Supporting Information

Unraveling the Photophysical Characteristics and Biological Applications of Vinyl Sulfones as Viscosity Sensors

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General information

Commercially available chemicals were purchased in high quality and were used without further purification. The reactions were monitored by thin layer chromatography (TLC) carried out on silica gel plates (60 F_{254}, MERCK, Germany) and visualized by a dual short (λ = 254 nm) / long (λ = 366 nm) wavelength UV lamp. Column chromatography was performed with silica gel 60 (particle size 0.06−0.2 mm; 70−230 mesh ASTM). ^{1}H-NMR and ^{13}C-NMR spectra were recorded with Bruker AVANCE 500 (500 MHz) spectrometer. Spectra were evaluated in first order and coupling constants *J* are reported in Hertz (Hz).Splitting patterns for the spin multiplicity of the signals in the spectra are given as the following: s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublet and combinations thereof. Chemical shifts for ^{1}H-NMR were reported as *δ*, parts per million (ppm), relative to the signal of $CHCl_3$ at 7.26 ppm and signal of DMSO-d_6 at 2.50. Chemical shifts for ^{13}C-NMR were reported as *δ*, parts per million (ppm), relative to the signal of $CHCl_3$ at 77.16 ppm and signal of DMSO-d_6 at 39.52. Mass spectrometry was measured under ESI conditions.

Materials and methods

Spectroscopic materials and methods

Absorption and fluorescence spectroscopic analyses

All UV-vis absorption and fluorescence spectra were recorded on a UV-vis spectrophotometer (Agilent Technologies Cary 300) and a spectrofluorometer (PerkinElmer LS55), respectively. In both analyses, stock solutions of vinyl sulfones-NO_2 (**3a**–**3d**) and vinyl sulfones-NH_2 (**4a**–**4d**) (3 mM) were prepared in DMSO. A suitable amount of the stock solution was added to various solvents including toluene, chloroform, tetrahydrofuran (THF), dichloromethane (DCM), dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), acetone, acetonitrile (MeCN), methanol (MeOH), DI water and 10 mM PBS buffer (pH 7.4) (3 mL) to obtain the final concentration of 10 μM in the working solutions. For fluorescence experiments, the emission spectra were recorded at the excitation wavelength of each compound's absorption maximum wavelength. The fluorescence quantum yields (Φ_f) were calculated relative to quinine sulfate in 0.1 M H_2SO_4 as a standard (Φ_f = 0.54).

Fluorescence response to solvent viscosity

Each vinyl sulfones-NH_2 (**4a**–**4d**) (5 μM) was dissolved in water/glycerol mixtures (0–100 % v/v). Then, a spectrofluorometer (PerkinElmer LS55) was used to analyze the prepared solutions. The emission spectra were recorded at an excitation wavelength of 315 nm. The fluorescence quantum yields (Φ_f) were estimated relative to quinine sulfate in 0.1 M H_2SO_4 as a standard (Φ_f = 0.54), using the equation below.

$$\Phi_f = \Phi_{std} \times \left(\frac{A_{sample}}{A_{std}}\right) \times \left(\frac{I_{std}}{I_{sample}}\right) \times \left(\frac{\eta_{sample}}{\eta_{std}}\right)^2$$

where Φ denotes fluorescence quantum yield, *A* = a peak area of emission, *I* = an absorbance at the excitation wavelength, and *η* stands for solvent reflective index.

pH effects of vinyl sulfones-NH_2 (4a–4d) by fluorescence spectroscopic analysis

Vinyl sulfones-NH_2 (**4a**–**4d**) (10 μM) was dissolved in commercial pH 1–12 buffers (Merck, St. Louis, MO, USA; phosphoric acid (H_3PO_4) for pH 1.08–3.05, acetic acid (CH_3COOH) for pH 4.05–5.02, potassium dihydrogen phosphate (KH_2PO_4) for pH 6.00–9.16, potassium hydrogen phosphate ($KH(PO_4)^{2-}$) for pH 10.08–12.00. The actual pH values of the prepared buffer solutions were measured by a pH meter. The resulting solutions were examined using a spectrofluorometer.

Photo-Stability test

Vinyl sulfones-NH_2 (**4a**–**4d**) (10 μM) was dissolved in DMSO for measurement of the absorption and emission intensity. The prepared solutions of **4a**–**4d** were irradiated by 100 W UV light (365 nm) at room temperature with a distance of 30 cm. The UV-vis absorption and fluorescence spectra of the solutions were measured at different time points including 0, 5, 10, 15, 20, and 30 min by UV-Vis microplate reader (Thermo Scientific Multiskan GO) and fluorescence microplate reader (ThermoScientific Varioskan LUX), respectively. The photo-stability was represented as photobleaching absorption and emission (%) determined from the change in intensity of absorption and emission at the maxima of absorption and emission before and after irradiation.

Computational calculation

The frontier molecular orbitals of **3a** and **4a**–**4d** are calculated by density functional theory (DFT) calculations at the B3LYP/6-311G level using the Turbomole program. The geometrical structure of the electronic ground states was optimized by considering the continuum solvation model (CSM) in the Conductor-like Screening Model (COSMO), using gas ($\varepsilon = 1$) and water ($\varepsilon = 78$) as the solvent.

Methods for in vitro assays

Cell culture

Human liver hepatocellular carcinoma (HepG2) and human embryonic kidney 293 (HEK-293) cell lines were cultured on 75 cm^3 culture flasks in Dulbecco's Modified Eagle's Media (DMEM, Hyclone) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin–streptomycin (Corning). The cells were maintained at 37 ˚C in a humidified atmosphere containing 5% of CO_2.

Cell viability

The cells were seeded on a 96-well cell culture plate at approximately 1.5×10^4 cells per/well for 24 h. Then, the cells were incubated with 0, 2.5, 5, 10, 20, 30, 40, and 50 μM of vinyl sulfones-NO_2 (**3a**–**3d**) and vinyl sulfones-NH_2 (**4a**–**4d**) for 24 h. After incubation, the cells were washed with 0.01 M of PBS pH 7.4 twice before adding 0.5 mg/mL of MTT reagent (Methylthiazolyldiphenyl-tetrazolium bromide, Sigma-Aldrich) in 0.01 M of PBS pH 7.4 solution for 2.5 h. After solution removal, DMSO was added to dissolve the formazan product and detected through UV-vis absorption of formazan at wavelength 560 nm (BMG Labtech/SPECTROstar Nano microplate reader).

Cell imaging

For the time-dependent assay, approximately 1×10^4 cells of HepG2 or HEK-293 were seeded on an 8-well Chambered Coverglass (LabTek, Nunc) for 24 h. Then, the cells were incubated with 20 μM of vinyl sulfones-NO_2 (**3a**–**3d**) and vinyl sulfones-NH_2 (**4a**–**4d**) for 0, 1, and 6 h. After incubation, the cells were washed with 0.01 M PBS buffer twice. The cells were

visualized under a 60x oil immersion objective lens by Laser Scanning Confocal Microscope (Nikon A1Rsi) with a 405 nm laser.

For the dose-dependent assay, approximately 1×10^4 cells of HepG2 were seeded on an 8-well Chambered Coverglass (LabTek, Nunc) for 24 h. Then, the cells were incubated with 0, 10, and 20 μM of vinyl sulfones-NO_2 (**3a**–**3d**) and vinyl sulfones-NH_2 (**4a**–**4d**) for 6 h. After incubation, the cells were washed with 0.01 M PBS buffer twice. The cells were visualized under a 60x oil immersion objective lens by Laser Scanning Confocal Microscope (Nikon A1Rsi) with 405 nm laser.

For the co-localization assay, approximately 1×10^4 cells of HepG2 were seeded on 8-well Chambered Coverglass (LabTek, Nunc) for 24 h. After incubation, the cells were incubated with 20 μM of **4c** and **4d** for 6 h. Then, the cells were washed with 0.01 M PBS buffer twice and were treated with media containing 1.0 μM of MitoTracker™ Green FM (Thermo Fisher Scientific), LysoTracker™ Green DND-26 (Thermo Fisher Scientific), ER-Tracker™ Green (BODIPY™ FL Glibenclamide, Thermo Fisher Scientific), and C6-NBD Ceramide (Golgi tracker, Avanti Polar Lipids) for 10 min. The cells were visualized under 60x oil immersion objective lens by Laser Scanning Confocal Microscope (Nikon A1Rsi) with 405 nm laser and 488 nm laser (MitoTracker, LysoTracker, Golgi tracker, and ER tracker), and Pearson's correlation coefficient for colocalization of **4c** and **4d** with organelles trackers was obtained from ImageJ.

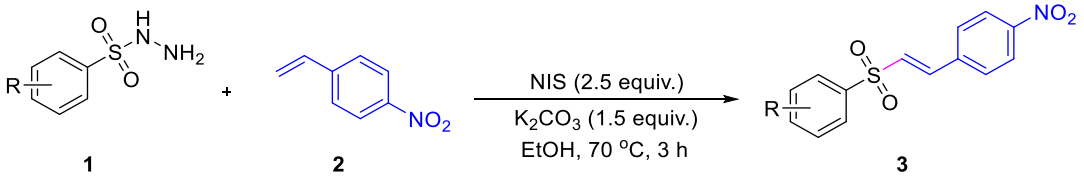
For the viscosity assay, approximately 1×10^4 cells of HepG2 were seeded on 8-well Chambered Coverglass (LabTek, Nunc) for 24 h. After incubation, the cells were incubated with 20 μM of **4c** and **4d** for 1 h. Then, the cells were washed with 0.01 M PBS buffer twice. To induce mitochondria swelling, the cells were pretreated with nystatin (20 μM) for 30 min at 37 °C before **4c** and **4d** (20 μM) staining and incubated for 1 h at 37 °C. The cells were visualized under a 60x oil immersion objective lens by Laser Scanning Confocal Microscope (Nikon A1Rsi) with a 405 nm laser.

Statistical analysis

The mean of three distinct observations (n = 3) and the standard deviation (mean ± SD) from three separate experiments are used to present the data. The Student's T-test was used for the

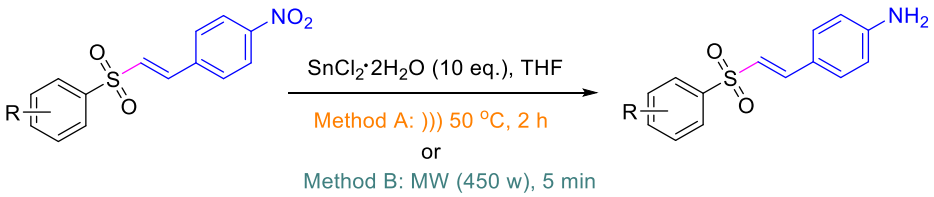
statistical analysis. P values of < 0.05 were considered significant (*P < 0.05, **P < 0.01, ***P < 0.001).

General procedures for the synthesis of vinyl sulfones-NO_2 (3)[1]



To a solution of sulfonyl hydrazide **1** (1.5 mmol, 1.5 equiv.), 4-nitrostyrene **2** (0.13 mL, 1.0 mmol, 1.0 equiv.), and K_2CO_3 (0.2073 g, 1.5 mmol, 1.5 equiv.) in EtOH (5 mL) were added slowly, portion-wise NIS (0.2073 g, 2.5 mmol, 2.5 equiv.) at room temperature. Then, the reaction mixture was allowed to stir at 70 °C for 3 h. The reaction was cooled to room temperature and EtOH was evaporated under reduced pressure. The crude mixture was diluted in EtOAc (5 mL) and quenched with sat. $Na_2S_2O_3$ (5 mL), and the resulting mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (20 mL), dried (anh. $MgSO_2$), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexanes) to yield the corresponding vinyl sulfones-NO_2 (**3a**–**3d)**.

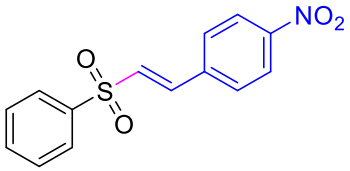
General procedure for the synthesis of vinyl sulfones (4)



Method A: To a vial filled with vinyl sulfones-NO_2 (**3**, 0.2 mmol, 1.0 equiv.) and $SnCl_2.2H_2O$ (451 mg, 2.0 mmol, 10.0 equiv.) was diluted with THF (1 mL). The reaction mixture was sonicated at 50 °C for 2 h, then extracted with EtOAc (3 × 10 mL) and washed with brine (20 mL). The organic layer was dried (anh. MgSO4), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexanes or DCM/MeOH) to provide the corresponding compounds **4**.

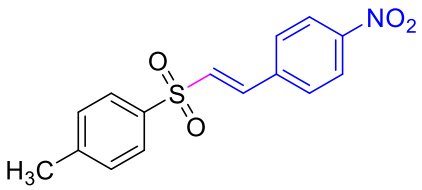
Method B: To crimp-sealed thick-walled glass tube containing vinyl sulfones-NO_2 (**3**, 0.2 mmol, 1.0 equiv.) and $SnCl_2.2H_2O$ (451 mg, 2.0 mmol, 10.0 equiv.) was diluted with THF (1 mL). The sealed reaction tube was irradiated by microwave (power 450 W) for 5 min. After completion of the reaction, the reaction mixture was extracted with EtOAc (3 × 10 mL) and washed with brine (20 mL). The organic layer was dried (anh. MgSO4), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexanes or DCM/MeOH) to provide the corresponding compounds **4**.

Characterization data of vinyl sulfones-NO_2 (3)



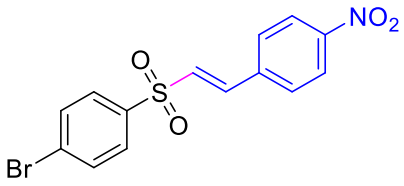
(*E*)-1-nitro-4-(2-(phenylsulfonyl)vinyl)benzene (3a)[2]

Purification by column chromatography (EtOAc/hexanes, 1:4 v/v) afforded **3a** (0.2257 g, 78%); pale yellow solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.24 (d, J = 8.7 Hz, 2H), 7.96 (d, J = 7.5 Hz, 2H), 7.72 (d, J = 15.5 Hz, 1H), 7.67−7.64 (m, 3H), 7.58 (t, J = 7.7Hz, 2H), 7.02 (d, J = 15.5 Hz, 1H); 13C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 149.1, 139.9, 139.4, 138.5, 134.0, 131.9, 129.7, 129.4, 128.0, 124.4 ppm.



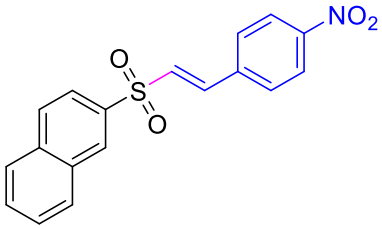
(*E*)-1-methyl-4-((4-nitrostyryl)sulfonyl)benzene (3b)[2]

Purification by column chromatography (EtOAc/hexanes, 1:4 v/v) afforded **3b** (0.1820 g, 60%); white solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.22 (d, J = 8.7 Hz, 2H), 7.83 (d, J = 8.3 Hz, 2H), 7.68 (d, J = 15.5 Hz, 1H), 7.63 (d, J = 8.7 Hz, 2H), 7.36 (d, J = 8.1 Hz, 2H), 7.01 (d, J = 15.5 Hz, 1H), 2.44 (1H, s); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 149.0, 145.2, 138.8, 138.7, 136.9, 132.2, 130.3, 129.3, 128.1, 124.4, 21.7 ppm.



(*E*)-1-bromo-4-((4-nitrostyryl)sulfonyl)benzene (3c)[2]

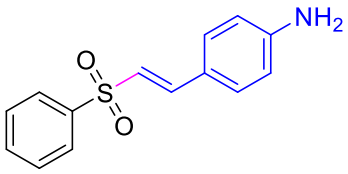
Purification by column chromatography (EtOAc/hexanes, 1:4 v/v) afforded **3c** (0.1510 g, 41%); white solid; ^{1}H NMR ($CDCl_3$, 500 MHz): 8.26 (d, J = 8.7 Hz, 2H), 7.82 (d, J = 8.6 Hz, 2H), 7.73−7.71 (m, 3H), 7.65 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 15.5 Hz, 1H); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 149.3, 139.9, 139.0, 138.3, 133.1, 131.5, 129.6, 129.5, 129.4, 124.5 ppm.



(*E*)-2-((4-nitrostyryl)sulfonyl)naphthalene (3d)

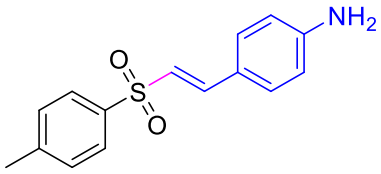
Purification by column chromatography (EtOAc/hexanes, 3:7 v/v) afforded **3d** (0.3360 g, 99%); pale yellow solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.57 (s, 1H), 8.24 (d, J = 8.8 Hz, 2H), 8.02 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 8.1 Hz, 1H), 7.89 (dd, J = 8.6, 1.7 Hz, 1H), 7.77 (d, J = 15.5 Hz, 1H), 7.70−7.67 (m, 1H), 7.66−7.64 (m, 3H), 7.07 (d, J = 15.5 Hz, 1H); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 149.2, 139.4, 138.6, 136.7, 135.6, 132.50, 132.0, 130.1, 130.0, 129.7, 129.6, 129.4, 128.2, 128.0, 124.4, 122.6 ppm.

Characterization data of vinyl sulfones-NH_2 (4)



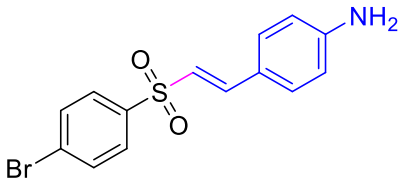
(*E*)-4-(2-(phenylsulfonyl)vinyl)aniline (4a)

Purification by column chromatography (EtOAc/hexanes, 1:1 v/v) afforded **4a** (25.9 mg, 50%); yellow solid; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.87 (d, J = 7.3 Hz, 2H), 7.67 (t, J = 7.3 Hz, 1H), 7.62 (t, J = 7.4 Hz, 2H), 7.41 (t, J = 12.0 Hz, 3H), 7.03 (d, J = 15.2 Hz, 1H), 6.54 (d, J = 8.5 Hz, 2H), 5.86 (s, 2H); 13C{^{1}H} NMR (DMSO-d_6, 125 MHz): δ 152.2, 143.2, 142.0, 132.9, 131.0, 129.4, 126.6, 120.2, 119.3, 113.4 ppm; HRMS (ESI) m/z [M + Na]$^+$ calcd for $C_{14}H_{13}NNaO_2S$ 282.0559, found 282.0567.



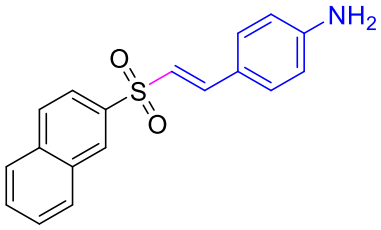
(*E*)-4-(2-tosylvinyl)aniline (4b)

Purification by column chromatography (DCM/MeOH, 99:1 v/v) afforded **4b** (26.2 mg, 48%); yellow solid; ^{1}H NMR (DMSO-d_6, 500 MHz): 7.74 (d, J = 8.2 Hz, 2H), 7.42 (d, J = 8.1 Hz, 2H), 7.39−7.36 (m, 3H), 6.99 (d, J = 15.2 Hz, 1H), 6.54 (d, J = 8.5 Hz, 2H), 5.84 (s, 2H), 2.38 (s, 3H); ^{13}C{^{1}H} NMR (DMSO-d_6, 125 MHz): δ 152.1, 143.4, 142.6, 139.2, 130.9, 129.8, 126.7, 120.6, 119.34, 113.4, 21.0 ppm; HRMS (ESI) m/z [M + Na]$^+$ calcd for $C_{15}H_{15}NNaO_2S$ 297.0716, found 297.0720.



(*E*)-4-(2-((4-bromophenyl)sulfonyl)vinyl)aniline (4c)

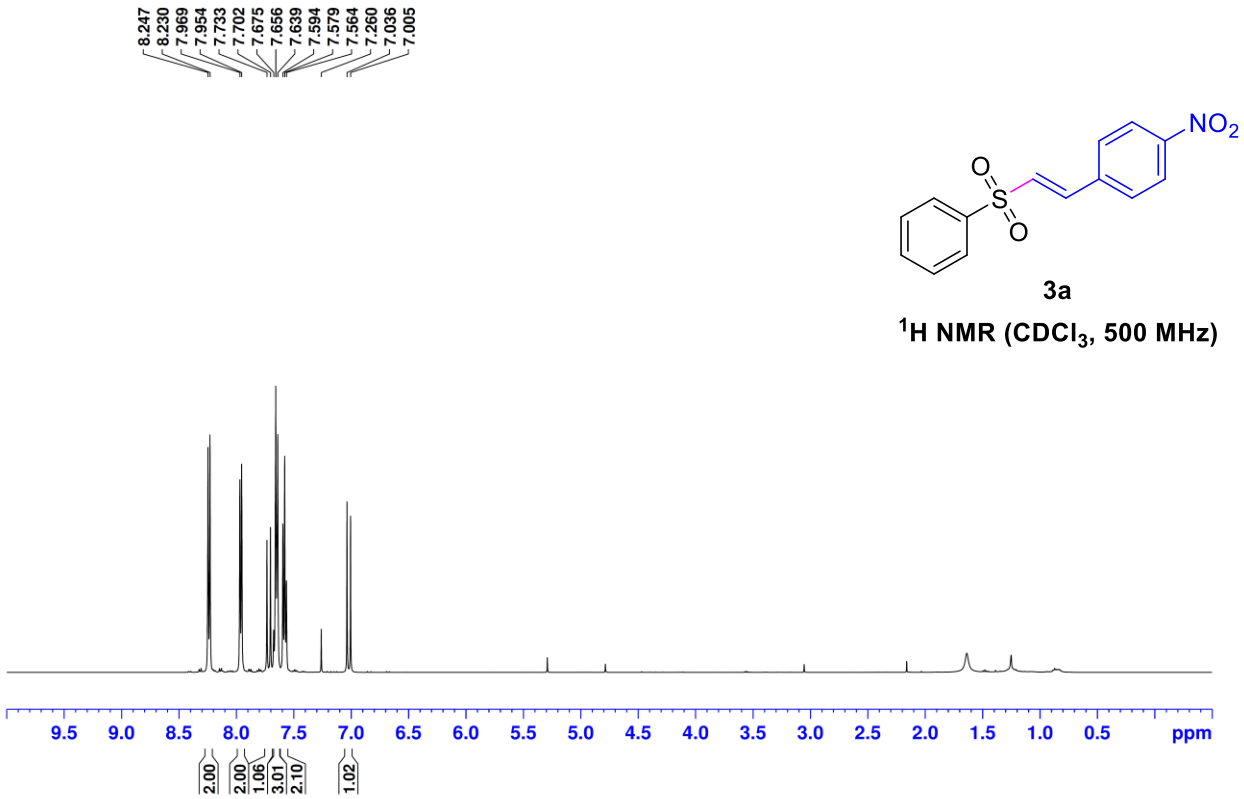
Purification by column chromatography (EtOAc/hexanes, 1:1 v/v) afforded **4c** (37.2 mg, 55%); red-brown solid; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.92−7.86 (m, 4H), 7.50 (d, J = 15.2 Hz, 1H), 7.47 (d, J = 8.6 Hz, 2H), 7.12 (d, J = 15.2 Hz, 1H), 6.62 (d, J = 8.6 Hz, 2H), 5.98 (s, 2H); ^{13}C{^{1}H} NMR (DMSO-d_6, 125 MHz): δ 152.3, 143.8, 141.4, 132.5, 131.1, 128.7, 126.9, 119.6, 119.2, 113.4 ppm; HRMS (ESI) m/z [M + Na]$^{+}$ calcd for $C_{14}H_{12}BrNNaO_2S$ 359.9664, found 359.9661.

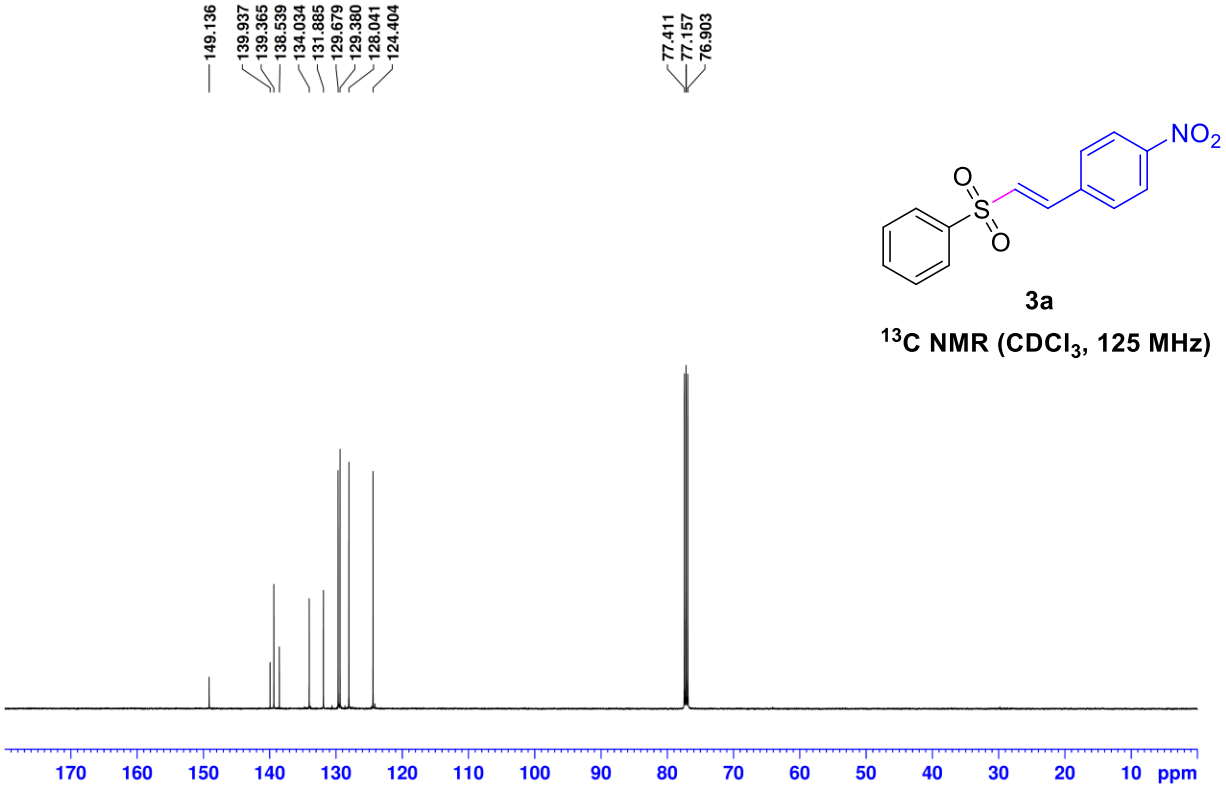


(*E*)-4-(2-(naphthalen-2-ylsulfonyl)vinyl)aniline (4d)

Purification by column chromatography (EtOAc/hexane, 3:7 v/v) afforded **4d** (61.3 mg, 99%); pale yellow solid; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.54 (s, 1H), 8.19 (d, J = 8.0 Hz, 1H), 8.14 (d, J = 8.7 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.86 (dd, J = 8.7, 1.8 Hz, 1H), 7.73−7.66 (m, 2H), 7.48 (d, J = 15.2 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H),), 7.09 (d, J = 15.2 Hz, 1H), 6.54 (d, J = 8.6 Hz, 2H), 5.87 (s, 3H); ^{13}C{^{1}H} NMR (DMSO-d_6, 125 MHz): δ 152.2, 143.3, 139.0, 134.4, 131.9, 131.0, 129.6, 129.3, 129.0, 127.8, 127.7, 127.5, 122.3, 120.2, 119.4, 113.4 ppm; HRMS (ESI) m/z [M + Na]$^{+}$ calcd for $C_{18}H_{15}NNaO_2S$ 332.0716, found 332.0735.

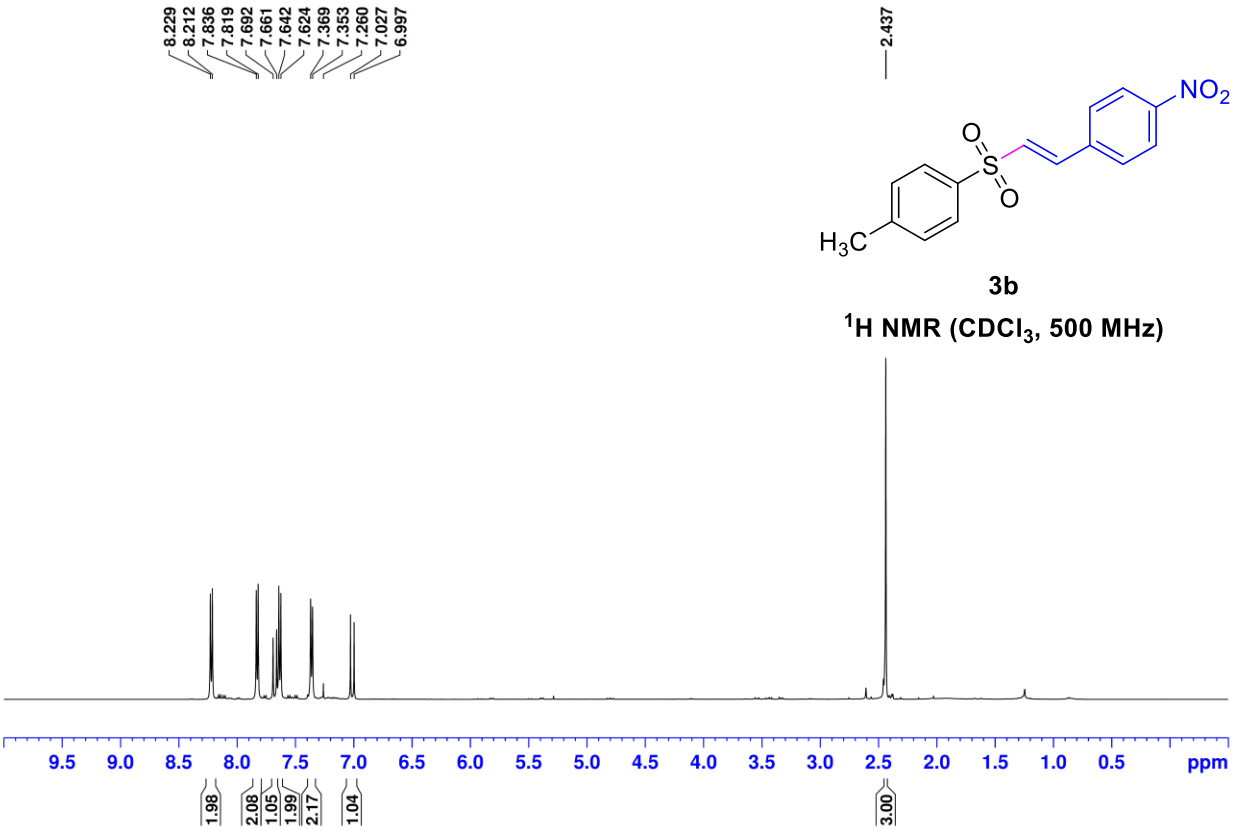
^{1}H and ^{13}C NMR spectra of vinyl sulfones-NO_2 (3)





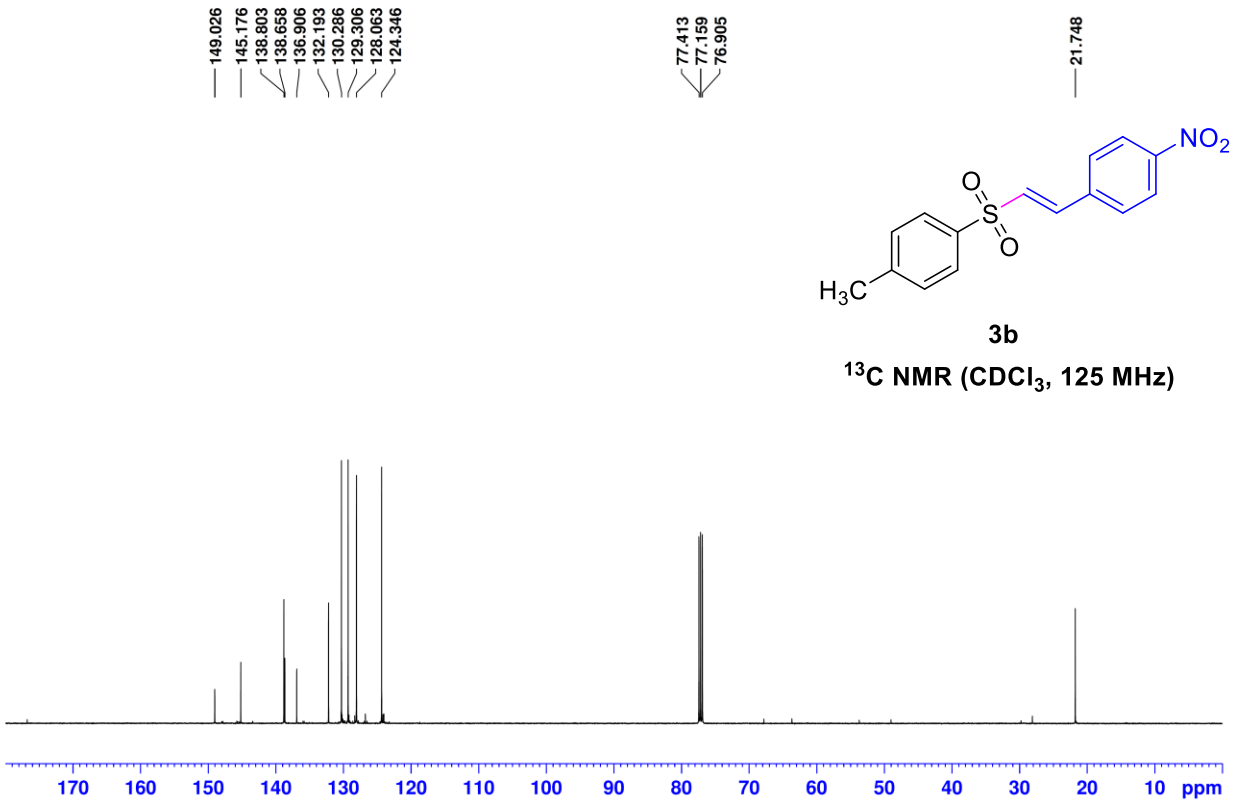
3a

^{13}C NMR ($CDCl_3$, 125 MHz)



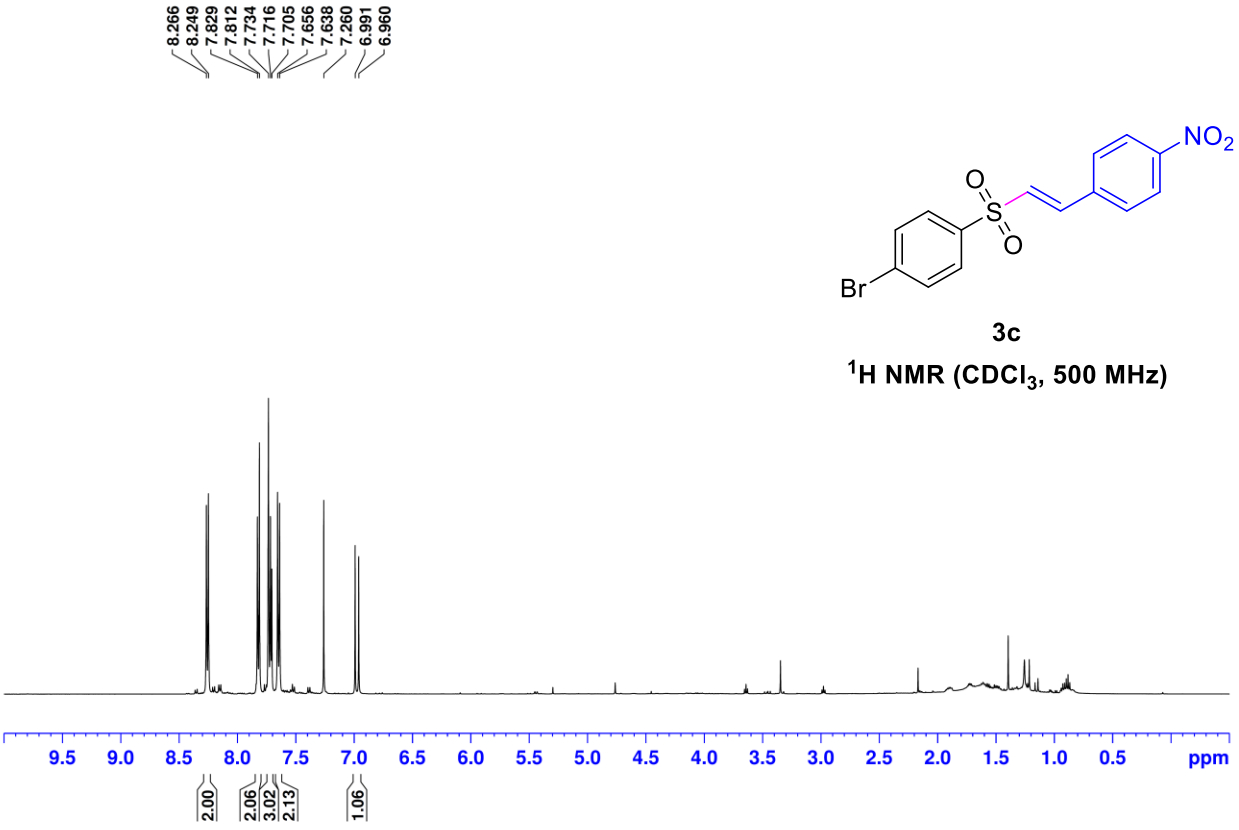
3b

^{1}H NMR ($CDCl_3$, 500 MHz)



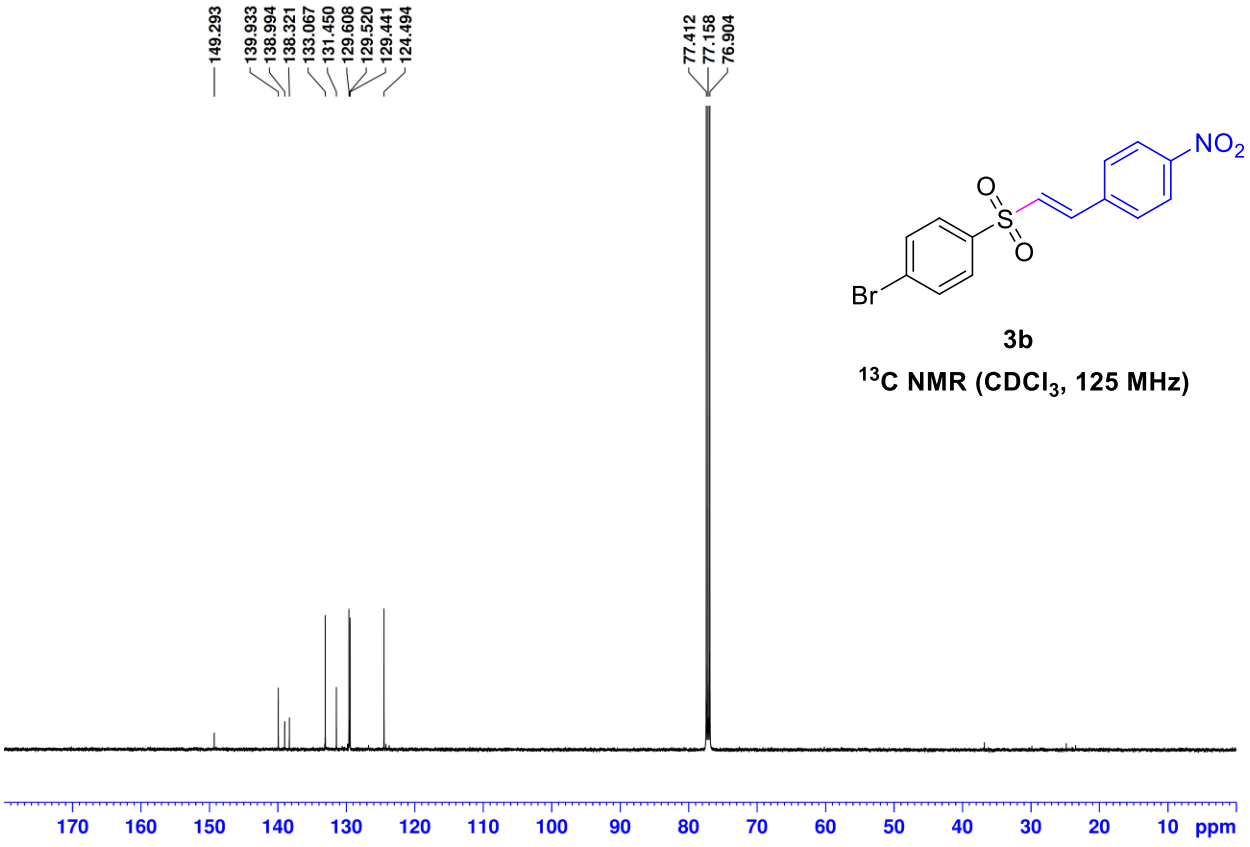
3b

^{13}C NMR ($CDCl_3$, 125 MHz)



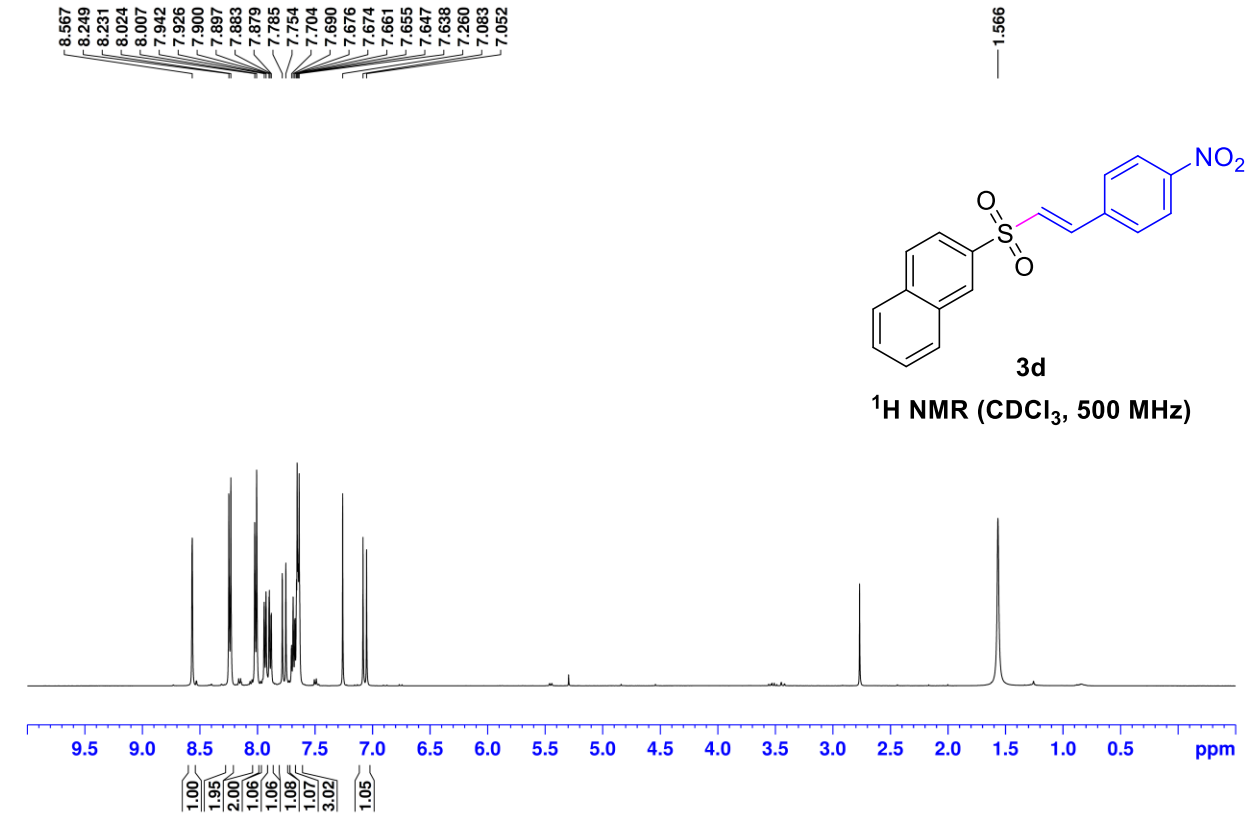
3c

^{1}H NMR ($CDCl_3$, 500 MHz)



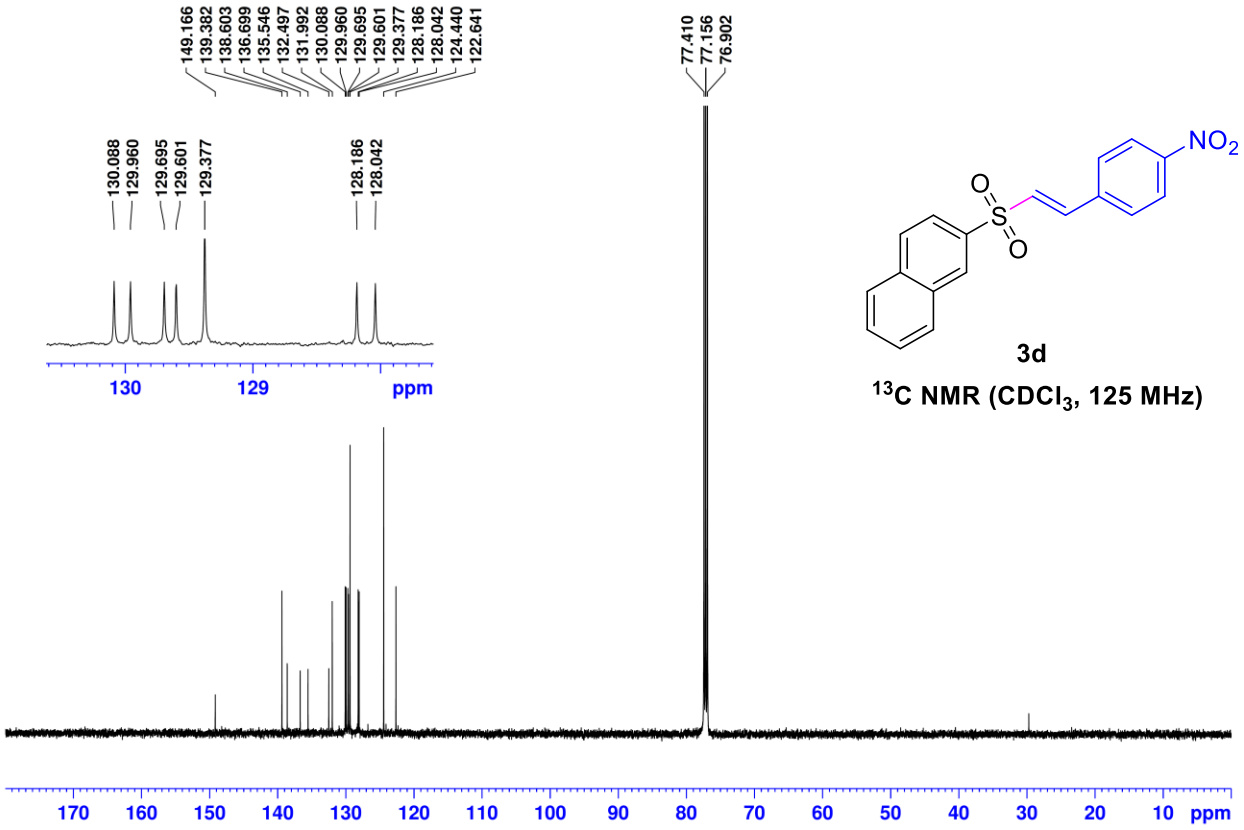
3b

^{13}C NMR ($CDCl_3$, 125 MHz)

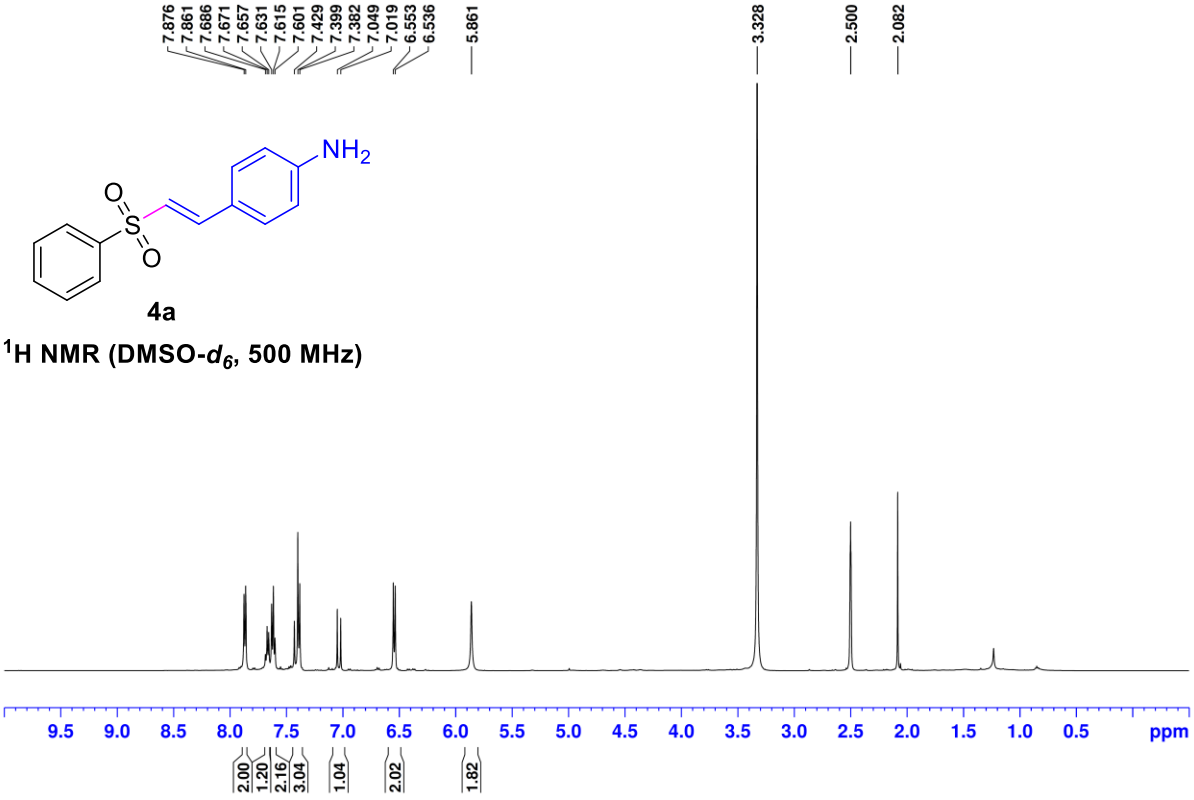


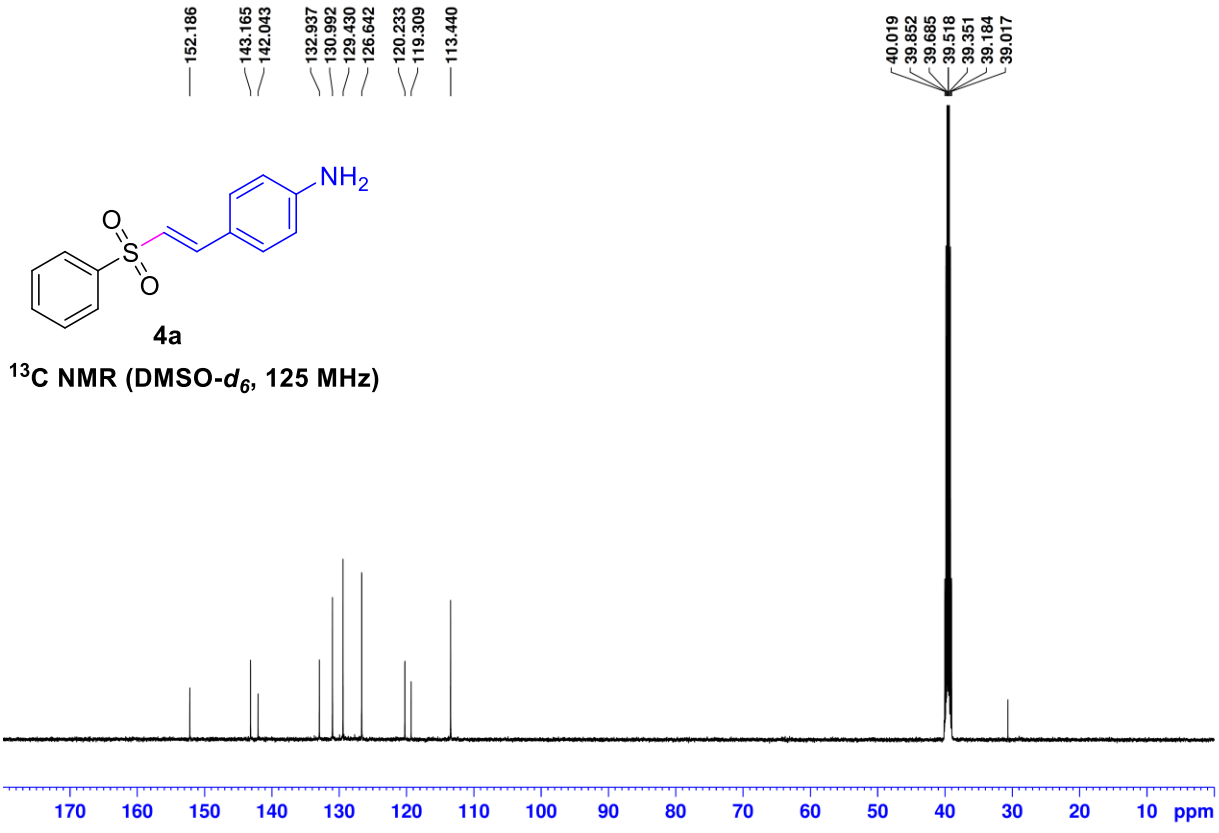
3d

^{1}H NMR ($CDCl_3$, 500 MHz)



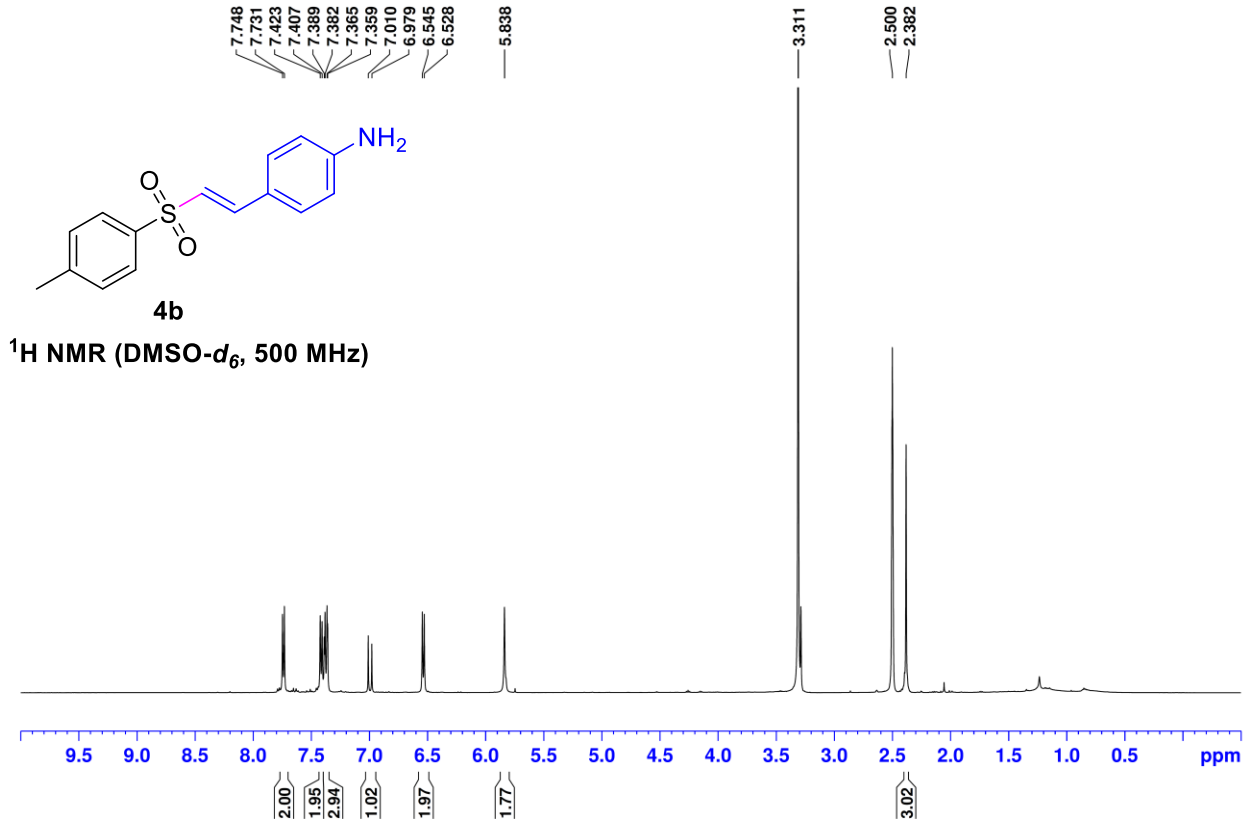
^{1}H and ^{13}C NMR spectra of vinyl sulfones-NH$_2$ (4)





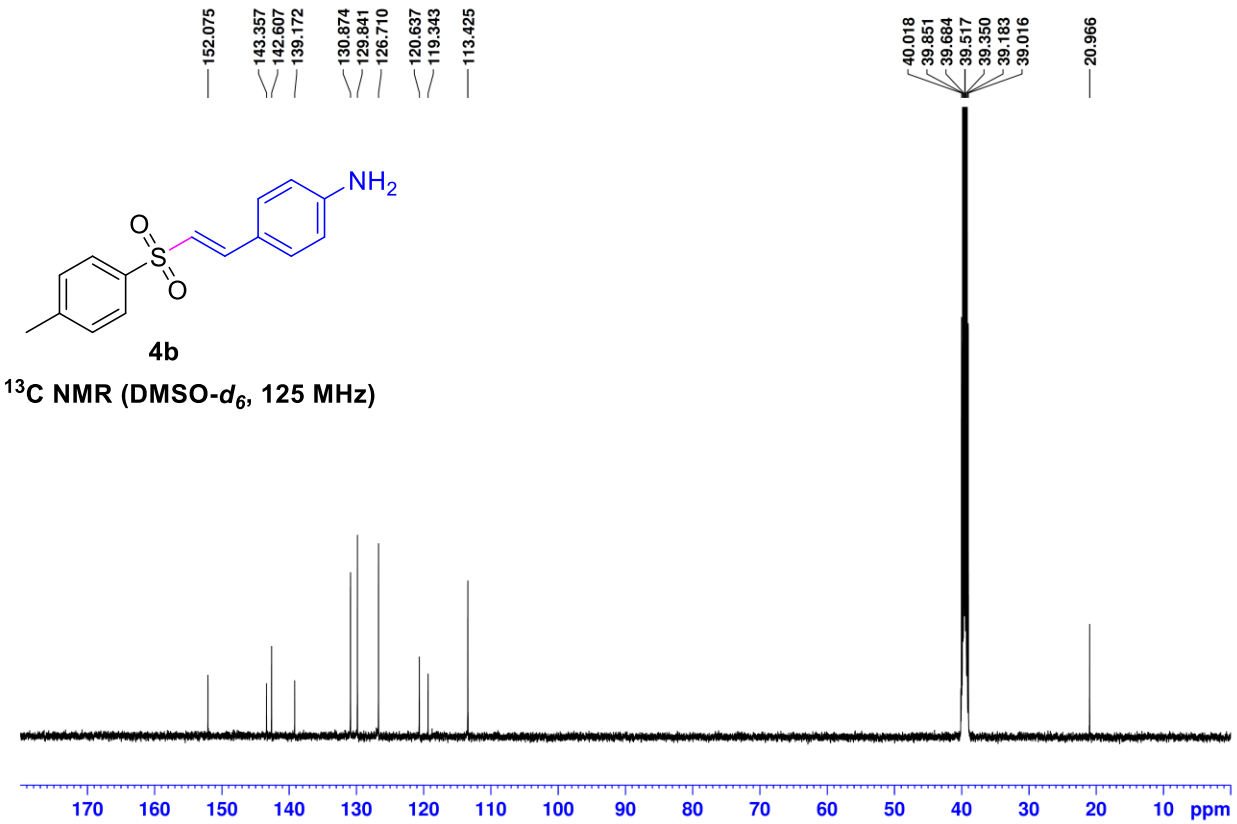
4a

^{13}C NMR (DMSO-d_6, 125 MHz)



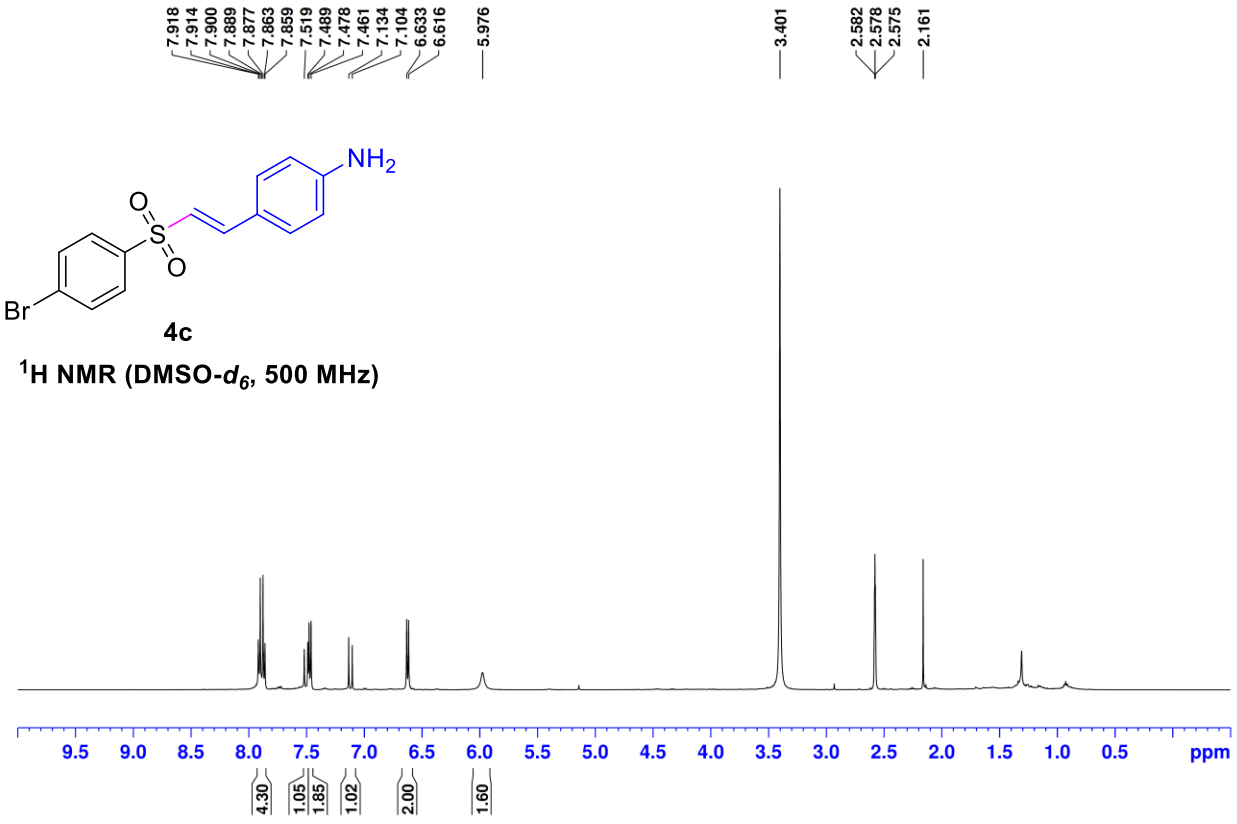
4b

^{1}H NMR (DMSO-d_6, 500 MHz)



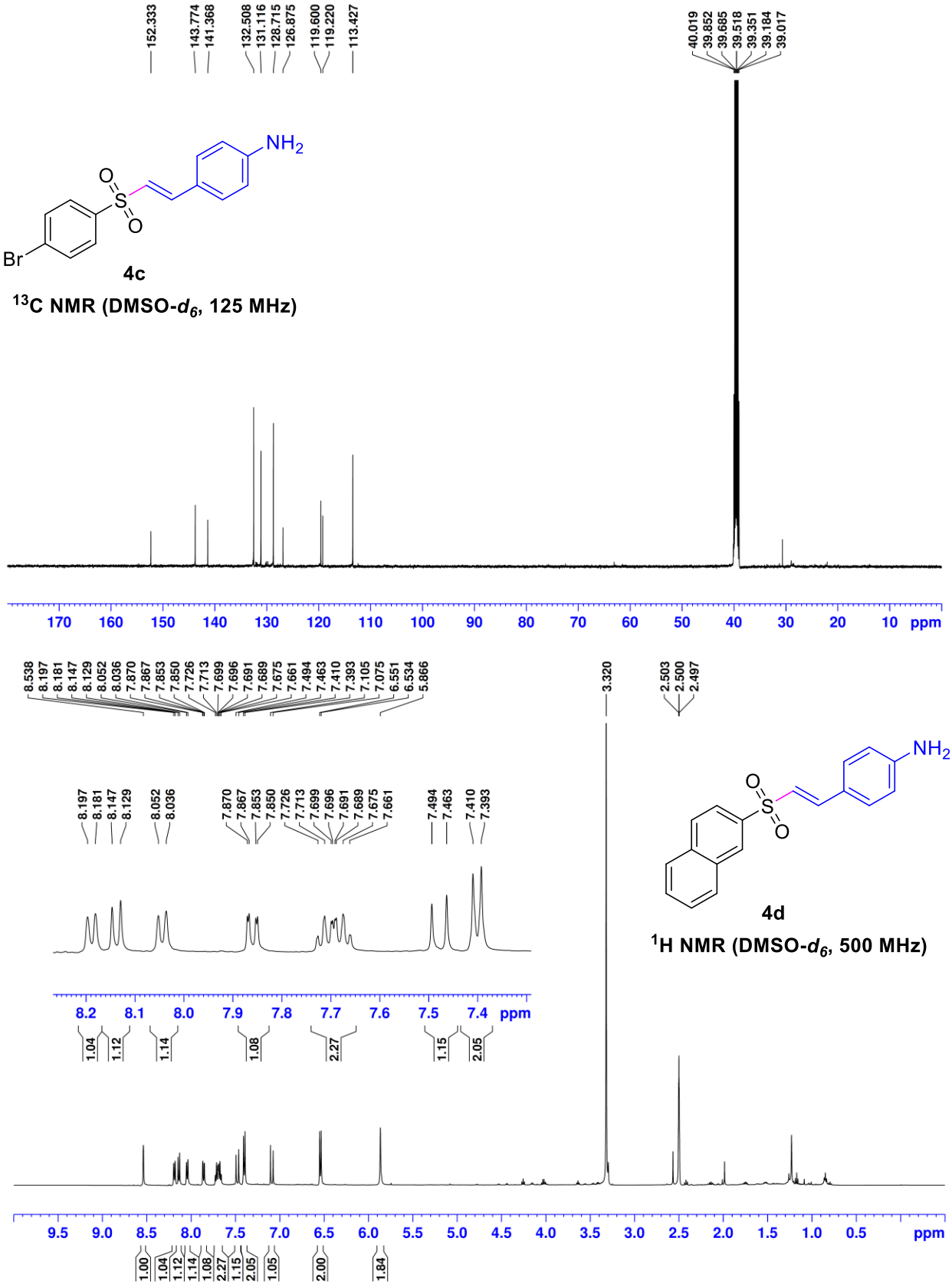
4b

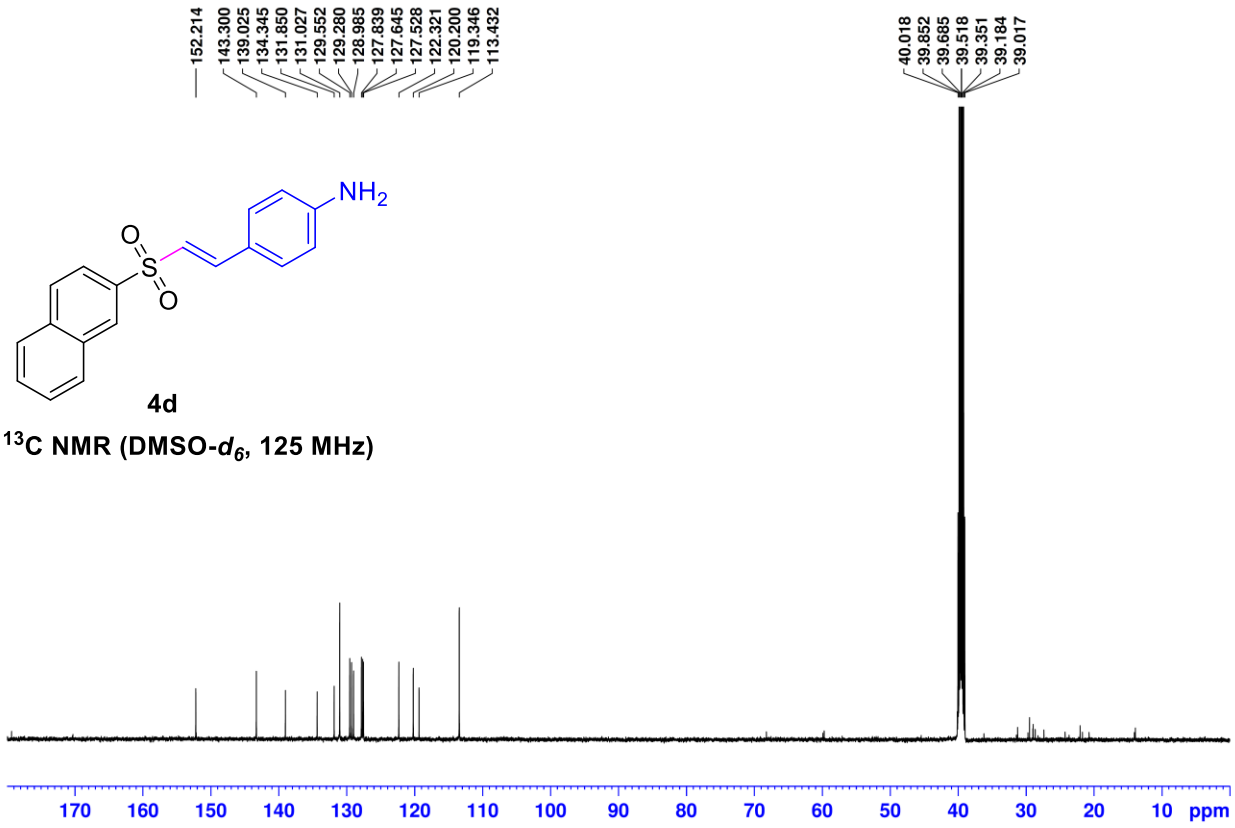
^{13}C NMR (DMSO-d_6, 125 MHz)



4c

^{1}H NMR (DMSO-d_6, 500 MHz)


152.333
143.774
141.368
132.508
131.116
128.715
126.875
119.600
119.220
113.427
40.019
39.852
39.685
39.518
39.351
39.184
39.017
NH2
O
S
O
Br
4c
13C NMR (DMSO-d6, 125 MHz)
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
8.538
8.197
8.181
8.147
8.129
8.052
8.036
7.870
7.867
7.853
7.850
7.726
7.713
7.699
7.696
7.691
7.689
7.675
7.661
7.494
7.463
7.410
7.393
7.105
7.075
6.551
6.534
5.866
3.320
2.503
2.500
2.497
NH2
O
S
O
4d
1H NMR (DMSO-d6, 500 MHz)
8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 ppm
1.04
1.12
1.14
1.08
2.27
1.15
2.05
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00
1.04
1.12
1.14
1.08
2.27
1.15
2.05
1.05
2.00
1.84



4d

^{13}C NMR (DMSO-d_6, 125 MHz)

HRMS spectra of vinyl sulfones-NH_2 (4)

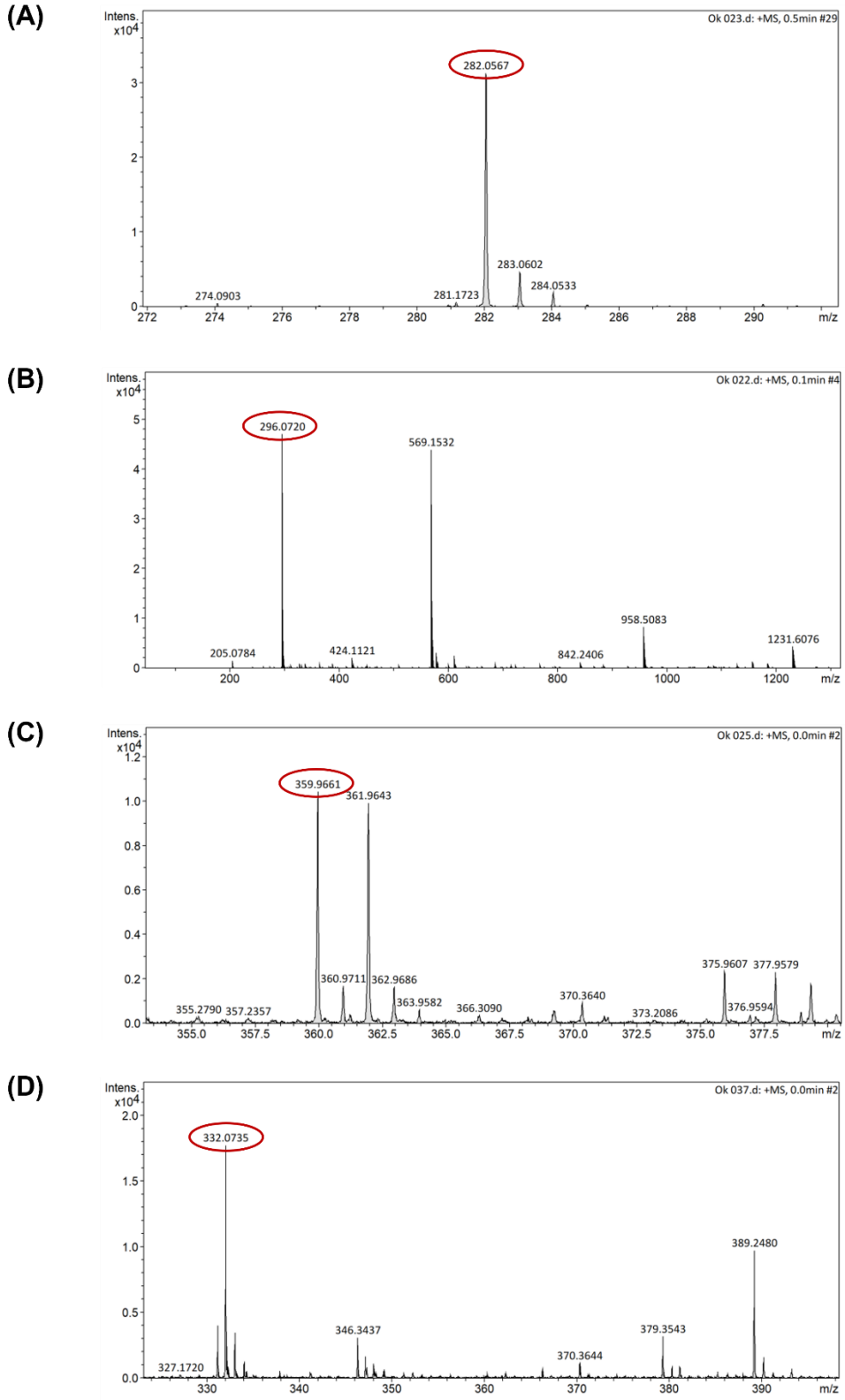


Fig. S1. HRMS spectra of (A) **4a**, (B) **4b**, (C) **4c**, and (D) **4d**.

Absorption and emission spectroscopic analyses

Table S1. The photophysical properties of vinyl sulfones-NO_2 (**3a**–**3d**) (10 μM) in different solvents.

Solvent	dye	[a]λ_{abs} (nm)	[b]λ_{em} (nm)	[c]$\Delta\upsilon$ (nm)	[d]Φ_f	[e]ε ($M^{-1}cm^{-1}$)
Toluene	**3a**	298	Non-fluorescence			2.38×10^4
	3b	285	Non-fluorescence			8.88×10^4
	3c	285	Non-fluorescence			1.05×10^5
	3d	285	Non-fluorescence			9.15×10^4
Chloroform	**3a**	297	Non-fluorescence			2.72×10^4
	3b	276	Non-fluorescence			1.28×10^5
	3c	277	Non-fluorescence			1.28×10^5
	3d	278	Non-fluorescence			1.27×10^5
DCM	**3a**	297	Non-fluorescence			2.87×10^4
	3b	281	Non-fluorescence			5.09×10^4
	3c	279	Non-fluorescence			8.30×10^4
	3d	279	Non-fluorescence			9.52×10^4
THF	**3a**	299	Non-fluorescence			2.71×10^4
	3b	281	Non-fluorescence			9.25×10^4
	3c	279	Non-fluorescence			7.88×10^4
	3d	306	Non-fluorescence			2.89×10^4
Acetone	**3a**	327	Non-fluorescence			5.40×10^3
	3b	332	Non-fluorescence			1.20×10^3
	3c	328	Non-fluorescence			5.20×10^3
	3d	328	Non-fluorescence			1.15×10^4
MeCN	**3a**	297	Non-fluorescence			2.90×10^4
	3b	279	Non-fluorescence			6.11×10^4
	3c	277	Non-fluorescence			8.70×10^4
	3d	279	Non-fluorescence			6.98×10^4
MeOH	**3a**	275	Non-fluorescence			8.11×10^4
	3b	297	Non-fluorescence			2.12×10^4
	3c	274	Non-fluorescence			8.70×10^4
	3d	275	Non-fluorescence			7.20×10^4
DMF	**3a**	301	Non-fluorescence			2.62×10^4
	3b	279	Non-fluorescence			7.91×10^4
	3c	280	Non-fluorescence			5.15×10^4
	3d	280	Non-fluorescence			5.78×10^4
DMSO	**3a**	318	Non-fluorescence			1.76×10^4
	3b	318	Non-fluorescence			1.76×10^4
	3c	319	Non-fluorescence			1.70×10^4
	3d	319	Non-fluorescence			2.03×10^4
DI water	**3a**	300	Non-fluorescence			2.46×10^4
	3b	307	Non-fluorescence			1.64×10^4
	3c	276	Non-fluorescence			2.59×10^4
	3d	313	Non-fluorescence			1.79×10^4
PBS	**3a**	299	Non-fluorescence			2.20×10^4
	3b	306	Non-fluorescence			1.66×10^4
	3c	265	Non-fluorescence			9.30×10^4
	3d	258	Non-fluorescence			3.28×10^4

[a]λ_{abs} = absorption maximum wavelength, [b]λ_{em} = emission maximum wavelength (Excitation wavelength at λ_{abs}), [c]$\Delta\upsilon$ = stokes shifts (λ_{em} - λ_{abs}), [d]Φ_f = fluorescence quantum yields calculated by using quinine sulfate in 0.1 M H_2SO_4 was used as a standard (Φ_f = 0.54), [e]ε = molar absorptivity.

Table S2. The photophysical properties of vinyl sulfones-NH_2 **4a**–**4d** (10 μM) in different solvents.

Solvent	dye	[a]λ_{abs} (nm)	[b]λ_{em} (nm)	[c]$\Delta\upsilon$ (nm)	[d]Φ_f	[e]ε ($M^{-1}cm^{-1}$)
Toluene	**4a**	335	403	68	0.0017	2.50×10^4
	4b	332	403	71	0.0012	2.21×10^4
	4c	341	409	68	0.0030	2.23×10^4
	4d	340	409	69	0.0035	2.64×10^4
Chloroform	**4a**	333	407	74	0.0062	2.38×10^4
	4b	330	407	77	0.0012	2.24×10^4
	4c	337	414	77	0.0029	2.24×10^4
	4d	338	415	77	0.0037	2.93×10^4
THF	**4a**	344	420	76	0.0040	2.85×10^4
	4b	344	420	76	0.0033	2.51×10^4
	4c	349	430	81	0.0062	2.33×10^4
	4d	350	440	90	0.0146	2.94×10^4
DCM	**4a**	334	413	79	0.0022	3.10×10^4
	4b	334	413	79	0.0030	2.86×10^4
	4c	338	420	82	0.0036	2.51×10^4
	4d	339	424	85	0.0053	3.00×10^4
DMSO	**4a**	357	445	88	0.1066	2.66×10^4
	4b	357	445	88	0.1100	2.32×10^4
	4c	361	455	94	0.0623	2.27×10^4
	4d	363	490	127	0.0641	3.02×10^4
DMF	**4a**	353	437	84	0.0076	2.88×10^4
	4b	353	437	84	0.0069	2.51×10^4
	4c	357	441	84	0.0089	2.37×10^4
	4d	358	472	114	0.0139	2.98×10^4
Acetone	**4a**	345	426	81	0.0040	2.76×10^4
	4b	345	426	81	0.0061	2.41×10^4
	4c	348	437	89	0.0219	2.21×10^4
	4d	349	461	112	0.0501	2.94×10^4
MeCN	**4a**	340	431	91	0.0031	2.75×10^4
	4b	340	431	91	0.0030	2.37×10^4
	4c	344	442	98	0.0055	2.32×10^4
	4d	343	468	125	0.0137	1.05×10^4
MeOH	**4a**	345	436	91	0.0039	2.35×10^4
	4b	345	436	91	0.0032	2.26×10^4
	4c	349	448	99	0.0086	2.39×10^4
	4d	349	459	110	0.0074	2.84×10^4
DI water	**4a**	331	452	121	0.0012	2.23×10^4
	4b	331	452	121	0.0024	1.96×10^4
	4c	336	455	119	0.0009	1.79×10^4
	4d	332	454	122	0.0005	1.10×10^4
PBS	**4a**	331	452	121	0.0017	2.23×10^4
	4b	328	452	124	0.0021	1.99×10^4
	4c	334	452	118	0.0010	1.78×10^4
	4d	334	453	119	0.00012	2.09×10^4

[a]λ_{abs} = absorption maximum wavelength, [b]λ_{em} = emission maximum wavelength (Excitation wavelength at λ_{abs}), [c]$\Delta\upsilon$ = stokes shifts (λ_{em} - λ_{abs}), [d]Φ_f = fluorescence quantum yields calculated by using quinine sulfate in 0.1 M H_2SO_4 was used as a standard (Φ_f = 0.54), [e]ε = molar absorptivity.

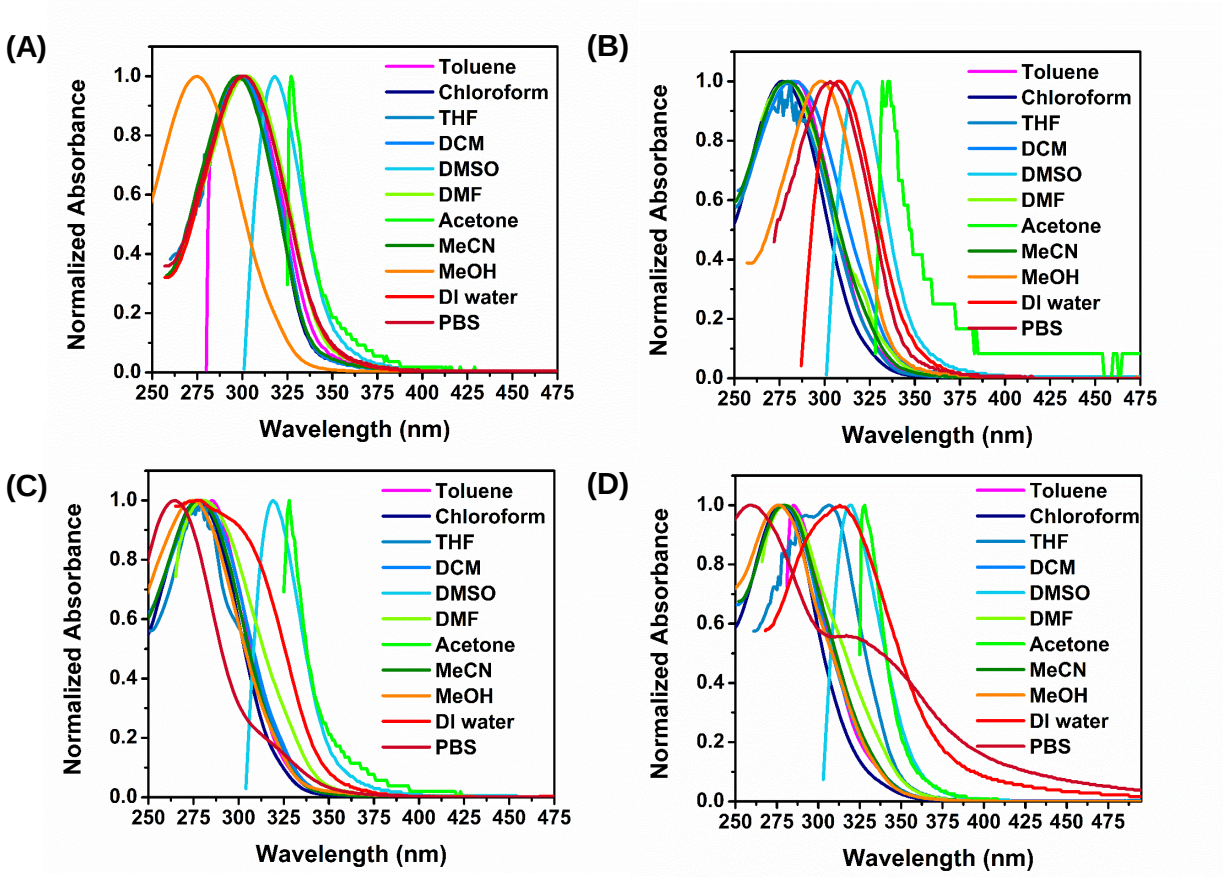


Fig. S2. Absorption spectra of (A) **3a**, (B) **3b**, (C) **3c**, and (D) **3d** (10 μM) in different solvents.

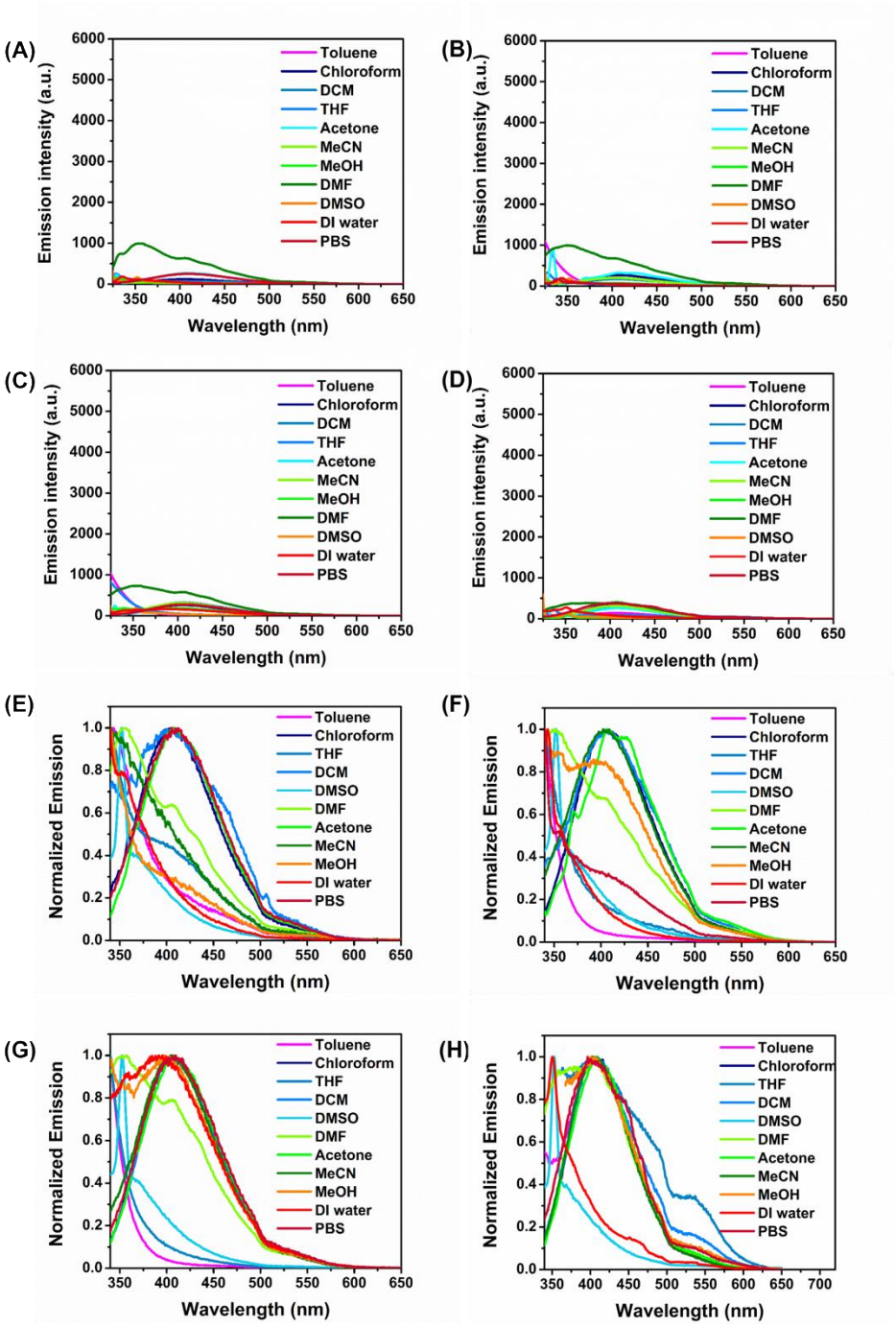


Fig. S3. Fluorescent spectra (excited at λ_{max} of each solvent) of (A) **3a**, (B) **3b**, (C) **3c**, and (D) **3d** (10 μM) in different solvents (y-axis is scaled to compare with Figure S5) and normalized fluorescent spectra (excited at λ_{max} of each solvent) of (E) **3a**, (F) **3b**, (G) **3c**, and (H) **3d** (10 μM) in different solvents.

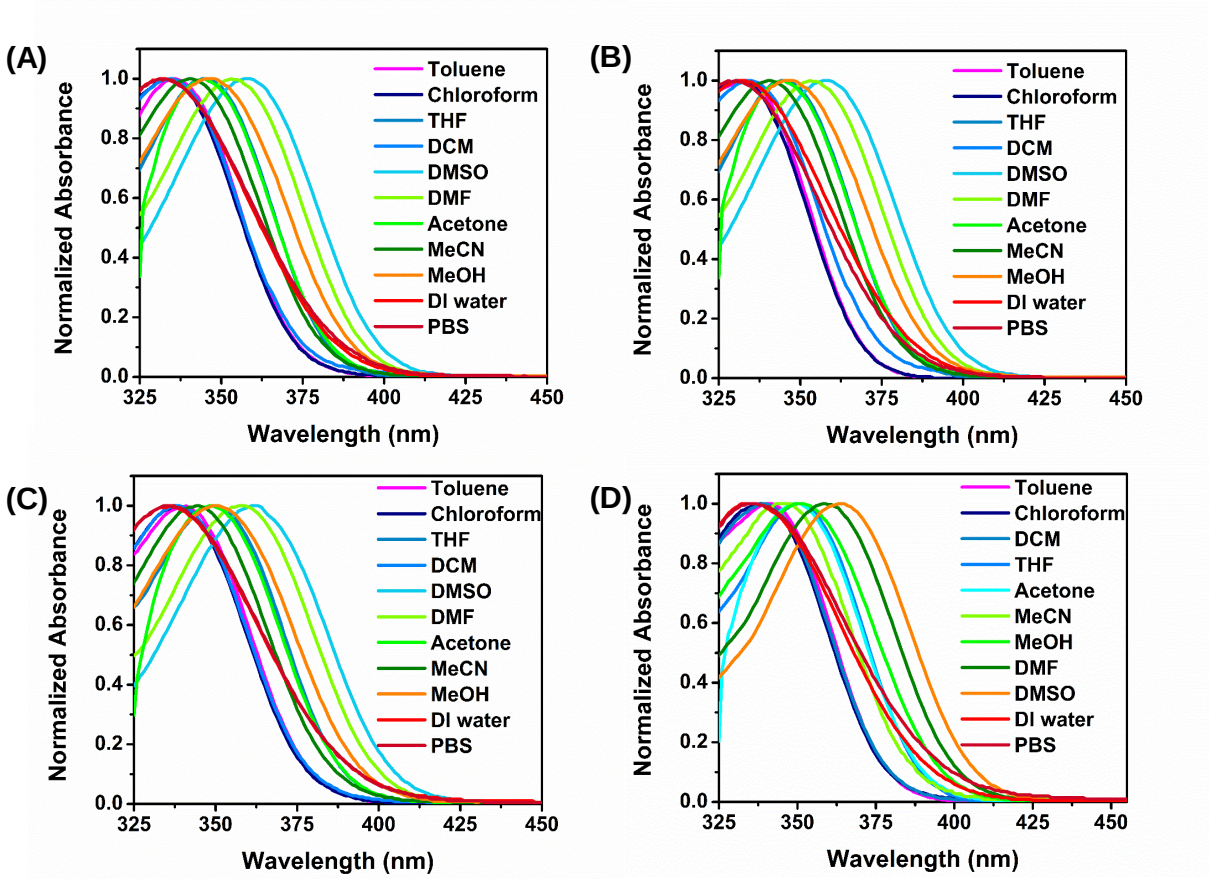


Fig. S4. Absorption spectra of (A) **4a**, (B) **4b**, (C) **4c**, and (D) **4d** (10 μM) in different solvents.

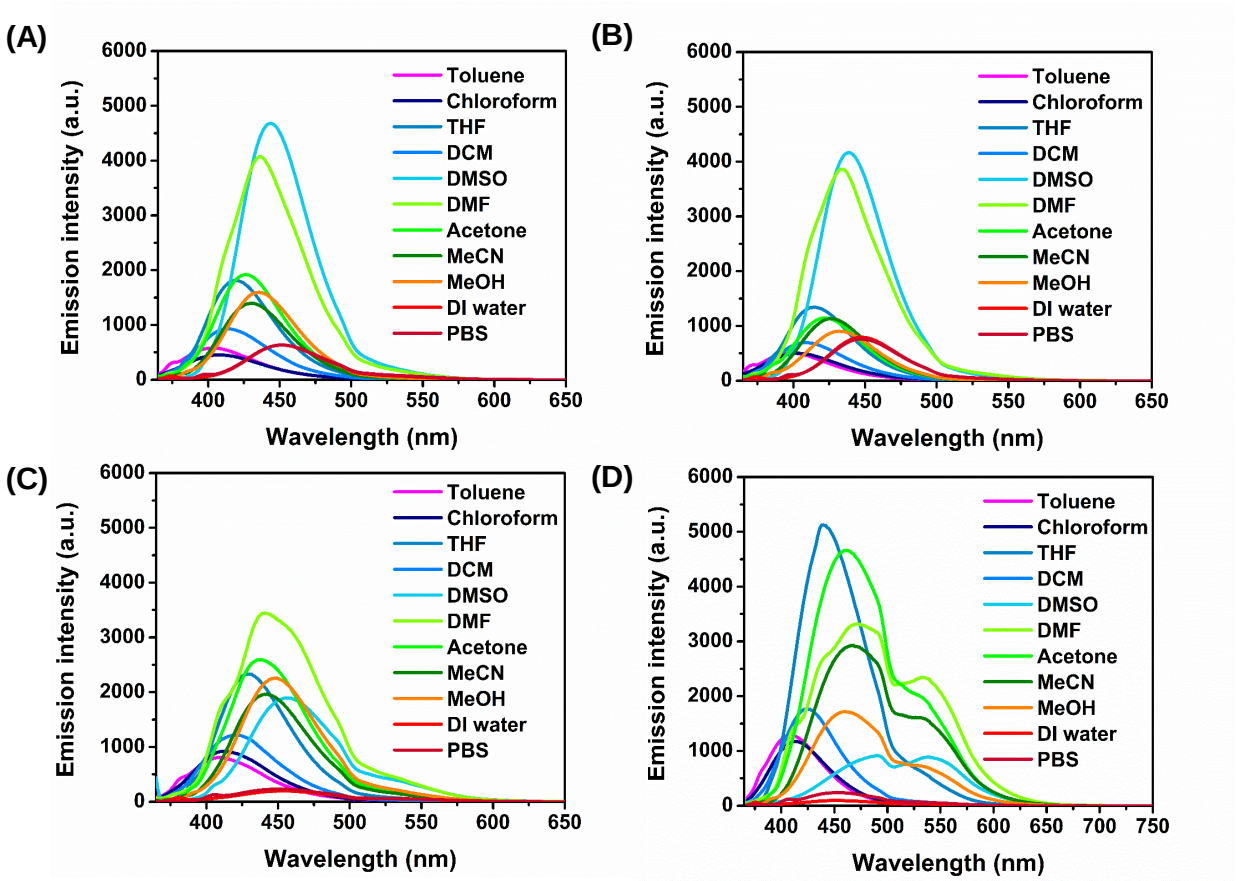


Fig. S5. Fluorescent spectra (excited at λ_{max} of each solvent) of (A) **4a**, (B) **4b**, (C) **4c**, and (D) **4d** (10 μM) in different solvents.

Solvatochromism

Table S3. The photophysical properties of vinyl sulfones-NH_2 (**4a**–**4d**) (10 µM) in different solvents.

Solvent	ε[a]	n[b]	Δf[c]	4a				4b				4c				4d			
				λ_{abs}[d]	λ_{em}[e]	$\Delta\upsilon$[f]	Φ_f[g]	λ_{abs}[d]	λ_{em}[e]	$\Delta\upsilon$[f]	Φ_f[g]	λ_{abs}[d]	λ_{em}[e]	$\Delta\upsilon$[f]	Φ_f[g]	λ_{abs}[d]	λ_{em}[e]	$\Delta\upsilon$[f]	Φ_f[g]
Toluene	2.37	1.496	0.0126	335	403	68	0.0017	332	403	71	0.0012	341	409	68	0.0030	340	409	69	0.0035
Chloroform	4.81	1.447	0.1482	333	407	74	0.0062	330	407	77	0.0012	337	414	77	0.0029	338	415	77	0.0037
THF	7.58	1.404	0.2107	344	420	76	0.0040	344	420	76	0.0033	349	430	81	0.0062	350	440	90	0.0146
DCM	10.37	1.424	0.2276	334	413	79	0.0022	334	413	79	0.0030	338	420	82	0.0036	339	424	85	0.0053
DMSO	47.2	1.477	0.264	357	445	88	0.1066	357	445	88	0.1100	361	455	94	0.0623	363	490	127	0.0641
DMF	36.7	1.431	0.2749	353	437	84	0.0076	353	437	84	0.0069	357	441	84	0.0089	358	472	114	0.0139
Acetone	20.7	1.358	0.2846	345	426	81	0.0040	345	426	81	0.0061	348	437	89	0.0219	349	461	112	0.0501
MeCN	37.5	1.344	0.3055	340	431	91	0.0031	340	431	91	0.0030	344	442	98	0.0055	343	468	125	0.0137
MeOH	32.7	1.328	0.3086	345	436	91	0.0039	345	436	91	0.0032	349	448	99	0.0086	349	459	110	0.0074
DI water	78.5	1.333	0.3193	331	452	121	0.0012	331	452	121	0.0024	336	455	119	0.0009	332	454	122	0.0005

[a]ε = solvent dielectric constant, [b]n = index of refraction, [c]$\Delta f = (\varepsilon-1)/(2\varepsilon+1)-(n^2-1)/(2n^2+1)$ accounted for the spectral shifts due to reorientation of solvents molecules, called the orientation polarizability, [d]λ_{abs} = absorption maximum wavelength (nm), [e]λ_{em} = emission maximum wavelength (nm), [f]$\Delta\upsilon$ = stokes shifts ($\lambda_{em} - \lambda_{abs}$) (nm), [g]Φf = fluorescence quantum yields calculated by using quinine sulfate in 0.1 M H_2SO_4 was used as a standard (Φ_f = 0.54).

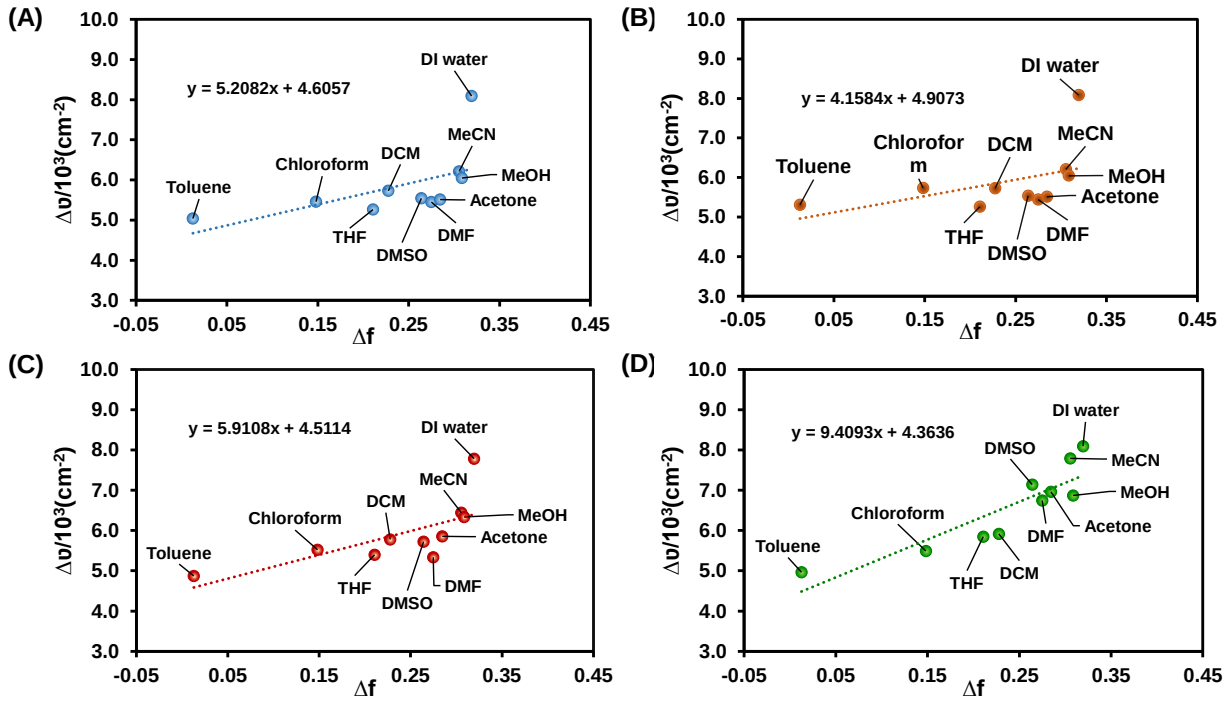


Fig. S6. Lippart-Mataga plots for (A) **4a**, (B) **4b**, (C) **4c**, and (D) **4d** (10 µM) in different solvents.

DFT calculation

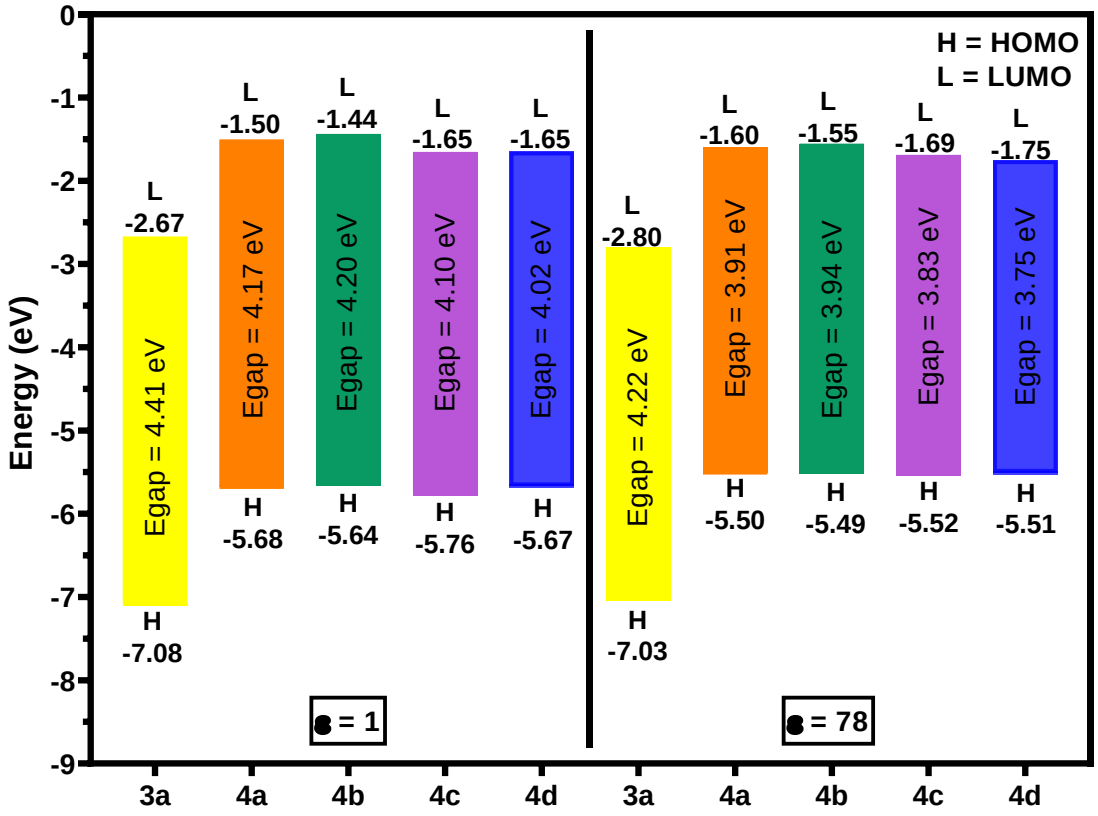


Fig. S7. HOMO-LUMO energy diagram in gas phase (ε = 1) and water phase (ε = 78) of **3a** and **4a**–**4d**.

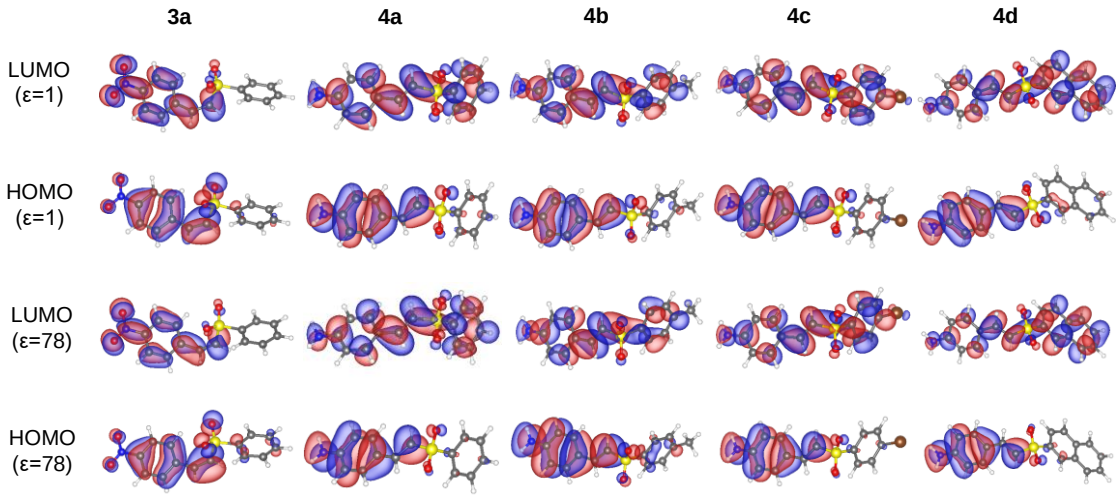


Fig. S8. Frontier molecular orbital (HOMO-LUMO) in gas phase (ε = 1) and water phase (ε = 78) of **3a** and **4a**–**4d**.

Cytotoxicity assays

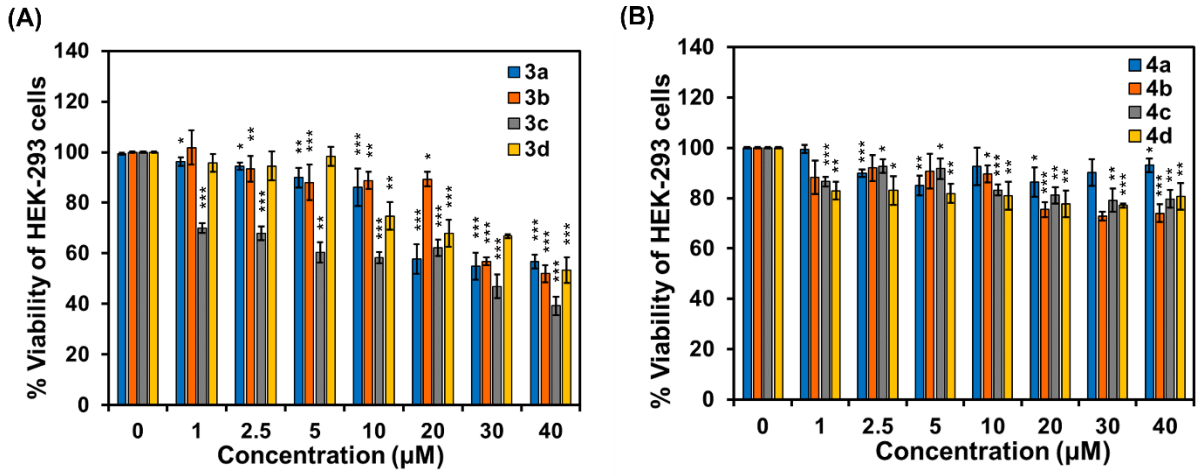


Fig. S9. Cytotoxicity assays of (A) vinyl sulfones-NO_2 (**3a**–**3d**) and (B) vinyl sulfones-NH_2 (**4a**–**4d**). HEK-293 cells were treated at different concentrations for 24 h (error bar represents standard deviation, $n = 3$). Statistical analysis is based on T-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$), the control and each concentration were compared.

Confocal images of HepG2 cells and corrected total cell fluorescence

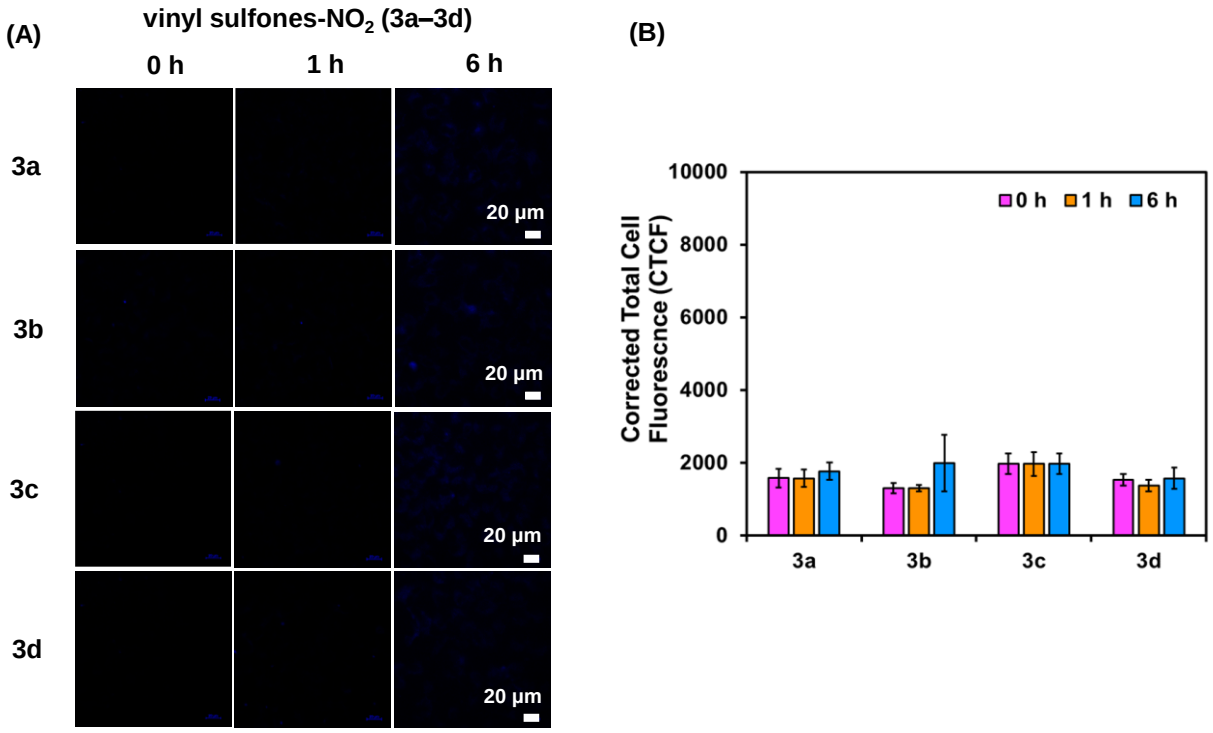


Fig. S10. (A) Confocal images of HepG2 cells incubated with vinyl sulfones-NO_2 (**3a**–**3d**) (20 µM) for 0–6 h. Scale bar = 20 µm. (B) Corrected total cell fluorescence data qualified using ImageJ and represent the mean ± SD (n = 30).

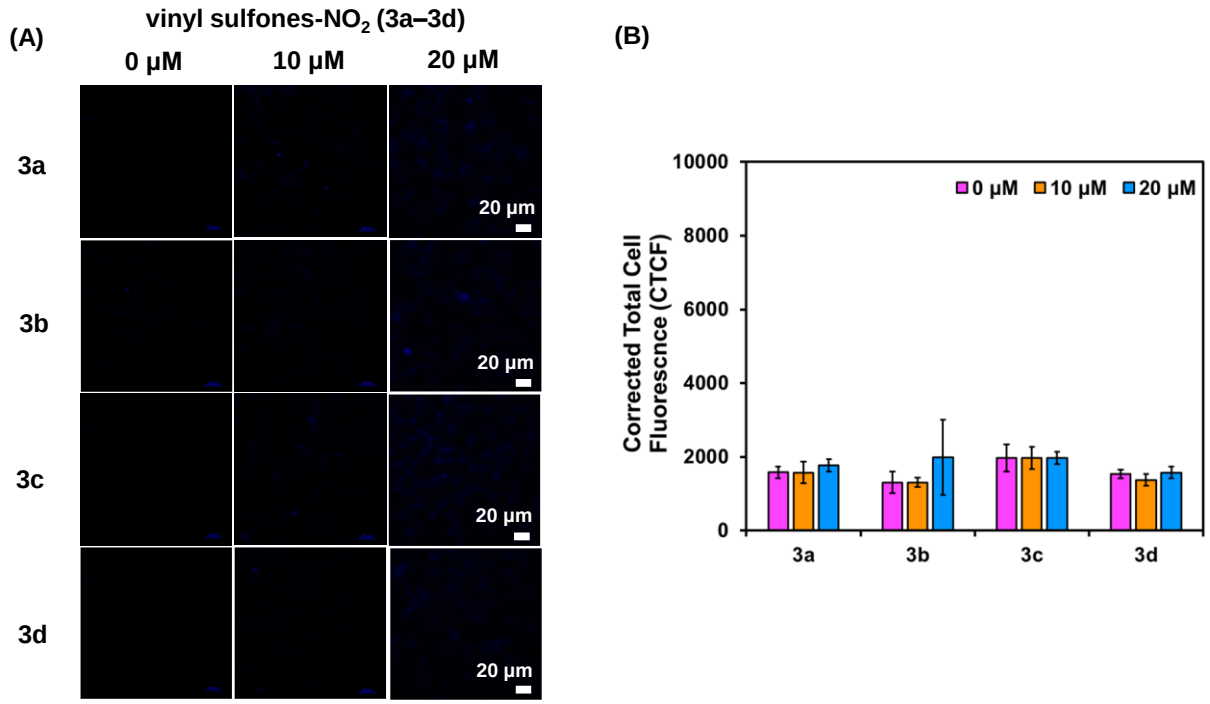


Fig. S11. (A) Confocal images of HepG2 cells incubated with vinyl sulfones-NO_2 (**3a**–**3d**) (0–20 µM) for 6 h and (B) Corrected total cell fluorescence data qualified using ImageJ and represent the mean ± SD (n = 30).

Confocal images of HEK-293

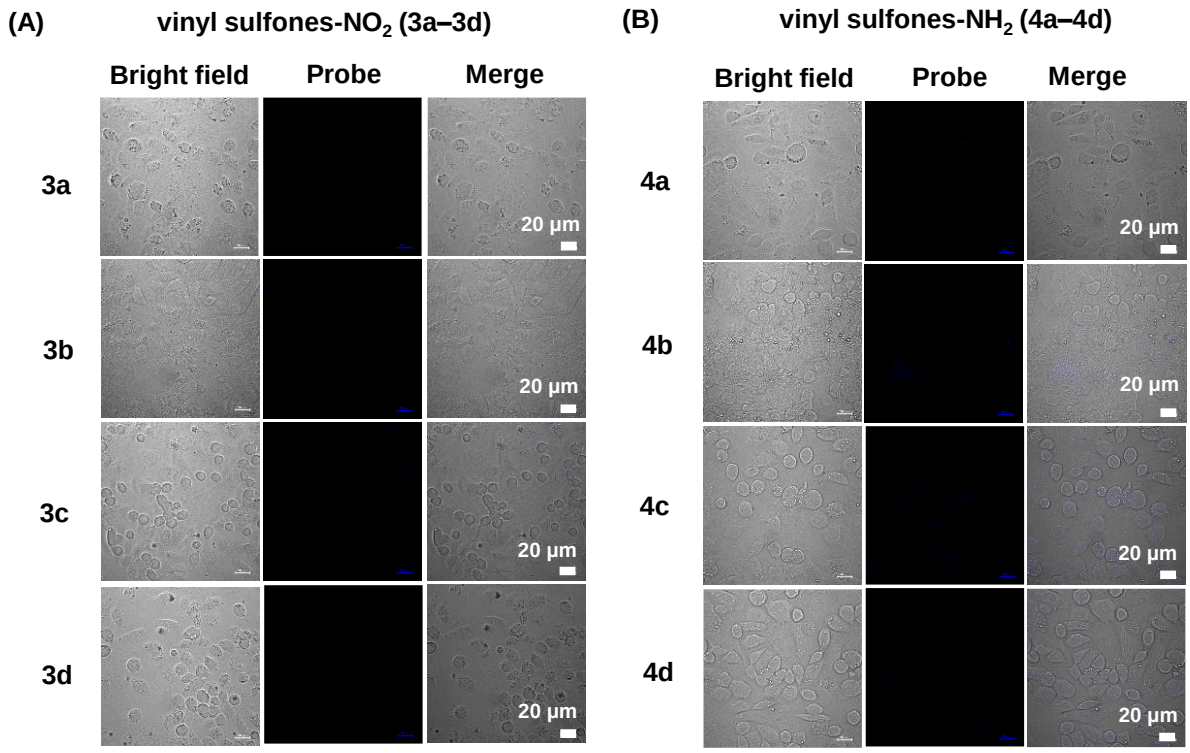


Fig. S12. Confocal images of HEK-293 cells incubated with (A) vinyl sulfones-NO_2 (**3a**–**3d**) and (B) vinyl sulfones-NH_2 (**4a**–**4d**) (20 μM) for 6 h.

References

1. M. Pramanik, K. Choudhuri and P. Mal, *Asian Journal of Organic Chemistry*, 2019, **8**, 144-150.
2. Y. Xu, J. Zhao, X. Tang, W. Wu and H. Jiang, *Adv. Synth. Catal.*, 2014, **356**, 2029-2039.