphate buffered solution at pH 7.4 (<0.1% DMSO).

Compound	λ_{max} (nm) [ε(M^{-1} cm^{-1})] (±10%) in phosphate buffer at pH 7.4 (<0.1% DMSO).	
1	360 [9500]	-----
2	345 [14100]	390[6180]
4	435 [13750]	-----
5	350 [6050]	415 [4600]
NB	390 [15580]	-----

Table S1: Absorption properties of **1**, **2**, **4**, **5** and **NB** in 10 mM phosphate buffered solution at pH 7.4 (<0.1% DMSO).

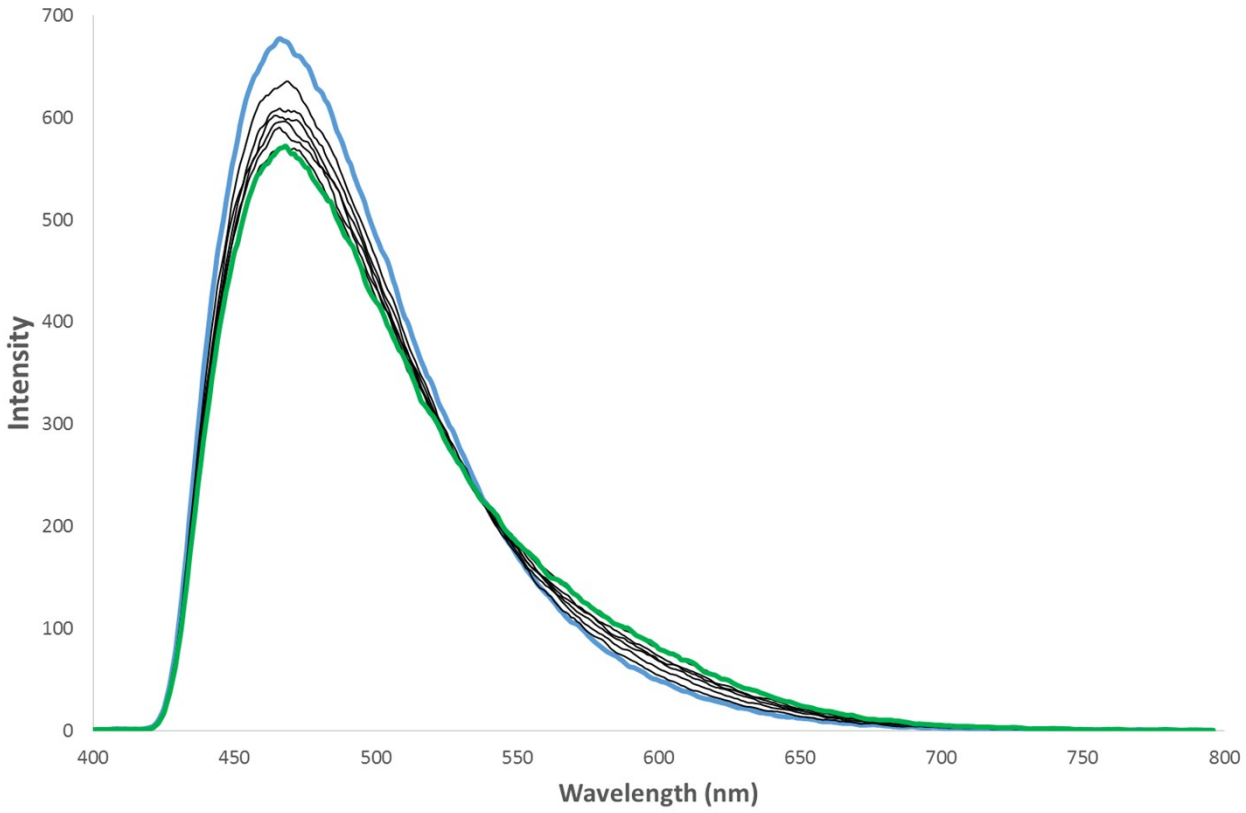


Figure S12: Changes in the emission spectrum of **2** (10 μM) at various time points (0 - 25 mins) after addition of NTR (1 μg mL^{-1}).

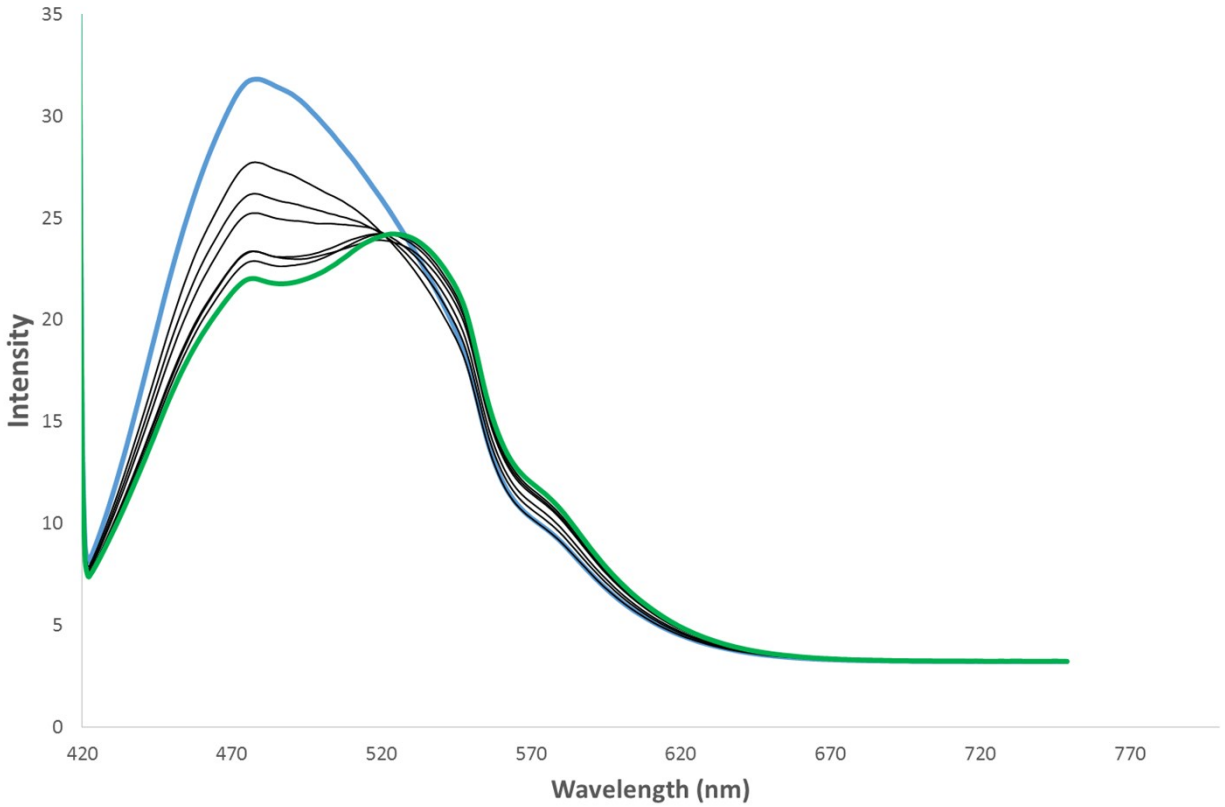


Figure S13: Changes in the emission spectrum of **NB** (10 μM) at various time points (0 - 25 mins) after addition of NTR (1 μg mL^{-1}).

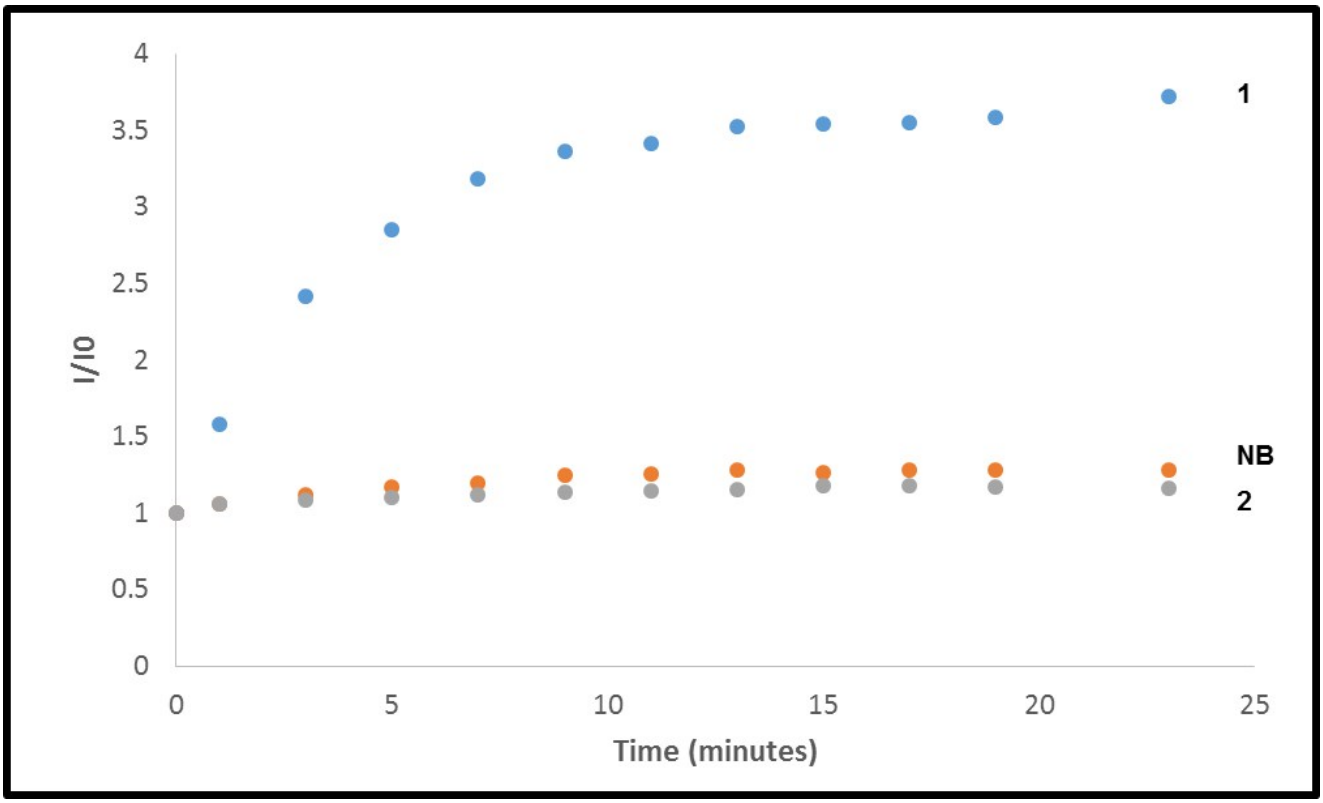


Figure S14: Comparison of the relative changes in emission (0 – 25 mins) (λ = 530 nm) of **1** (blue), **2** (grey) and **NB** (orange) (10 μM) upon treatment with NTR (1 μg mL-1).

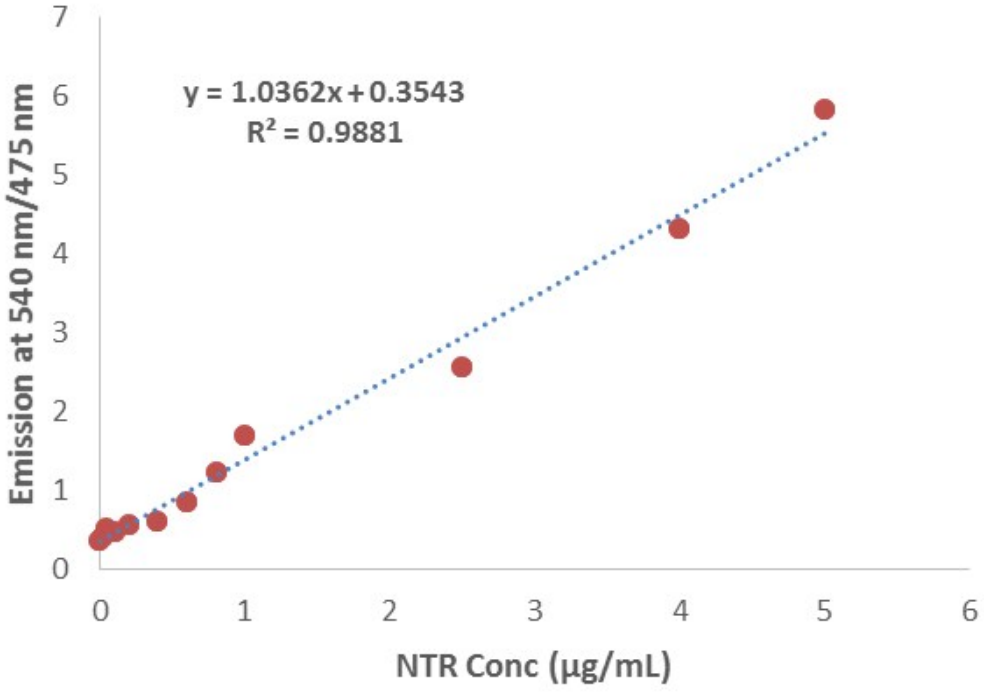


Figure S15: Calibration plot obtained from ratiometric emission of **1** at 475 nm and 540 nm from over the concentration range 0.02 – 5 μg mL^{-1} NTR. The detection limit (3S/m, in which S is the standard deviation of blank measurements, n = 11, and m is the slope of the linear equation) was determined to be 0.77 μg mL^{-1}.

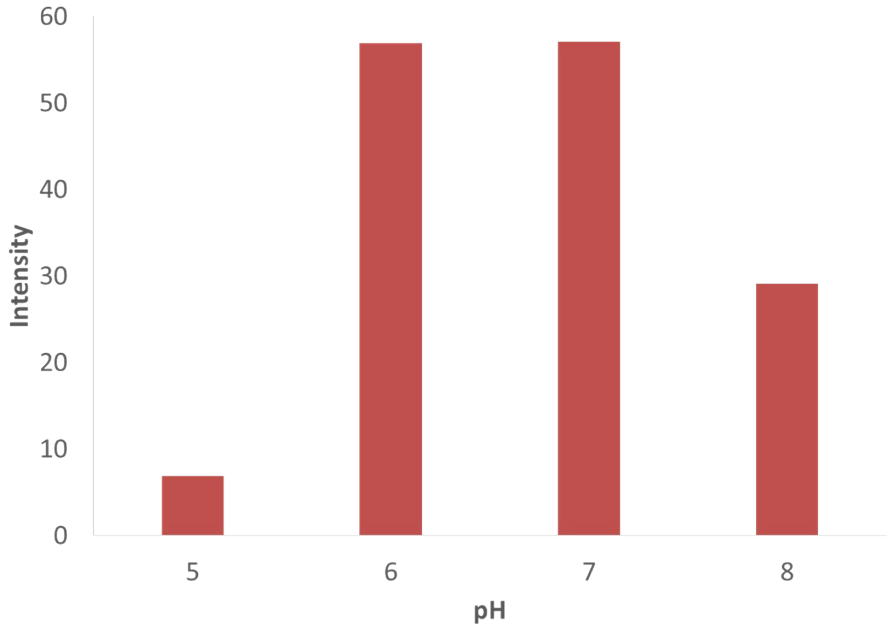


Figure S16: Changes in the emission spectrum of 1 (10 μM) (λ_{ex} 345 nm) at various pH values.

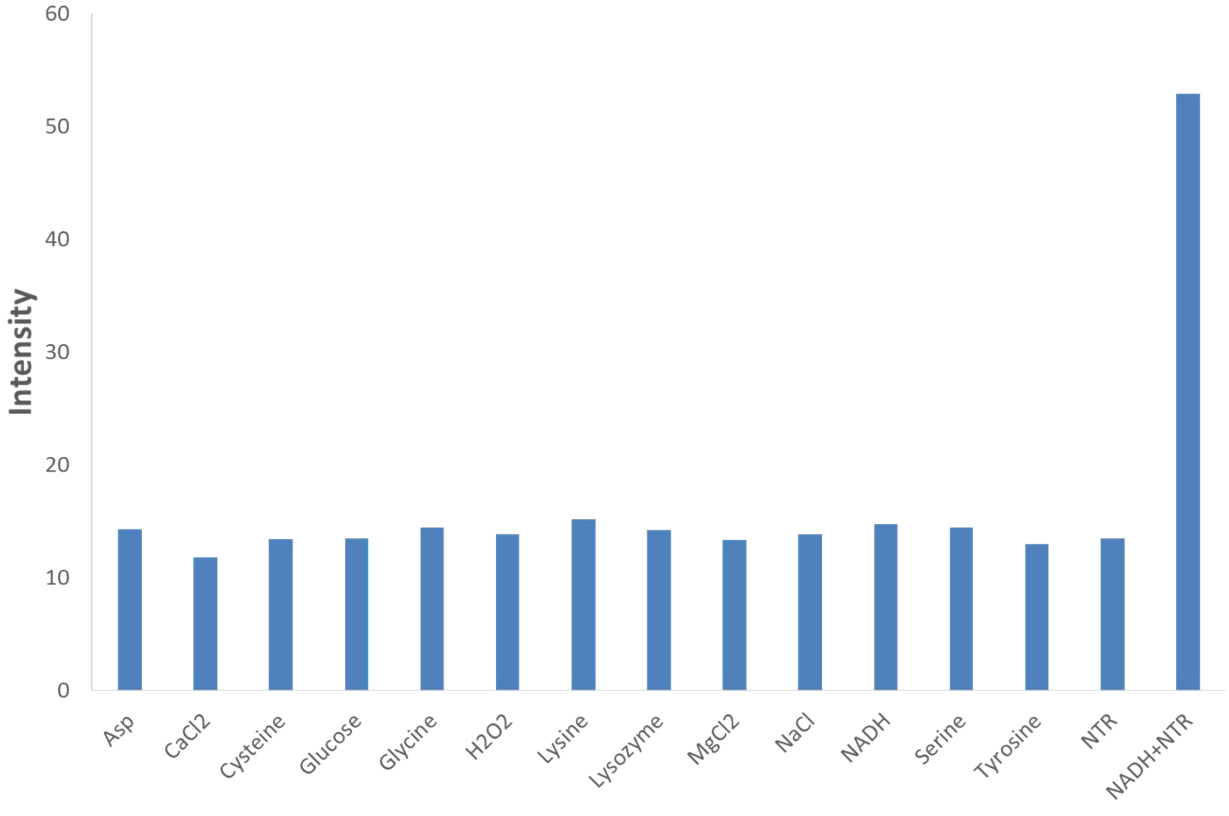


Figure S17: Changes in the emission maximum of **1** (10 μM) (λ_{ex} 345 nm) upon addition of various biological interferants.

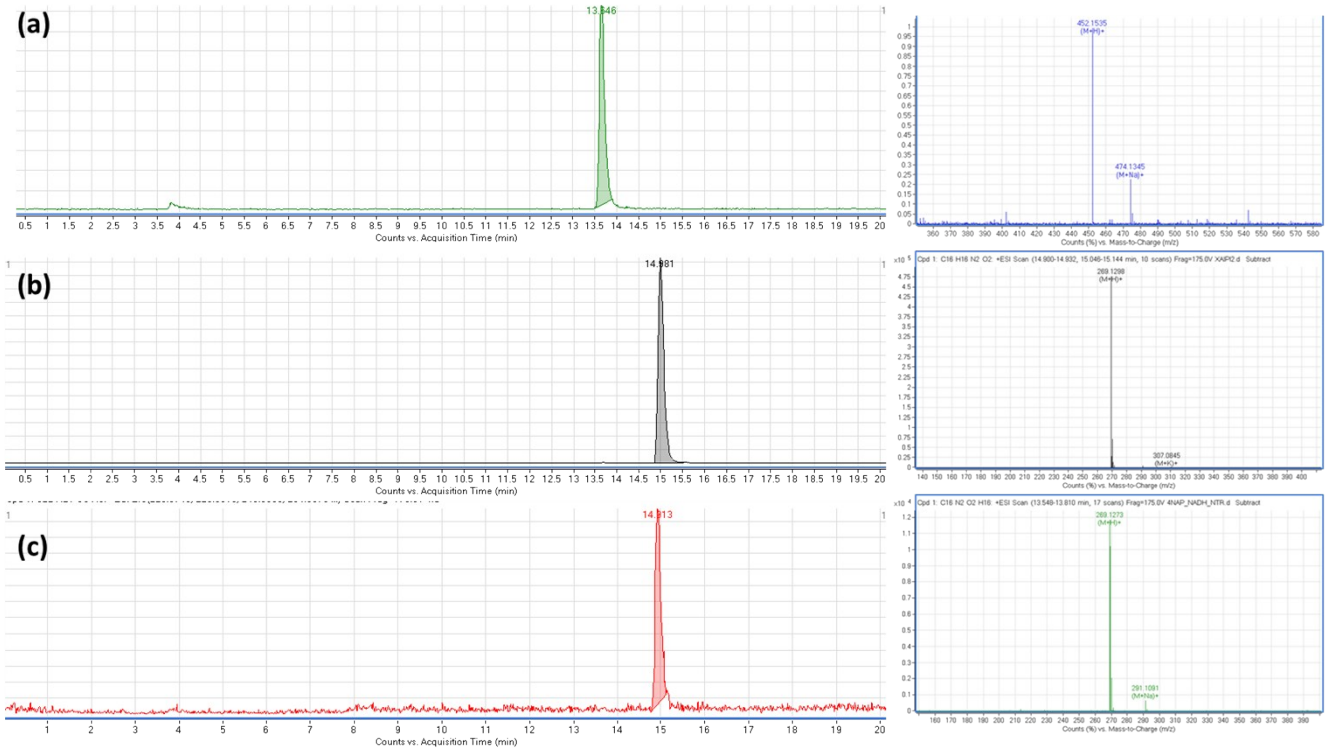


Figure S18: Analytical LCMS traces of (a) **1**, (b) **4** and (c) **1** after treatment with NTR (1µg/mL) in the presence of NADH (500 µM);(0-100% acetonitrile over 20 min, 0.1% Formic Acid, λ = 254 nm); Calculated Mass of **1** $[M+H]^+$: 452.1565; Calculated Mass of **2** $[M+H]^+$: 269.1285

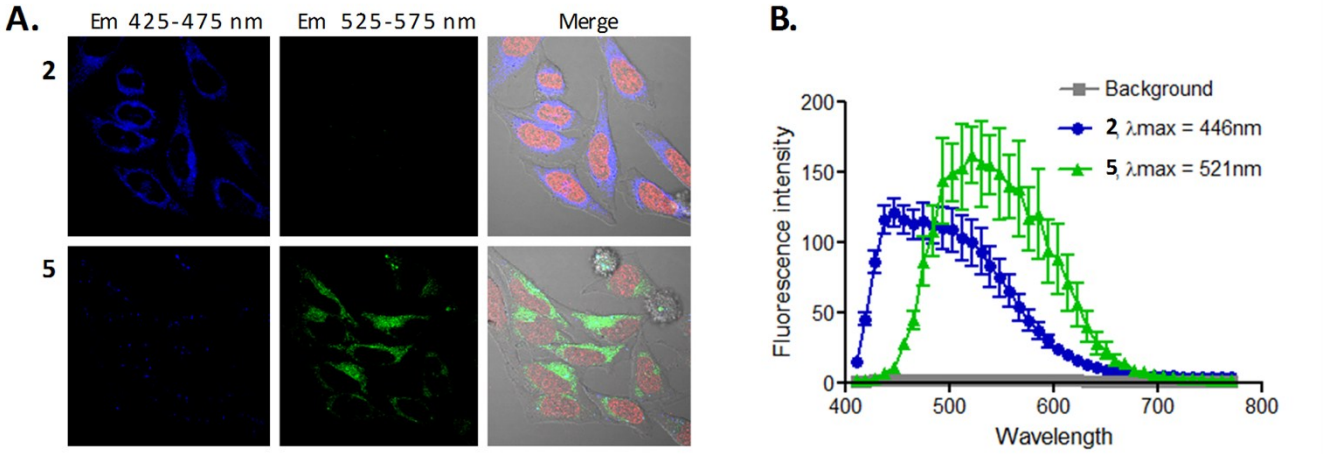


Figure S19: Uptake of **2** and **5** (10 µM) by HeLa cells measured using confocal laser scanning microscopy (A) cellular localisation of **2** and **5** at 37°C, (B) emission spectra of treated cells measured using confocal microscopy.

References:

(1) O'Connor, L. J.; Cazares-Korner, C.; Saha, J.; Evans, C. N. G.; Stratford, M. R. L.; Hammond, E. M.; Conway, S. J.,*Org. Chem. Front.,* **2015**, *2*, 1026-1029.

(2) An, F.-F.; Ye, J.; Zhang, J.-F.; Yang, Y.-L.; Zheng, C.-J.; Zhang, X.-J.; Liu, Z.; Lee, C.-S.; Zhang, X.-H., *J. Mater. Chem. B* **2013**, *1*, 3144-3151.