Electronic Supplementary Information

New Fluorescent Probes for Carbonic Anhydrases

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Synthesis of the CA probes:

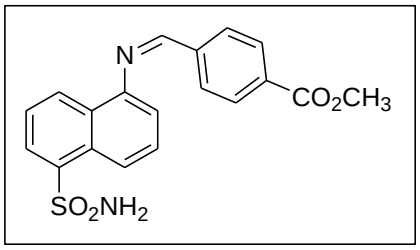
Reagents were purchased from Alfa Aesar or Aldrich or TCI America. Dichloromethane (CH_2Cl_2) was distilled under N_2 from P_2O_5. All other solvents used for reactions were HPLC grade and were used without further purification. Experiments were conducted under an atmosphere of dry nitrogen or using a guard tube.

Melting points were determined on a micro melting point apparatus. ^1H and ^{13}C NMR spectra were recorded using 400 or 300 MHz spectrometers. Solvents used for NMR spectra were one of the following $CDCl_3$, DMSO-d_6 with TMS as the internal standard. Many of the compounds reported here contained exchangeable protons (from amine, amide and hydroxyl groups). To remove these signals from the ^1H NMR spectra, one drop of D_2O was added to the solvent, DMSO-d_6. TLC was performed with Adsorbosil plus IP, 20 x 20 cm plate, 0.25 mm (Altech Associates, Inc.). Flash chromatography was carried out with Merck Silica Gel 60. Chromatography plates were visualized either by UV light or in an iodine chamber.

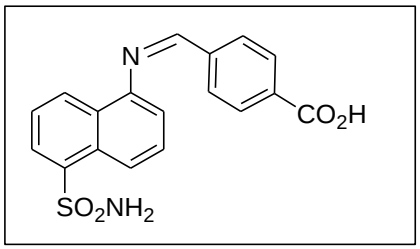
Synthetic details for the synthesis of **2** have been reported in the literature previously.[1]

Compound 3: Triphenylphosphine (3.19 g, 12.17 mmol) in CH_2Cl_2 (100 mL) was cooled in an ice bath to 0 ^oC, thionylchloride (0.97 mL, 13.3 mmol) was added, and the reaction mixture was stirred for 20 minutes. The ice bath was removed, and compound **2** (2.50 g, 5.80 mmol) was added. The reaction mixture was stirred for 1.5 hours at room temperature and then cooled to 0 ^oC. Tert-butylamine (1.75 mL, 16.5 mmol) was added dropwise by a syringe pump over 5 minutes followed by dropwise addition of triethylamine (2.3 mL, 16.5 mmol) over 5 minutes. The solution was stirred at room temperature for 6 hours. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with 0.5 N HCl, brine, dried over $MgSO_4$ and evaporated to give the crude product as viscous oil. This was purified by silica gel column chromatography (eluant: 30% ethyl acetate in hexane, R_f = 0.3) to obtain the pure product as a white solid. Yield: 1.29 g (59%), mp: 182-183 ^oC ; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.41-8.32 (m, 2H), 8.16 (d, 1H, J = 8.8 Hz), 7.90 (s, broad, 1H), 7.65-7.54 (m, 2H), 6.80 (s, broad, 1H), 4.60 (s, 1H), 1.55 (s, 9 H), 1.14 (s, 9 H) . ^{13}C NMR ($CDCl_3$, 100 MHz): δ 153.91, 139.05, 134.29, 129.61, 129.27, 128.41, 127.03, 124.70, 121.76, 121.10, 81.47, 55.41, 30.41, 28.71.

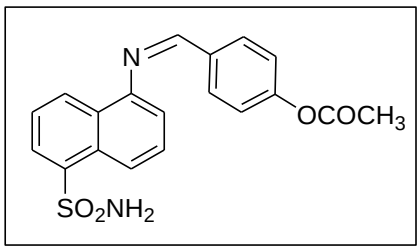
Compound 4: To a solution of compound **3** (1.50 g, 3.96 mmol) in CH_2Cl_2 (3 mL) trifluoroacetic acid (13.1 mL) was added dropwise at 0 ^oC. The reaction mixture was stirred for 24 hours at room temperature. The reaction mixture was then evaporated three times with dichloromethane. A white residue was obtained after evaporation. The white solid was triturated by heating with a mixture of dichloromethane and ethyl acetate. After cooling to room temperature, the solid was filtered and dried in vacuum. Yield: 0.88 g, (99 %) mp: 248-250 ^oC ; ^1H NMR (DMSO-d_6 + D_2O, 400 MHz): δ 8.30 (d, 1H, J = 8.5 Hz), 8.12 (d, 1H, J = 7.2 Hz), 7.83, (d, 1H, 8.87 Hz), 7.57-7.43 (m, 2H), 6.91 (d, 1H, J = 7.7 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 139.38, 128.81, 128.37, 127.32, 126.58, 124.13, 122.62, 115.06, 110.91.



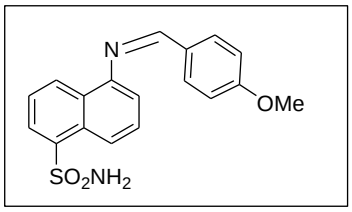
Compound 5a: To a solution of terephthaldehyde acid methyl ester (0.088 g, 0.54 mmol) in absolute ethanol (3 mL), 1-2 drops of glacial acetic acid was added. After stirring for 10 minutes at room temperature this solution was added to a suspension of **4** (0.10 g, 0.45 mmol), in 2 mL of absolute ethanol and the mixture was stirred for 12 hours at room temperature. The yellow precipitate formed was filtered, washed with ice-cold absolute ethanol and dried in vacuum. Yield: 0.100g (60%), mp: 238-240 ^{o}C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.87 (s, 1H), 8.63-8.55 (m, 2H), 8.25-8.14 (m, 5 H), 7.76-7.66 (m, 4H), 7.45 (d, 1H, J = 6.5 Hz), 3.92 (s, 3H). δ ^{13}C NMR (DMSO-d_6, 100MHz) : δ 166.36, 161.44, 148.83, 140.44, 139.88, 132.56, 130.22, 129.75, 128.92, 128.54, 128.49, 127.52, 125.05, 123.96, 114.57, 52.95. HRMS calcd for $C_{19}H_{16}N_2O_4S$ (M + Na): 391.0726, found: 391.1197. Anal. Calcd. for $C_{19}H_{16}N_2O_4S$: C, 61.94; H, 4.38; N, 7.60. Found: C, 62.03; H, 4.50; N, 7.74.



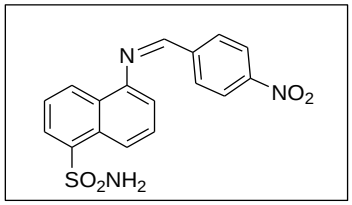
Compound 5b: To a solution of 4-carboxybenzaldehyde (0.081g, 0.54 mmol) in absolute ethanol (4 mL), 1 drop of acetic acid was added. After stirring for 10 minutes at room temperature the solution was added to the suspension of **4** (0.10 g, 0.45 mmol), in 3 mL of absolute ethanol and the reaction mixture was stirred 12 hours at room temperature. The yellow solid obtained was filtered, washed with ice cold absolute ethanol and dried in vacuum. Yield: 0.07 g (47%), mp: 253-255 ^{o}C ; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.86 (s, 1H), 8.63-8.55 (m, 2H), 8.22-8.12 (m, 5H), 7.76-7.66 (m, 4H), 7.45 (d, 1H, J = 7.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.43, 161.58, 148.90, 140.12, 139.86, 133.86, 130.35, 129.76, 129.63, 128.94, 128.53, 127.51, 125.04, 123.88, 114.56. HRMS calcd for $C_{18}H_{14}N_2O_4S$ (M + H): 355.0753, found: 355.1540. Anal. Calcd. for $C_{18}H_{14}N_2O_4S$: C, 61.01; H, 3.98; N, 7.90. Found: C, 61.33; H, 4.18; N, 7.81.



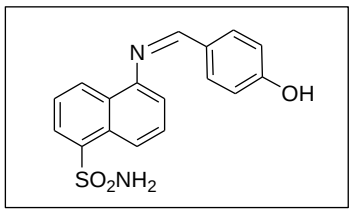
Compound 5c: To a solution of 4-acetoxybenzaldehyde (0.096 g, 0.58 mmol) in 2-propanol (6 mL), one drop of acetic acid was added. After stirring for 10 minutes at room temperature the solution was added to a suspension of **4** (0.10 g, 0.45 mmol), in 3 mL of 2-propanol and the reaction mixture was stirred for 12 hours at room temperature. The precipitate was filtered, washed with ice cold 2-propanol and dried in vacuum. Yield: 0.078 g (47%), mp: 216-218 ^{o}C ; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.76 (s, 1H), 8.60 (d, 1H, J = 8.5 Hz), 8.52 (d, 1H, J = 8.9 Hz), 8.20-8.13 (m, 3H), 7.75-7.63 (m, 4H), 7.40-7.34 (m, 3H), 2.33 (s, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 169.28, 161.11, 153.38, 148.94, 139.50, 133.91, 130.59, 129.44, 128.25, 127.16, 124.61, 122.78, 114.22, 21.18. HRMS calcd for $C_{19}H_{17}N_2O_4S$ (M + H): 369.0909, found: 369.2863. Anal. Calcd. for $C_{19}H_{16}N_2O_4S$: C, 61.94; H, 4.38; N, 7.60. Found: C, 61.67; H, 4.44; N, 7.42.



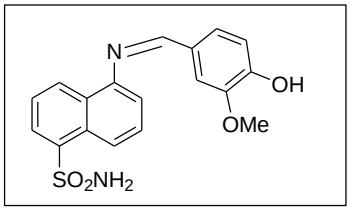
Compound 5d: To a suspension of **4** (0.125 g, 0.56 mmol) in methanol (7 mL), 4-methoxybenzaldehyde (0.10 g, 0.73 mmol) was added and the mixture was stirred at room temperature for 24 hours. The precipitate formed was filtered, washed with ice cold methanol and dried in vacuum. Yield: 0.057 g (30%), mp: 194-196 ^{o}C ; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.66 (s, 1H), 8.60 (d, 1H, J = 8.5 Hz), 8.50 (d, 1H, J = 8.5 Hz), 8.19 (dd, 1H, J = 7.3, 1.28 Hz), 8.05-8.03 (m, 2H), 7.72-7.63 (m, 4H) 7.34 (d, 1H J = 7.4 Hz), 7.15-7.12 (m, 2H), 3.87 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 162.80, 161.48, 149.67, 139.78, 131.45, 129.85, 129.52, 129.06, 128.57, 127.40, 124.72, 122.98, 114.94, 114.34, 50.04. HRMS calcd for $C_{18}H_{16}N_2O_3S$ (M + H): 341.0960, found: 341.0888. Anal. Calcd. for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23. Found: C, 63.31; H, 4.59; N, 7.98.



Compound 5e: To a suspension of **4** (0.125 g, 0.56 mmol), in methanol (7 mL), 4-nitrobenzaldehyde (0.09 g, 0.62 mmol) was added and the mixture was stirred at room temperature for 12 hours. The bright yellow precipitate was filtered, washed with ice cold methanol and dried in vacuum. Yield: 0.108 g (54%), mp: 240-242 ^{o}C ; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.96-8.95 (broad s, 1H), 8.66-8.57 (m, 2H), 8.45-8.34 (m 3H), 8.20 (m, 1H), 7.79-7.67 (m, 4H), 7.50 (d, 1H, J = 7.7 Hz). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 160.65, 149.61, 148.44, 141.94, 139.88, 130.61, 129.77, 128.90, 128.52, 128.48, 127.56, 125.19, 124.66, 124.32, 114.69. HRMS calcd for $C_{17}H_{13}N_3O_4S$ (M + Na): 378.0524, found: 378.1308. Anal. Calcd. for $C_{17}H_{13}N_3O_4S$: C, 57.46; H, 3.69; N, 11.82. Found: C, 57.78; H, 3.57; N, 12.01.

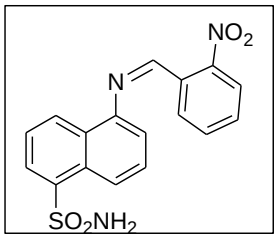


Compound 5f: To a suspension of **4** (0.10 g. 0.45 mmol) in absolute ethanol (10 mL), 4-hydroxybenzaldehyde (0.07 g, 0.57 mmol) was added and the reaction mixture was heated to reflux for 24 hours. The reaction mixture then was cooled down to 0 ^{o}C and the precipitated solid was filtered, washed with ice cold absolute ethanol and dried in vacuum. Yield: 0.062 g (39%), mp: 237-239 ^{o}C : ^{1}H NMR (DMSO-d_6/D_2O, 400 MHz): δ 8.30 (d, 1H, J = 8.2 Hz), 8.14 (d, 1H, J = 7.1 Hz), 7.85 (d, 1H, J = 8.5 Hz), 7.68 (d, 1H, J = 8.5 Hz), 7.58-7.53 (m, 1H), 7.48 (t, 1H, J = 8.1 Hz), 6.95-6.88 (m, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 166.47, 160.52, 146.17, 139.51, 129.34, 129.25, 128.95, 127.86, 126.74, 124.51, 123.73, 122.22, 115.36, 113.04, 108.84. HRMS calcd for $C_{17}H_{14}N_2O_3S$ (M + H): 327.0803, found: 327.0804. Anal. Calcd. for $C_{17}H_{14}N_2O_3S$: C, 62.56; H, 4.32; N, 8.58. Found: C, 62.33; H, 4.55; N, 8.72.

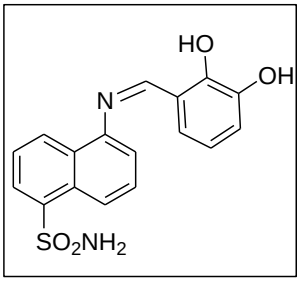


Compound 5g: To a suspension of **4** (0.11g, 0.50 mmol) in absolute ethanol (10 mL), 4-hydroxy-3-methoxybenzaldehyde (0.08 g, 0.54 mmol) was added and the reaction mixture was heated to reflux overnight. The reaction mixture was then cooled to 0 ^{o}C. The bright yellow solids were filtered, washed with ice cold absolute ethanol and dried in vacuum. Yield: 0.109 g (62%), mp: 223-224 ^{o}C : ^{1}H NMR (DMSO-d_6/D_2O, 400 MHz): δ 8.61 (d, 1H, J = 8.5 Hz), 8.57 (s, 1H,), 8.48 (d, 1H, J = 8.8 Hz), 8.19 (d, 1H, J = 7.4 Hz), 7.71-7.63 (m, 5H), 7.48 (d, 1H, J = 8.2 Hz), 7.31 (d, 1H J = 7.4 Hz), 6.95 (d, 1H, J = 7.9 Hz) 3.91 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 161.82, 151.20, 149.90, 148.64, 139.73, 129.81, 129.14, 128.59, 128.53,

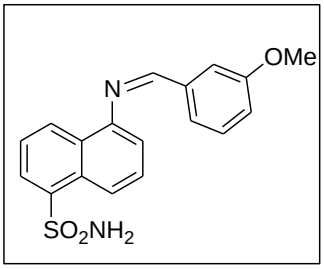
128.44, 127.37, 125.03, 124.63, 122.75, 116.00, 114.32, 111.48, 56.20. HRMS calcd for $C_{18}H_{16}N_2O_4S$ (M + H): 357.0909, found: 357.1347. Anal. Calcd. for $C_{18}H_{16}N_2O_4S$: C, 60.66; H, 4.53; N, 7.86. Found: C, 60.47; H, 4.69; N, 7.99.



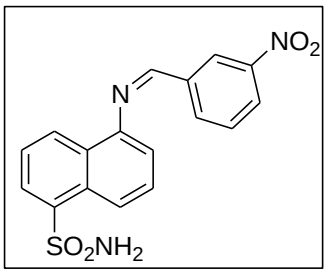
Compound 5h: To a suspension of **4** (0.11g, 0.50 mmol) in absolute ethanol (10 mL), 2-nitrobenzaldehyde (0.08 g, 0.53 mmol) was added and the reaction mixture was heated to reflux overnight. The reaction mixture was then cooled to 0^o C. The precipitated yellow solid was filtered, washed with ice cold absolute ethanol and dried in vacuum. Yield: 0.098 g (56%), mp: 235-237 ^{o}C : ^{1}H NMR (DMSO-*d*$_6$, 400MHz): δ 8.97 (s, 1H), 8.53 (dd, 2H, J = 7.7 Hz, 1.4 Hz), 8.33 (dd, 1H, J = 7.7 Hz, 1.4 Hz), 8.17 (dd, 1H, J = 7.4, 1.1 Hz), 8.11 (dd, 1H, J = 8.1, 1.3 Hz), 7.93-7.88 (m, 1H), 7.79 (td, 1H, J = 7.7, 1.6 Hz), 7.74-7.63 (m, 4H), 7.36 (d, 1H, J= 6.5 Hz). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ 159.01, 150.05, 148.95, 140.21, 134.54, 133.06, 131.26, 130.62, 129.93, 129.13, 128.82, 127.87, 125.49, 125.30, 124.51, 115.05. HRMS calcd for $C_{17}H_{13}N_3O_4S$ (M + H): 356.0705, found: 356.1091. Anal. Calcd. for $C_{17}H_{13}N_3O_4S$: C, 57.46; H, 3.69; N, 11.82. Found: C, 57.65; H, 3.78; N, 11.73.



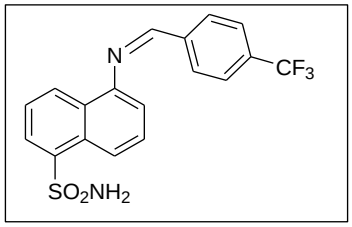
Compound 5i: To a suspension of **4** (0.10g, 0.45 mmol) in absolute ethanol (10 mL), 2,3-dihydroxybenzaldehyde (0.07 g, 0.50 mmmol) was added and the reaction mixture was heated to reflux overnight. A red precipitate was obtained on cooling down to 0 ^{o}C for half an hour. The red precipitate was filtered and washed with ice cold absolute ethanol and dried in vacuum. Yield: 1.17 g (76%), mp: 220-222 ^{o}C : ^{1}H NMR (DMSO-*d*$_6$; 400 MHz): δ 9.00 (s, 1H), 8.59 (d, 1H, J = 8.5 Hz), 8.45 (d, 1H, J = 8.5 Hz), 8.22 (dd, 1H, J = 7.1, 1.1 Hz), 7.77-7.67 (m, 4H), 7.56 (d, 1H, J = 6.8 Hz), 7.24 (dd, 1H, J = 7.9, 1.7 Hz), 7.02 (dd, 1H, J = 7.8, 1.6 Hz), 6.85 (t, 1H, J = 7.8 Hz). ^{13}C NMR (DMSO-*d*$_6$; 100 MHz): δ 165.61, 149.64, 146.85, 146.27, 140.19, 129.11, 128.55, 127.95, 127.53, 125.61, 124.16, 123.18, 120.53, 119.97, 119.67, 115.89. HRMS calcd for $C_{17}H_{14}N_2O_4S$ (M + H): 343.0753, found: 343.1653. Anal. Calcd. for $C_{17}H_{14}N_2O_4S$: C, 59.64; H, 4.12; N, 8.18. Found: C, 59.77; H, 4.26; N, 8.33.



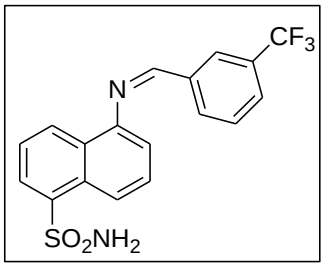
Compound 5j: To a solution of 3-methoxybenzaldehyde (0.08 g, 0.59 mmol) in 2-propanol (7 mL), 1-2 drops of acetic acid were added. After stirring for 10 minutes at room temperature the solution was added to a suspension of **4** (0.10g , 0.45 mmol) in 3 mL of 2-propanol. The reaction mixture was stirred for 14 hours at room temperature. The precipitate formed was filtered, washed with ice cold 2-propanol and dried in vacuum. Yield: 0.09 g (59%), mp: 192-194 ^{0}C ; ^{1}H NMR (DMSO-*d*$_6$; 400 MHz): δ 8.71 (s, 1H), 8.58 (d, 1H, J = 8.2 Hz), 8.52 (d, 1H, J = 8.8 Hz), 8.18 (dd, 1H, J = 7.3, 1.3 Hz), 7.72-7.63 (m, 6H), 7.49 (t, 1H, J = 8.1 Hz), 7.36 (d, 1H, J = 6.5 Hz), 7.17 (dt, 1H, J = 7.7, 1.7 Hz), 3.86 (s, 1H). ^{13}C NMR (DMSO-*d*$_6$; 100 MHz): δ 162.28, 160.21, 149.28, 139.82, 137.96, 130.61, 129.71, 129.02, 128.53, 127.47, 124.90, 123.46, 122.46, 118.62, 114.49, 113.66, 55.88. HRMS calcd for $C_{18}H_{16}N_2O_3S$ (M + H): 341.0960, found: 341.1555. Anal. Calcd. for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23. Found: C, 53.60; H, 4.81; N, 8.12.



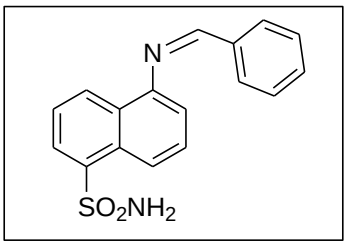
Compound 5k: To a suspension of **4** (0.1 g, 0.45 mmol) in absolute ethanol (10 mL), 3-nitrobenzaldehyde (0.08 g, 0.54 mmol) was added and the reaction mixture was heated to reflux overnight. The reaction mixture was then cooled to 0^{o} C and the yellow precipitate was filtered, washed with ice cold absolute ethanol and dried in vacuum. Yield: 0.12 g (75%), mp: 239-241^{o} C ; ^{1}H NMR (DMSO-*d*$_6$; 400 MHz): δ 8.96 (s, 1H), 8.89-8.87 (m, 1H), 8.64-8.53 (m, 3H), 8.46-8.42 (m, 1H), 8.21 (dd, 1H, J = 7.4 Hz), 7.89 (t, 1H, J = 7.8 Hz), 7.77-7.67 (m, 4H), 7.47 (d, 1H, J = 6.5 Hz). ^{13}C NMR (DMSO-*d*$_6$; 100 MHz): δ 160.62, 148.53, 139.89, 138.03, 135.42, 131.16, 131.16, 129.69, 128.92, 128.53, 128.48, 127.55, 126.58, 125.13, 124.08, 123.81, 114.72. HRMS calcd for $C_{17}H_{13}N_3O_4S$ (M + H): 356.0705, found: 356.0632. Anal. Calcd. for $C_{17}H_{13}N_3O_4S$: C, 57.46; H, 3.69; N, 11.82. Found: C, 57.37; H, 3.81; N, 11.69.



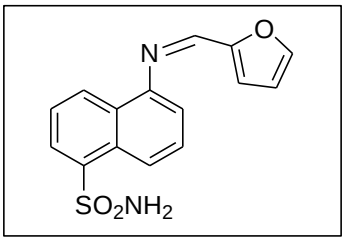
Compound 5l: To a solution of 4-trifluoromethylbenzaldehyde (0.1 g, 0.45 mmol) in 2-propanol (7 mL), 1-2 drops of acetic acid was added. After stirring for 10 minutes at room temperature, the solution was added to a suspension of **4** (0.102 g, 0.58 mmol) in 4 mL of 2-propanol and the reaction mixture was stirred for 24 hours at room temperature. The precipitate formed was filtered, washed with ice cold 2-propanol and dried in vacuum. Yield: 0.047 g (28%), mp: 208-210 ^{o}C ; ^{1}H NMR (DMSO-*d*$_6$; 400 MHz): δ 8.89 (s, 1H), 8.59 (m, 2H), 8.31 (d, 1H, J = 8.2 Hz), 8.21 (dd, 1H, J = 7.4,1.1 Hz), 7.96 (d, 2H, J = 8.2 Hz), 7.76-7.66 (m, 4H), 7.46 (d, 1H, J = 7.4 Hz). ^{13}C NMR (DMSO-*d*$_6$; 100 MHz): δ 161.18, 148.70, 140.06, 139.89, 131.67, 130.20, 129.72, 128.90, 128.53, 128.48, 127.52, 126.41, 125.08, 124.01, 114.64. HRMS calcd for $C_{18}H_{13}F_3N_2O_2S$ (M + H): 379.0728, found: 379.1324. Anal. Calcd. for $C_{18}H_{13}F_3N_2O_2S$: C, 57.14; H, 3.46; N, 7.40. Found: C, 57.28; H, 3.67; N, 7.55.



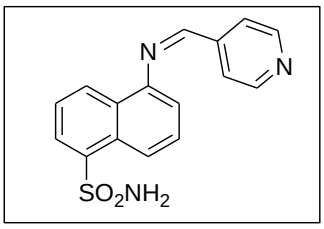
Compound 5m: To a solution of 3-trifluoromethylbenzaldehyde (0.102 g, 0.58 mmol) in 2-propanol (7 mL), 1-2 drops of acetic acid was added. After stirring for 10 minutes at room temperature, the solution was added to a suspension of **4** (0.10 g, 0.45 mmol) in 4 mL of 2-propanol. The reaction mixture was stirred for 24 hours at room temperature. The precipitate obtained was filtered, washed with ice cold 2-propanol and dried in vacuum. Yield: 0.076 g, (45%), mp: 233-235 ^{o}C ; ^{1}H NMR (DMSO-*d*$_6$; 300 MHz): δ 8.89 (s, 1H), 8.59 (m, 2H), 8.43 (s, 2H), 8.21 (d, 1H), 7.98 (d, 1H, J = 7.2 Hz), 7.86 (d, 1H, J = 8.1 Hz), 7.78-7.66 (m, 5H), 7.44 (d, 1H, J = 7.2 Hz). ^{13}C NMR (DMSO-*d*$_6$; 125 MHz): δ 161.17, 148.82, 139.86, 137.43, 133.19, 130.73, 129.67, 128.98, 128.52, 128.49, 127.52, 125.94, 125.07, 123.89, 114.65. HRMS calcd for $C_{18}H_{13}F_3N_2O_2S$ (M + H): 379.0728, found: 379.1387. Anal. Calcd. for $C_{18}H_{13}F_3N_2O_2S$: C, 57.14; H, 3.46; N, 7.40. Found: C, 57.44; H, 3.29; N, 7.61.



Compound 5n: To a solution of benzaldehyde (0.062 g, 0.58 mmol) in t-butanol (5 mL), 1-2 drops of acetic acid was added. After stirring for 10 minutes at room temperature, the solution was added to a suspension of **4** (0.100 g, 0.45 mmol), in 2 mL of t-butanol and stirred for 8 hours at room temperature. The yellowish white precipitate formed was filtered, washed with ice cold 2-propanol and dried in vacuum. Yield: 0.085 g, (61%), mp: 192-194 ^{o}C ; ^{1}H NMR (DMSO-d_6; 400 MHz): δ 8.76 (s, 1H), 8.60 (d, 1H, J = 8.5 Hz), 8.53 (d, 1H, J = 8.8 Hz), 8.20 (dd, 1H, J = 7.3, 1.3 Hz), 8.10 (dd, 2H, J = 7.3, 2.4 Hz), 7.74-7.64 (m, 4H), 7.62-7.57 (m, 3H), 7.39 (d, 1H, J = 7.4 Hz). ^{13}C NMR (DMSO-d_6; 100 MHz): δ 162.39, 149.34, 139.83, 136.54, 132.43, 129.75, 129.60, 129.49, 128.98, 128.53, 127.45, 124.89, 123.42, 114.48. HRMS calcd for $C_{17}H_{14}N_2O_2S$ (M + Na): 333.0674, found: 333.1072. Anal. Calcd. for $C_{17}H_{14}N_2O_2S$: C, 65.79; H, 4.55; N, 9.03. Found: C, 65.77; H, 4.29; N, 8.87.



Compound 5o: To a solution of 2-furaldehyde (0.05 g, 0.58 mmol) in 2-propanol (6 mL), 1-2 drops of acetic acid was added. After stirring for 10 minutes at room temperature, the solution was added to a suspension of **4** (0.1 g, 0.45 mmol) in 2 mL of 2-propanol and stirred overnight at room temperature. The reaction mixture was cooled to 0^{o} C, the precipitate formed was filtered and washed with ice cold 2-propanol and dried in vacuum. Yield: 0.065 g, (48%), mp: 188-190 ^{o}C ; ^{1}H NMR (DMSO-d_6; 400 MHz): δ 8.58-8.49 (m, 3H), 8.19 (dd, 1H, J = 7.3,1.3 Hz), 8.04 (d, 1H, J = 1.7 Hz), 7.72-7.63 (m, 4H), 7.36 (d, 1H, J = 7.4 Hz), 7.30 (d, 1H, J = 3.4 Hz), 6.78 (m, 1H). ^{13}C NMR (DMSO-d_6; 100 MHz): δ 152.50, 149.87, 149.21, 147.48, 139.79, 129.83, 128.95, 128.55, 127.45, 124.85, 123.39, 118.58, 114.25, 113.28. HRMS calcd for $C_{15}H_{12}N_2O_3S$ (M + H): 301.0647, found: 301.1367. Anal. Calcd. for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33. Found: C, 60.14; H, 4.17; N, 9.26.



Compound 5p: To a suspension of **4** (0.10 g, 0.45 mmol) in absolute ethanol (10 mL), pyridine-4-carbaldehyde (0.06 g, 0.56 mmol) was added and the reaction mixture was heated to reflux overnight. A yellow solution was obtained which was cooled to room temperature and the solvent was evaporated to give a yellow solid. The yellow residue was treated with diethyl ether, filtered and washed with ice cold ether and finally dried in vacuum. Yield: 0.084 g, (60%), mp: 188-190 ^{o}C ; ^{1}H NMR (DMSO-d_6; 400 MHz) δ 8.82-8.81 (m, 3H), 8.59 (t, 2H, J = 9.4 Hz) 8.21 (d, 1H, J = 7.4 Hz), 8.02-8.00 (m, 2H), 7.76-7.66 (m, 4H), 7.47 (d, 1H, J = 7.5 Hz). ^{13}C NMR (DMSO-d_6; 400 MHz) δ 161.02, 151.08, 148.41, 142.99, 139.89, 129.67, 128.88, 128.52, 128.45, 127.56, 125.19, 124.34, 123.06, 114.71. HRMS calcd for $C_{16}H_{13}N_3O_2S$ (M + H): 312.0807, found: 312.0836. Anal. Calcd. for $C_{16}H_{13}N_3O_2S$: C, 61.72; H, 4.21; N, 13.50. Found: C, 61.83; H, 4.49; N, 13.66.

Integrity of the probes before and after binding with CA:

In order to determine the stability of the probes in aqueous solutions before binding to CA, we have measured the ^{1}H NMR spectra of the synthesized probes in 1:1 DMSO-d_6-D_2O solutions. We prepared 5 mM solutions of the probes in aqueous DMSO-d_6 and monitored the ^{1}H NMR spectra for 6 hours at room temperature. We did not detect any aldehydes (δ = 10.249 ppm) in the probe solutions by NMR spectroscopy. As an illustrative example, the ^{1}H NMR spectra for the probe **5h** is shown here (Figure S1). *Clearly, within the detection limits of ^{1}H NMR spectroscopy, the probes are not in equilibrium with the aldehydes in aqueous DMSO solutions*. It should be noted that although imines are usually hydrolyzed in water, there are reports in the literature of imines stable in aqueous solution for more that 6 hours.[6]

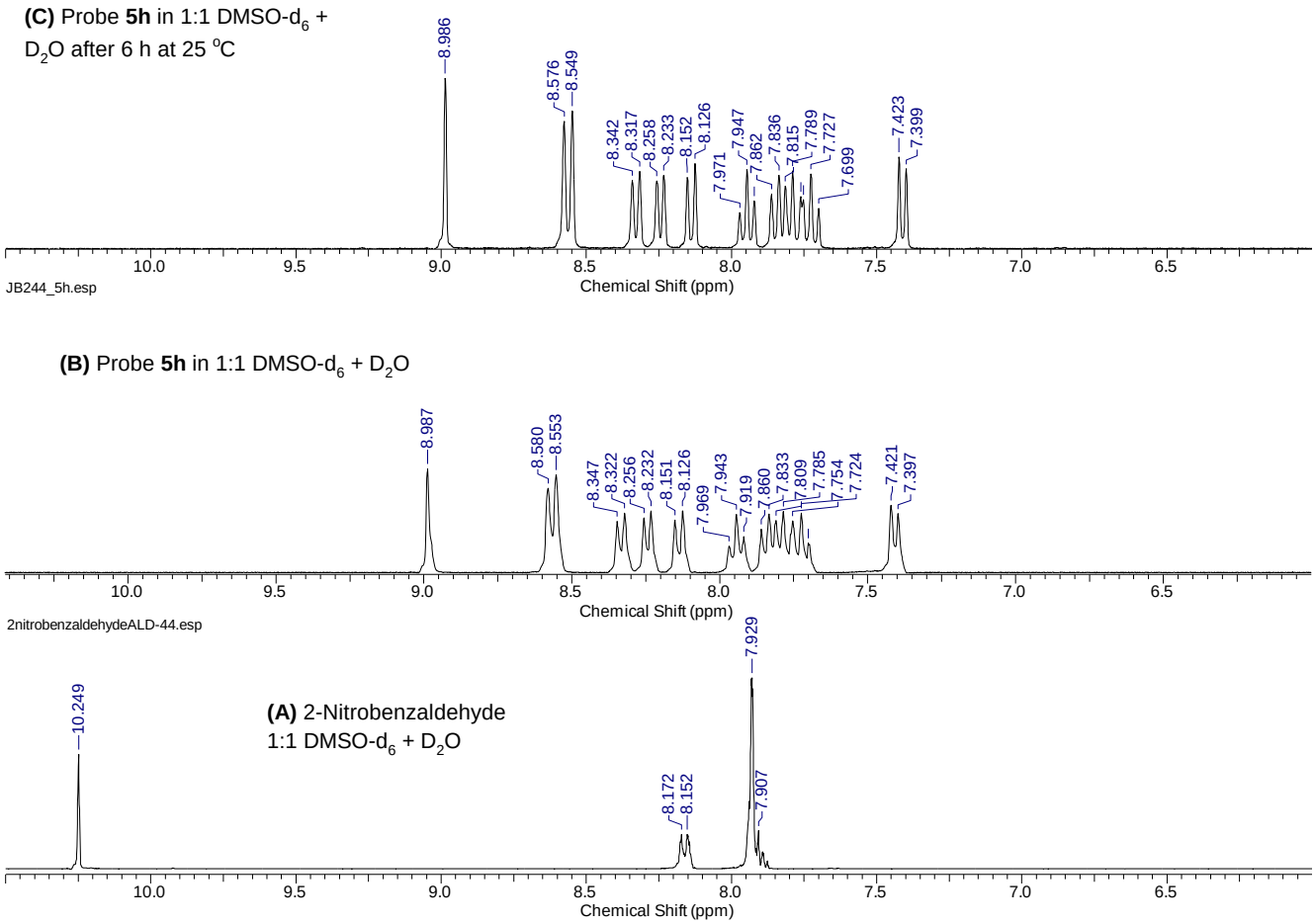


Figure S1. The ^{1}H NMR spectra for 2-nitrobenzaldehyde (**A**), probe **5h** immediately after preparing the solution (**B**) and after storing for 6 hours at room temperature (**C**) are shown. The aldehyde proton is present in panel **A** (δ = 10.249 ppm) and is clearly absent in the spectra of the probe **5h** in aqueous DMSO-d_6 solution (Panel **B**), even after storing the aqueous solution for 6 h at room temperature (Panel **C**).

We also monitored the time dependent changes in UV-Vis spectral profiles upon mixing probe **5h** (initially present in 100% DMSO) with water. The idea was that if the Schiff's base is hydrolyzed at a

typical literature reported[7] rate of 0.32 – 3.2 min^{-1}, we would be able to observe the time dependent spectral changes. However, in contrast to this expectation, we did not observe any UV-Vis spectral changes of the compound in water up to 2 hours (Figure S2). This data is in accord with the ^{1}H NMR studies (Figure S1) of the compound **5h** in the presence of D_2O. Please note that there are other reports in the literature of imines stable in aqueous solution for more that 6 hours.[6]

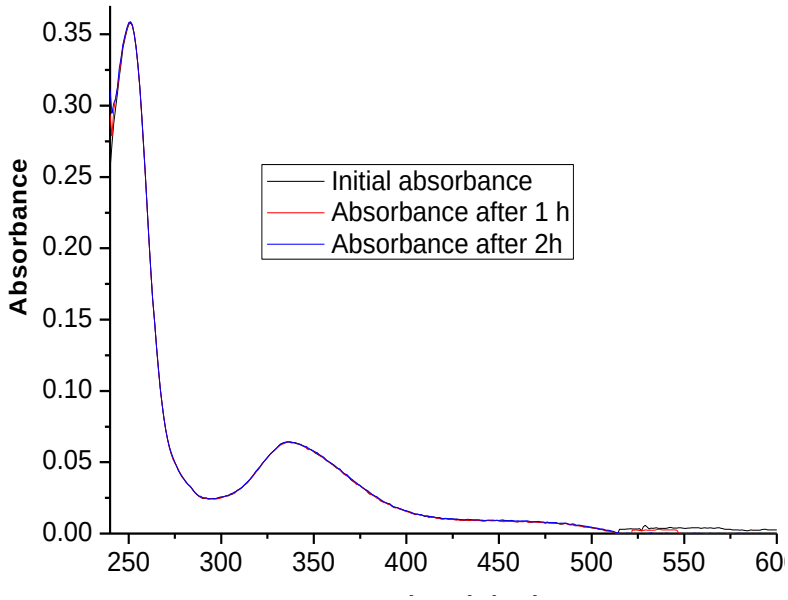


Figure S2. UV-Vis spectra of probe **5h** (5 μM in water containing 10% DMSO) is shown at various time intervals (black: immediately after dissolution; red: after 1 h at room temperature; blue: after 2 h at room temperature).

Transient kinetics for the binding of probe 5h to CA II:

It has been known that the hydration equilibrium of Schiffs base and its associated aldehyde and amine components is favored to the former,[7] and the rate constant for the breakdown of Schiff's base (from aromatic amines and aromatic aldehydes) is typically in the range 0.32 – 3.2 min^{-1}.[7] Taking into consideration of these features, we purported to determine whether the changes in fluorescence intensity upon binding of fluorophores originates from the binding of parent (Schiff's base) compound or its hydrolyzed amine product. Figure S3 shows the stopped flow trace for the binding of **5h** with CA II under pseudo-first order condition. The analysis of the experimental data yield a rate constant of 25 s^{-1}, which is at least 450 times faster than that expected rate for the hydrolysis of the Schiff's base from aromatic aldehydes and aromatic amines.[7] Clearly, the observed fluorescence changes in Figure S3 is a consequence of binding of the parent compound rather than its dissociated (hydrolyzed) amine product. In this regard, it should be pointed out that arylsulfonamide moiety substituted with amino groups are poor inhibitors for CAs (particularly CA II).[8] On the other hand when the p-amino group is derivatized to form the amide moiety, its binding affinity increases by 1-2 orders of magnitude.[9] Hence, even if a mixture of Schiff's base and free amine (albeit in low concentration) present at equilibrium, the mass action would favor the binding of Schiff's base (in preference to the amine

derivative) to CA II. Therefore, we conclude that the observation fluorescence changes upon binding of probe **5h** to CA II is due to the binding of the parent compound not its hydrolytic product.

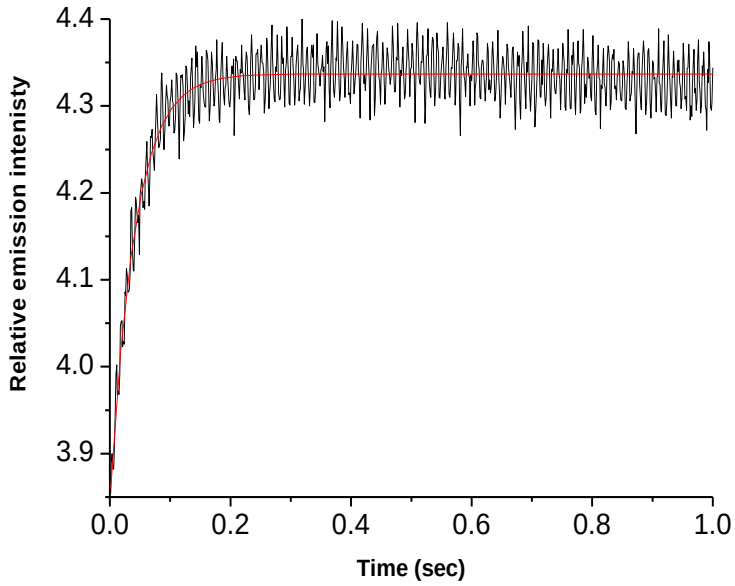


Figure S3. Stopped flow trace for the binding of **5h** (90 μM in 25 mM HEPES buffer, pH = 7.0 containing 10% DMSO) with CA II (4 μM in 25 mM HEPES buffer, pH = 7.0) under pseudo-first order condition is shown. Excitation: 330 nm, cut off filter at 395 nm. Fluorescence Emission was monitored at 470 nm. The red trace is the fitted curve, yielding a first order rate constant of 25 sec^{-1}.

Dependence of the fluorescence emission spectra of the probes on zinc coordination:

Sulfonamides in aqueous solution are known to be poor binders for Zn^{2+} ions.[10] Hence, we did not anticipate the fluorescence emission spectra of the probes to be modulated by Zn^{2+} ions in aqueous solution. Poor solubility of $ZnSO_4$ in HEPES buffer at pH = 7.0 prevented us from studying the fluorescence spectra of the probes in the presence of various concentrations of Zn^{2+} ions at pH = 7.0. Figure S4 shows the fluorescence emission spectra of **5h** (5 μM, λ_{ex} = 336 nm) in the absence (black trace) and presence of 25 mM (red trace) and 50 mM (blue trace) $ZnSO_4$ in water containing 10% DMSO. There is no shift in the emission maximum of the fluorophore and there is no increase in the fluorescence emission intensity in the presence of Zn^{2+} ions. *Clearly, the binding of Zn^{2+} to probe **5h** does not produce spectral shifts or emission intensity increases as observed upon binding to CAs.* We believe the fluorescence spectral changes upon binding of different fluorophores to CAs originate from a combined effect of the hydrophobicity of the active site pocket as well as high "effective" concentration of Zn^{2+} at the sulfonamide binding region of the enzyme. We do not foresee any experimental approach to segregate the influence of Zn^{2+} coordination (to the sulfonamide moiety of the fluorophore) in the enzyme active site and the polarity of the enzyme's active site phase in assembling the observed fluorescence changes.

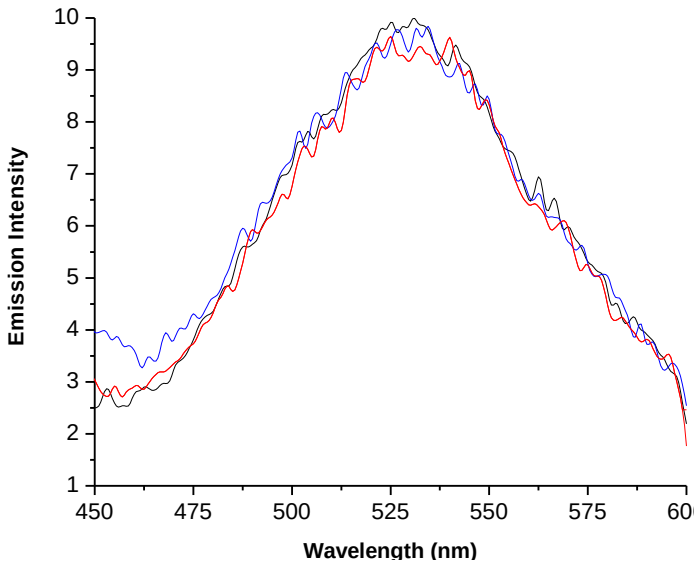


Figure S4. Fluorescence emission intensities of the probe **5h** (5 μM, λ_{ex} = 336 nm) in the absence (black) and presence of 25 mM (red) and 50 mM (blue) $ZnSO_4$ in water containing 10% DMSO.

Cloning, expression and purification of human carbonic anhydrases were performed by following procedures developed in our laboratory previously.[4]

Spectrophotometric studies:

All the absorption studies were performed on a Beckman DU 7400 spectrophotometer. The concentration of the probes and the buffer were the same as described for the spectrofluorometric studies. Stock solutions of all the probes and the standard were made in 100% DMSO, and were diluted in 25 mM HEPES buffer (pH = 7.0) containing 10% DMSO. The stock solutions were diluted to 5 mM and the absorption spectra were recorded.

Spectrofluorimetric studies:

All the spectrofluorimetric studies were performed on Perkin–Elmer lambda 50-B spectrofluorometer equipped with a magnetic stirrer and thermostatic water bath. Stock solutions of all the probes and the standard were made in 100% DMSO, and were diluted in 25 mM HEPES buffer (pH = 7.0) containing 10% DMSO. The final concentration of the probes and CA were 5 μM and 10 μM respectively. Protein concentration was determined by standard BCA method using BSA as a standard (BCA protein assay kit from Sigma Chemical Company was used). The emission spectra were determined by fixing the excitation wavelength at the excitation maxima of the respective probes. The emission and the excitation slits were maintained at 9 mm and 2.5 mm. The PMT voltage was kept constant throughout the experiment. Quinine was used as the standard fluorescent probe since its absorption maxima overlapped with almost all the probes.

Determination of the dissociation constants of the probes with CA I and CA II:

The stock solution of the probes were prepared in 100% DMSO and was diluted in 25 mM HEPES buffer (pH = 7.0) containing 10% DMSO. The emission spectra of the probes in the absence and presence of the enzymes were acquired by fixing the excitation wavelength of the probes at the absorption maxima. The dissociation constant of the enzyme-probe complex was determined by titrating a fixed concentration of the enzyme (5 μM) with increasing concentrations of the probe (0-15 μM) in 25 mM HEPES containing 10% DMSO. The enhancement of the intensity at the emission maxima of the probes were monitored, as described in the literature.[5] A typical titration curve for the titration of probe **5f** with CA II is shown in Figure S5.

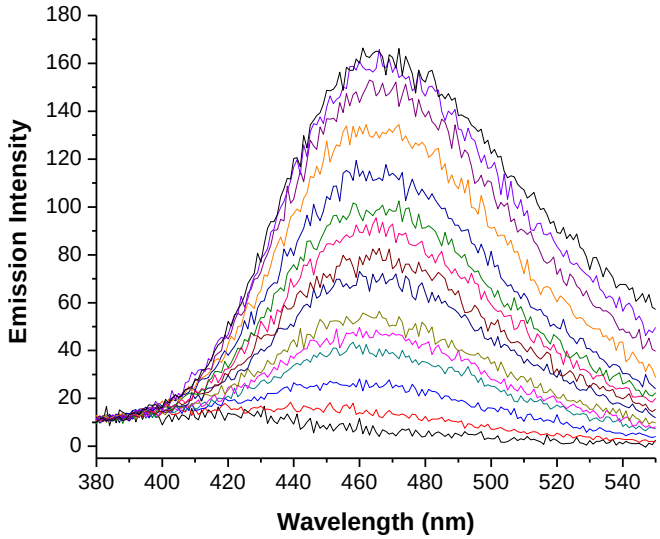


Figure S5. Titration of the probe **5f** (0 – 15 μM) with recombinant human CA II (5 μM) in 25 mM HEPES buffer (pH = 7.0) containing 10% DMSO. The emission intensity at 466 nm (λ_{ex} = 336 nm) was followed to determine the dissociation constant.

Determination of the quantum yields:

Quantum yield is the ratio of the amount of light emitted from a sample to the amount of light absorbed by the sample.

Φ = [# of fluorescence photons emitted /[# of incident photons absorbed]

1. Absorption spectrum of the sample was taken after blanking with the buffer containing 10% DMSO.

2. The optical density of the sample at the excitation wavelength was recorded.

3. The emission spectrum of the solvent was recorded.

4. The emission spectrum of the sample was taken.

5. Since the emission spectrum of solvent was almost near to zero, it was neglected.

6. The area under the emission peak was calculated.

7. The same procedure was repeated for the standard.

8. Quantum yield was determined using the following formula:

$$\Phi = \Phi_S * I * OD_s * n_2/I_s * OD * n_{2s}$$

$$Q = Q_R \frac{I}{I_R} \frac{OD_R}{OD} \frac{n^2}{n_R^2}$$

Where ϕ is the quantum yield of the sample, I is the integrated intensity, OD is the optical density. The subscript 's' refers to the standard flurophore (quinine) with a quantum yield of 0.54.

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