Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Electronic supplementary information

Protein assisted fluorescence enhancement of a dansyl containing fluorescent reagent: Detection of Hg^{2+} ion in aqueous medium

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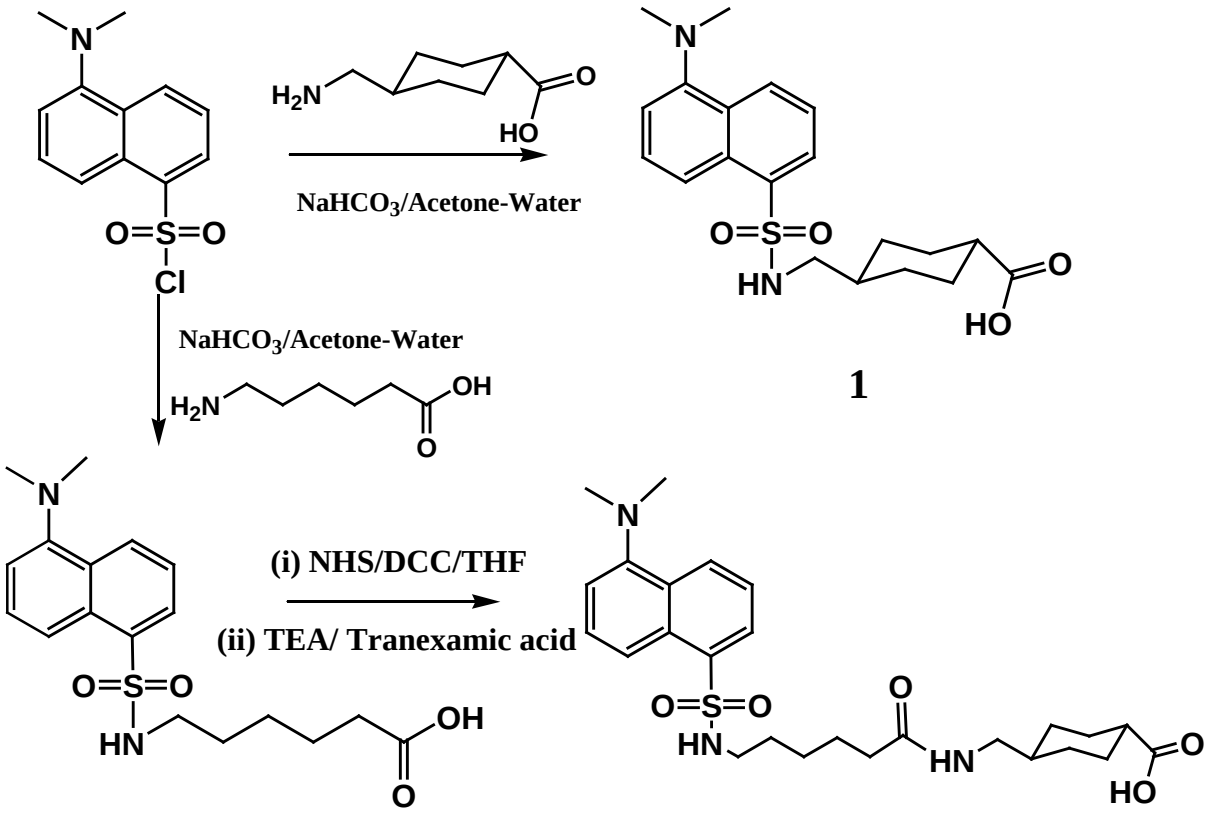
Experimental:

All the reagents and solvents were purchased from Merck and Sigma-Aldrich and were used without any further purification. IR spectra in KBr were recorded on a Varian-3100 FTIR spectrometer. ^{1}H and ^{13}C NMR spectra (chemical shifts in δ ppm) were recorded on a JEOL AL 300 FT–NMR (300 MHz) spectrometer, using TMS as internal standard. The UV-Vis absorption spectra were recorded on Shimadzu 1700 spectrophotometer using a quartz cuvette (path length = 1cm). Fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrophotometer (Varian). Sodium salt of probe is prepared by treating the compound with 1N NaOH solution. Stock solution of probe (c = $1x10^{-3}$M) was prepared in 50 mM AcONa solution in water. For each experiment 80µL of stock solution was taken and diluted to 2mL to make the concentration of probe for each experiment is in 50mM AcONa buffer solution. In emission titration experiments 12 µL of 0.1 M solution of different metal ions were used. In ^{1}H NMR titration experiment solution of probe ($1x10^{-2}$ M) and $HgClO_4$ in DMSO-d_6 solution was prepared. A stock solution of bovine serium albumin (BSA) (c = $1x\ 10^{-3}$ M) was prepared in AcONa (50 mM) buffer.



Scheme 1: Synthesis of Fluorescent reagents.

Characterization data:

4-Aminomethyl-N-dansylsulphonamidocyclohexane-1-carboxylic acid (1). Yield = 50%. R_f = 0.67 (EtOAc). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 12 (Br, 1H, -COOH), 8.48 (d, 1H, J = 8.4Hz, H2), 8.33 (d, 1H , J = 8.7Hz, H8), 8,08 (d, 1H , J = 6.9 Hz, H4), 7,9 (s, -NH), 7.64 (m, 2H, H3, H7), 7.29 (d, 1H , H6), 2.83 (s, 8H, -N(CH_3)$_2$, and –SO_2NHCH_2-), 0.75-2.61 (m, 10H, cyclohexane); ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.5, 151, 136, 129, 129, 128 , 127.7 , 123.5 , 119., 115.0 , 48.7 , 45.0 , 42.3, 30.8, 29.0, 28.1; FT-IR (KBr) ν(cm^{-1}) = 526, 570**,** 660, 790 (Ar, C-H stretch), 1061 (S=O stretch), 1141 (S=O)$_2$ stretch), 1204 (C-O stretch), 1307 (C-N stretch), 1458, 1523, 1647 (C=C stretch), 1696 (C=O stretch), 2860, 2937 (C-H stretch), 3447 (N-H stretch); Anal. Calc. For $C_{20}H_{26}N_2O_4S$: C, 61.52; H, 6.71; N, 7.17%. Found: C, 61.41; H, 6.81; N, 7.27%.

6-Amino-N-dansylsulphonamido-1-hexanoic acid (2). Yield = 50%. R_f = 0.58 (EtOAc). ^{1}H NMR (300 MHz, DMSO-d_6)**:** δ (ppm) 11.9 (Br, 1H, -COOH), 8.46 (d, 1H, J = 8.5Hz, H2), 8.3 (d, 1H, J = 8.7Hz, H8), 8.09 (d, 1H, J = 7.5Hz, H4), 7.87 (t, -NH, J_1 = 5.4; J_2 = 5.7Hz), 7.63 (m, 2H, J = 8.1Hz, H3, H7), 7.26 (d, 1H, J = 7.2Hz, H6), 2.87 (s, 6H, H4), 1.10- 2.76 (m,10H, (-CH_2)$_5$); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 174.3 , 151.3 , 136.1 , 129.0 , 129.0 , 128.2 , 127.7 , 123, 119.1 , 115.1 , 45.0 (6C, N-(CH_3)$_2$), 42.2 , 33.4 , 28.7 , 25.4 , 23.8; FT-IR (KBr) υ_{max}(cm^{-1}) = 510, 570, 610, 658, 790 (Ar, C-H stretch), 1033 (S=O), 1204 (C-O stretch), 1397 (C-N stretch), 1459, 1491, 1519 (C=C), 1651 (C=O), 3054 (C-H aromatic), 3448 (N-H stretch); Anal. Calc. For $C_{18}H_{24}N_2O_4S$: C, 59.32; H, 6.64; N, 7.69%. Found: C, 59.21; H, 6.71; N, 7.72%.

6-Amino-(N-dansylsulphonamido)-N-carboxamido-4-methylcyclohexane carboxylic acid (3). Yield = 75%. R_f = 0.28 (EtOAc); ^{1}H NMR (300 MHz, DMSO-d_6)**:** δ (ppm) 12.0 (Br, 1H, -COOH), 8.46 (d, 1H, J = 8.4Hz, H2), 8.30 (d, 1H, J = 8.7Hz, H8), 8.09 (d, 1H, J = 7.2Hz, H4), 7.86 (s, 1H, NH), 7.67 (s, 1H, -CONH), 7.60 (m, 1H, H3,H7), 7.26 (d, 1H, H6), 2.82 (m, 8H, -N(CH_3)$_2$ and –SO_2NHCH_2-), 0.85-2.72 (10H, cyclohexane, 10H, -(CH_2)$_5$); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 189, 176, 152.9, 138.9 , 129.3, 129.1, 126.9, 125.7 , 123.6 , 122.6, 119.1, 116.2, 45.7, 45.0, 42.3, 40.3, 29.4, 28.3, 25.2, 24.8, 18.3; FT-IR (KBr) υ_{max}(cm^{-1}) = 574, 653, 794 (aromatic C-H stretch), 1082 (S=O stretch), 1146 (S=O)$_2$ stretch), 1229 (C-O stretch), 1312 (C-N stretch), 1455, 1542, 1631 (C=C stretch), 1710 (C=O stretch), 2854, 2929 (C-H stretch in CH_2), 3389 (N-H strectch); Anal. Calc. For $C_{26}H_{37}N_3O_5S$: C, 62.00; H, 7.40; N, 8.34%. Found: C, 59.81; H, 7.47; N, 8.42%.

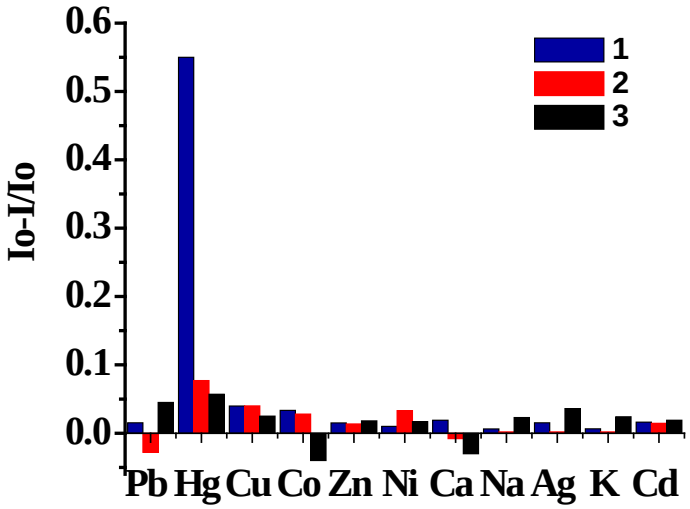


Figure S1: Interaction of reagents with different metal ions in AcONa buffer solution (50 mM; pH = 6.7).

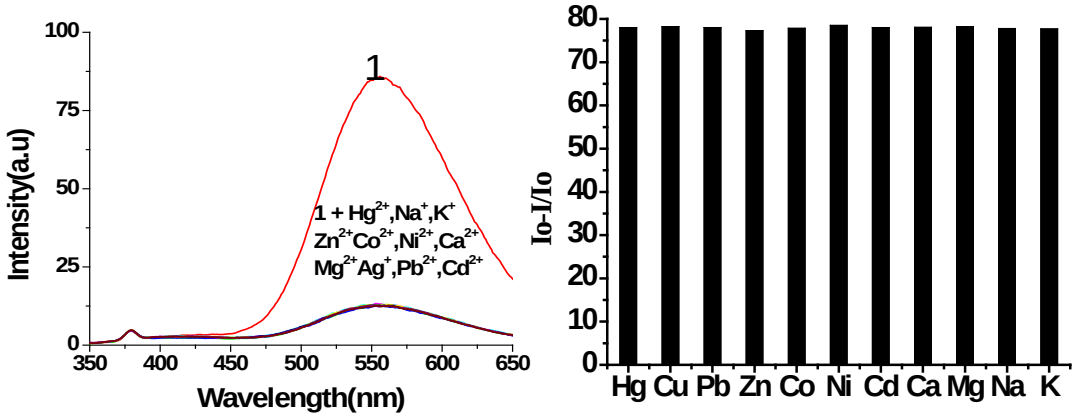


Figure S2: (a) Competitive metal ion study after addition of tested metal ions to the solution of **1**+Hg^{2+} in AcONa buffer (50mM; pH = 6.7). Bar diagram shows change in relative fluorescence intensity of **1** in presence of different metal ions.

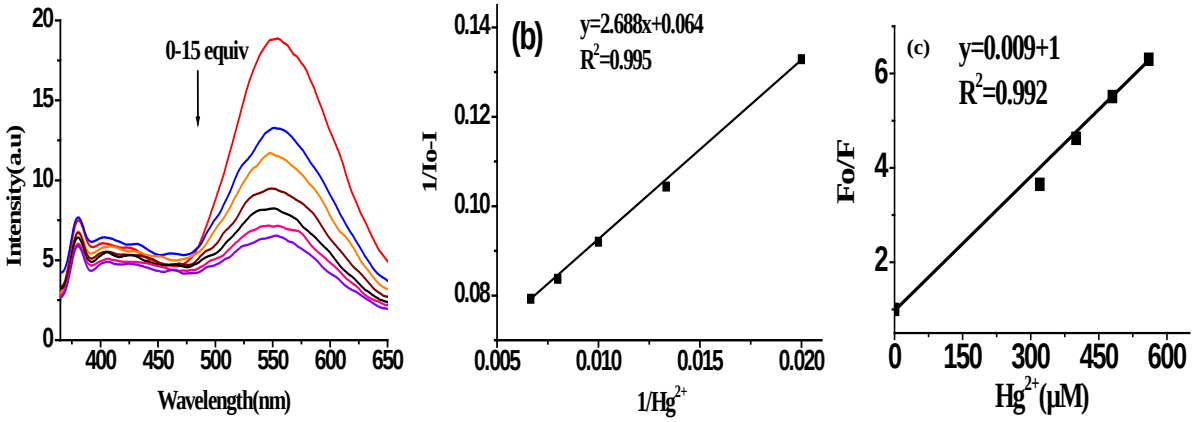


Figure S3: Fluorescence titration spectra of 1 (10 μM) with Hg^{2+} ion (0-15 equiv). (b) B-H plot for 1:1 stoichiometry between 1 and Hg^{2+} ion. (c) *S-V* plot for **1** with Hg^{2+} in AcONa buffer.

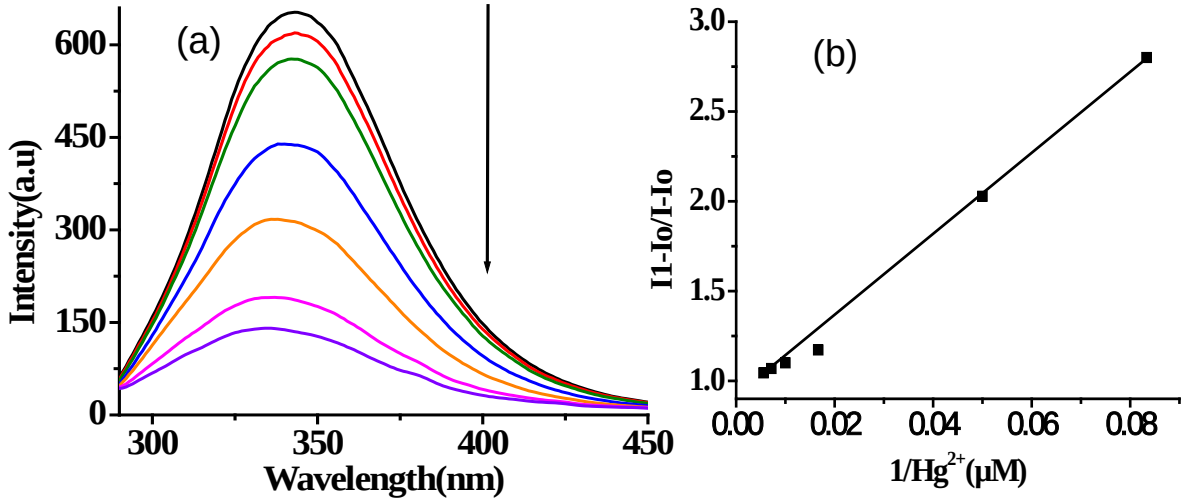


Figure S4: (a) Fluorescence titration spectra of BSA (5 μM) upon interaction with Hg^{2+} ion in AcONa buffer (50 mM; pH=6.7). (b) Benesi-Hildebrand plots of BSA for Hg^{2+} metal ion shows $K_{ass.}$ = 4.34 x 10^3 M.

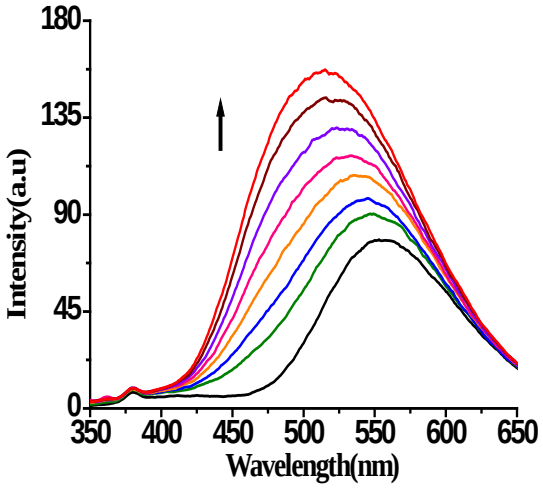


Figure S5: Fluorescence titration spectra of **1** with FBS in AcONa buffer (50 mM; pH = 6.7).

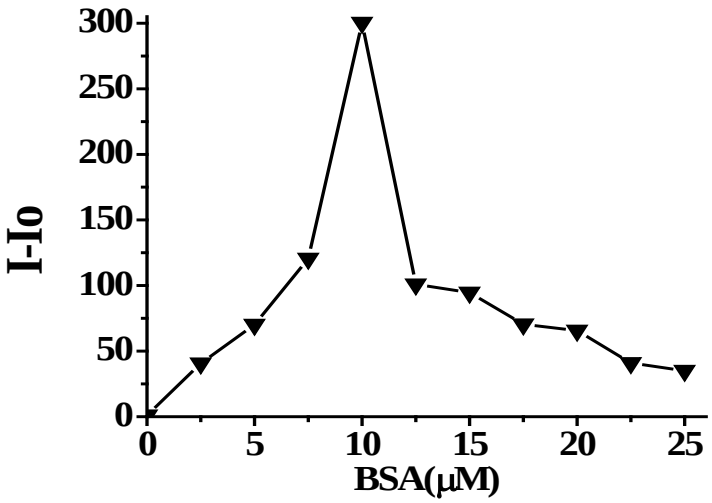


Figure S6: Optimum concentration of BSA for **1** to detect Hg^{2+} ion.

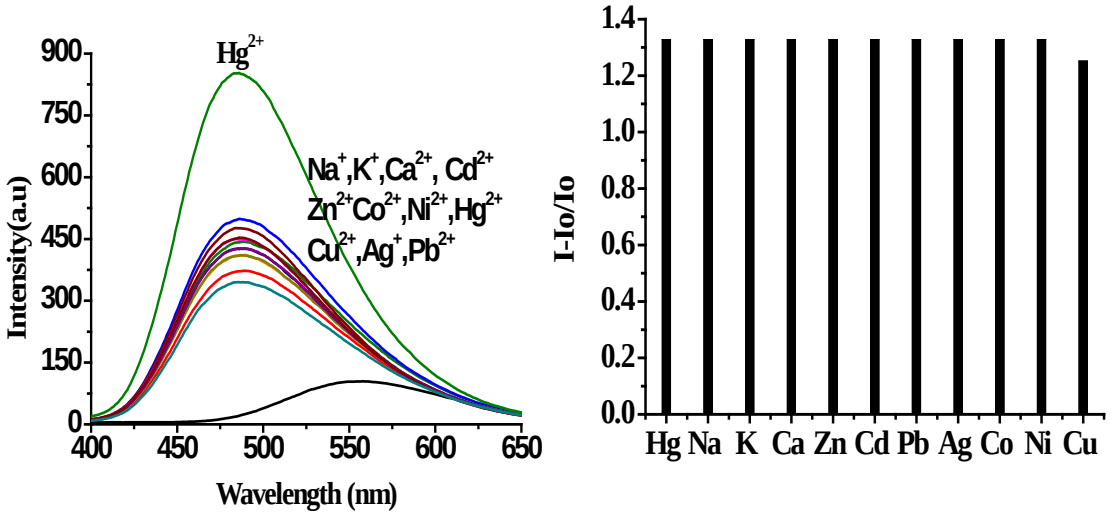


Figure S7: Fluorescence spectra of **1** upon interaction with tested metal ions in BSA and bar diagram shows interference study after addition of tested metal ions to the solution of **1**+BSA+Hg^{2+}.

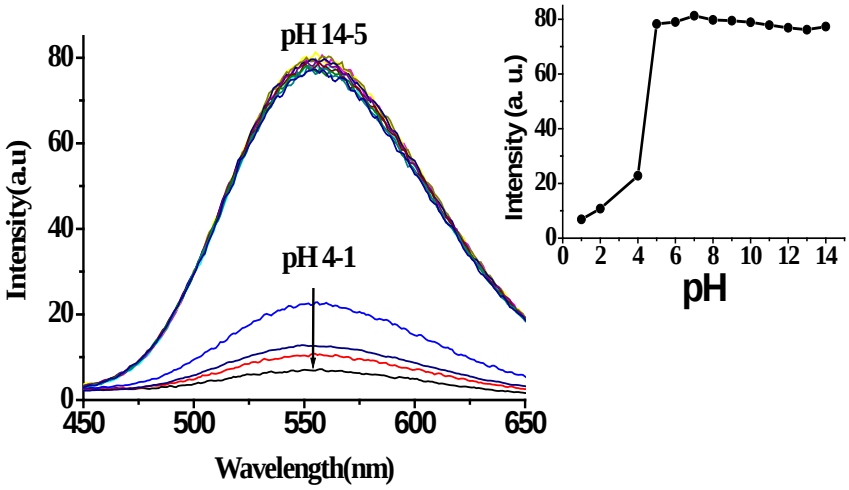


Figure S8: Change in emission spectra at different pHs in AcONa buffer (50mM; pH = 6.7). Inset shows change in intensity *Vs* pH.

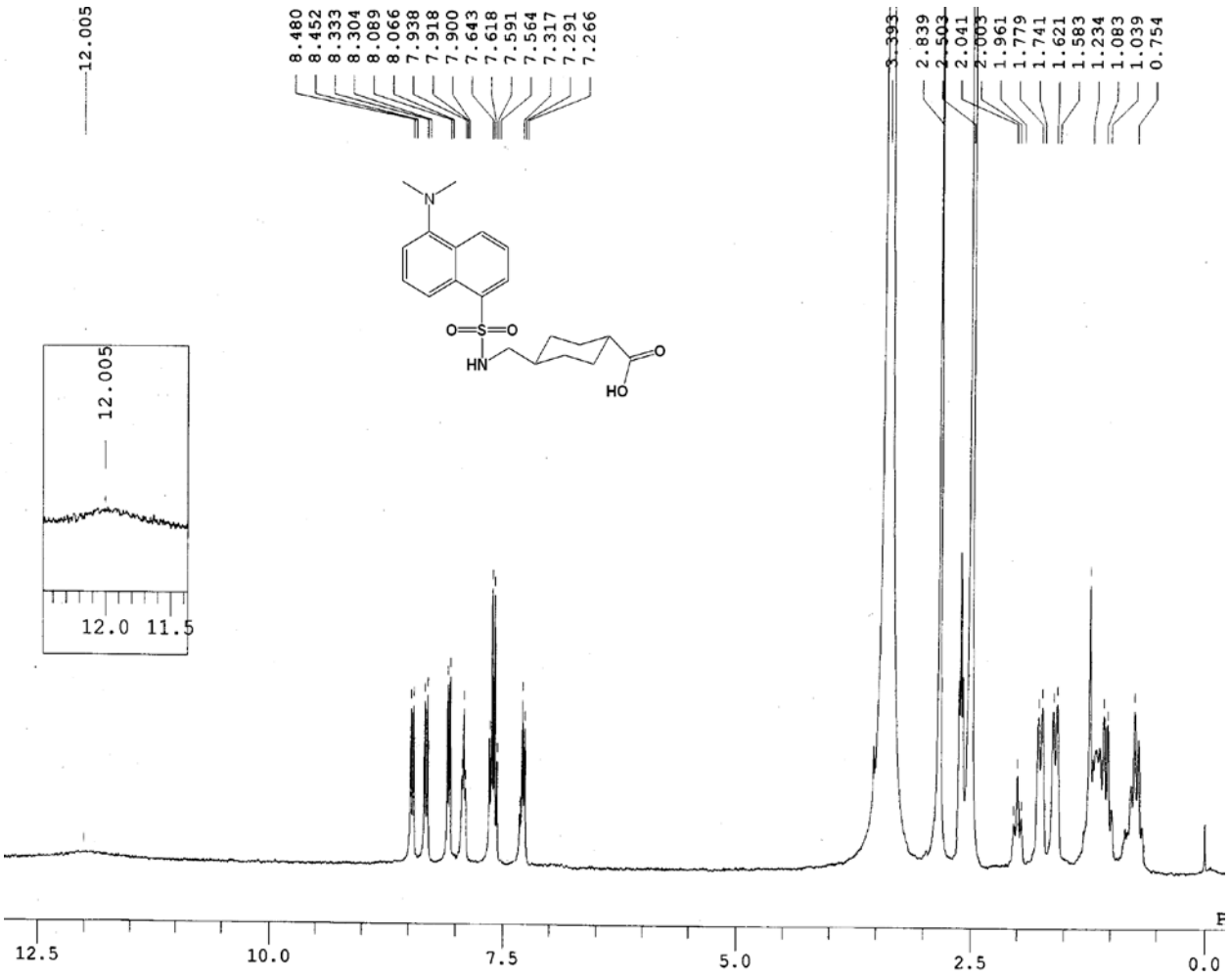


Figure S9: ^{1}H NMR Spectrum of **1** in DMSO-d_6

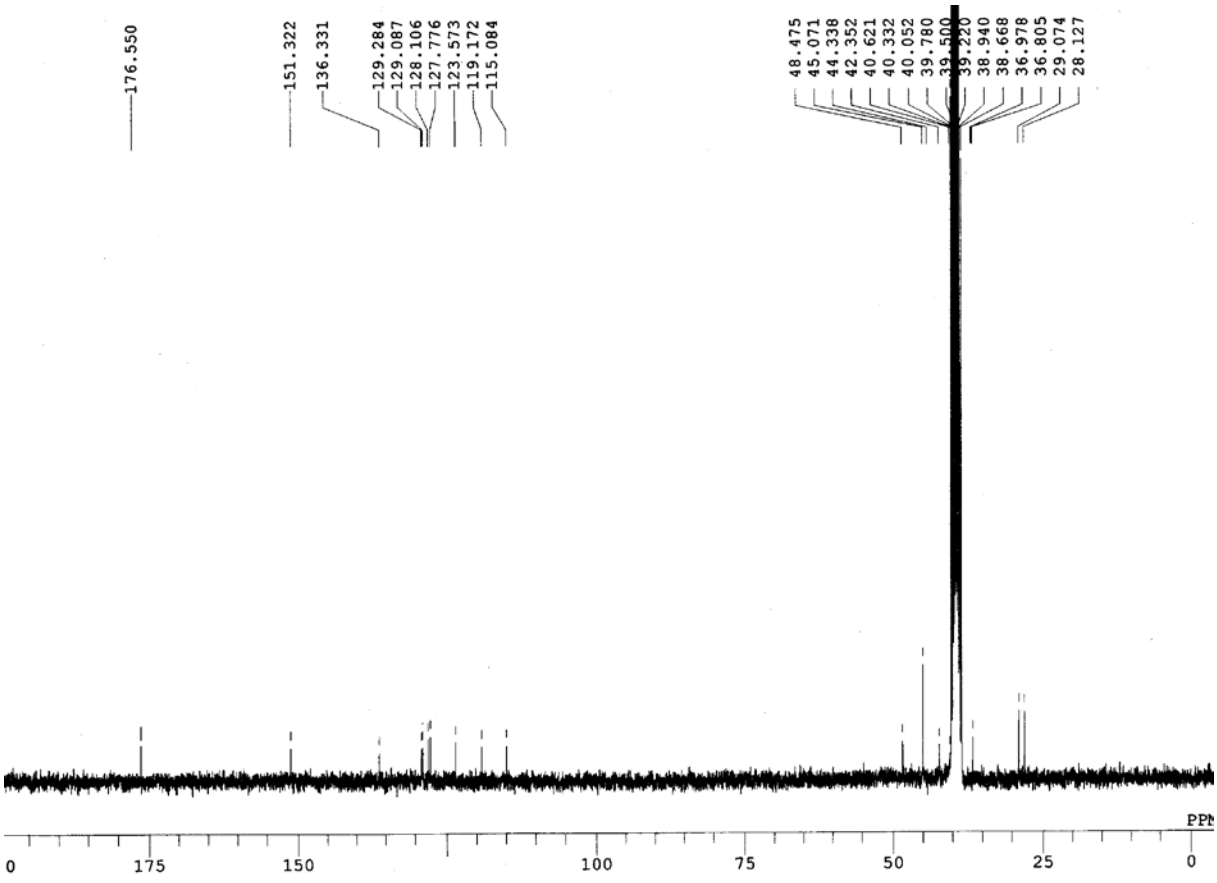


Figure S10: ^{13}C NMR Spectrum of **1**in DMSO-d_6**.**

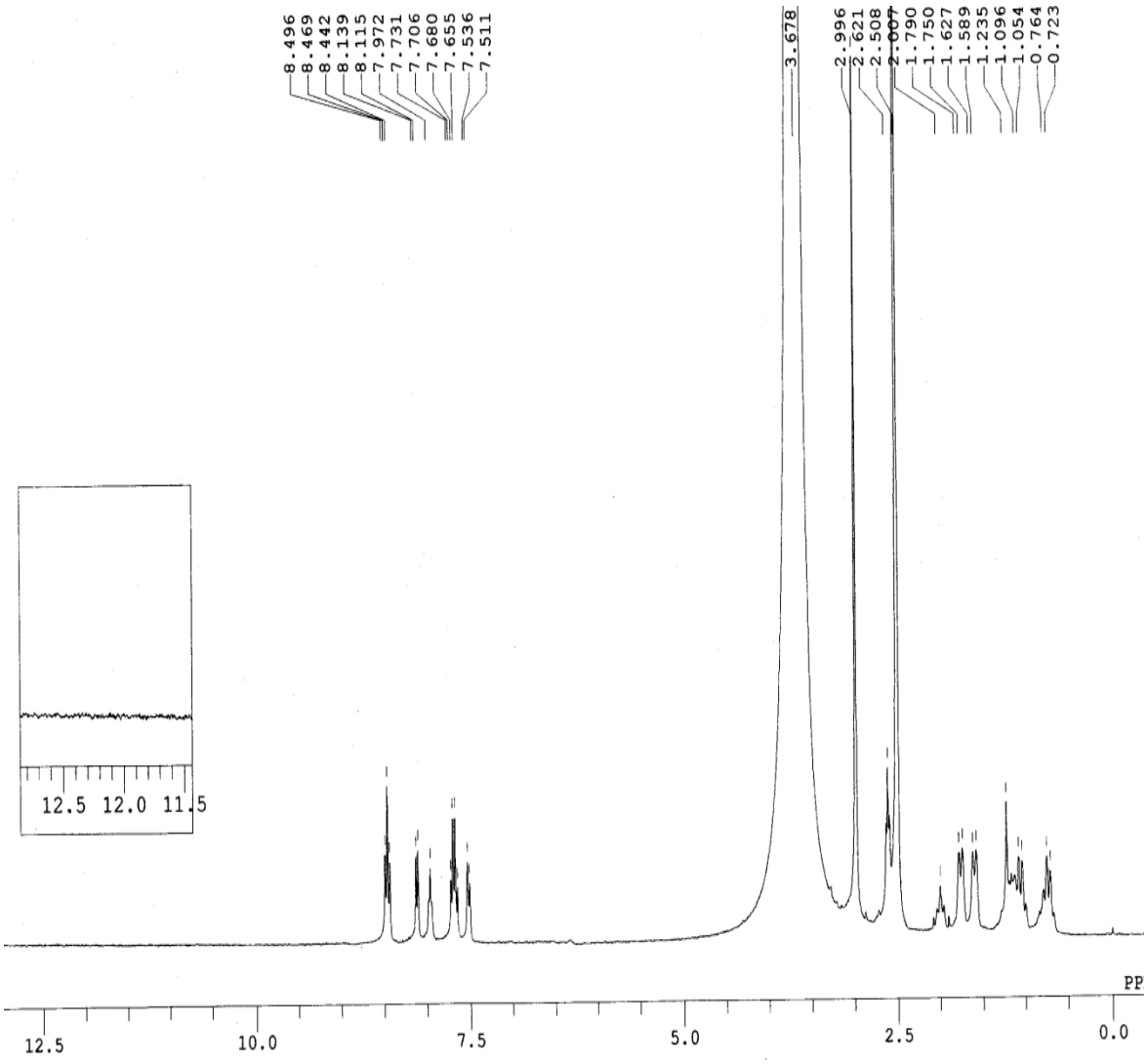


Figure S11: ^{1}H NMR spectrum of **1** in DMSO-d_6 after 0.5 equiv addition of mercury.

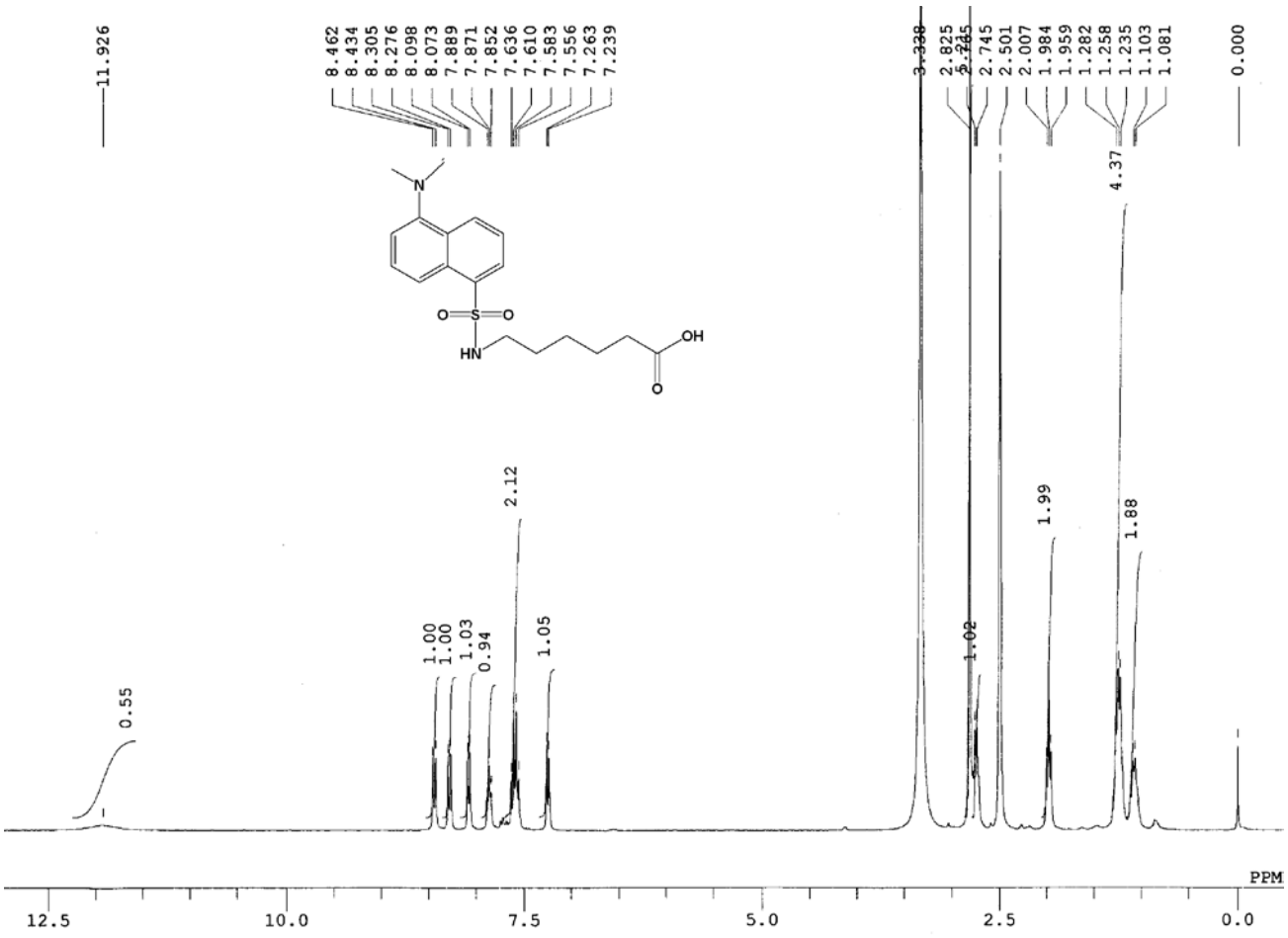


Figure S12: ^{1}H NMR Spectrum of **2** in DMSO-d_6

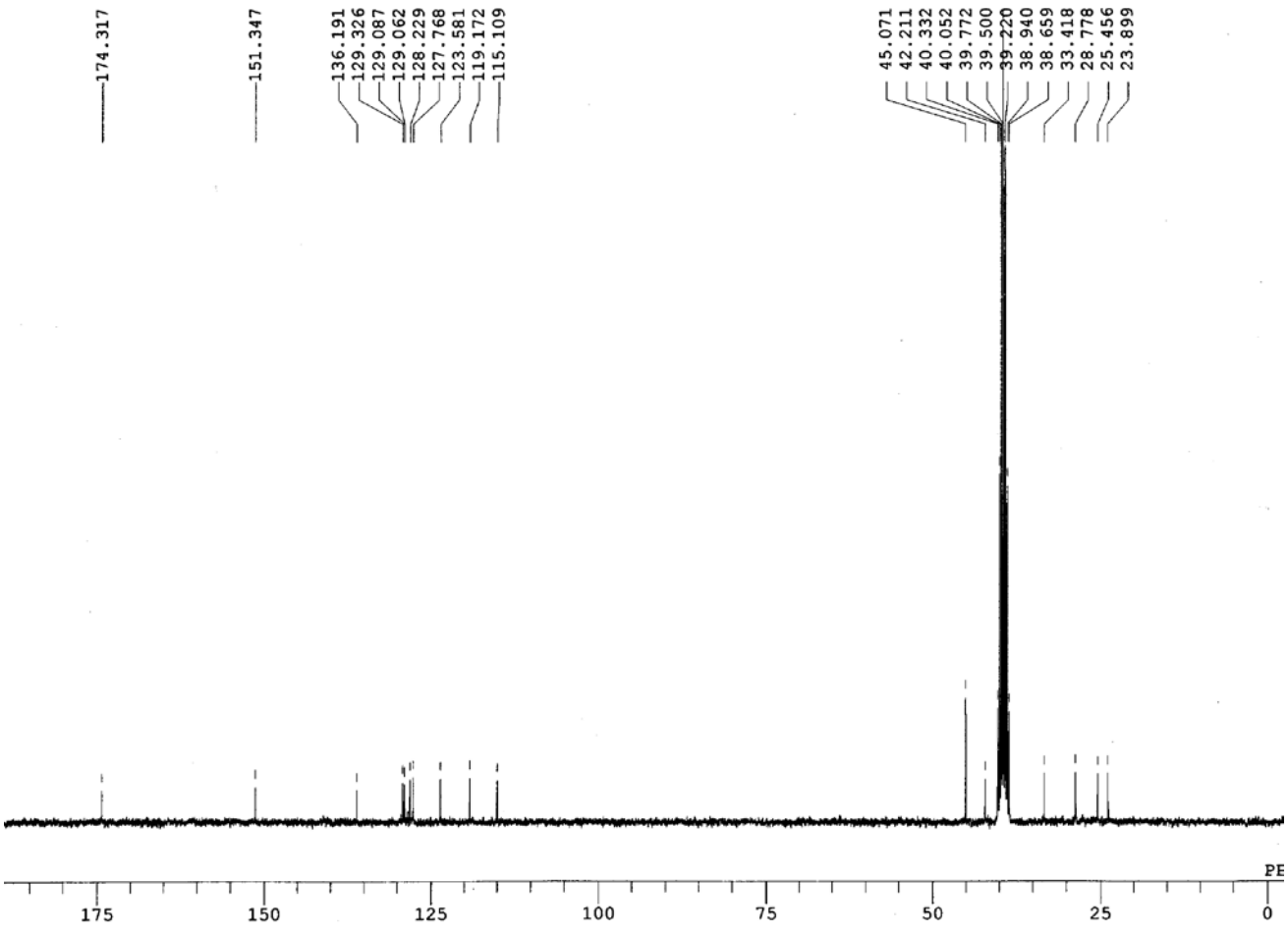


Figure S13: ^{13}C NMR Spectrum of **2** in DMSO-d_6.

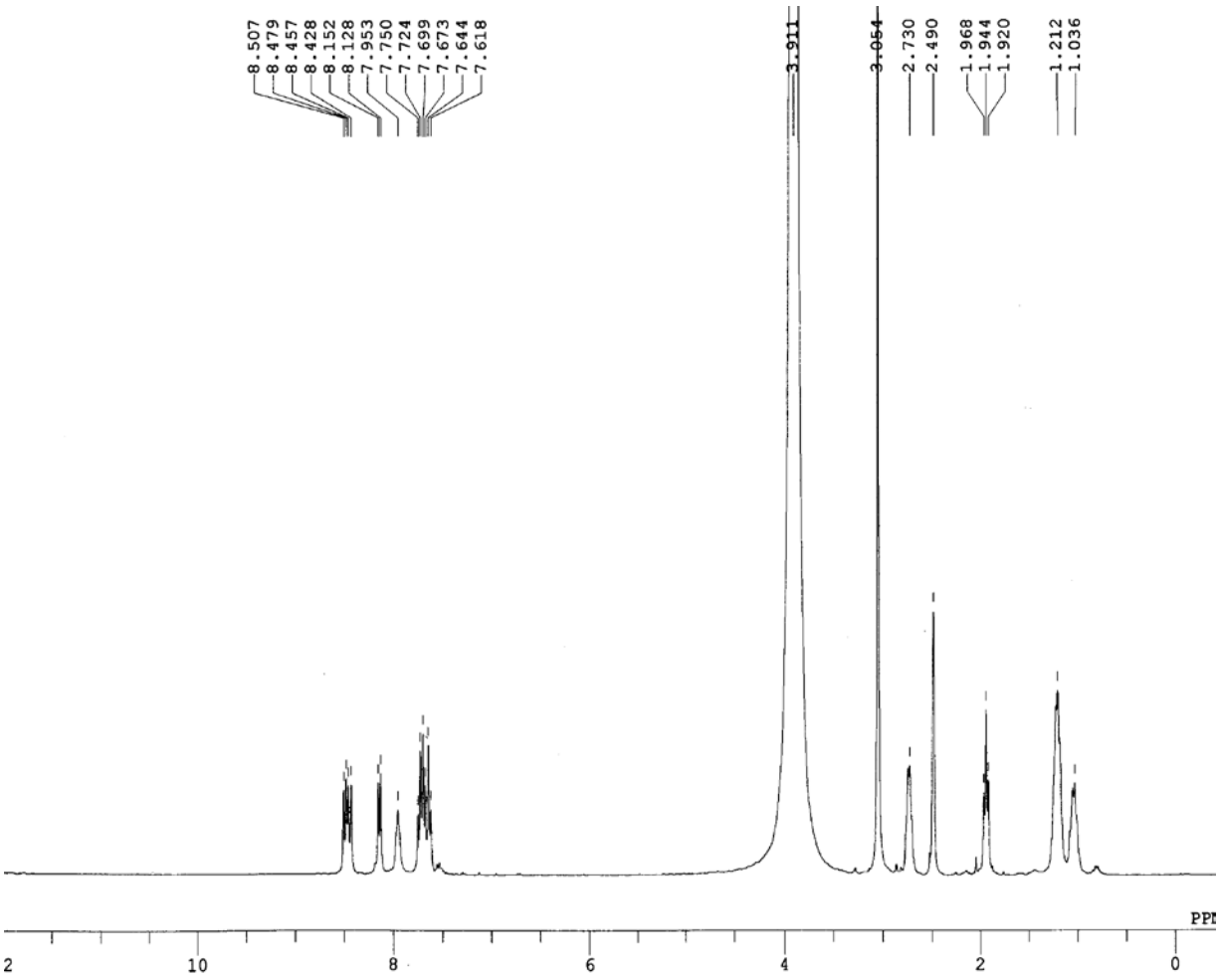


Figure S14: ^{1}H NMR spectrum of **2** in DMSO-d_6 after 2 equiv addition of mercury.

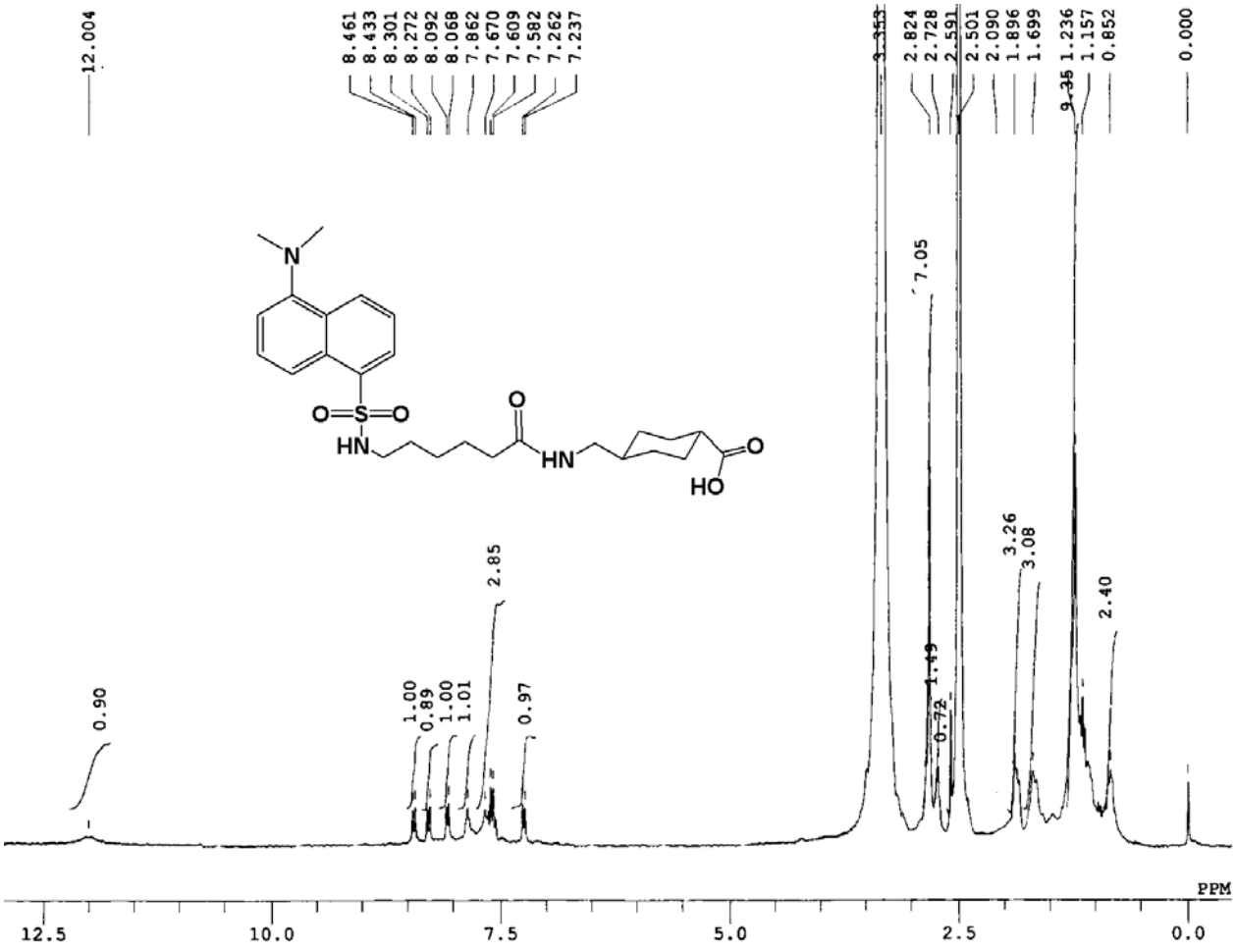


Figure S15:[1] H NMR Spectrum of **3** in DMSO-d_6.

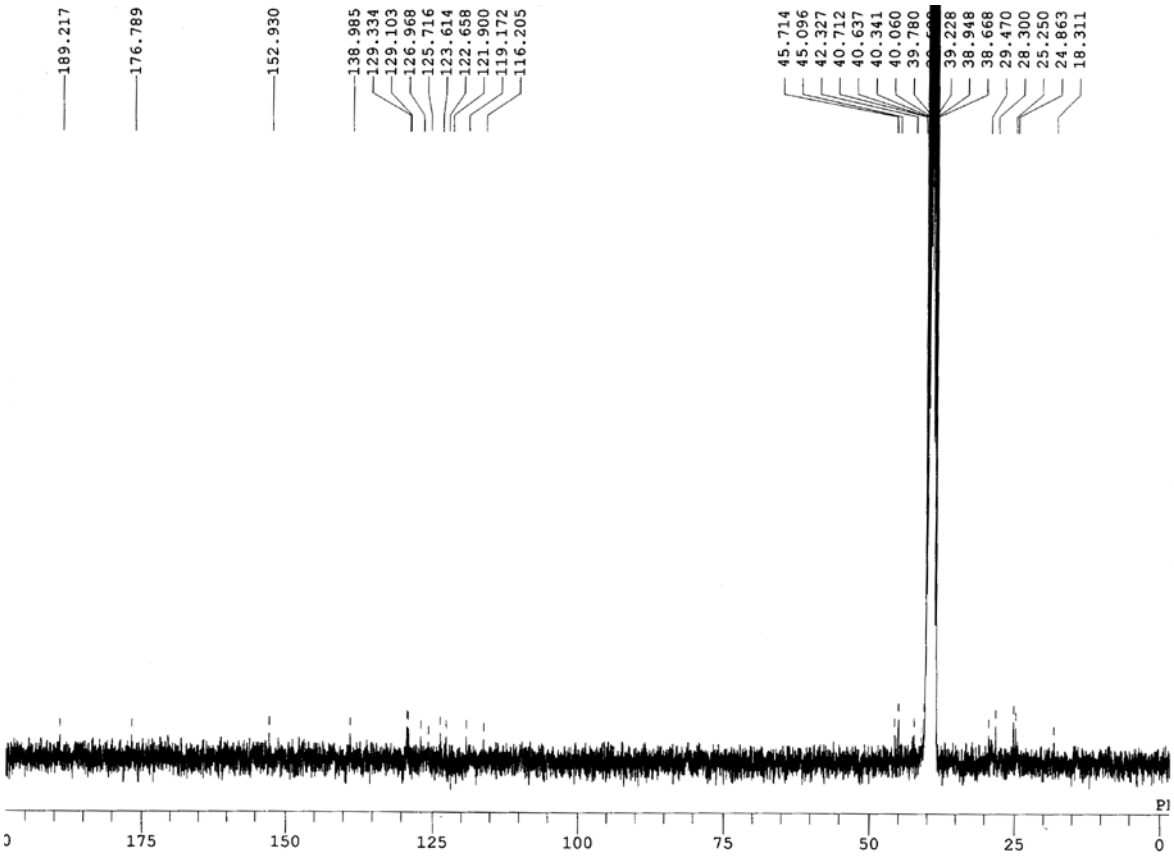


Figure S16: ^{13}C NMR Spectrum of **3** in DMSO-d_6.