Electronic Supplementary Information

Photophysical properties of quinoxalin-2(1H)-ones: application to the preparation of an azide-based fluorogenic probe for the detection hydrogen sulfide

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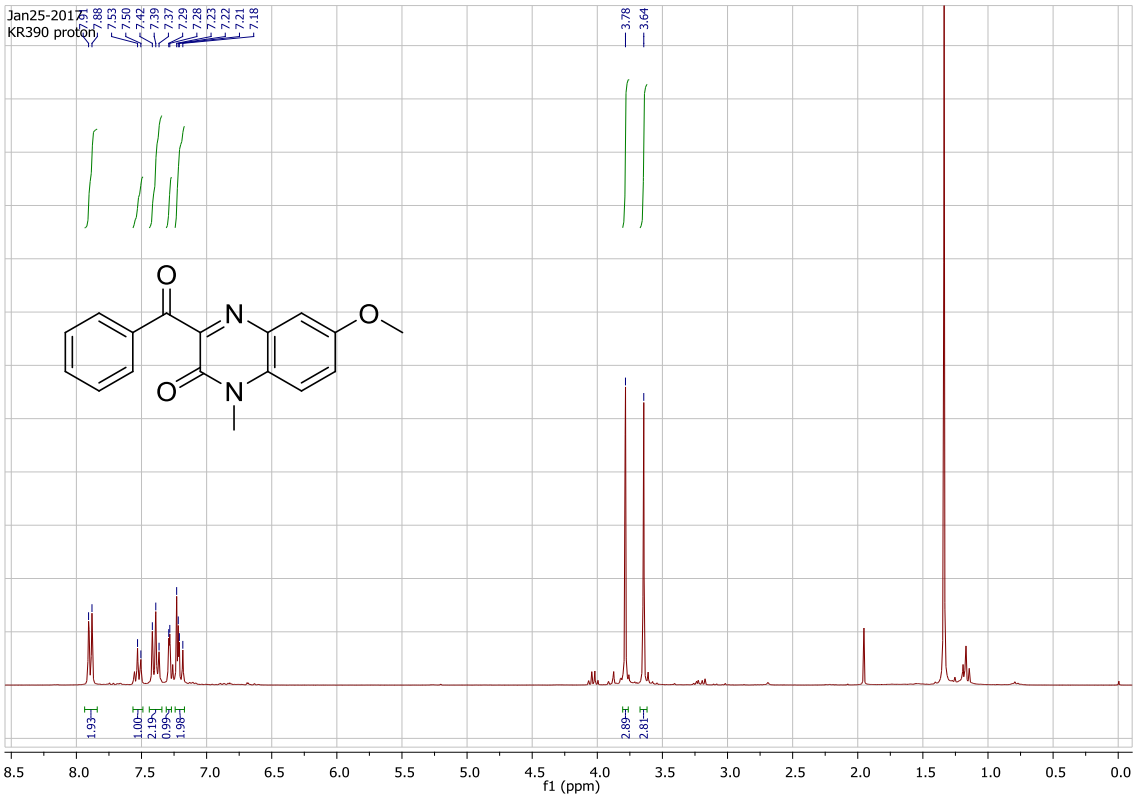
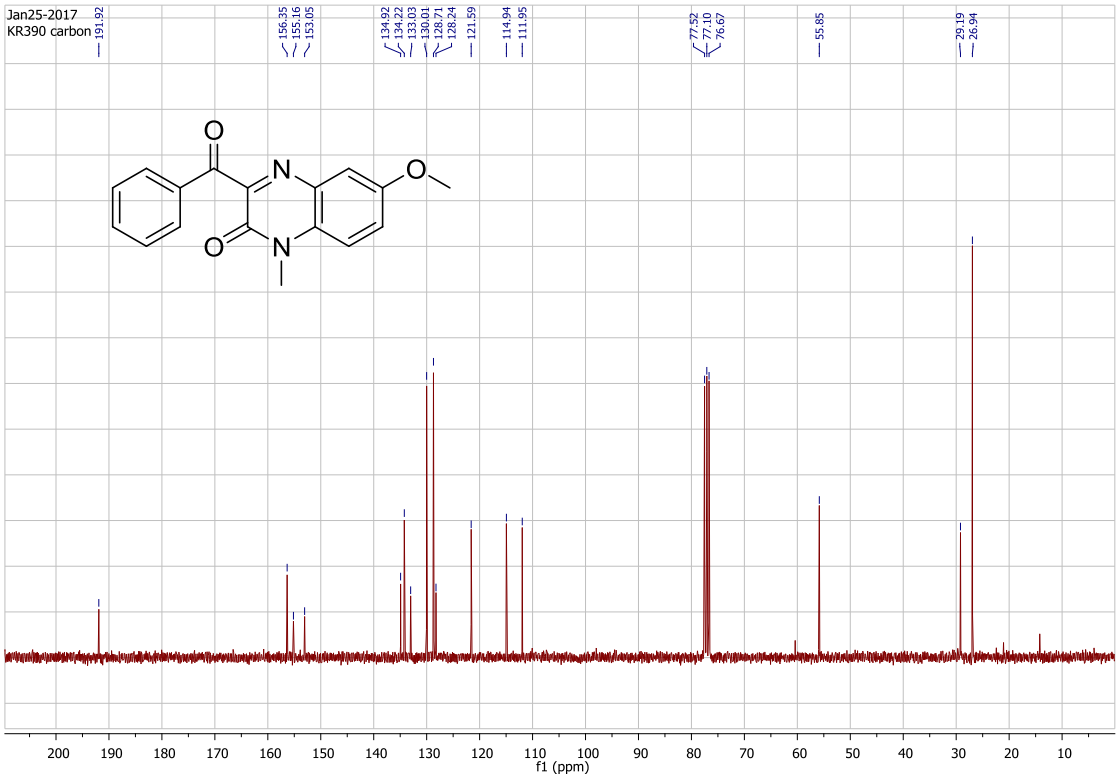
I. Copies of ^{1}H, and ^{13}C NMR

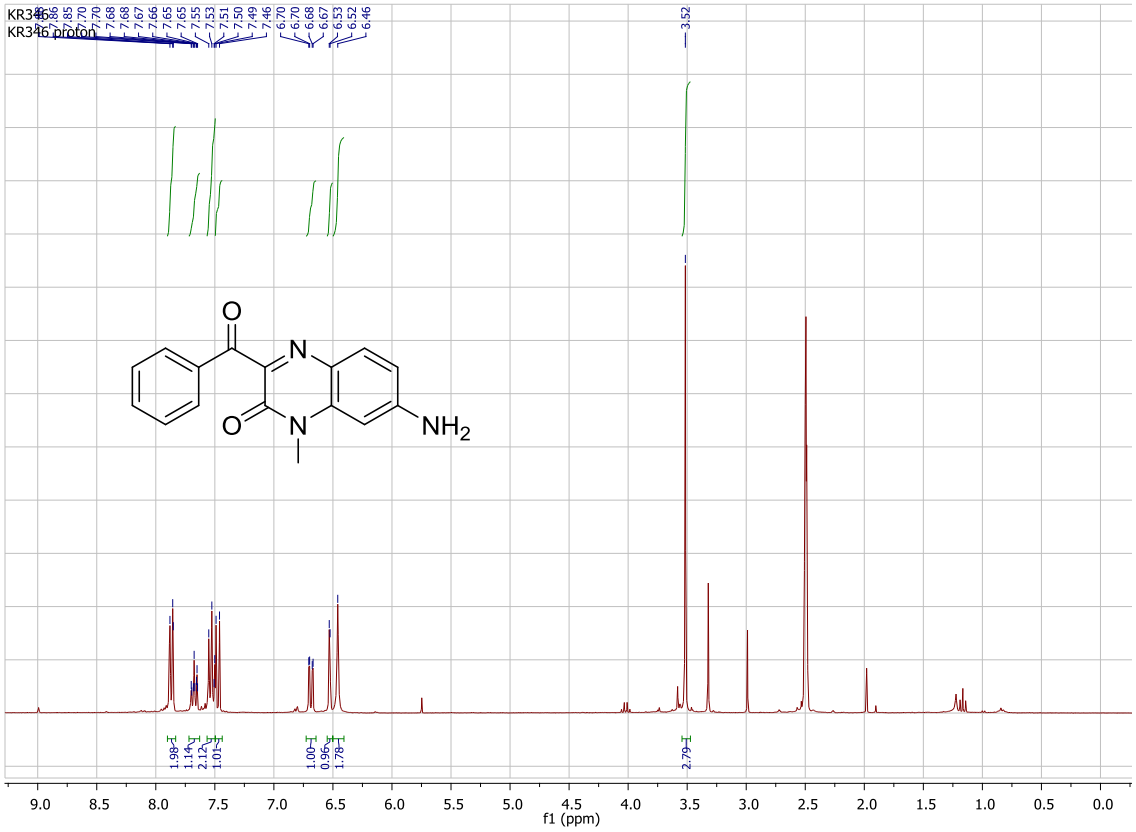
^{1}H NMR spectrum of compound **4** recorded in $CDCl_3$ at 300 MHz



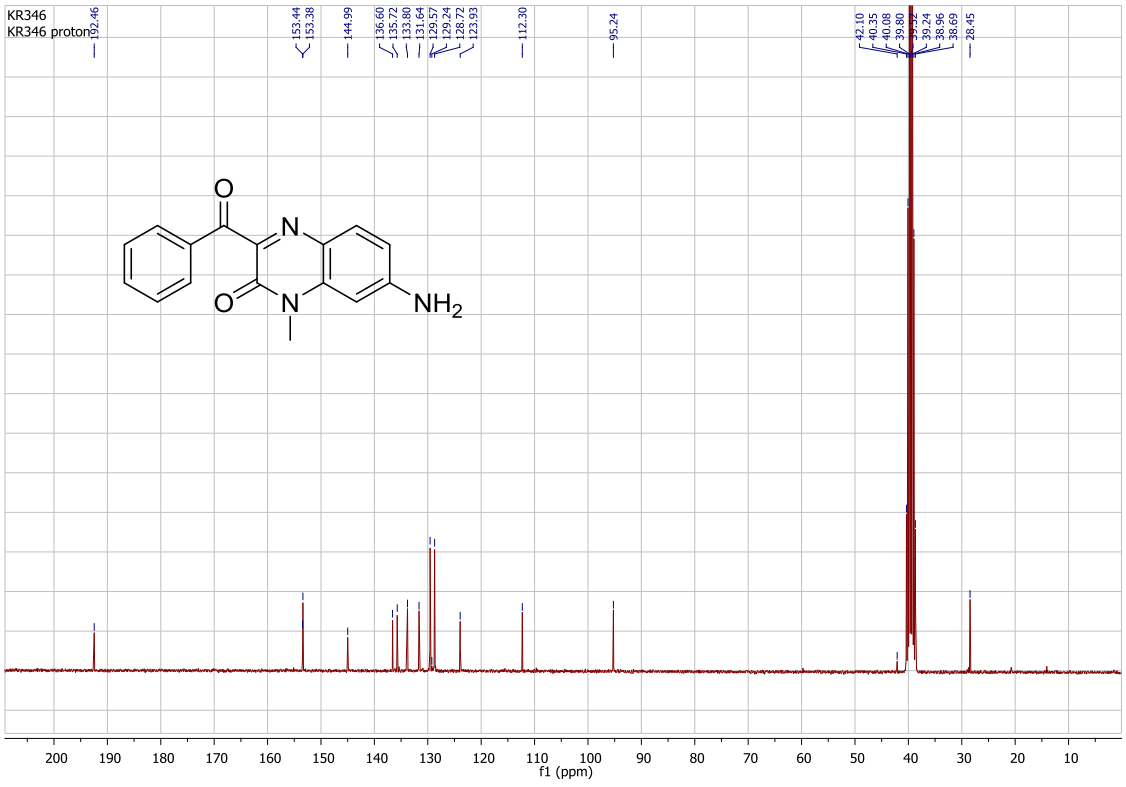
^{13}C NMR spectrum of compound **4** recorded in $CDCl_3$ at 75 MHz



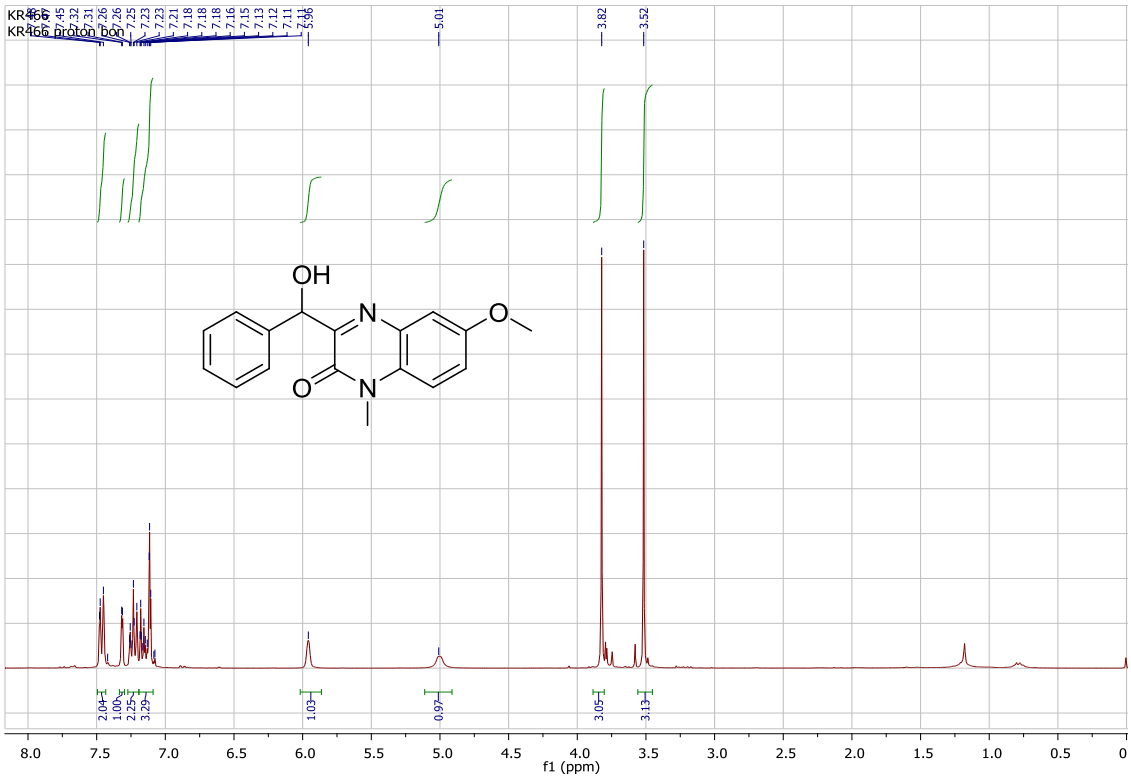
^{1}H NMR spectrum of compound **7** recorded in DMSO-D_6 at 300 MHz



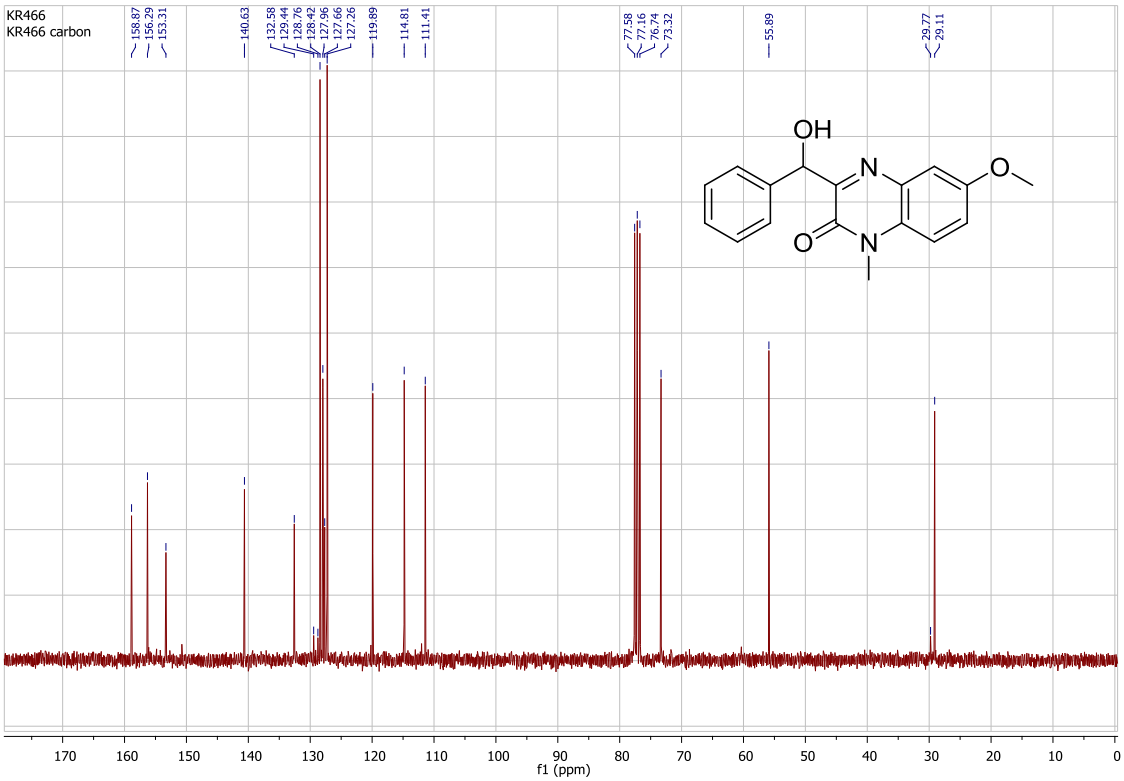
^{13}C NMR spectrum of compound **7** recorded in DMSO-D_6 at 75 MHz



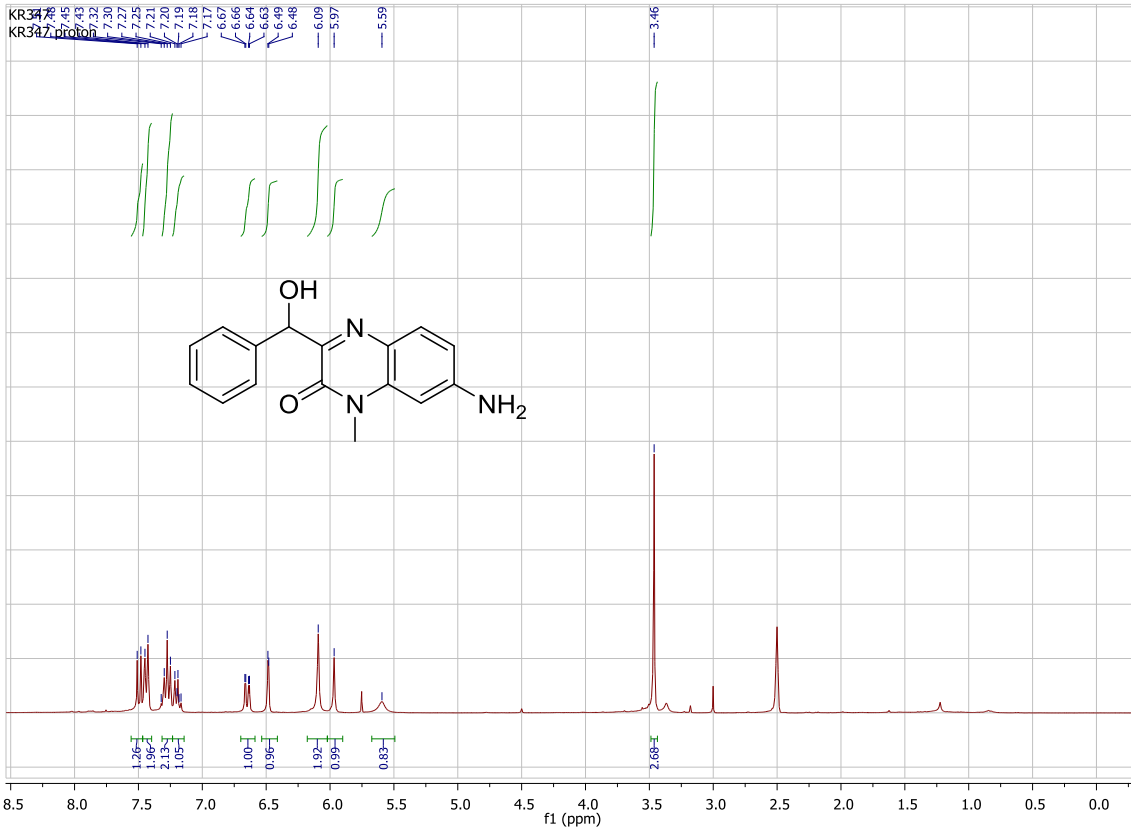
^{1}H NMR spectrum of compound **8** recorded in $CDCl_3$ at 300 MHz



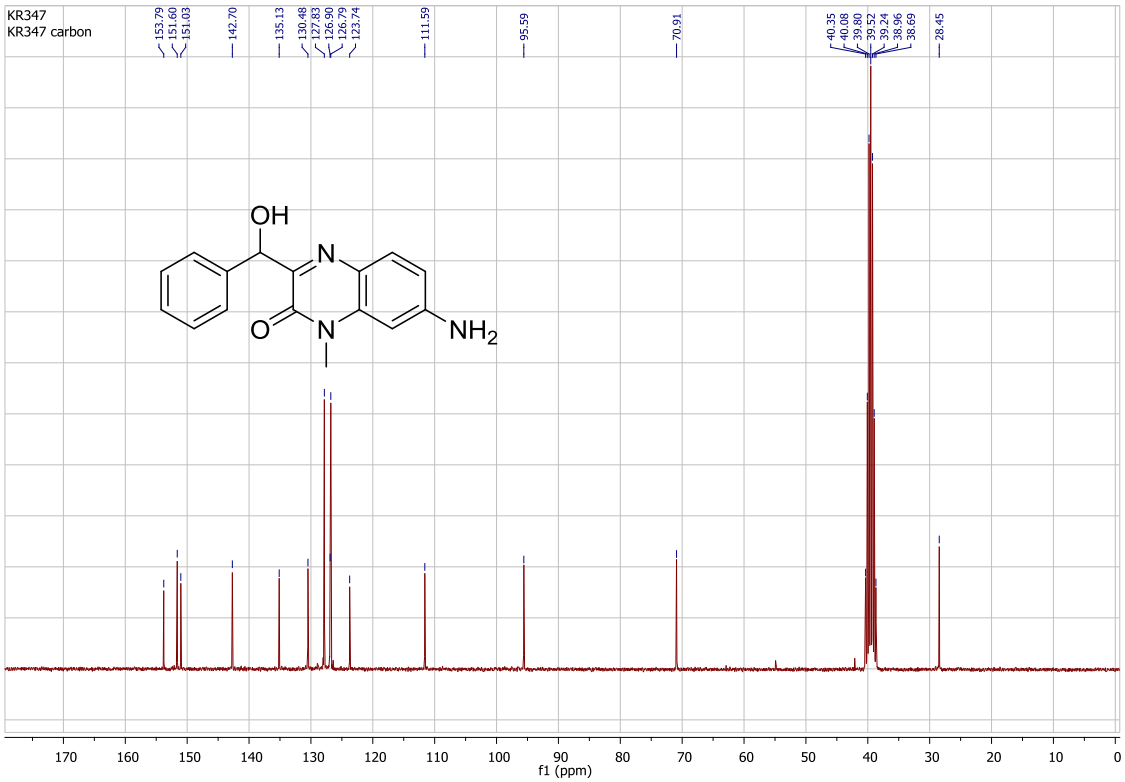
^{13}C NMR spectrum of compound **8** recorded in $CDCl_3$ at 75 MHz



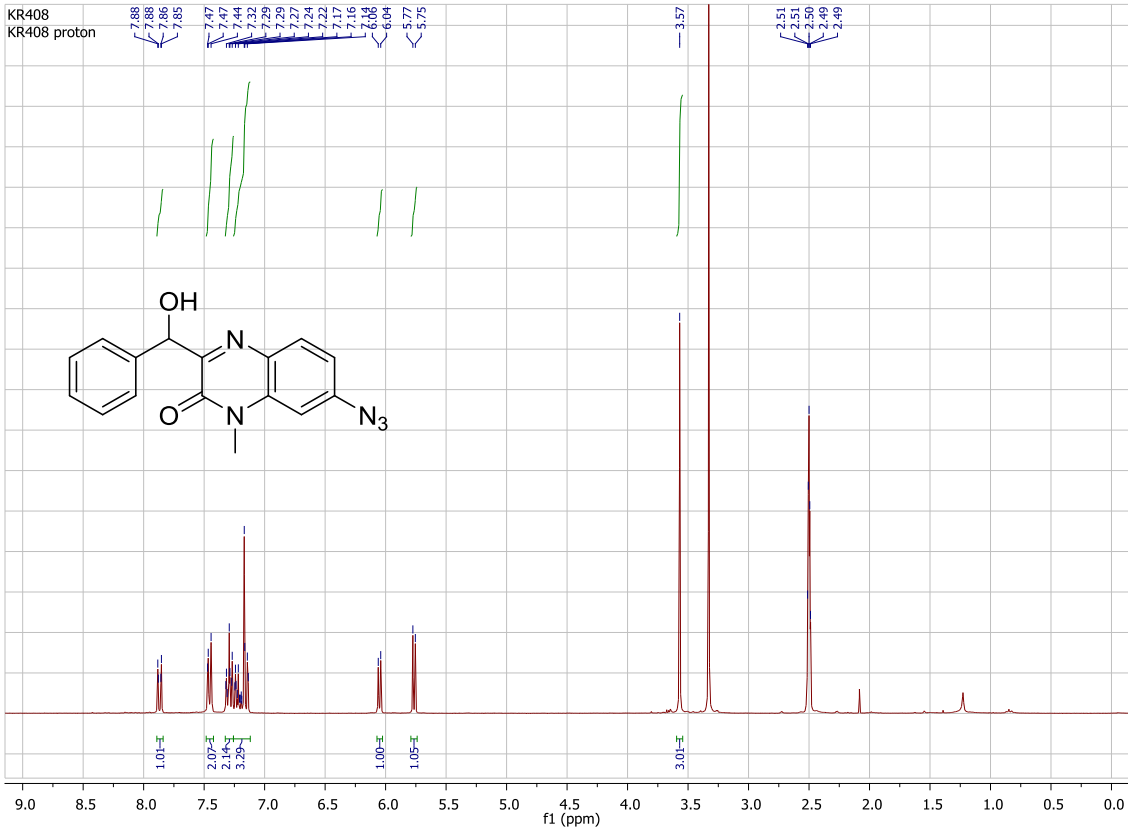
^{1}H NMR spectrum of compound **10** recorded in DMSO-D_6 at 300 MHz



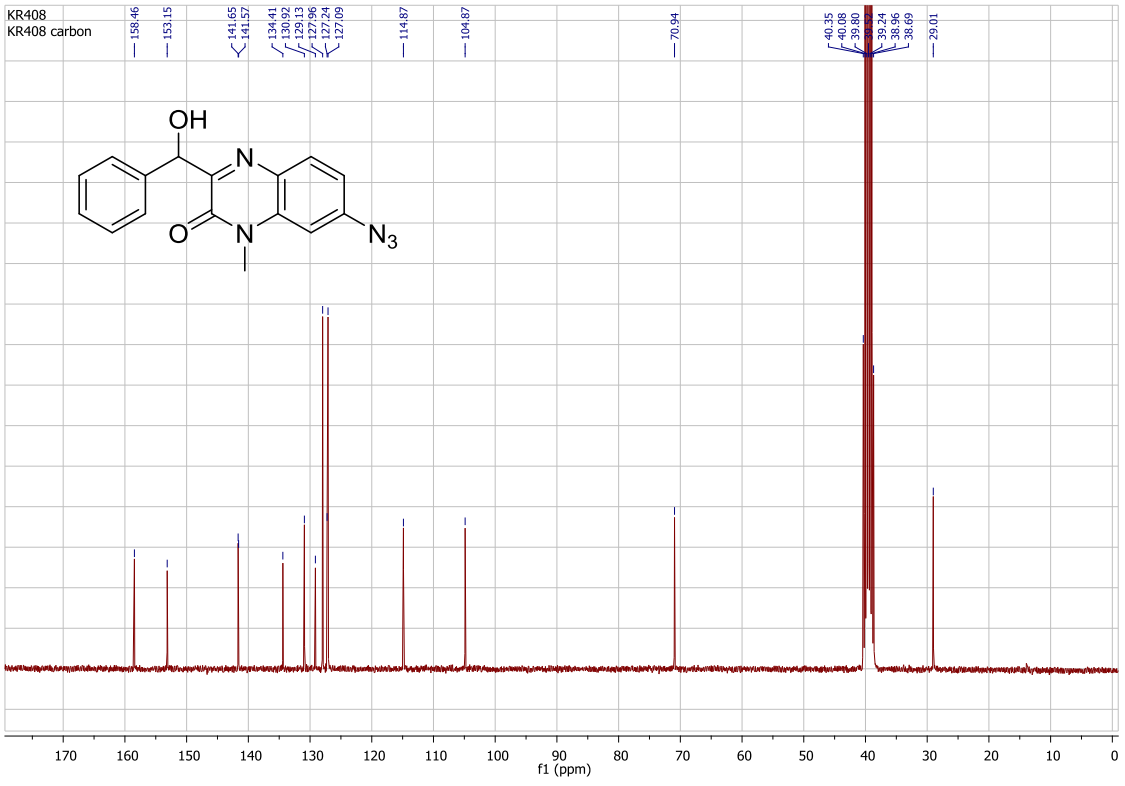
^{13}C NMR spectrum of compound **10** recorded in DMSO-D_6 at 75 MHz



^{1}H NMR spectrum of compound **11** recorded in DMSO-D_6 at 300 MHz



^{13}C NMR spectrum of compound **11** recorded in DMSO-D_6 at 75 MHz



II. Absorbance, excitation and emission spectra of 7-aminoquinoxalinone 10 in different solvents

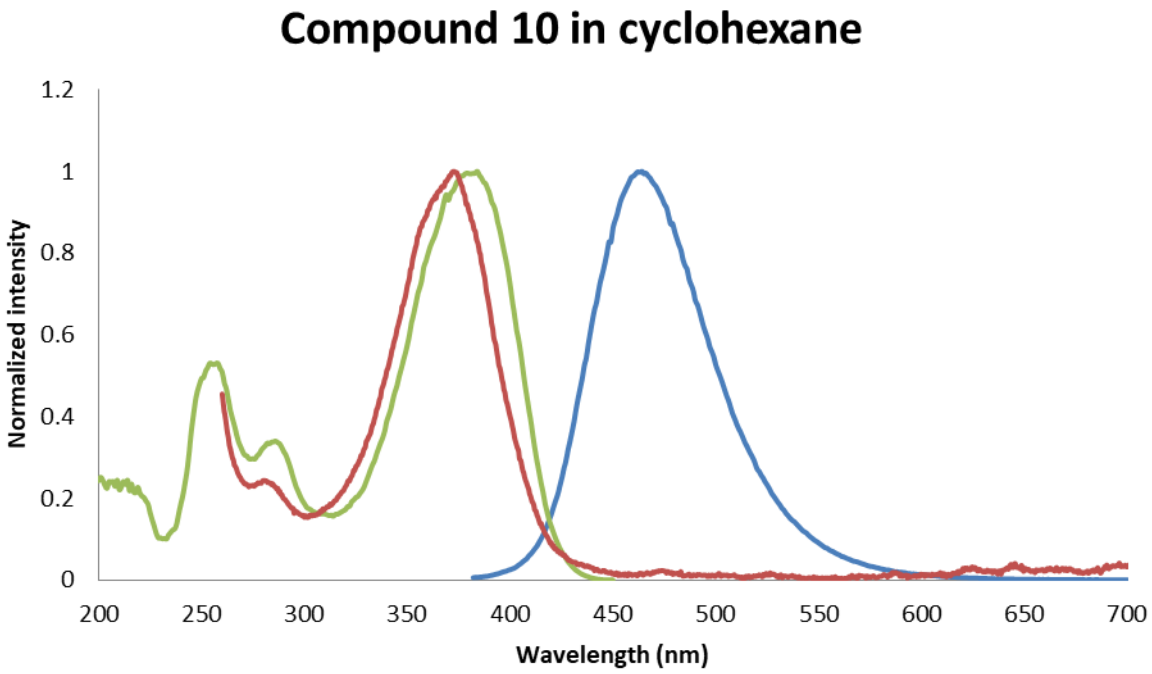


Figure S1 Normalized absorption (red trace), emission (blue trace, λ_{ex} = 366 nm) and excitation (green trace, λ_{em} = 464 nm) spectra for compound **10** at 25 °C in cyclohexane.

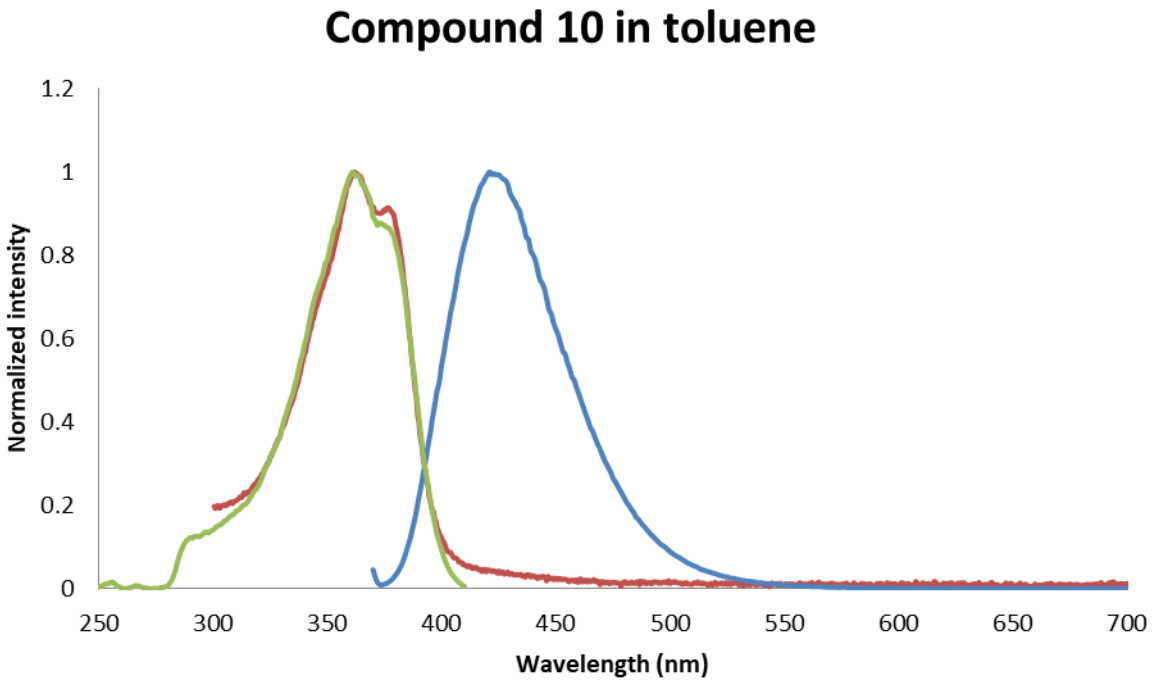


Figure S2 Normalized absorption (red trace), emission (blue trace, λ_{ex} = 366 nm) and excitation (green trace, λ_{em} = 421 nm) spectra for compound **10** at 25 °C in toluene.

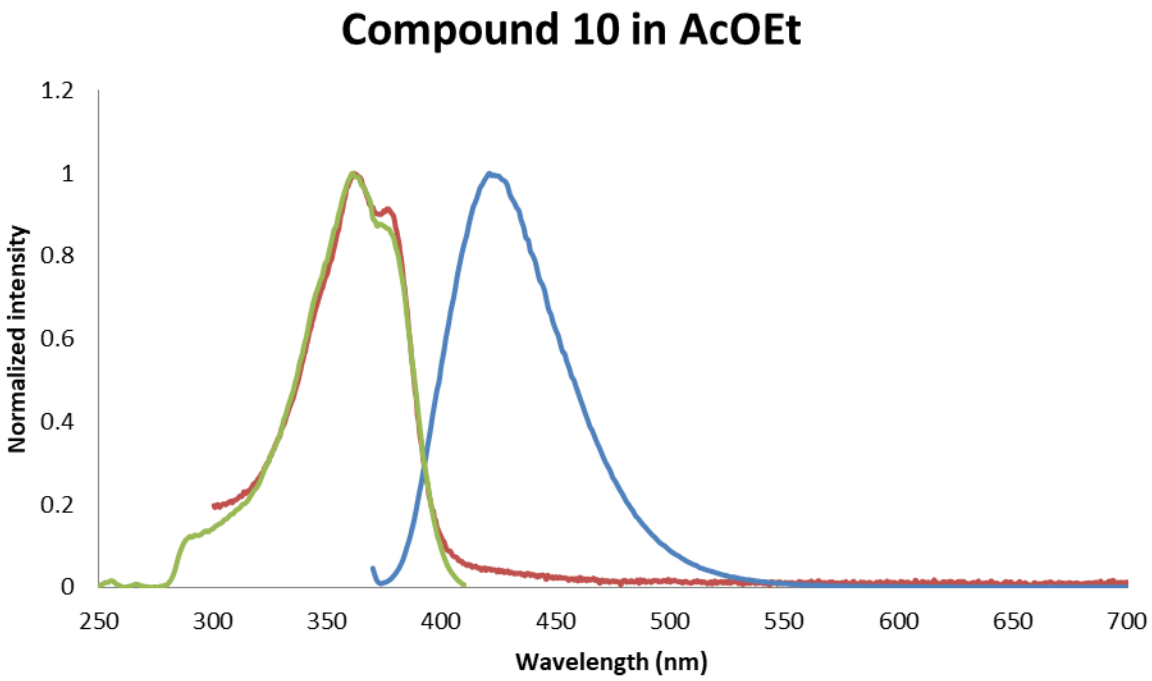


Figure S3 Normalized absorption (red trace), emission (blue trace, λ_{ex} = 366 nm) and excitation (green trace, λ_{em} = 434 nm) spectra for compound **10** at 25 °C in AcOEt.

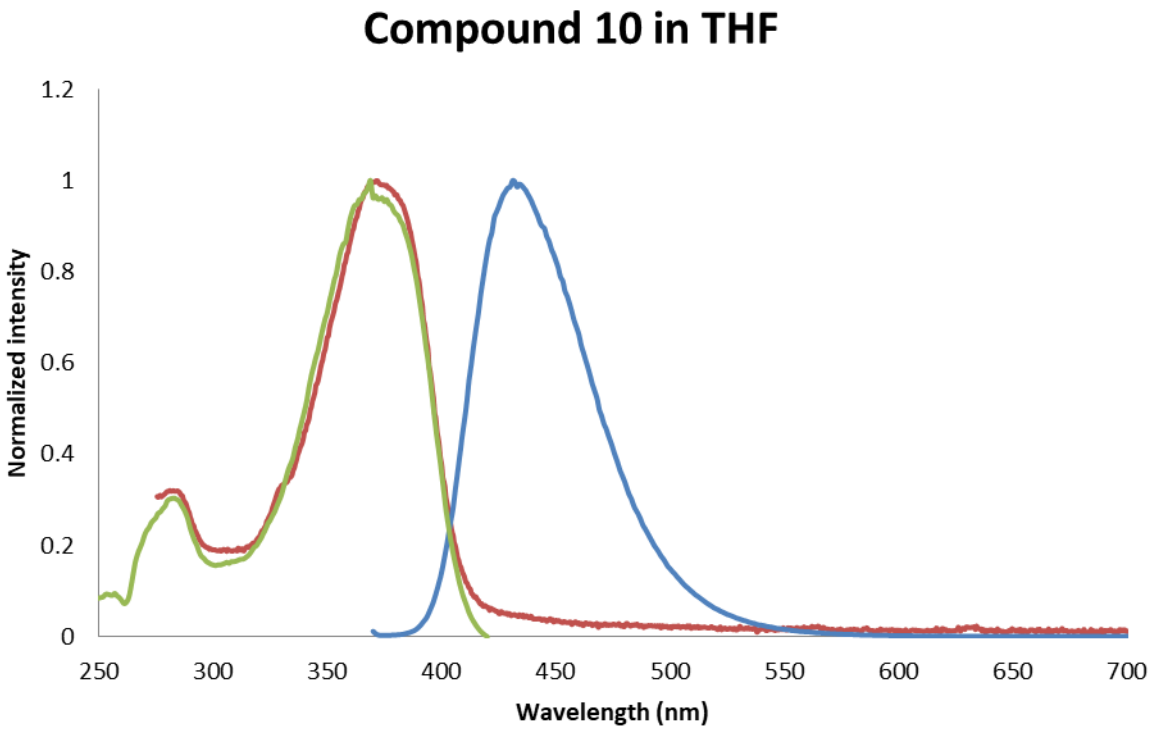


Figure S4 Normalized absorption (red trace), emission (blue trace, λ_{ex} = 366 nm) and excitation (green trace, λ_{em} = 431 nm) spectra for compound **10** at 25 °C in THF.

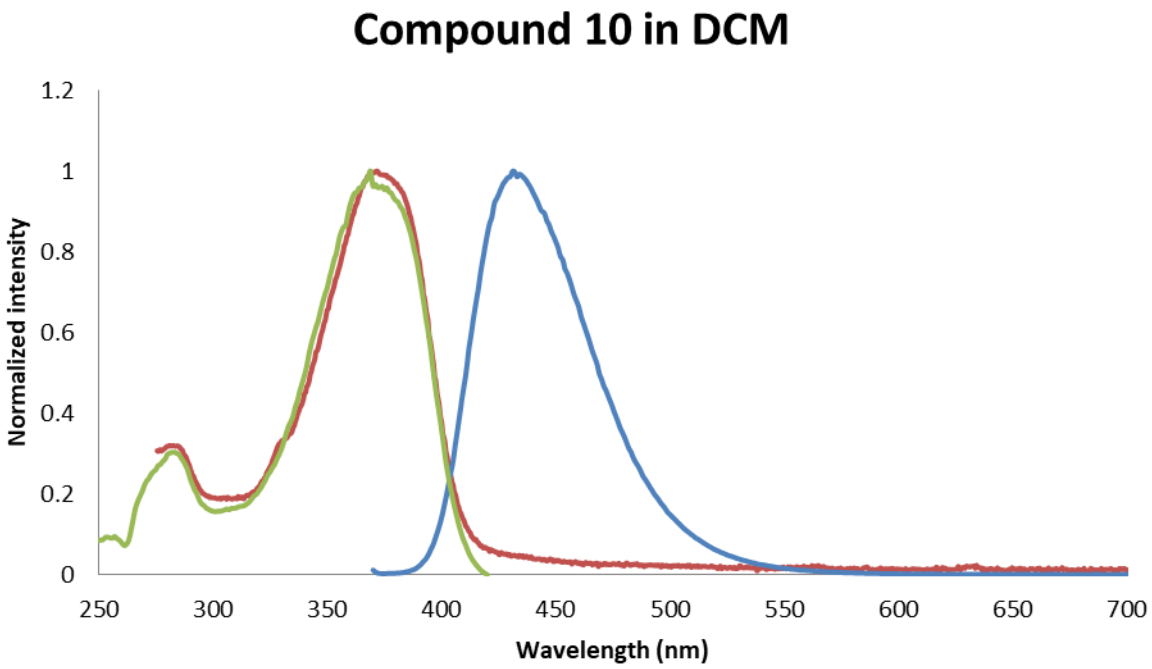


Figure S5 Normalized absorption (red trace), emission (blue trace, λ_{ex} = 366 nm) and excitation (green trace, λ_{em} = 429 nm) spectra for compound **10** at 25 °C in DCM.

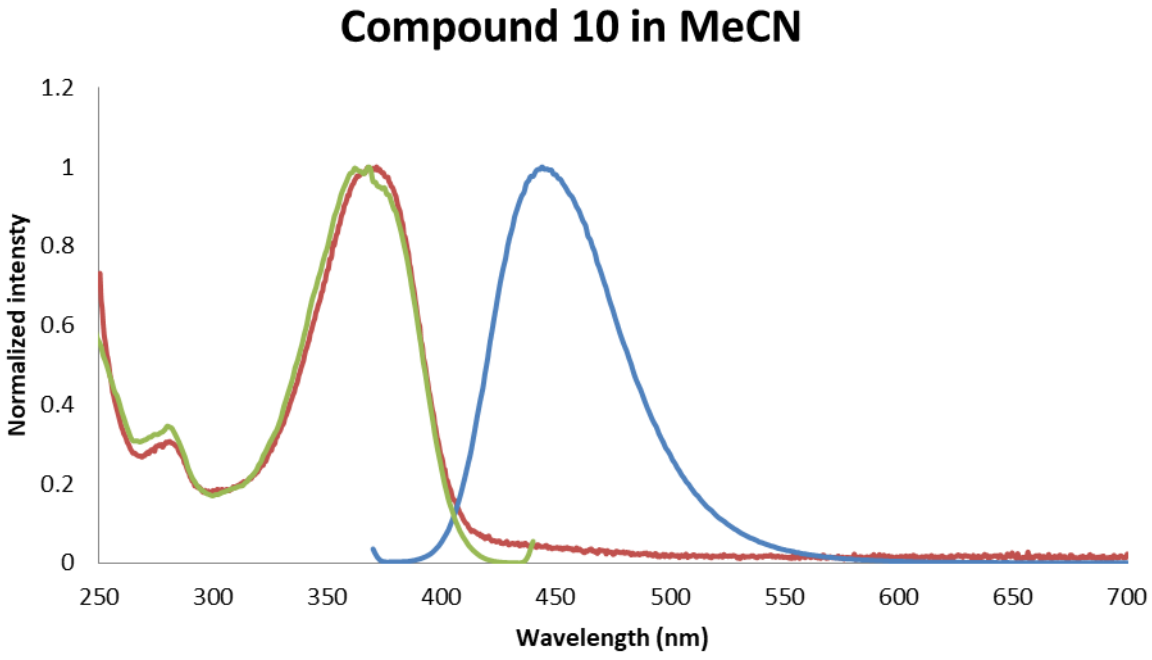


Figure S6 Normalized absorption (red trace), emission (blue trace, λ_{ex} = 366 nm) and excitation (green trace, λ_{em} = 444 nm) spectra for compound **10** at 25 °C in MeCN.

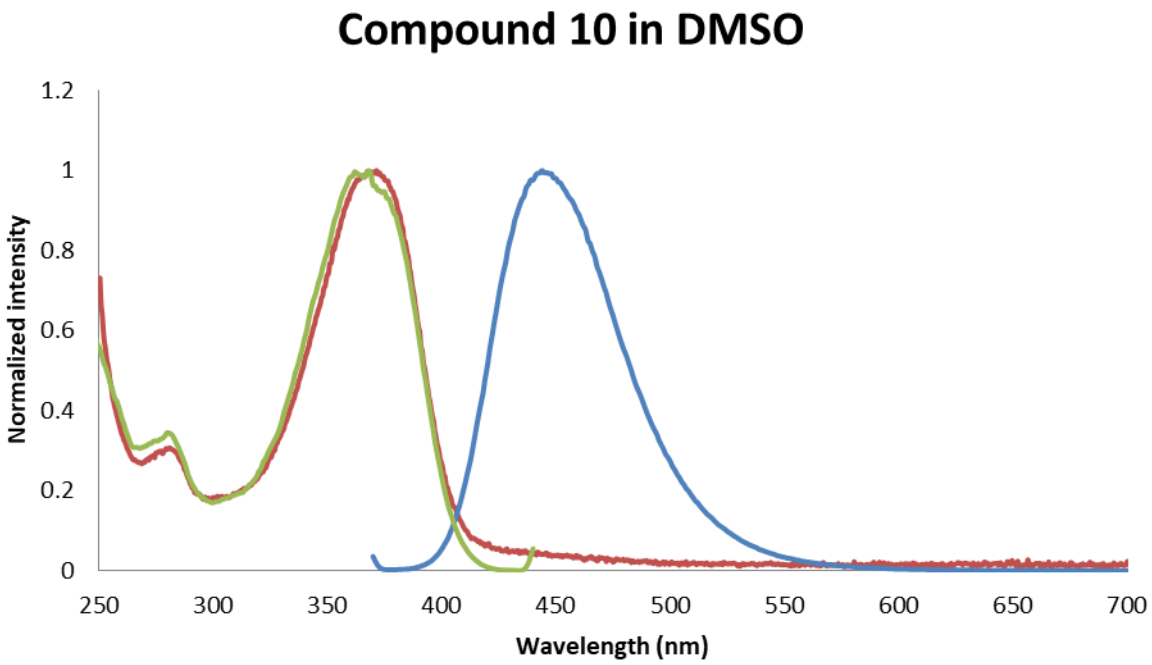


Figure S7 Normalized absorption (red trace), emission (blue trace, λ_{ex} = 366 nm) and excitation (green trace, λ_{em} = 459 nm) spectra for compound **10** at 25 °C in DMSO.

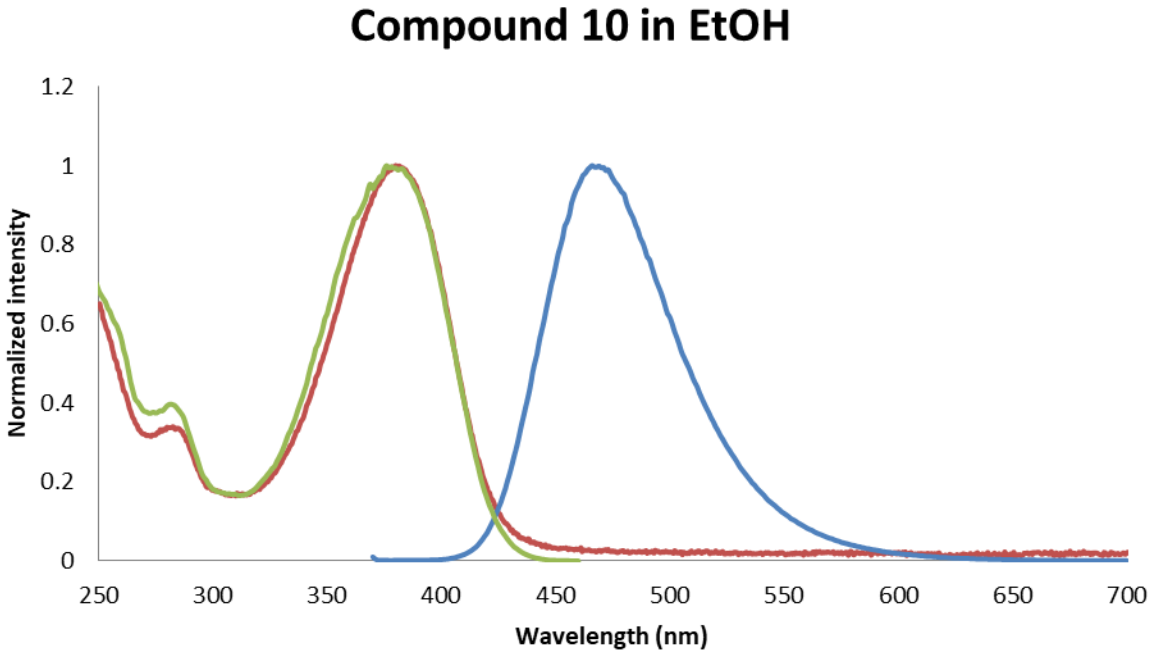


Figure S8 Normalized absorption (red trace), emission (blue trace, λ_{ex} = 366 nm) and excitation (green trace, λ_{em} = 467 nm) spectra for compound **10** at 25 °C in EtOH.

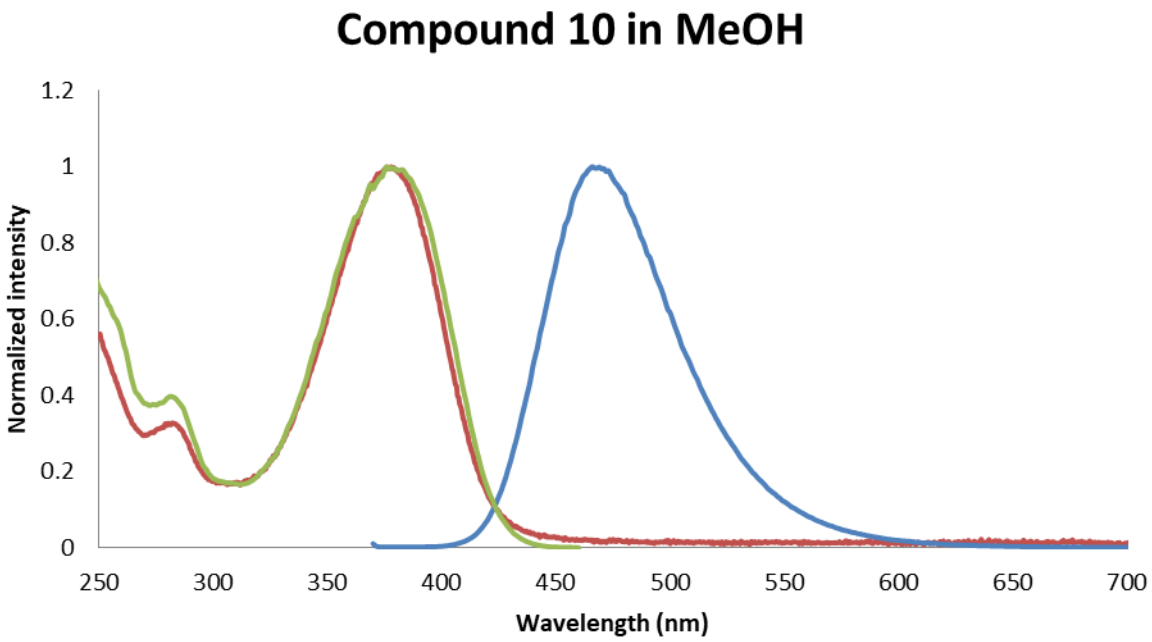


Figure S9 Normalized absorption (red trace), emission (blue trace, λ_{ex} = 366 nm) and excitation (green trace, λ_{em} = 474 nm) spectra for compound **10** at 25 °C in MeOH.

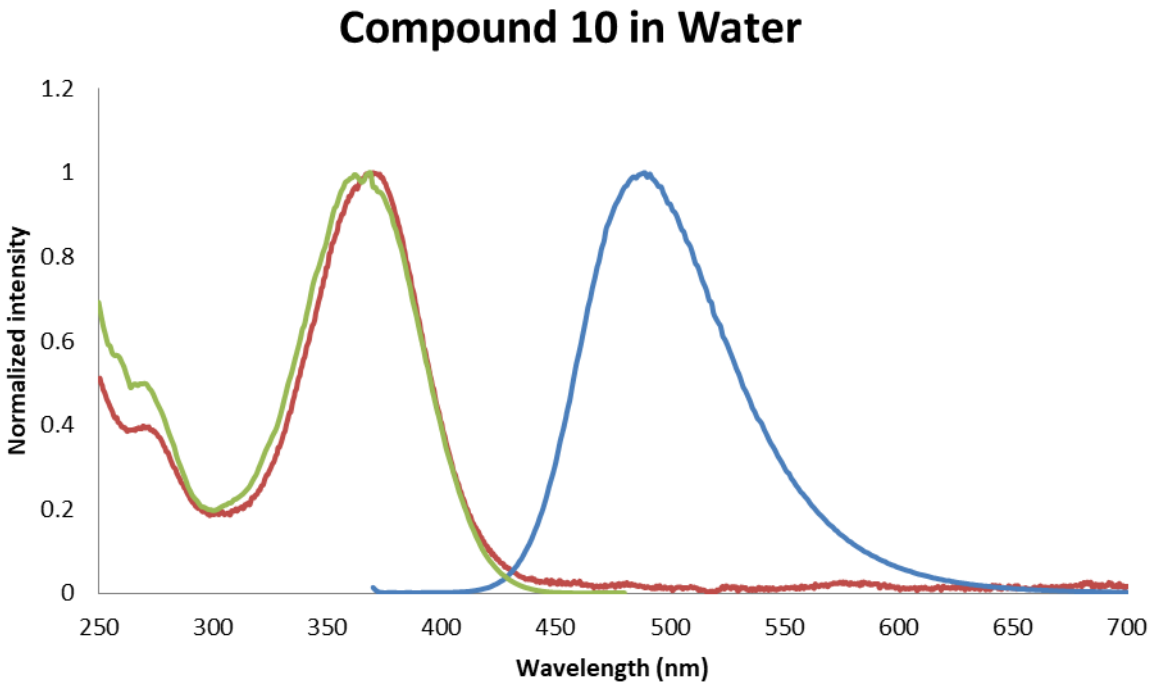


Figure S10 Normalized absorption (red trace), emission (blue trace, λ_{ex} = 366 nm) and excitation (green trace, λ_{em} = 489 nm) spectra for compound **10** at 25 °C in Water.

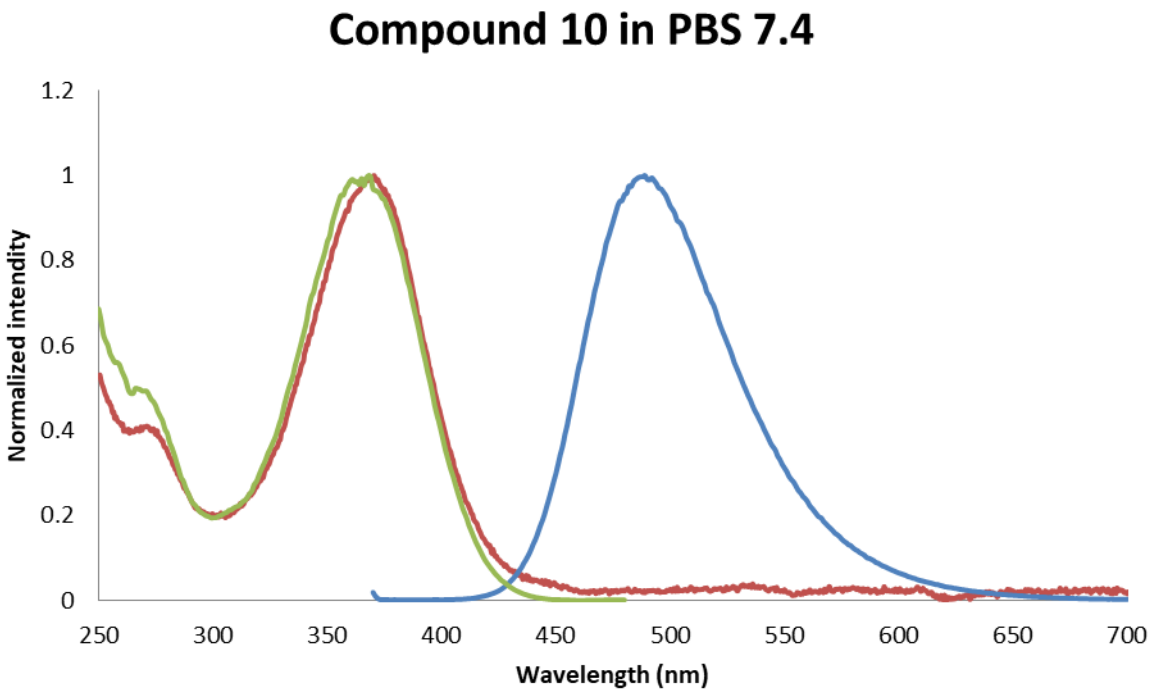


Figure S11 Normalized absorption (red trace), emission (blue trace, λ_{ex} = 366 nm) and excitation (green trace, λ_{em} = 489 nm) spectra for compound **10** at 25 °C in PBS buffer.

III. pH dependence of the emission of 7-aminoquinoxalinone 10

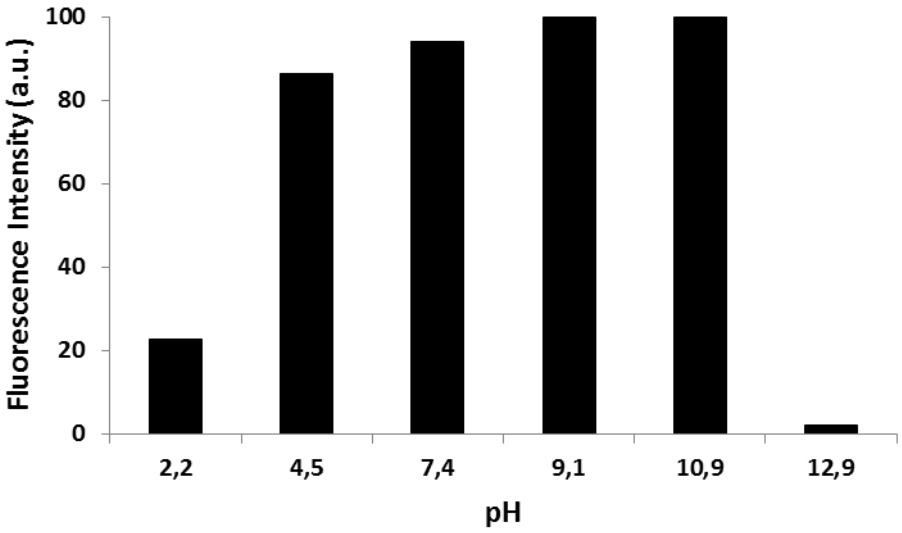


Figure S12 Fluorescence responses of 7-aminoquinoxalinone **10** in aq. HCl (10 mM, pH 2.2), sodium acetate buffer (0.1 M, pH 4.5), in PBS (0.1 M, pH 7.4), in sodium borate buffer (0.1 M, 9.1), in sodium carbonate buffer (0.1 M, pH 10.9), or in aq. NaOH (2.0 M, pH = 12.9) at 25 °, λ_{ex}= 366 nm, λ_{em}= 489 nm.

IV. *In vitro* fluorescence assays

A 10 µM solution of the corresponding fluorogenic probe (compound **11** or 4-methyl-7-azidocoumarin) was prepared in 3 mL of PBS Buffer (0.1 M pH 7.4) and transferred into the quartz fluorescence cell (Hellma, 104F-QS, 10 x 4 mm, Chamber volume 3.5 mL). A 0.3 M solution of bio-analytes (5 µL, 50 equiv.) was added and the resulting mixture was incubated at 25 °C for 60 min or 240 min. After excitation at 370 nm (for compound 11) or 330 nm (for the 4-methyl-7-azidocoumarin), the fluorescence emission at 489 nm (for compound **11**) or 450 nm (4-methyl-7-azidocoumarin) was simultaneously monitored over time with measurements recorded every 0.5 s. Emission spectra of the probe was recorded before and after cleavage in order to determine the quenching efficiency (QE), which was calculated on the basis of the following equation: QE = 100 × [1-(fluorescence emission intensity of the probe)/(fluorescence emission intensity of the probe after compete reaction with NaHS)]

For probe **11**: QE = 100 x [1-(1114.4)/(80925)] = 98.6 %

For 4-methyl-7-azido-coumarin: QE = 100 x [1-(261)/(58828)] = 99.6 %

V. HPLC monitoring of the reduction of 11 by hydrogen sulfide

A 0.1 mg/mL solution of probe **11** in DMSO/ACN/H_2O (1 mL, 1:1:8) containing NaHS (50 equiv.) was monitoring by RP-HPLC at t = 5 min and t = 1 h.

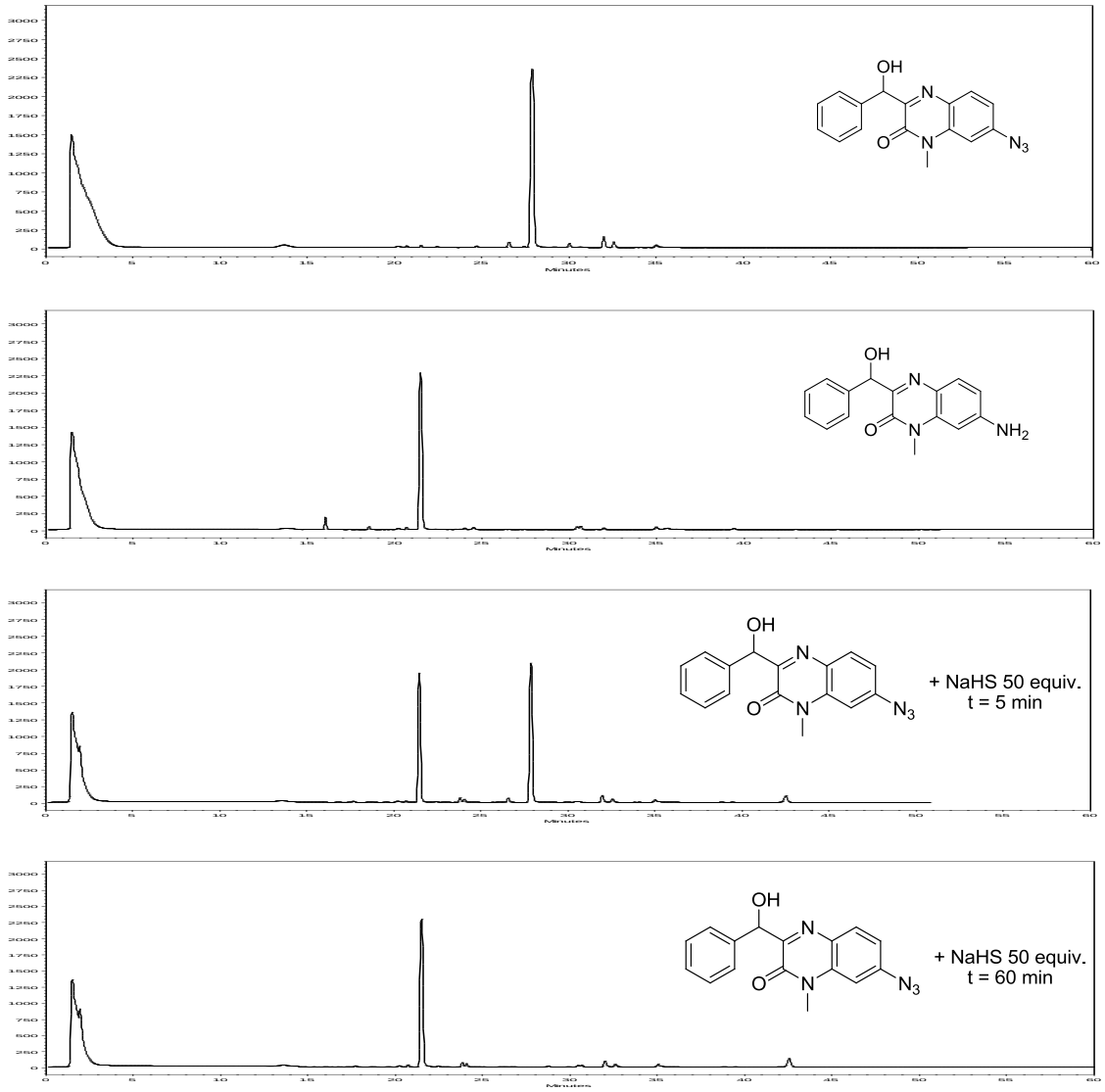


Figure S13 RP-HPLC elution profiles (analytical system) of the reduction of probe **11** by NaHS (50 equiv.) (detection in "MaxPlot" mode).

VI. Detection limit of probe 11

The detection limit of probe **11** towards NaHS analyte was calculated on the basis of the linear relationship between the emission intensity at 489 nm and the concentration of NaHS. The detection limit was calculated with the following equation: detection limit = 3σ/k, with σ the standard deviation of blank measurement, k the slope between the fluorescence intensity (F) at 489 nm versus the concentration of NaHS. σ was found to be 1.023 and k = 2.4249, therefore a detection limit of 1.30 µM was found.

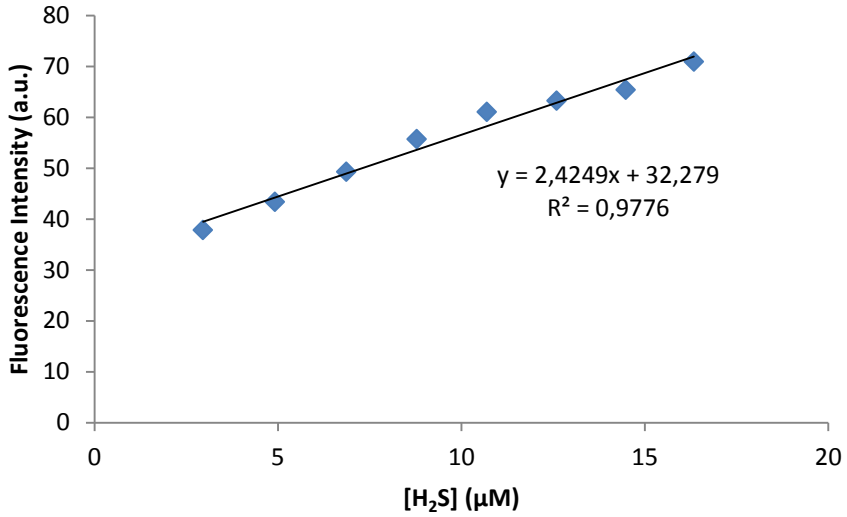


Figure S14 Fluorescence intensity changes of probe **11** (20 µM) in PBS (0.1 M, pH = 7.4) at 25 °C for NaHS [3-17 µM], λ_{ex}= 366 nm, λ_{em}= 489 nm (fluorescence intensity values were measured when the intensity reached a plateau *i.e.*t = 5-15 min).