Discovery of a new class of 1-(4-sulfamoylbenzoyl)piperidine-4-carboxamides as human Carbonic Anhydrase inhibitors

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Content

S1. Materials and methods

All commercially available solvents and reagents were used without further purification. ^{1}H NMR spectra were recorded using an Inova 500 spectrometer (Varian, Palo Alto, CA, USA). ^{13}C NMR spectra were recorded using a Bruker Advance III HD 600 (Bruker, Bremen, Germany) at 126 MHz. The chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane (TMS), which was used as an internal standard. The spectra were recorded in hexadeuteriodimethylsulphoxide (DMSO-d_6). Infrared spectra were recorded using a Vector 22 spectrometer (Bruker, Bremen, Germany) in Nujol mulls. The main bands are given in cm^{-1}. Positive-ion electrospray ionization (ESI) mass spectra were recorded using a double-focusing MAT 95 instrument (Finnigan, Waltham, MA, USA) with BE geometry. Melting points (mp) were determined with a SMP1 Melting Point apparatus (Stuart Scientific, Stone, UK) and are uncorrected. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. The purity of the tested compounds was determined by combustion elemental analyses conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara with a MT-5 CHN recorder elemental analyzer (Yanagimoto, Kyoto, Japan) and the values found were within 0.4% of theoretical values. All compounds are >95% pure by HPLC analysis. Ethyl 1-(4-sulfamoylbenzoyl)piperidine-4-carboxylate (3) was synthesized as previously reported [1-3].

S2 Procedures for the synthesis of intermediate 4

1-(4-sulfamoylbenzoyl)piperidine-4-carboxylic acid (4) To a solution of ethyl 1-(4-sulfamoylbenzoyl)piperidine-4-carboxylate (4.9 g 15 mmol) in EtOH (50 mL), aqueous 5N solution of sodium hydroxide (NaOH) (25 mL) and water (20 mL) were added. The resulting mixture was stirred at r.t. for 24h, then EtOH was removed under vacuum. The resulting residue was ice added and acidified with 6N aqueous solution of hydrochloric acid until pH 3-4. The formed precipitate was filtered, washed with water, dried and used without further purification. Yield 88%. M.p. 162-163°C. ^{1}H NMR (DMSO-d_6) 1.52 (s, 2H, CH), 1.77 (s, 1H, CH), 1.91 (s, 1H, CH), 2.36 (s, 1H, CH), 2.96 (s, 1H, CH), 3.08 (s, 1H, CH), 3.42 (s, 1H, CH), 4.31 (s, 1H, CH), 7.49 (s, 2H, NH_2), 7.93 (d, *J* = 8.5 Hz, 2H, Ar); 8.09 (d, *J* = 8.5 Hz, 2H, Ar); 13.32 (s, 1H, OH). IR (Nujol) 3359, 3259, 1730, 1687 cm^{-1}. Elemental analysis: calculated for $C_{13}H_{16}N_2O_5S$ (312.34) %C 49.99, %H 5.16, %N 8.97, found %C 50.04, %H 5.15, %N 8.94. m/z 313.

S3 General procedure for the synthesis and characterization data of final compounds 5-24

1-(4-sulfamoylbenzoyl)piperidine-4-carboxylic acid **4** (0.31g, 1 mmol), EDCI (0.19 g, 1 mmol) and HOBt (0.13 g, 1 mmol) were dissolved in anhydrous CH_3CN (5 mL). The resulting mixture was stirred at r.t. for 30 minutes, then the substituted amine was added. The mixture was stirred at r.t. overnight. The solvent was removed under vacuum and the residue was dissolved in ethyl acetate (10 mL) and washed sequentially with water (2 x 15 mL), saturated $NaHCO_3$ aqueous solution (2 x 15 mL), 10% citric acid (2 x 15 mL) and brine (2x10 mL). The organic layer was dried over anhydrous sodium sulfate (Na_2SO_4), filtered and evaporated under reduced pressure. The residues were treated with diisopropyl ether (iPr_2O) and the formed solids were filtered off and recrystallized from EtOH to give the corresponding amides **5-24**.

4-(4-(4-(3-methoxyphenyl)piperazine-1-carbonyl)piperidine-1-carbonyl)benzenesulfonamide 5. Following the general procedure, the title compound was prepared starting from 3-methoxyphenylpiperazine. Yield 39% M.p. 134-135°C. ^{1}H NMR (DMSO-d_6) δ 1.55 (m, 2H, CH); 3.14 (m, 6H, CH); 3.41 (m, 4H, CH); 3.71 (m 8H, CH, OCH_3); 6.47 (m, 3H, Ar); 7.12 (s, 1H, Ar); 7.45 (s, 2H, NH_2); 7.63 (m, 2H, Ar); 7.89 (m 2H, Ar). ^{13}C NMR (DMSO-d_6) 172.6, 168.2, 160.7, 152.6, 145.1, 140.0, 130.2 (2C), 128.1 (2C), 127.7 (2C), 126.4 (2C), 108.9, 105.1, 102.5, 55.4 (2C), 49.4, 48.8, 46.9, 45.1, 37.4. IR (Nujol) 3100, 1612 cm^{-1}. Elemental analysis: calculated for $C_{24}H_{30}N_4O_5S$ (486.58) %C 59.24, %H 6.21, %N 11.51, found %C 59.29, %H 6.20, %N 11.49. m/z 487.

4-(4-(4-(4-methoxyphenyl)piperazine-1-carbonyl)piperidine-1-carbonyl)benzenesulfonamide 6. Following the general procedure, the title compound was prepared starting from 4-methoxyphenylpiperazine. Yield 64% M.p. 139-140 °C. ^{1}H NMR (DMSO-d_6) δ 1.55 (m, 2H, CH); 3.00 (m, 7H, CH); 3.40 (m, 2H, CH); 3.50 (m, 2H, CH); 3.78 (m, 7H, CH, OCH_3); 6.85 (m, 4H, Ar); 7.45 (s, 2H, NH_2); 7.60 (m, 2H, Ar), 7.89 (m, 2H, Ar). ^{13}C NMR (DMSO-d_6) 172.5, 168.2, 153.8, 145.6, 145.1, 139.9, 139.5, 128.0 (2C), 127.7, 126.3, 126.1, 118.8, 118.6, 118.5, 114.8 (2C) 55.7 (2C), 51.0, 50.4, 45.3, 41.7, 37.4. IR (Nujol) 3311, 1614 cm^{-1}. Elemental analysis: calculated for $C_{24}H_{30}N_4O_5S$ (486.58) %C 59.24, %H 6.21, %N 11.51, found %C 59.19, %H 6.23, %N 11.53. m/z 487.

4-(4-(4-(o-tolyl)piperazine-1-carbonyl)piperidine-1-carbonyl)benzenesulfonamide 7. Following the general procedure, the title compound was prepared starting from 3-methylphenylpiperazine. Yield 71% M.p. 134-135 °C. ^{1}H NMR (DMSO-d_6) δ 1.56 (m, 2H, CH), 2.26 (s, 3H, CH_3); 2.84 (m , 7H, CH); 3.01 (m, 1H, CH), 3.15 (m, 1H, CH); 3.43 (m, 2H, CH); 3.61 (m, 4H, CH); 6.96 (m, 2H, Ar), 7.13 (d, *J* = 7.4 Hz, 2H, Ar), 7.45 (s, 2H, NH_2), 7.58 (d, *J* = 8.0 Hz, 2H, Ar), 7.88 (d, *J* = 8.5 Hz, 2H, Ar). ^{13}C NMR (DMSO-d_6) 172.6, 168.2, 151.4, 139.9, 132.4, 131.3 (2C), 129.8 (2C), 128.0, 127.7, 127.1, 126.3, 126.1, 123.7 (2C), 119.5 (2C), 52.4, 51.9, 45.9, 42.1, 37.4, 18.0. IR (Nujol) 3304, 1617 cm^{-1}. Elemental analysis: calculated for $C_{24}H_{30}N_4O_4S$ (470.58) %C 61.26, %H 6.43, %N 11.91, found %C 61.27, %H 6.42, %N 11.92. m/z 471.

4-(4-(4-(2,3-dimethylphenyl)piperazine-1-carbonyl)piperidine-1-carbonyl)benzenesulfonamide 8. Following the general procedure, the title compound was prepared starting from 2,3-dimethylphenylpiperazine. Yield 52% M.p. 137-138 °C. ^{1}H NMR (DMSO-d_6) δ 1.58 (m, 2H, CH), 2.26 (s, 3H, CH_3); 2.29 (s, 3H, CH_3); 2.86 (m , 7H, CH); 3.04 (m, 1H, CH), 3.12 (m, 1H, CH); 3.45 (m, 2H, CH); 3.60 (m, 4H, CH); 6.98 (m, 2H, Ar), 7.15 (d, *J* = 7.4 Hz, 2H, Ar), 7.47 (s, 2H, NH_2), 7.56 (d, *J* = 8.0 Hz, 2H, Ar), 7.89 (m, 1H, Ar). ^{13}C NMR (DMSO-d_6) 175.0, 172.6, 171.7, 168.2, 151.4, 145.1, 139.9 (2C), 137.9 (2C), 131.1 (2C), 129.8 (2C), 126.4, 126.3, 125.4 (2C), 117.2 (2C), 72.9, 43.2, 37.4, 20.7, 14.2. IR (Nujol) 3314, 1620 cm^{-1}. Elemental analysis: calculated for $C_{25}H_{32}N_4O_4S$ (484.61) %C 61.96, %H 6.66, %N 11.56, found %C 61.97, %H 6.65, %N 11.55. m/z 485.

4-(4-(4-(4-fluorophenyl)piperazine-1-carbonyl)piperidine-1-carbonyl)benzenesulfonamide 9. Following the general procedure, the title compound was prepared starting from 4-fluorophenylpiperazine. Yield 54% M.p. 130-132°C. ^{1}H NMR (DMSO-d_6) δ 1.55 (m, 2H, CH); 3.06 (m, 7H, CH); 3.42 (m, 4H, CH); 3.60 (m, 2H, CH); 3.72 (m, 2H, CH); 6.99 (m, 2H, Ar); 7.08 (m, 2H, Ar); 7.47 (s, 2H, NH_2); 7.64 (d, *J* = 7.5 Hz, 2H, Ar), 7.91 (d, *J* = 7.5 Hz, 2H, Ar). ^{13}C NMR (DMSO-d_6) 172.6, 168.2, 157.6, 156.1, 148.1, 145.3, 145.1, 139.9, 139.5, 128.1, 127.7, 126.2,

126.1 118.4, 118.3, 118.2, 115.8, 50.3, 49.7, 47.4, 45.1, 41.2, 37.4. IR (Nujol) 3211, 1621, 1597 cm^{-1}. Elemental analysis: calculated for $C_{23}H_{27}FN_4O_4S$ (474.55) %C 58.21, %H 5.74, %N 11.81, found %C 58.26, %H 5.75, %N 11.78. m/z 475.

4-(4-(4-(4-chlorophenyl)piperazine-1-carbonyl)piperidine-1-carbonyl)benzenesulfonamide 10. Following the general procedure, the title compound was prepared starting from 4- chlorophenylpiperazine. Yield 52% M.p. 142-143 °C. ^{1}H NMR (DMSO-d_6) δ 1.52 (m, 2H, CH); 1.67 (s, 1H, CH); 1.87 (s, 1H, CH); 2.81 (m, 2H, CH); 2.91 (m, 1H, CH); 3.15 (m, 5H, CH); 3.65 (m, 5H, CH); 6.97 (m, 2H, Ar); 7.16 (s, 2H, NH_2); 7.23 (m, 2H, Ar); 7.65 (m, 4H, Ar). ^{13}C NMR (DMSO-d_6) 171.8, 154.8, 150.1, 144.5, 137.0, 129.2 (2C), 126.7 (2C), 123.3 (2C), 119.0 (2C), 117.8 (2C), 49.2, 48.6, 47.2, 45.0, 38.4, 28.2, 24.2. IR (Nujol) 3223, 1622, 1599 cm^{-1}. Elemental analysis: calculated for $C_{23}H_{27}ClN_4O_4S$ (491.00) %C 56.26, %H 5.54, %N 11.41, found %C 56.33, %H 5.55, %N 11.38. m/z 491, 492.

4-(4-(4-benzylpiperazine-1-carbonyl)piperidine-1-carbonyl)benzenesulfonamide 11. Following the general procedure, the title compound was prepared starting from N-benzylpiperazine. Yield 44% M.p. 127-129°C. ^{1}H NMR (DMSO-d_6) δ 1.52 (m, 2H, CH); 2.61 (m, 4H, CH); 2.93 (m, 2H, CH); 3.11 (m, 1H, CH); 3.50 (m, 10H, CH, CH_2); 7.28 (m, 5H, Ar), 7.45 (s, 2H, NH_2), 7.57 (m, 2H, Ar), 7.87 (m, 2H, Ar). ^{13}C NMR (DMSO-d_6) 172.4, 168.2, 145.1, 139.9, 138.3, 129.4 (2C), 128.7 (2C), 127.7 (2C), 127.5 (2C), 126.4 (2C), 126.3, 126.1 (2C), 62.3, 53.6, 52.8, 45.3, 41.6, 37.4. IR (Nujol) 3311, 1614, 1512 cm^{-1}. Elemental analysis: calculated for $C_{24}H_{30}N_4O_4S$ (470.58) %C 61.26, %H 6.43, %N 11.91, found %C 61.30, %H 6.41, %N 11.94. m/z 471.

4-(4-(4-heptylpiperazine-1-carbonyl)piperidine-1-carbonyl)benzenesulfonamide 12. Following the general procedure, the title compound was prepared starting from *n*-heptylpiperazine. Yield 46% M.p. 88-89 °C. ^{1}H NMR (DMSO-d_6) δ 0.85 (m, 3H, CH_3); 1.24 (m, 8H, CH_2); 1.42 (m, 3H, CH); 1.53 (m, 3H, CH); 1.42 (m, 1H, CH); 2.25 (m 7H, CH); 2.92 (m, 2H, CH); 2.97 (m, 1H, CH); 3.45 (m, 4H, CH); 7.45 (s, 2H, NH_2); 7.57 (d, *J* = 8.0 Hz, 2H, Ar), 7.89 (d, J = 8.0 Hz, 2H, Ar). ^{13}C NMR (DMSO-d_6) 172.4, 168.2, 145.1, 139.9, 138.3, 127.9 (2C), 127.7, 126.3 (2C), 58.2, 53.9, 53.0, 45.3, 37.3, 31.7 (2C), 29.1 (2C), 27.4, 26.7, 22.5 (2C), 14.4. IR (Nujol) 3307, 3213, 1632, 1455 cm^{-1}. Elemental analysis: calculated for $C_{24}H_{38}N_4O_4S$ (478.65) %C 60.22, %H 8.00, %N 11.71, found %C 60.16, %H 8.01, %N 11.75. m/z 479.

4-(4-(4-octylpiperazine-1-carbonyl)piperidine-1-carbonyl)benzenesulfonamide 13. Following the general procedure, the title compound was prepared starting from *n*-octylpiperazine. Yield 37% M.p. 77-78°C. ^{1}H NMR (DMSO-d_6) δ 0.85 (m, 3H, CH_3), 1.25 (m, 10H, CH_2); 1.41 (m, 2H, CH); 1.52 (m, 2H, CH); 1.72 (m, 1H, CH); 2.27 (m, 5H, CH); 2.94 (m, 2H, CH), 3.20 (m, 1H, CH); 3.55 (m, 8H, CH); 7.45 (s, 2H, NH_2), 7.58 (d, *J* = 7.5 Hz, 2H, Ar), 7.89 (d, *J* = 8.0 Hz, 2H, Ar). ^{13}C NMR (DMSO-d_6) 172.4, 168.2, 145.1, 139.9, 127.9 (2C), 127.7, 126.3 (2C), 58.2, 53.9, 53.0, 46.9, 45.3, 41.6, 37.3, 31.7 (2C), 29.4, 29.1 (2C), 27.4, 26.6, 22.5, 14.4. IR (Nujol) 3198, 1614 cm^{-1}. Elemental analysis: calculated for $C_{25}H_{40}N_4O_4S$ (492.67) %C 60.95, %H 8.18, %N 11.37, found %C 60.87, %H 8.20, %N 11.34. m/z 493.

N-benzyl-1-(4-sulfamoylbenzoyl)piperidine-4-carboxamide 14. Following the general procedure, the title compound was prepared starting from N-benzylamine. Yield 90% M.p. 168-170°C. ^{1}H NMR (DMSO-d_6) δ 1.40 (m, 1H, CH); 1.66 (m, 2H, CH); 1.90 (m, 1H, CH); 2.41 (m, 1H, CH); 2.79 (t, *J* = 11.5 Hz, 1H, CH); 2.93 (t, *J* = 12.0 Hz, 1H, CH); 4.10 (m, 1H, CH); 4.20 (m, 1H, CH); 4.28 (m, 2H, CH_2); 7.16 (m, 2H, Ar); 7.26 (m, 3H, Ar NH_2); 7.31 (m, 2H, Ar); 7.66 (m, 4H, Ar); 9.05 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) 173.3, 154.8, 144.5, 140.0, 136.9 (2C), 128.7 (2C), 127.6 (2C), 127.2, 126.7 (2C), 119.0, 47.2, 44.6, 42.7, 42.3, 28.2, 24.8. IR (Nujol) 3351, 3220, 1644 cm^{-1}. Elemental analysis: calculated for $C_{20}H_{23}N_3O_4S$ (401.48) %C 59.83, %H 5.77, %N 10.47, found %C 59.87, %H 5.76, %N 10.44. m/z 402.

N-(2-methylbenzyl)-1-(4-sulfamoylbenzoyl)piperidine-4-carboxamide 15. Following the general procedure, the title compound was prepared starting from 2-methylbenzylamine. Yield 58% M.p. 219-220 °C. ^{1}H NMR (DMSO-d_6) δ 1.54 (m, 2H, CH); 2.31 (m, 4H, CH CH_3); 3.07 (m, 1H, CH); 3.51 (m, 1H, CH); 4.25 (m, 1H, CH); 4.48

(m, 2H, CH_2); 7.16 (m, 4H, Ar); 7.49 (m, 3H, Ar NH_2); 7.92 (m, 2H, Ar); 8.06 (m, 2H, Ar); 9.10 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) 174.1, 168.3, 165.7, 146.8, 145.1, 139.9, 137.7, 137.3, 136.1, 130.4, 128.5, 127.9, 127.7, 127.4, 126.4, 126.2, 126.1, 42.1, 41.4, 19.2, 19.1. IR (Nujol) 3314, 3232, 3103, 1631, 1542 cm^{-1}. Elemental analysis: calculated for $C_{21}H_{25}N_3O_4S$ (415.51) %C 60.70, %H 6.06, %N 10.11, found %C 60.78, %H 6.04, %N 10.07. m/z 416.

N-(4-methylbenzyl)-1-(4-sulfamoylbenzoyl)piperidine-4-carboxamide 16. Following the general procedure, the title compound was prepared starting from 4-methylbenzylamine. Yield 54% M.p. 140-142°C. ^{1}H NMR (DMSO-d_6) δ 1.85 (m, 3H, CH); 2.28 (s, 3H, CH_3); 3.02 (m, 2H, CH); 3.51 (m, 3H, CH); 4.24 (m, 1H, CH); 4.46 (s, 2H, CH_2); 7.14 (m, 3H, Ar), 7.23 (s, 2H, NH_2), 7.93 (m, 2H, Ar), 8.05 (m, 2H, Ar), 9.18 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) 175.9, 174.1, 168.3, 165.6, 146.8, 145.1, 139.9, 137.8, 137.0, 136.7, 136.4, 130.4, 129.7, 129.3, 128.4, 127.5, 126.4, 126.1, 43.0, 42.1, 21.1. IR (Nujol) 3389, 1630, 1548 cm^{-1}. Elemental analysis: calculated for $C_{21}H_{25}N_3O_4S$ (415.51) %C 60.70, %H 6.06, %N 10.11, found %C 60.65, %H 6.05, %N 10.14. m/z 416.

N-(3,5-dimethylbenzyl)-1-(4-sulfamoylbenzoyl)piperidine-4-carboxamide 17. Following the general procedure, the title compound was prepared starting from 3,5-dimethylbenzylamine. Yield 86% M.p. 208-210 °C. ^{1}H NMR (DMSO-d_6) δ 1.60 (m, 3H, CH); 1.84 (m, 1H, CH); 2.24 (s, 6H, CH_3); 2.85 (m, 1H, CH); 3.07 (m, 1H, CH); 3.51 (m, 1H, CH); 4.20 (m, 2H, CH_2); 4.45 (m, 2H, CH); 6.86 (m, 3H, Ar); 7.46 (m, 2H, Ar); 7.58 (s, 2H, NH_2), 7.90 (m, 2H, Ar), 8.29 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) 174.1, 168.2, 145.1, 140.0, 137.7 (2C), 128.7, 128.6, 128.4 (2C), 127.7 (2C), 126.4, 126.2 (2C), 125.6, 125.4, 42.4, 42.1, 29.3, 28.7, 21.4. IR (Nujol) 3292, 1633 cm^{-1}. Elemental analysis: calculated for $C_{22}H_{27}N_3O_4S$ (429.53) %C 61.52, %H 6.34, %N 9.78, found %C 61.47, %H 6.33, %N 9.82. m/z 430.

N-(2-methoxybenzyl)-1-(4-sulfamoylbenzoyl)piperidine-4-carboxamide 18. Following the general procedure, the title compound was prepared starting from 2-methoxybenzylamine. Yield 55% M.p. 152-153°C. ^{1}H NMR (DMSO-d_6) δ 1.86 (m, 3H, CH); 2.98 (m, 2H, CH); 3.40 (m, 3H, CH); 3.83 (m, 4H, CH OCH_3); 4.46 (m, 2H, CH_2); 6.92 (m, 1H, Ar), 7.01 (m, 1H, Ar), 7.22 (m, 2H, Ar), 7.49 (s, 2H, NH_2), 7.94 (d, *J* = 8.0 Hz, 2H, Ar), 8.06 (d, *J* = 8.5 Hz, 2H, Ar), 9.03 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) 174.3, 168.2, 165.9, 157.1, 146.8, 145.1, 140.0, 137.8, 129.8, 128.4, 127.9, 127.8, 127.7, 127.0, 126.4, 126.1. 120.6, 55.8, 42.1, 38.3, 37.5. IR (Nujol) 3318, 3196, 3097, 1619, 1562 cm^{-1}. Elemental analysis: calculated for $C_{21}H_{25}N_3O_5S$ (431.51) %C 58.45, %H 5.84, %N 9.74, found %C 58.50, %H 5.86, %N 9.71. m/z 432.

N-(2-chlorobenzyl)-1-(4-sulfamoylbenzoyl)piperidine-4-carboxamide 19. Following the general procedure, the title compound was prepared starting from 2-chlorobenzylamine. Yield 51% M.p. 160-161°C. ^{1}H NMR (DMSO-d_6) δ 1.81 (m, 3H, CH); 2.98 (m, 2H, CH); 3.51 (m, 3H, CH); 4.24 (m, 1H, CH); 4.58 (m, 2H, CH_2); 7.33 (m, 2H, Ar); 7.40 (d, *J* = 6.5 Hz, 1H, Ar); 7.43 (d, *J* = 6.0 Hz, 1H, Ar); 7.50 (s, 2H, NH_2); 7.94 (d, *J* = 7.5 Hz, 2H, Ar), 8.09 (d, *J* = 7.5 Hz, 2H, Ar), 9.24 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) 174.4, 168.2, 165.9, 146.9, 145.1, 140.0, 137.5, 136.8, 136.5, 132.5, 129.8, 129.3, 129.0, 128.5, 127.7, 126.4, 126.1, 42.1, 41.2, 21.4. IR (Nujol) 3314, 3211, 3069, 1632 cm^{-1}. Elemental analysis: calculated for $C_{20}H_{22}ClN_3O_4S$ (435.92) %C 55.10, %H 5.09, %N 9.64, found %C 55.14, %H 5.08, %N 9.60. m/z 436.

N-(4-chlorobenzyl)-1-(4-sulfamoylbenzoyl)piperidine-4-carboxamide 20. Following the general procedure, the title compound was prepared starting from 4-chlorobenzylamine. Yield 46% M.p. 198-200 °C. ^{1}H NMR (DMSO-d_6) δ 1.41 (m, 1H, CH); 1.66 (m, 2H, CH); 1.92 (m, 1H, CH); 2.37 (m, 1H, CH), 2.82 (t, *J* = 12.0, 1H, CH); 2.94 (t, , *J* = 12.5, 1H, CH); 4.07 (m, 1H, CH); 4.15 (m, 1H, CH); 4.26 (m, 2H, CH_2); 7.15 (s, 2H, NH_2); 7.27 (d, *J* = 7.5 Hz, 2H, Ar); 7.38 (d, *J* = 8.0 Hz, 2H, Ar); 7.61 (d, *J* = 8.5 Hz, 2H, Ar), 7.68 (d, *J* = 8.0 Hz, 2H, Ar), 8.89 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) 173.4, 154.8, 144.4, 139.1 (2C), 137.0 (2C), 131.7, 129.5 (2C), 128.7 (2C), 126.8, 119.0, 46.9, 44.6, 42.7, 41.7, 28.4, 24.8. IR (Nujol) 3320, 3223, 1626, 1543 cm^{-1}. Elemental analysis: calculated for $C_{20}H_{22}ClN_3O_4S$ (435.92) %C 55.10, %H 5.09, %N 9.64, found %C 55.16, %H 5.10, %N 9.60. m/z 436.

N-(2,4-dichlorobenzyl)-1-(4-sulfamoylbenzoyl)piperidine-4-carboxamide 21. Following the general procedure, the title compound was prepared starting from 2,4-dichlorobenzylamine. Yield 57% M.p. 123-124°C. ^{1}H NMR (DMSO-d_6) δ 1.42 (m, 1H, CH); 1.65 (m, 2H, CH); 1.90 (m, 1H, CH); 2.36 (m, 1H, CH), 2.84 (t, *J* = 12.0, 1H, CH); 2.92 (t, , *J* = 12.5, 1H, CH); 4.04 (m, 1H, CH); 4.13 (m, 1H, CH); 4.29 (m, 2H, CH_2); 7.16 (m, 3H, Ar NH_2); 7.56 (m, 2H, Ar); 7.68 (m, 4H, Ar); 8.50 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) 175.1, 173.6, 154.8, 144.4, 143.3, 136.0, 130.6, 129.1, 128.3, 127.8, 127.5, 126.8, 124.8, 119.5, 119.0, 110.2, 46.8, 44.6, 27.5, 24.8. IR (Nujol) 3185, 1614 cm^{-1}. Elemental analysis: calculated for $C_{20}H_{21}Cl_2N_3O_4S$ (470.37) %C 51.07, %H 4.50, %N 8.93, found %C 51.12, %H 4.49, %N 8.89. m/z 471.

N-(3,4-dichlorobenzyl)-1-(4-sulfamoylbenzoyl)piperidine-4-carboxamide 22. Following the general procedure, the title compound was prepared starting from 3,4-dichlorobenzylamine. Yield 55% M.p. 136-137°C. ^{1}H NMR (DMSO-d_6) δ 1.81 (m, 3H, CH); 2.98 (m, 2H, CH); 3.50 (m, 3H, CH); 4.24 (m, 1H, CH); 4.49 (m, 2H, CH_2); 7.55 (m, 5H, Ar NH_2); 7.94 (m, 2H, Ar); 8.05 (m, 2H, Ar); 9.27 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) 175.2, 173.4, 154.6, 144.1, 143.4, 136.5, 131.0, 130.2, 129.7, 129.5, 128.5, 127.2, 127.9, 126.4, 124.2, 119.5, 119.0, 42.3, 42.1, 41.4. IR (Nujol) 3333, 3213, 3105, 1630, 1550 cm^{-1}. Elemental analysis: calculated for $C_{20}H_{21}Cl_2N_3O_4S$ (470.37) %C 51.07, %H 4.50, %N 8.93, found %C 51.02, %H 4.51, %N 8.96. m/z 471.

N-(3,5-dichlorobenzyl)-1-(4-sulfamoylbenzoyl)piperidine-4-carboxamide 23. Following the general procedure, the title compound was prepared starting from 2,5-dichlorobenzylamine. Yield 34% M.p. 185-187°C. ^{1}H NMR (DMSO-d_6) δ 1.53 (m, 2H, CH); 1.68 (m, 1H, CH); 1.88 (m, 1H, CH); 2.80 (m, 2H, CH), 2.91 (t, *J* = 11.0, 1H, CH); 3.67 (m, 2H, CH); 4.13 (m, 2H, CH_2); 6.95 (dd, *J* = 8.5 2.5 Hz, 1H, Ar); 7.15 (m, 3H, Ar NH_2); 7.41 (d, *J* = 9.0 Hz, 1H, Ar); 7.63 (d, *J* = 9.5 Hz, 2H, Ar); 7.68 (d, *J* = 9.5 Hz, 2H, Ar); 8.87 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) 171.8, 154.8, 144.4, 137.0, 132.0 (2C), 131.0 (2C), 126.8, 120.5 (2C), 119.0, 117.1, 116.11, 48.6, 47.9, 47.1, 38.4, 28.2, 24.8. IR (Nujol) 3331, 3290, 1620, 1543 cm^{-1}. Elemental analysis: calculated for $C_{20}H_{21}Cl_2N_3O_4S$ (470.37) %C 51.07, %H 4.50, %N 8.93, found %C 51.13, %H 4.51, %N 8.88. m/z 471.

N-(4-fluorobenzyl)-1-(4-sulfamoylbenzoyl)piperidine-4-carboxamide 24. Following the general procedure, the title compound was prepared starting from 4-fluorobenzylamine. Yield 67% M.p. 204-205 °C. ^{1}H NMR (DMSO-d_6) δ 1.11 (m, 3H, CH); 3.08 (m, 2H, CH); 3.41 (m, 3H, CH); 4.24 (m, 1H, CH); 4.48 (m, 2H, CH_2); 7.16 (m, 2H, NH_2); 7.37 (m, 2H, Ar); 7.48 (m, 2H, Ar); 7.91 (d, *J* = 8.5 Hz, 2H, Ar), 8.04 (d, *J* = 8.0 Hz, 2H, Ar), 9.23 (m, 1H, NH). ^{13}C NMR (DMSO-d_6) 174.2, 168.2, 165.7, 162.5, 160.9, 146.8, 145.1, 140.0, 137.6, 136.3, 136.0, 135.9, 129.7, 128.4, 126.2, 115.6, 115.4, 42.6, 42.1, 41.7. IR (Nujol) 3349, 3187, 1096, 1644, 1549 cm^{-1}. Elemental analysis: calculated for $C_{20}H_{22}FN_3O_4S$ (419.47) %C 57.27, %H 5.29, %N 10.02, found %C 57.21, %H 5.30, %N 10.06. m/z 420.

S4 Carbonic Anhydrase inhibition assay protocol

An Applied Photophysics stopped-flow instrument was used for assaying the CA-catalyzed CO_2 hydration activity[4]. Phenol red (at a concentration of 0.2 mM) was used as an indicator, working at the absorbance maximum of 557 nm, with 20 mM HEPES (pH 7.5) as the buffer, and 20 mM Na_2SO_4 (for maintaining a constant ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction were used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in 10% DMSO aqueous solution and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to the assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier[5-7] and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier. Their concentrations in the assay system were 5.7–11.9 nM[8-10].

S5 Computational studies

Molecular docking

The crystal structures of hCA I (PDB ID 3W6H)[11], hCA II (PDB ID 3HS4)[12], hCA IX (PDB ID 3IAI)[13] and hCA XII (PDB ID 1JD0)[14] in complex with AAZ were retrieved from the RCSB Protein Data Bank and used for the following computational studies. The protocols employed for protein and ligands preparation were described in our previous paper[1]. Molecular docking was carried out by means of GOLD V 5.8.1[15]. The binding site was defined in order to include all the residues within 10 Å from the native ligand. A scaffold constraint was applied to the sulfonamide moiety to restrict the docking solutions to those in which this group assumes a binding orientation similar to that observed for the co-crystallized ligand AAZ in the crystal structure. The option "never dock a ligand when a constraint is physically impossible" was checked. Ten poses were generated for each ligand and ChemPLP was chosen as scoring function for ligands ranking. The top ranked docking pose was selected as initial conformation for molecular dynamics (MD) simulations.

Molecular dynamics simulations

MD simulations were performed by means of AMBER 18[16]. The General Amber force field (GAFF)[17] was used for ligands parametrization while the charges were assigned by using the AM1-BCC method as implemented in Antechamber[18]. The ff14SB force field[19] was used for protein parametrization. The dummy atom model for tetrahedral Zn^{2+} ion developed by Pang[20] was adopted to model the metal centre of hCAs active site which is composed of a Zn^{2+} ion tetrahedrally coordinated by three histidine residues with the fourth site occupied in this case by the deprotonated nitrogen of the sulfonamide moiety of the inhibitor. The systems were first solvated in a box of TIP3P water molecules placed 10 Å away from the complex, and then neutralised adding a proper number of Na^+ e Cl^- ions to reproduce the physiological salt concentration of 0.15 M. The so prepared systems underwent three steps of energy minimization involving first the hydrogen atoms, then the water molecules and finally the residues side chains. 20 ps of heating phase was carried out gradually increasing the temperature to 300 K exploiting the Langevin thermostat and applying positional restraints (5 kcal/mol) to the Cα and the Zn^{2+} ion. Two equilibration steps were executed using first the NVT ensemble for 50 ps, retaining the positional restraints on the Cα and the Zn^{2+} ion, and then for 70 ps employing the NPT ensemble keeping the pressure around 1 atm by means of the Berendsen barostat and gradually reducing the weight of the restraints. Finally, a production run of 200 ns was performed at constant pressure without any restraint. All the bonds involving hydrogen atoms were restrained by the SHAKE algorithm allowing the use of a timestep of 2 fs. Electrostatic interactions were computed by particle-mesh Ewald (PME) method and periodic boundary conditions were applied. Trajectories analysis was carried out by means of the Cpptraj module[21] of AmberTools 18. Cluster analysis was performed by using the average linkage hierarchical agglomerative method implemented in Cpptraj setting a minimum distance between the clusters greater than 2.

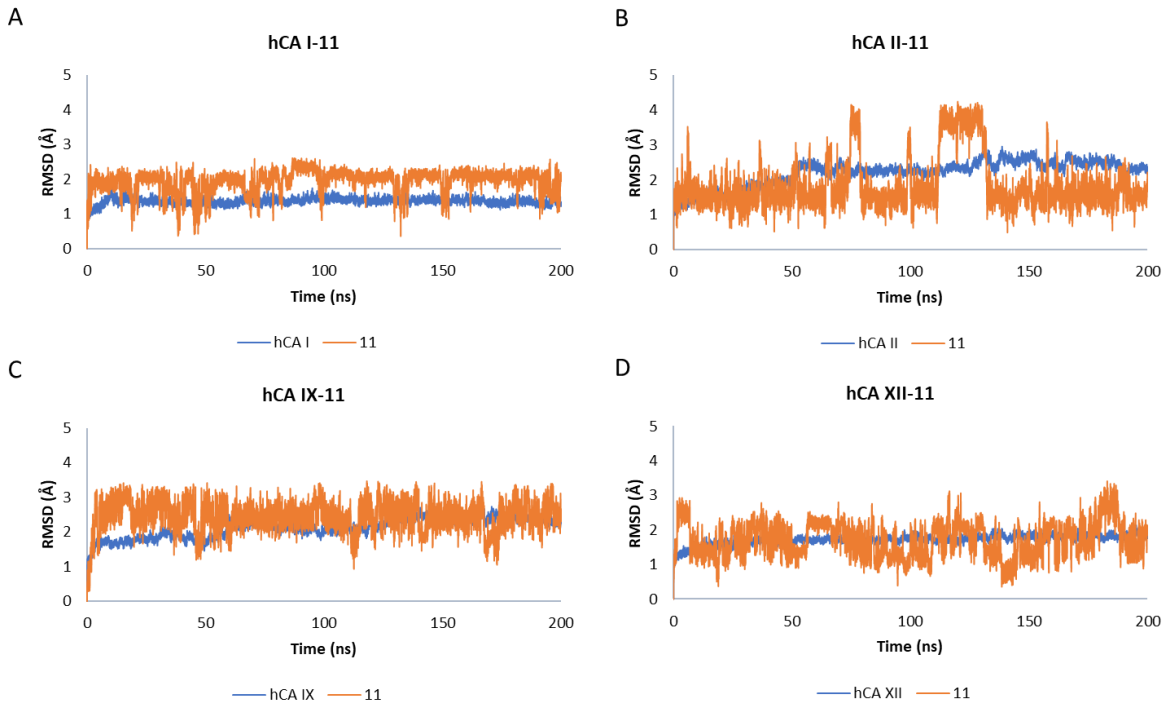


Figure S1. RMSD profiles obtained from the MD simulation of compound **11** in complex with A) hCA I, B) hCA II, C) hCA IX and D) hCA XII.

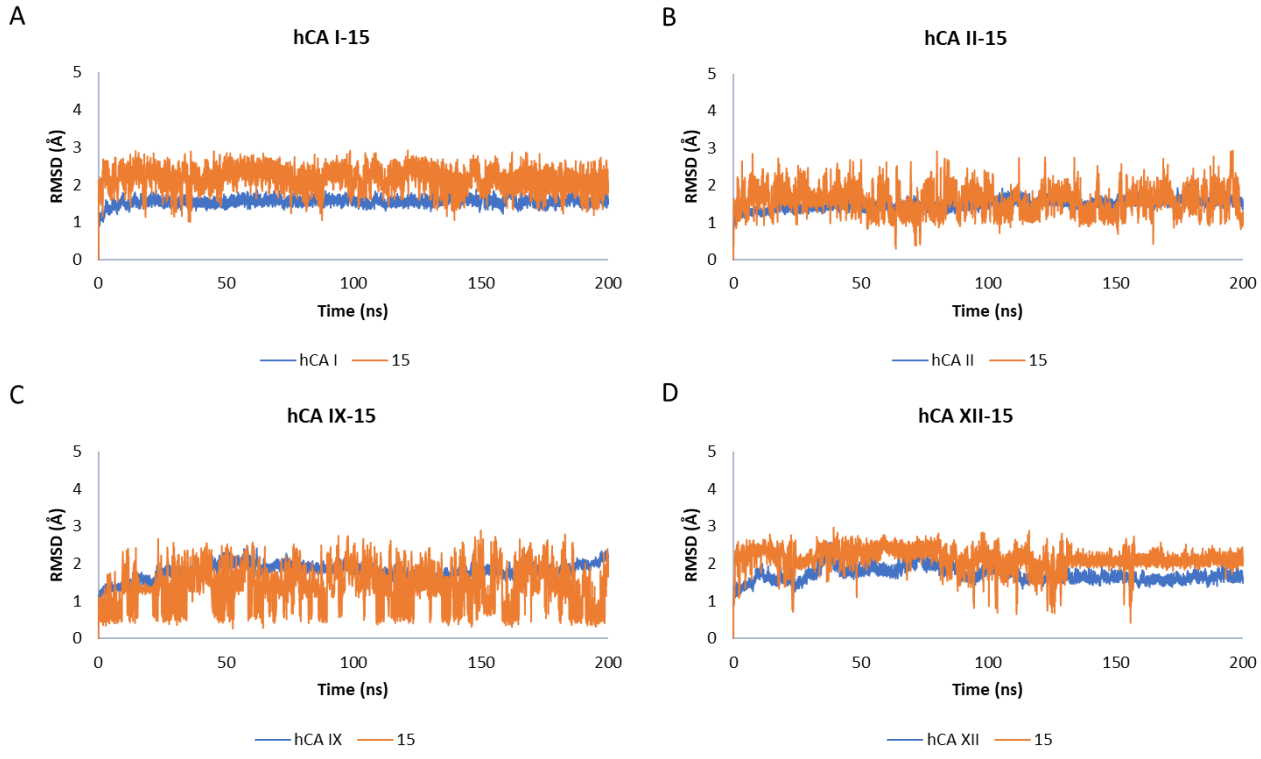
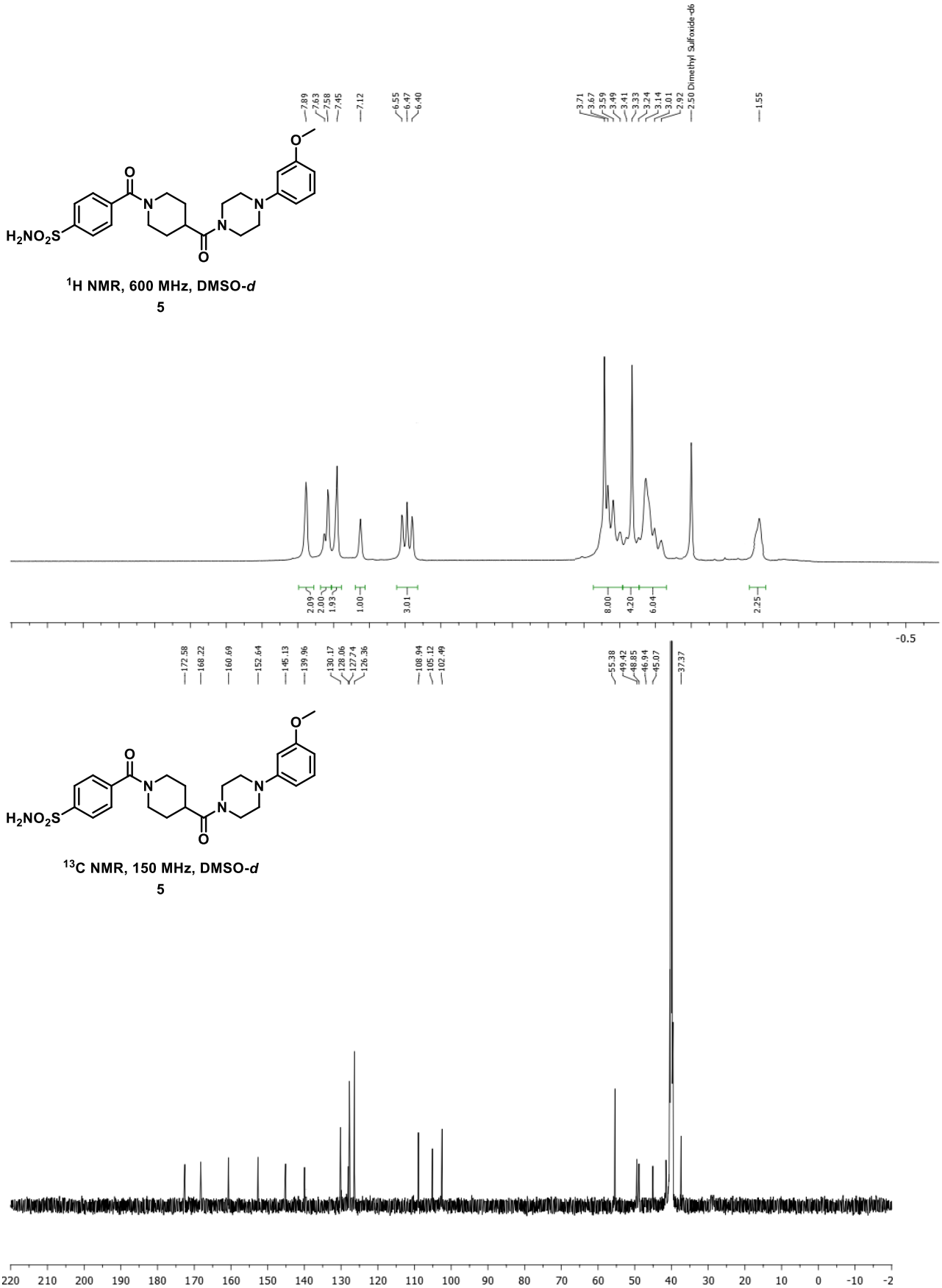


Figure S2. RMSD profiles obtained from the MD simulation of compound **15** in complex with A) hCA I, B) hCA II, C) hCA IX and D) hCA XII.

S6. 1H and 13C NMR spectra of final compounds 5-24

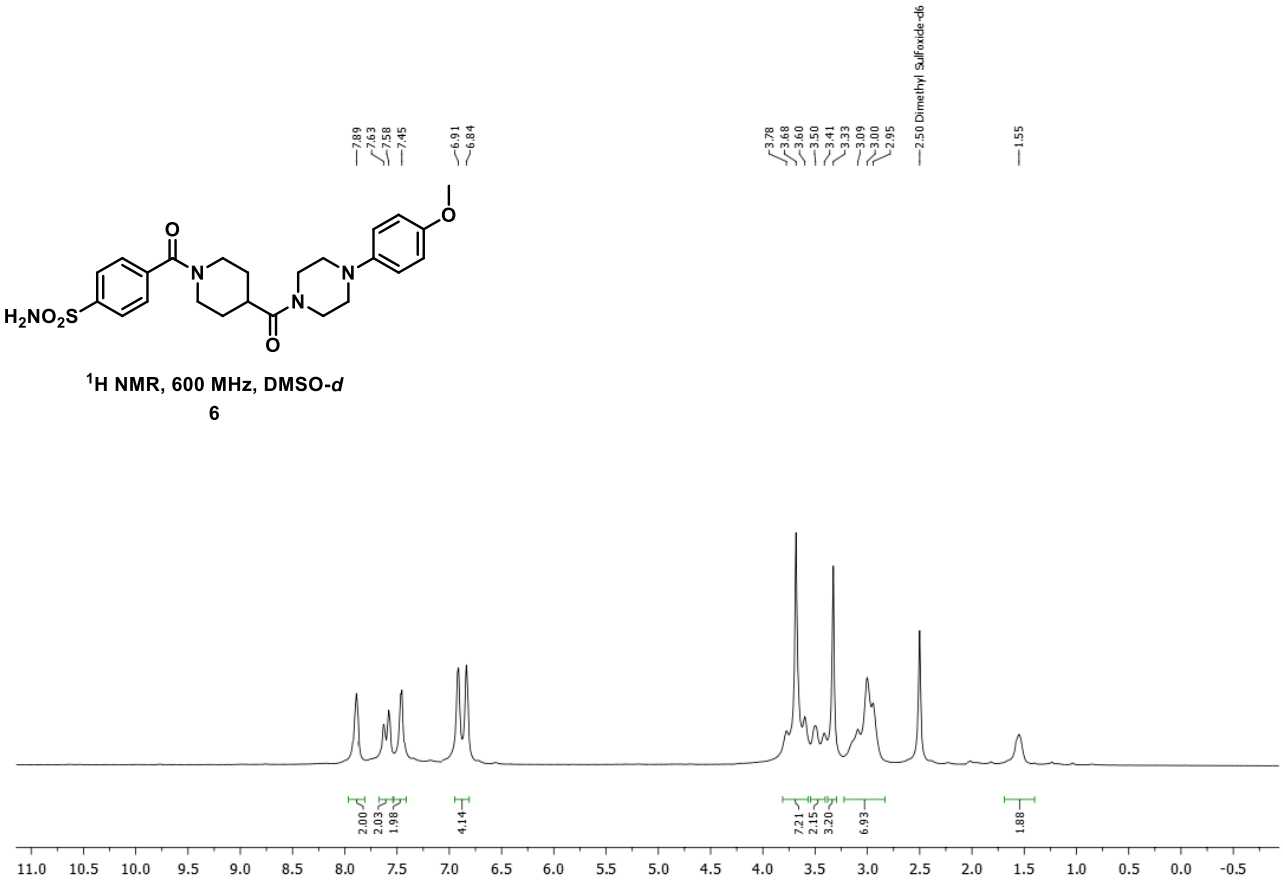


^{1}H NMR, 600 MHz, DMSO-*d*

5

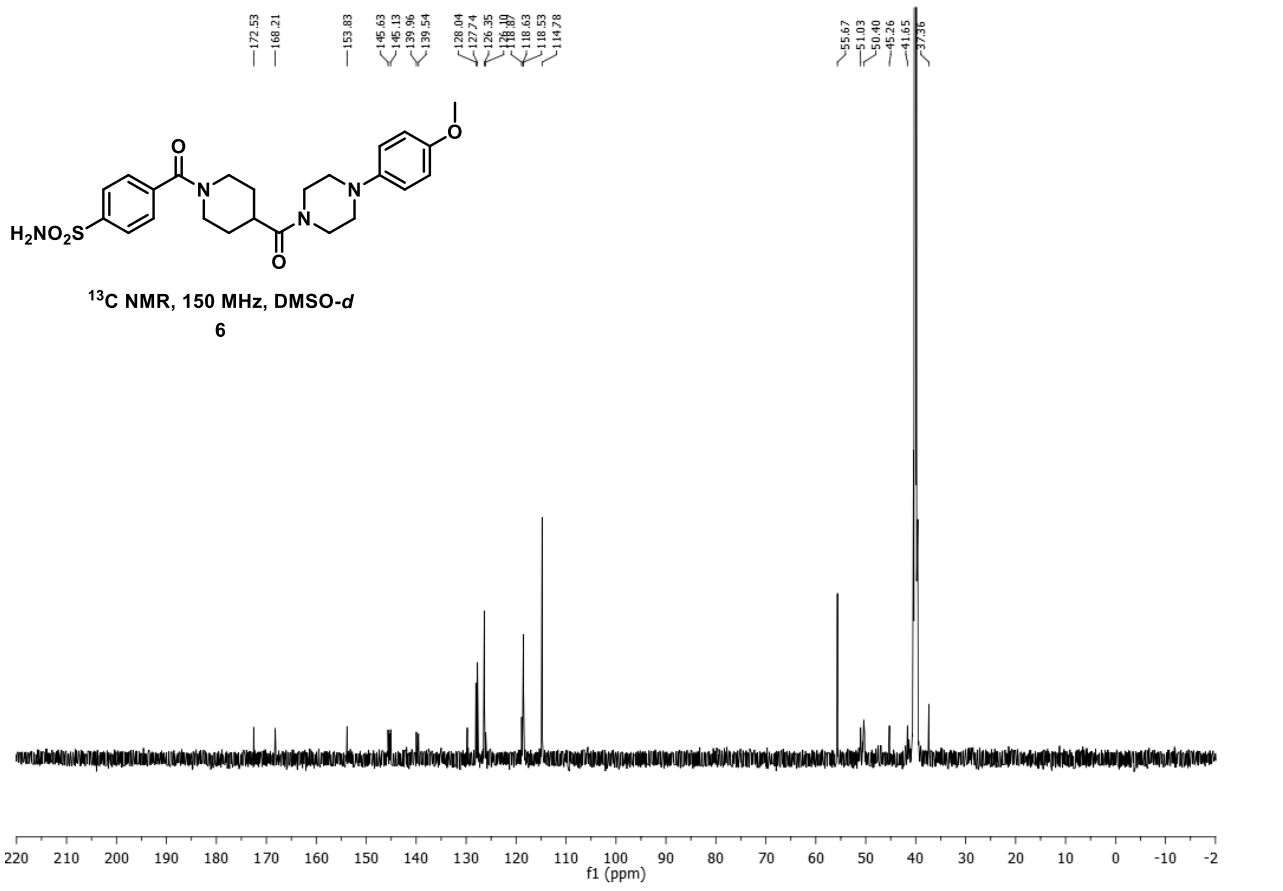
^{13}C NMR, 150 MHz, DMSO-*d*

5



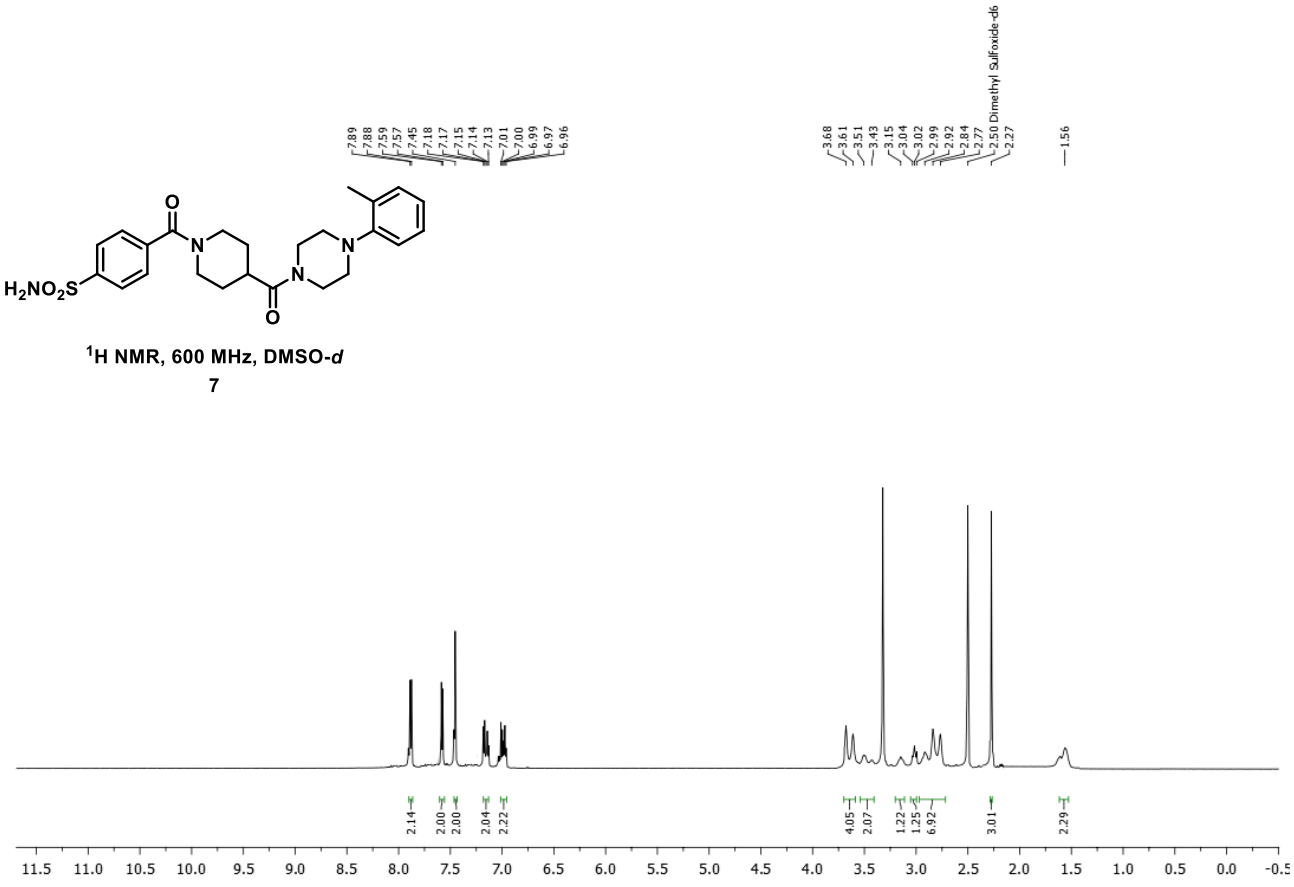
^{1}H NMR, 600 MHz, DMSO-*d*

6



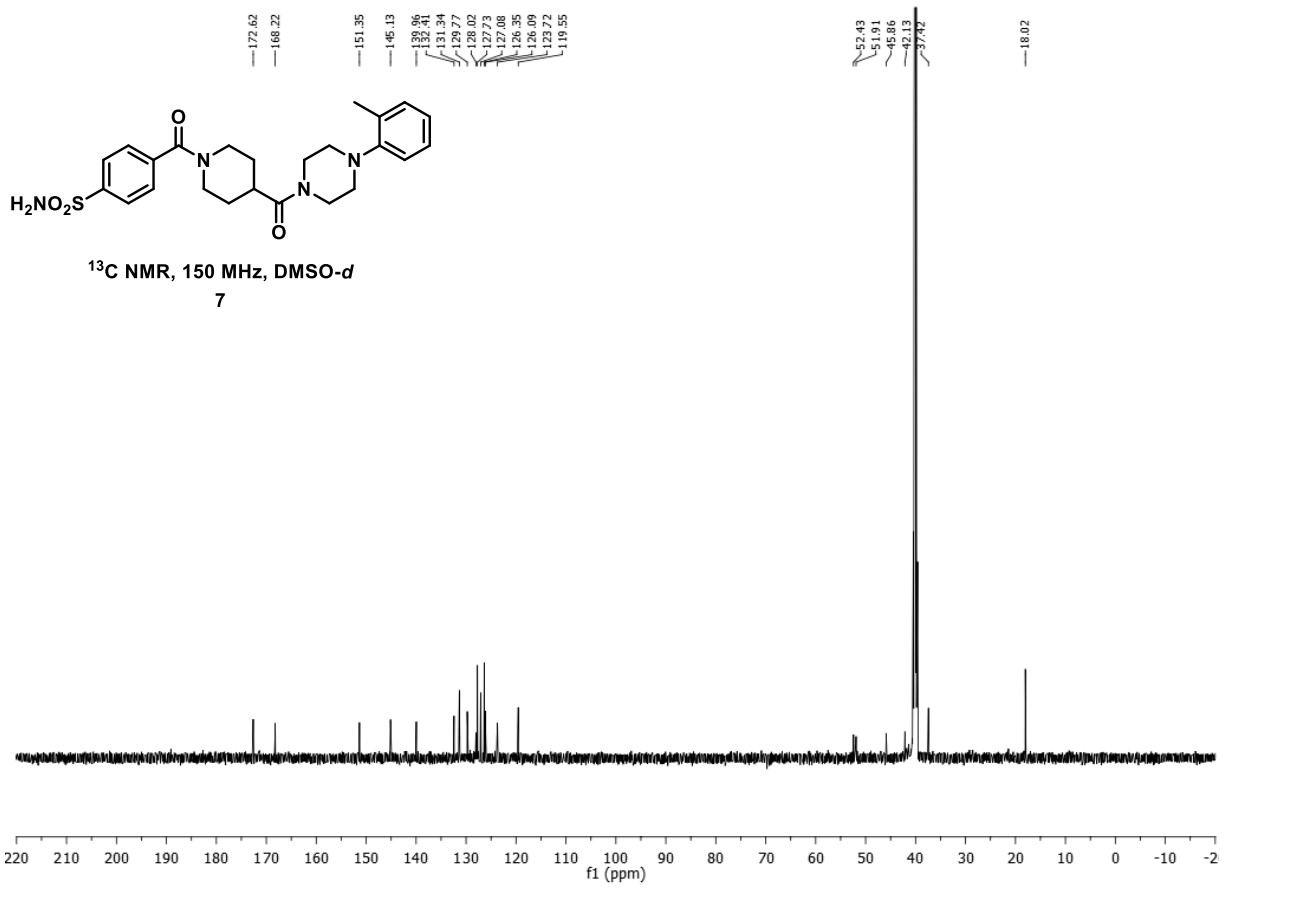
^{13}C NMR, 150 MHz, DMSO-*d*

6



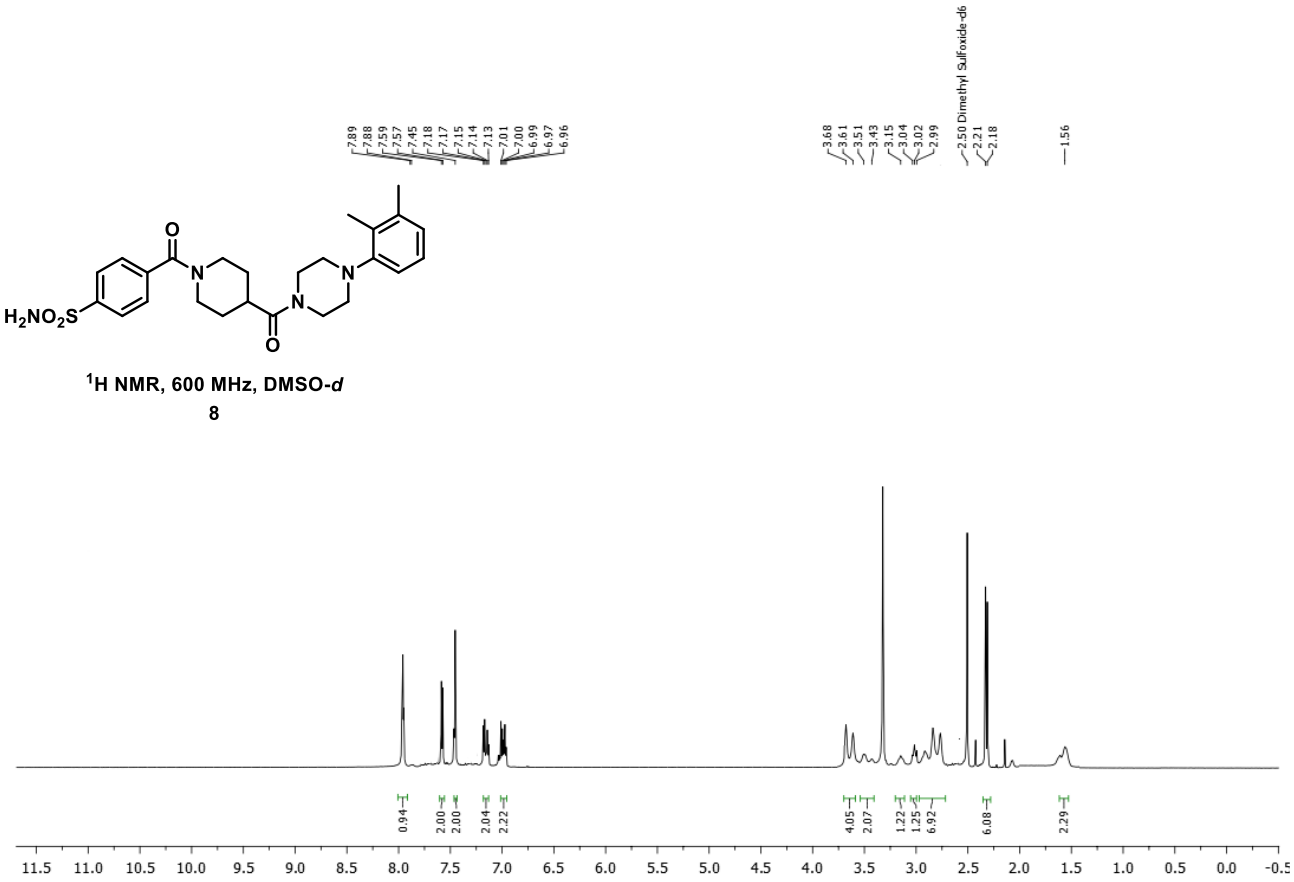
^{1}H NMR, 600 MHz, DMSO-*d*

7



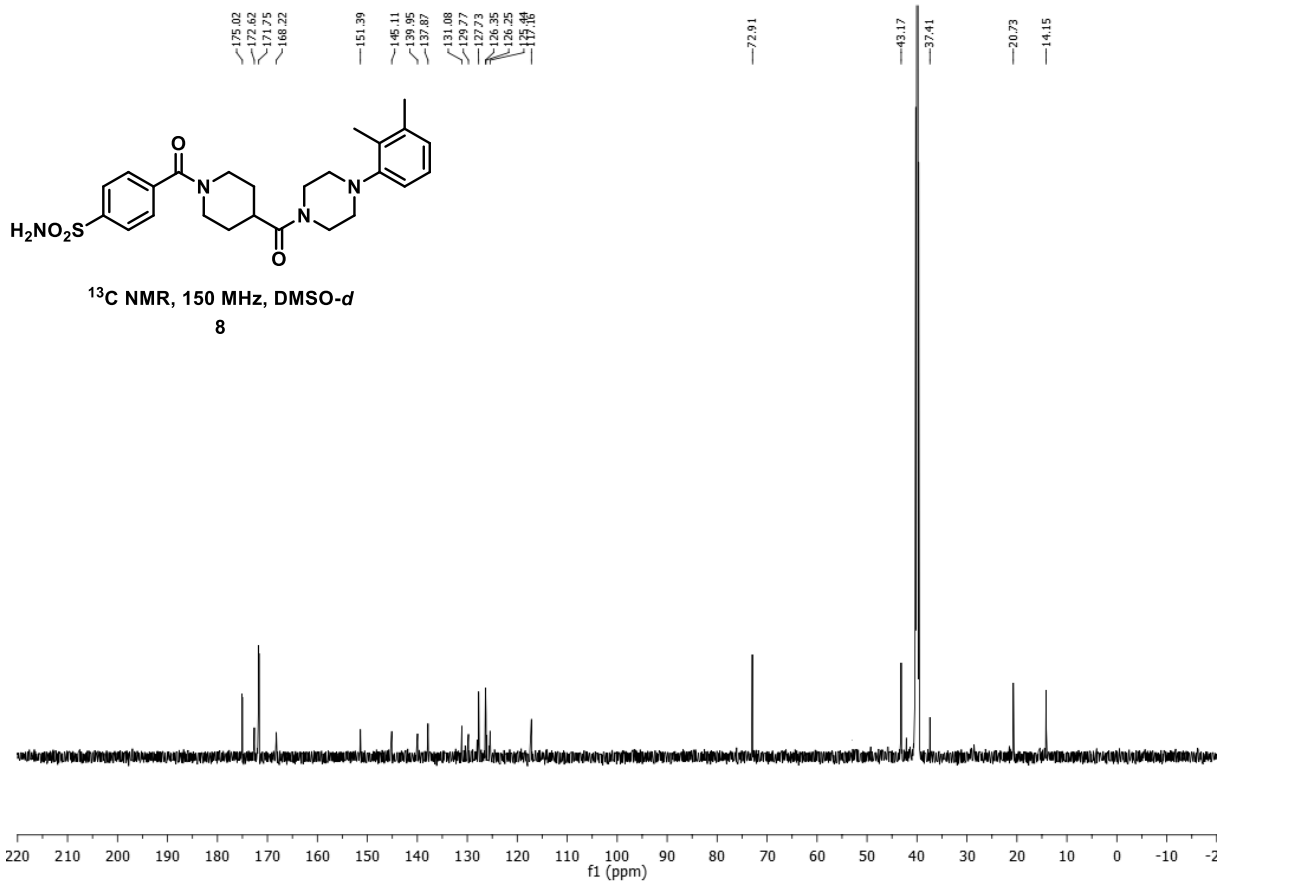
^{13}C NMR, 150 MHz, DMSO-*d*

7



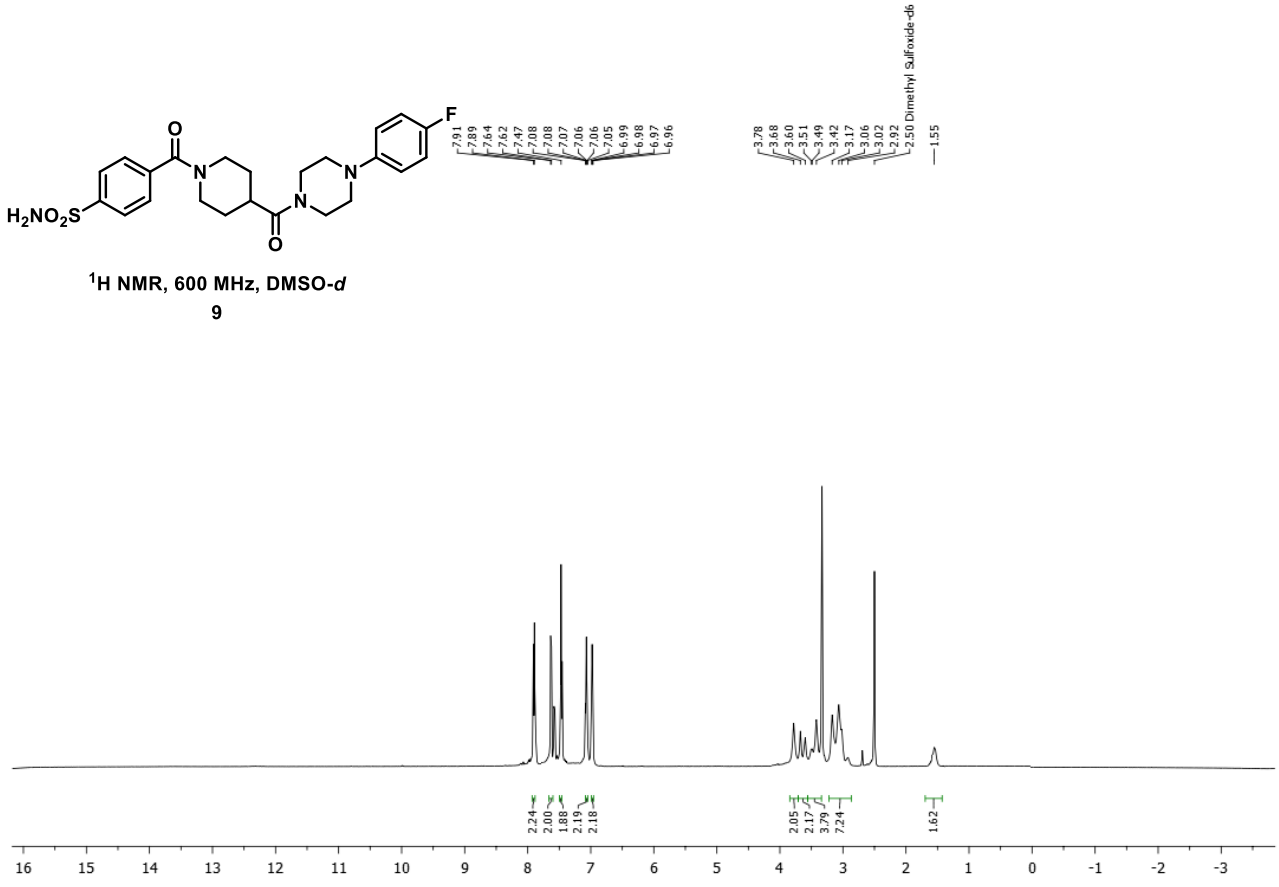
^{1}H NMR, 600 MHz, DMSO-*d*

8



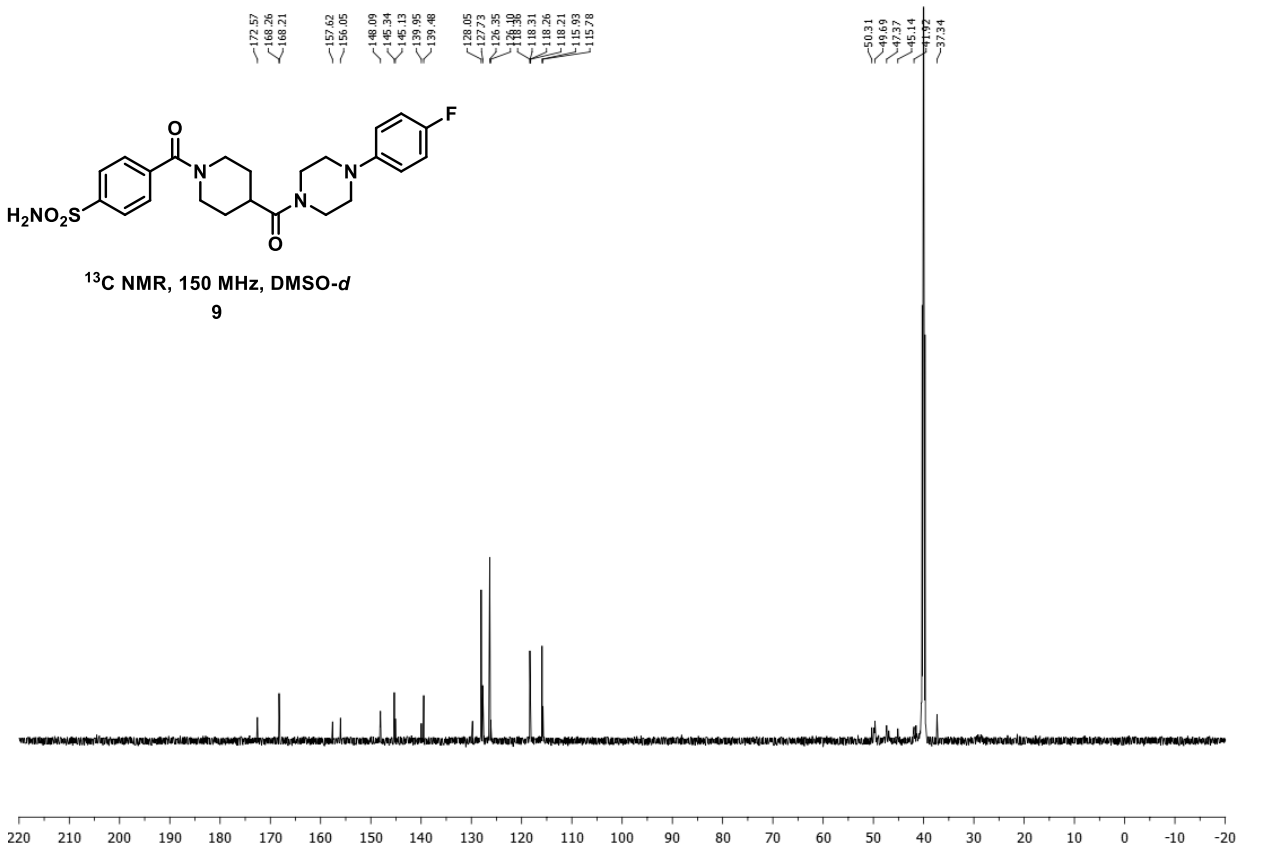
^{13}C NMR, 150 MHz, DMSO-*d*

8



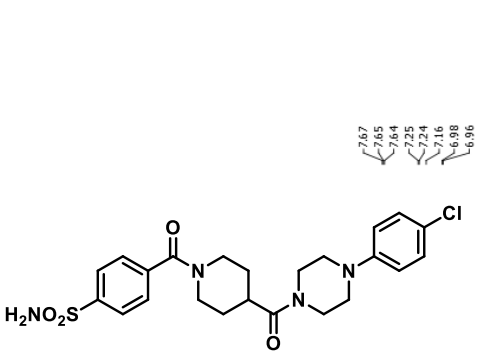
^{1}H NMR, 600 MHz, DMSO-*d*

9



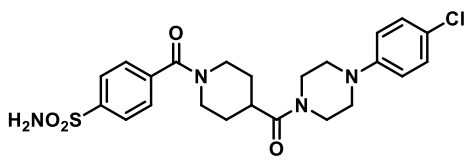
^{13}C NMR, 150 MHz, DMSO-*d*

9



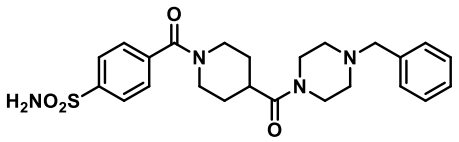
^{1}H NMR, 600 MHz, DMSO-*d*

10



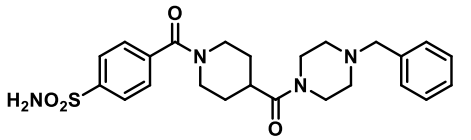
^{13}C NMR, 150 MHz, DMSO-*d*

10



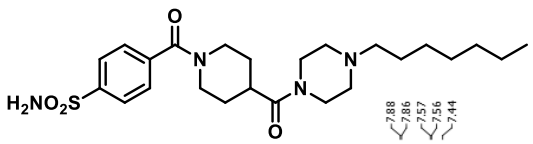
^{1}H NMR, 600 MHz, DMSO-*d*

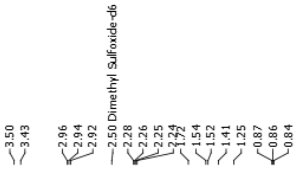
11



^{13}C NMR, 150 MHz, DMSO-*d*

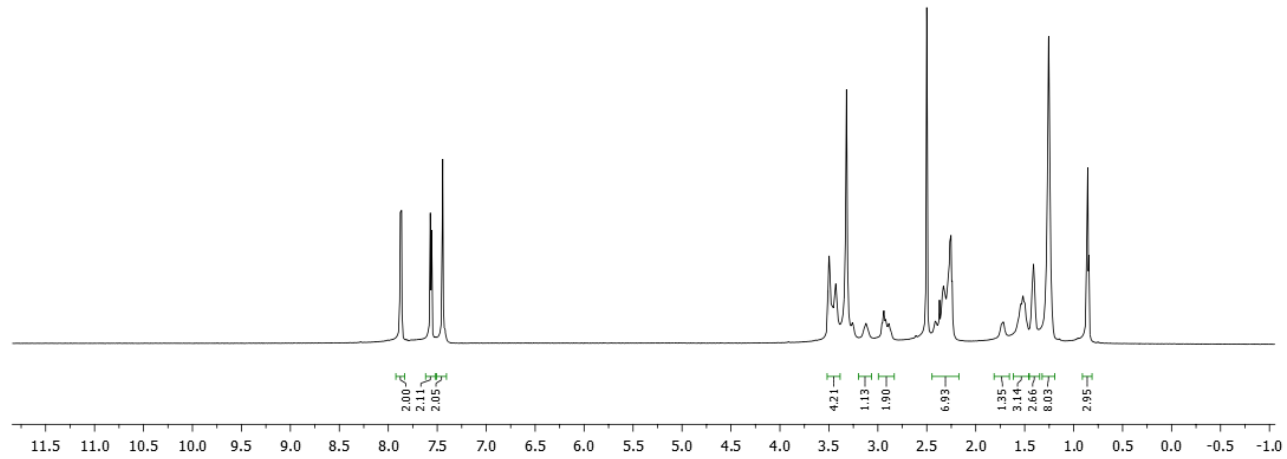
11

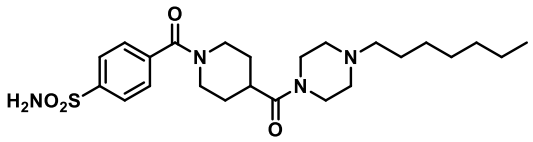




^{1}H NMR, 600 MHz, DMSO-*d*

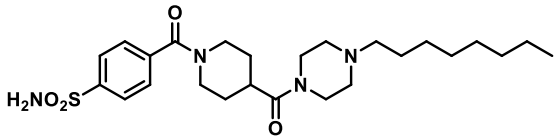
12





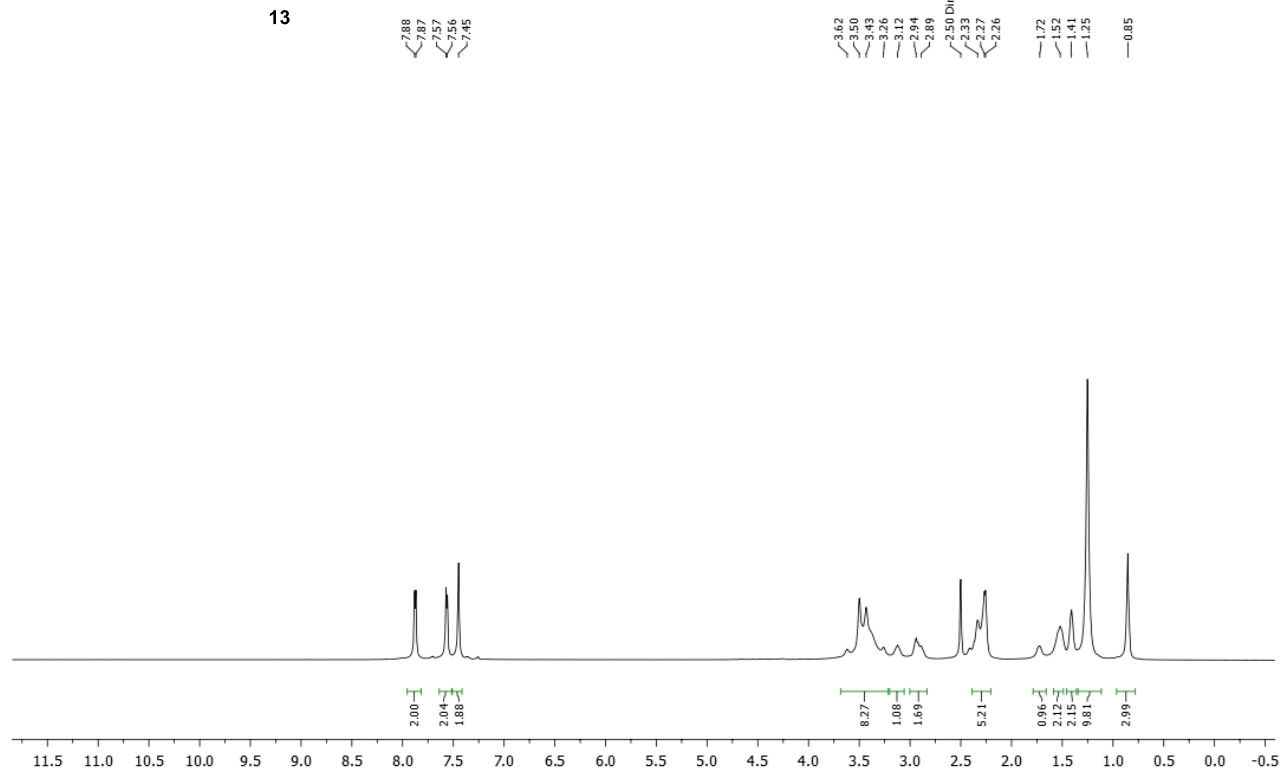
^{13}C NMR, 150 MHz, DMSO-*d*

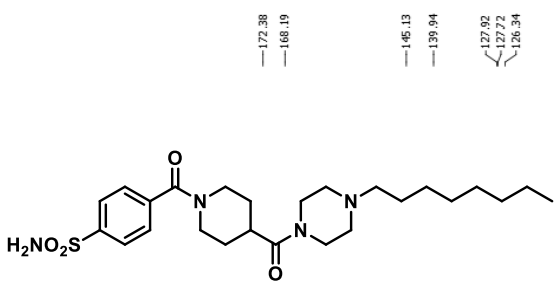
12



^{1}H NMR, 600 MHz, DMSO-*d*

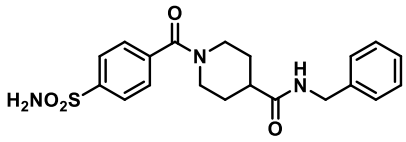
13





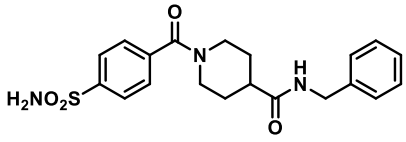
^{13}C NMR, 150 MHz, DMSO-*d*

13



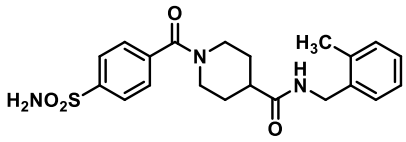
^{1}H NMR, 600 MHz, DMSO-*d*

14



^{13}C NMR, 150 MHz, DMSO-*d*

14



^{1}H NMR, 600 MHz, DMSO-*d*

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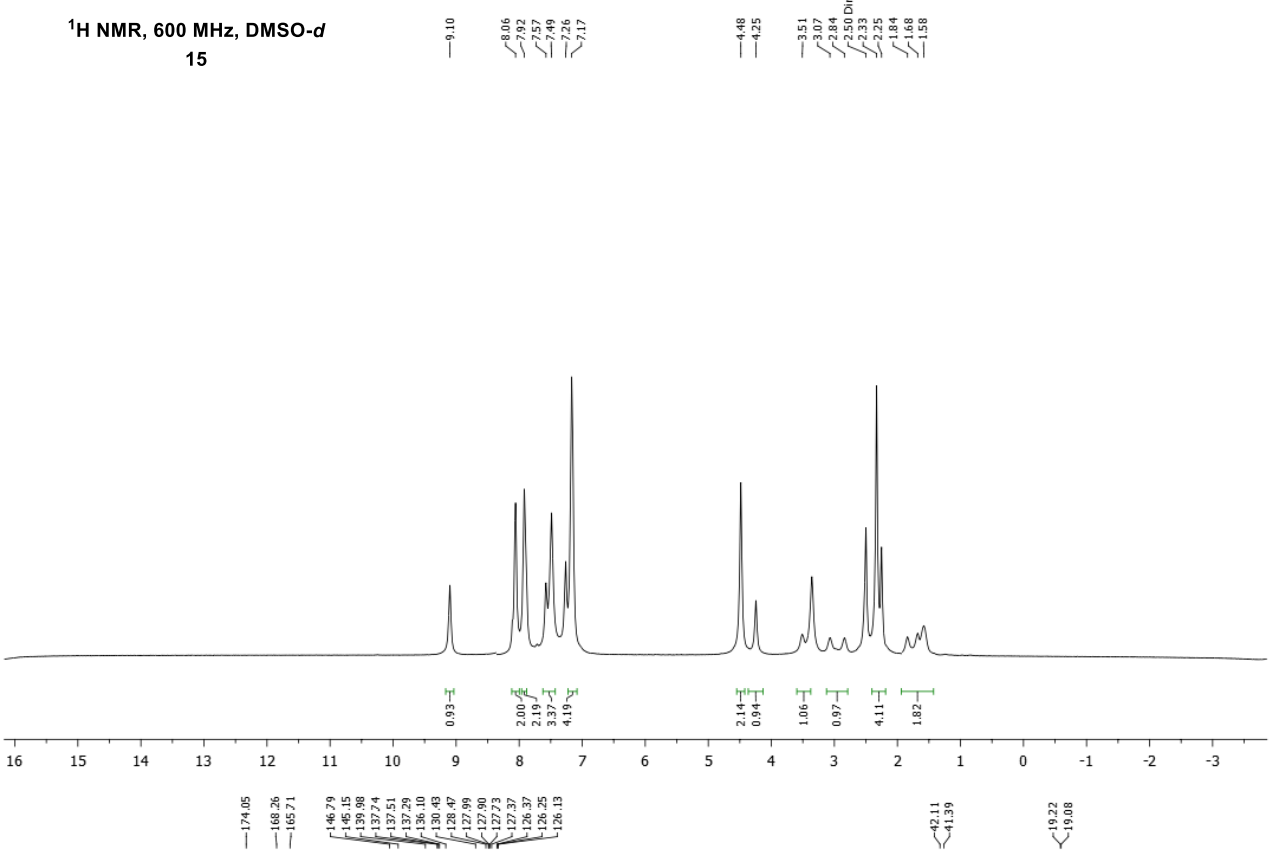

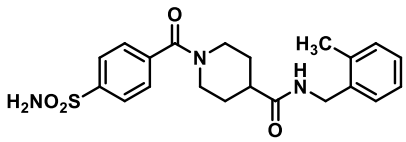

^{13}C NMR, 150 MHz, DMSO-*d*

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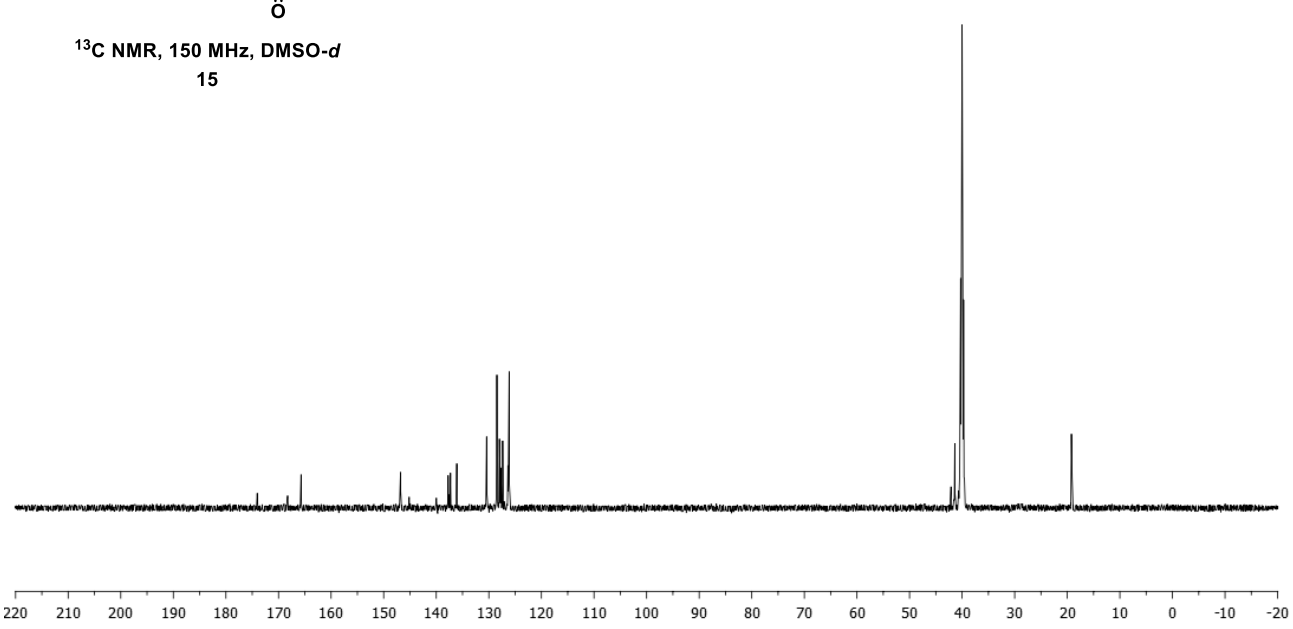
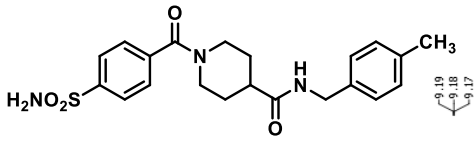
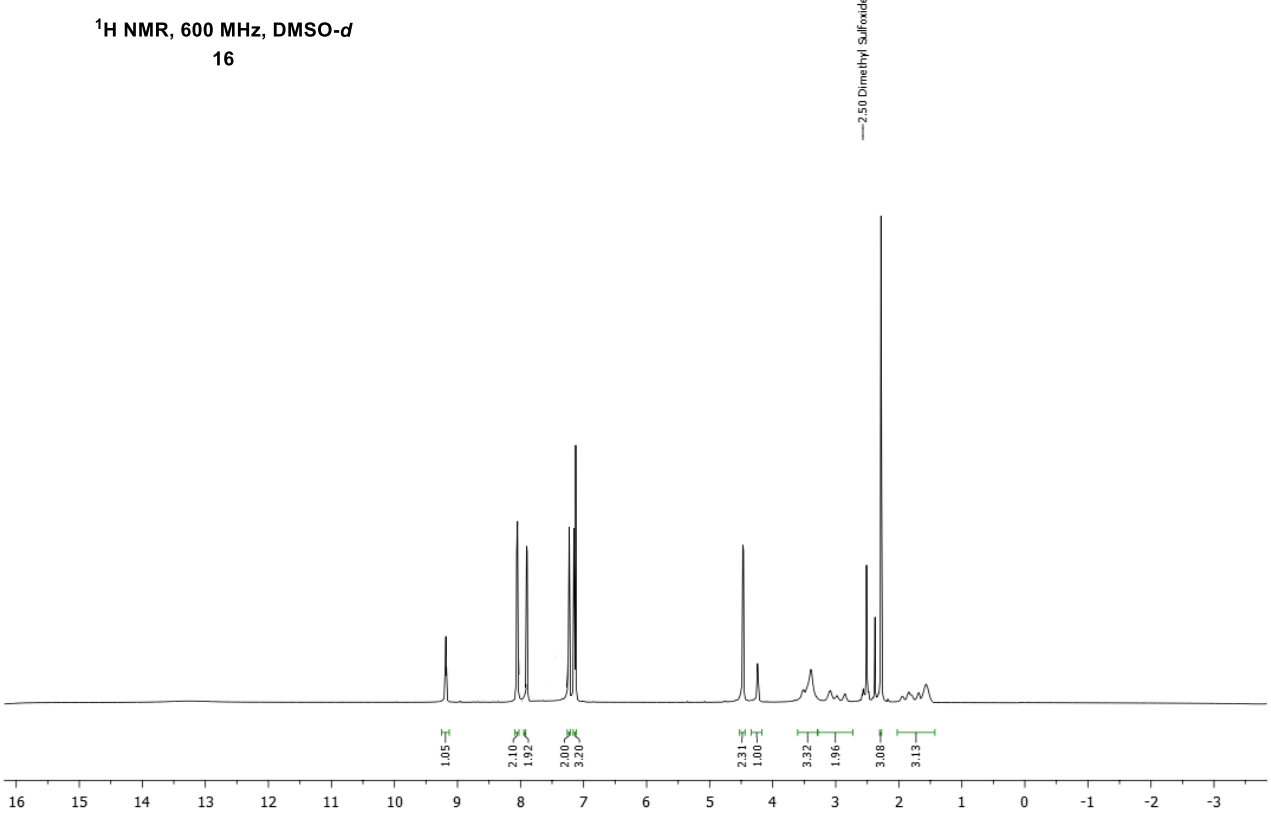
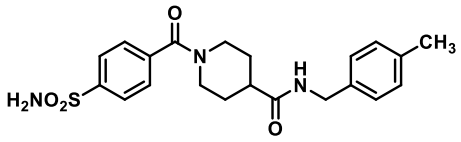




^{1}H NMR, 600 MHz, DMSO-*d*

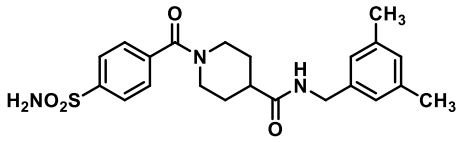
16





^{13}C NMR, 150 MHz, DMSO-*d*

16

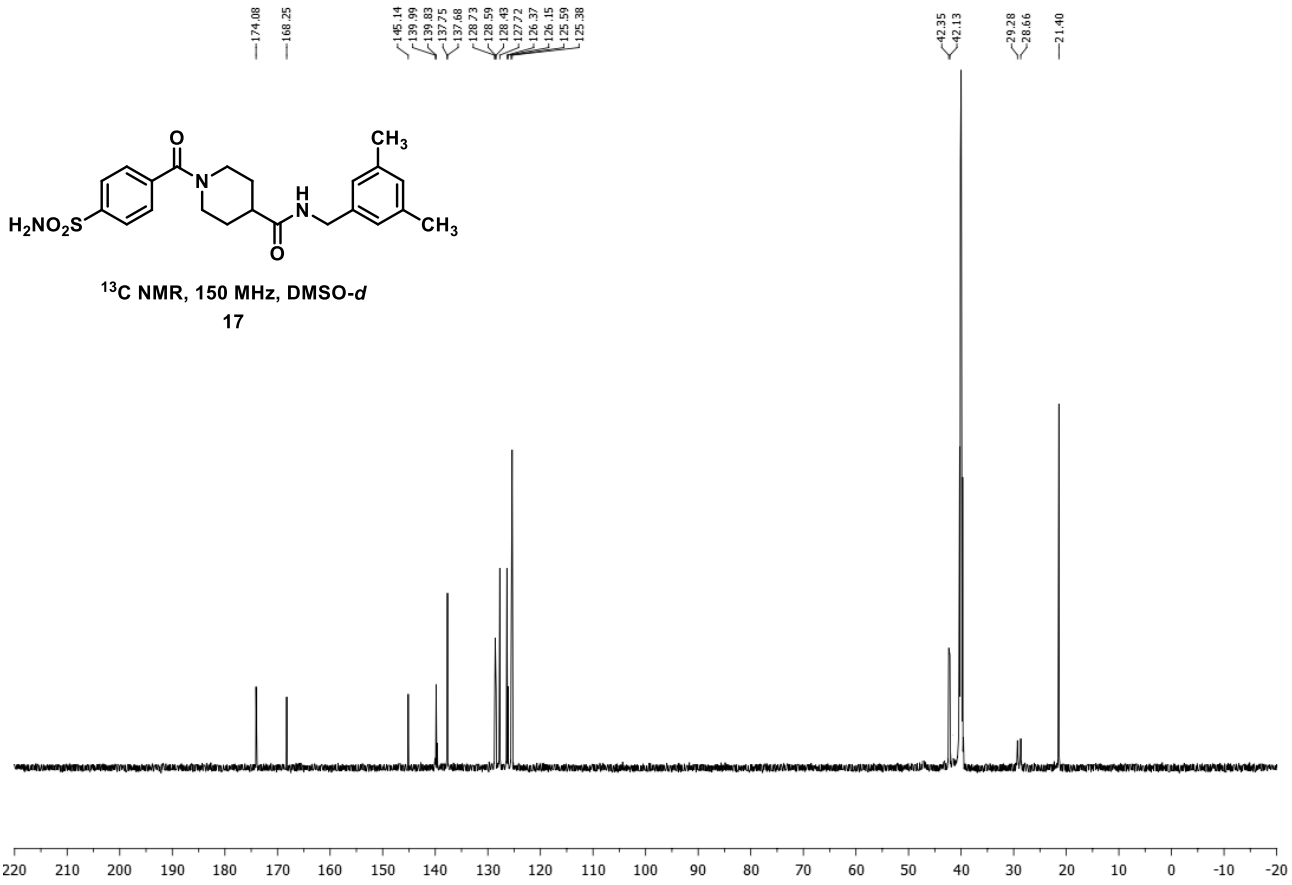


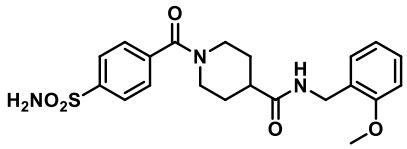
^{1}H NMR, 600 MHz, DMSO-*d*

17

^{13}C NMR, 150 MHz, DMSO-*d*

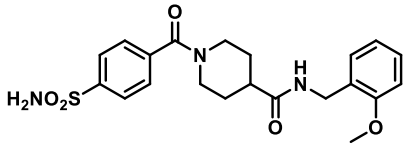
17





^{1}H NMR, 600 MHz, DMSO-*d*

18



^{13}C NMR, 150 MHz, DMSO-*d*

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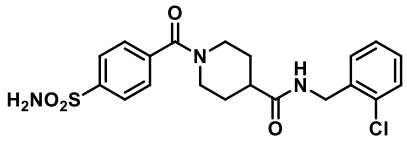
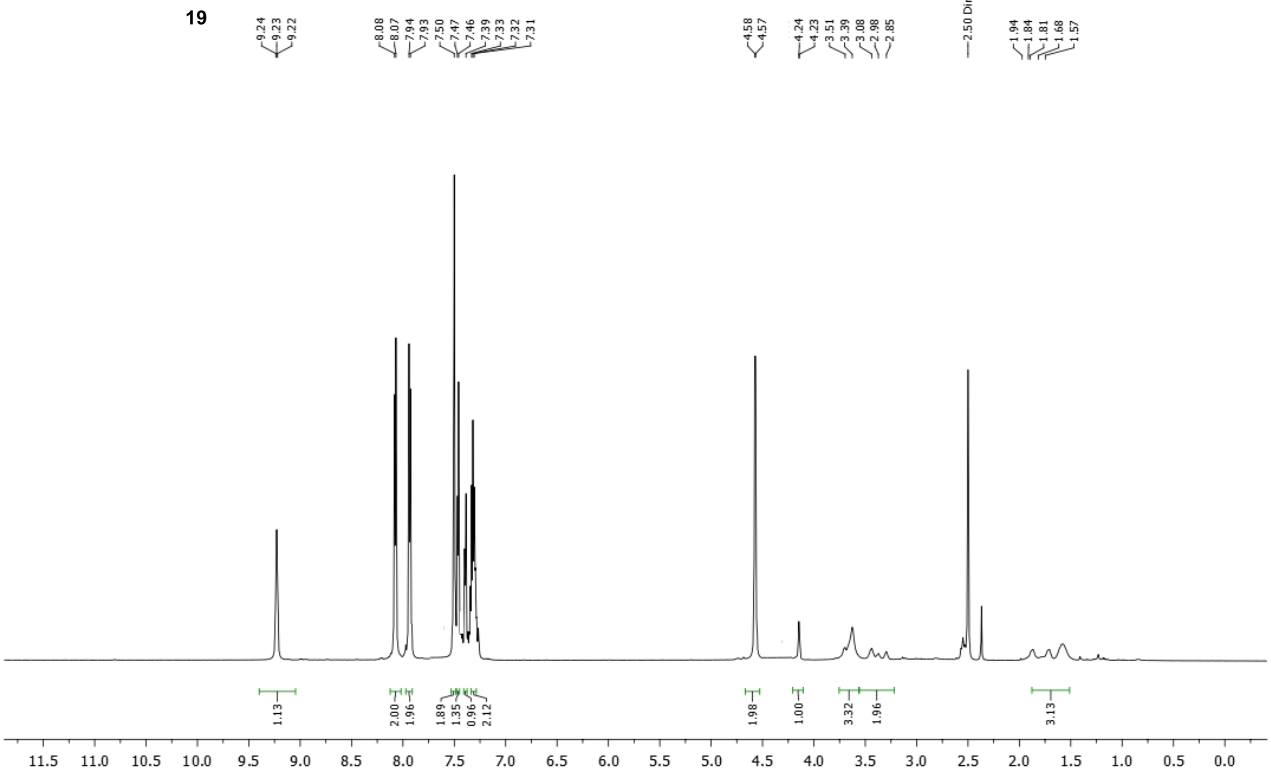
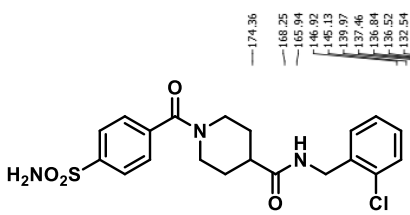


^{1}H NMR, 600 MHz, DMSO-*d*

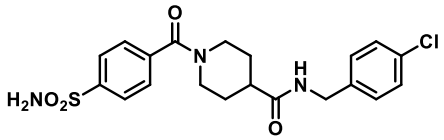
19





^{13}C NMR, 150 MHz, DMSO-*d*

19



^{1}H NMR, 600 MHz, DMSO-*d*

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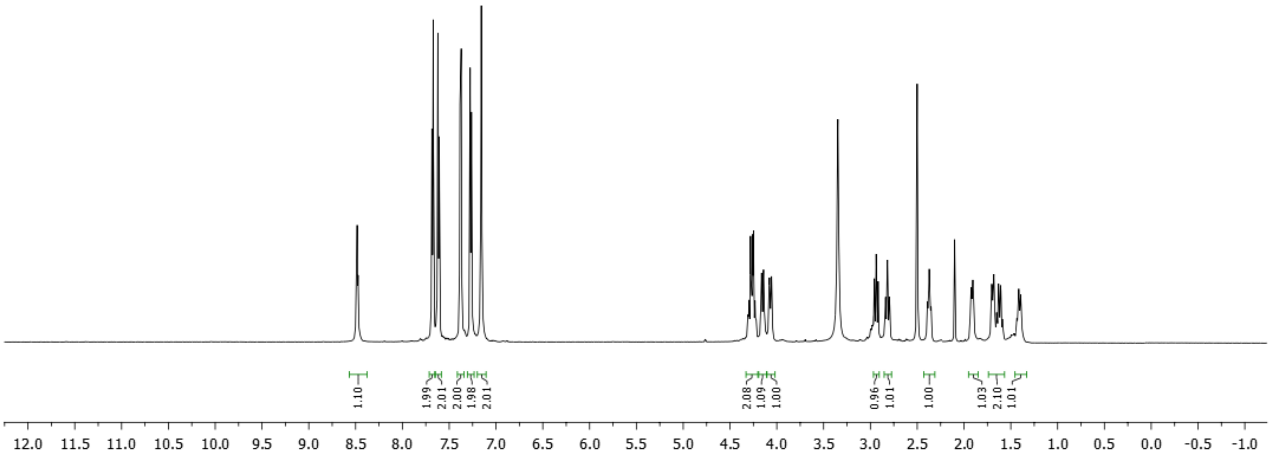
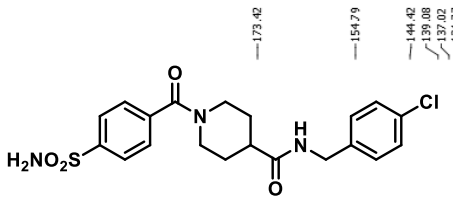
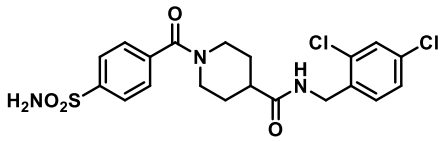




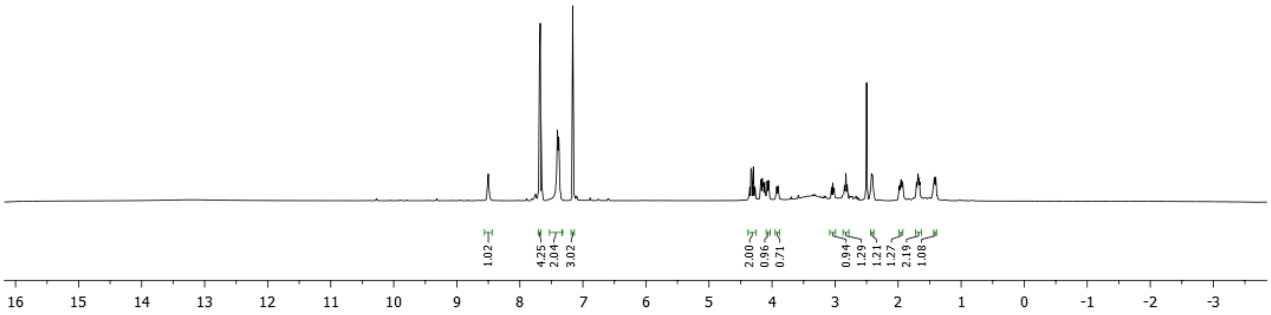
^{13}C NMR, 150 MHz, DMSO-*d*

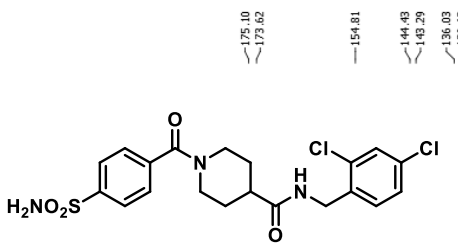
20



^{1}H NMR, 600 MHz, DMSO-*d*

21





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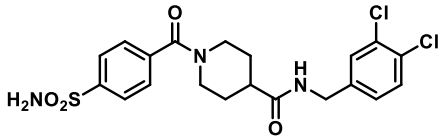
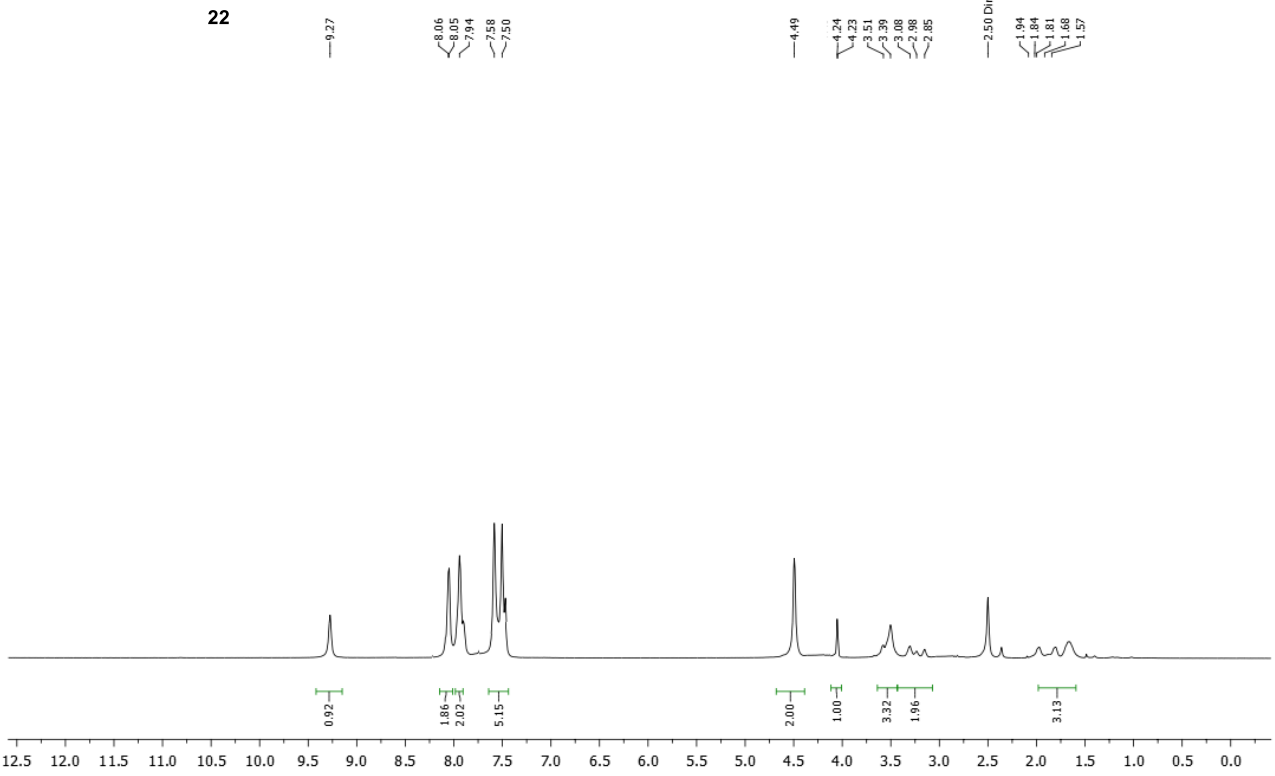
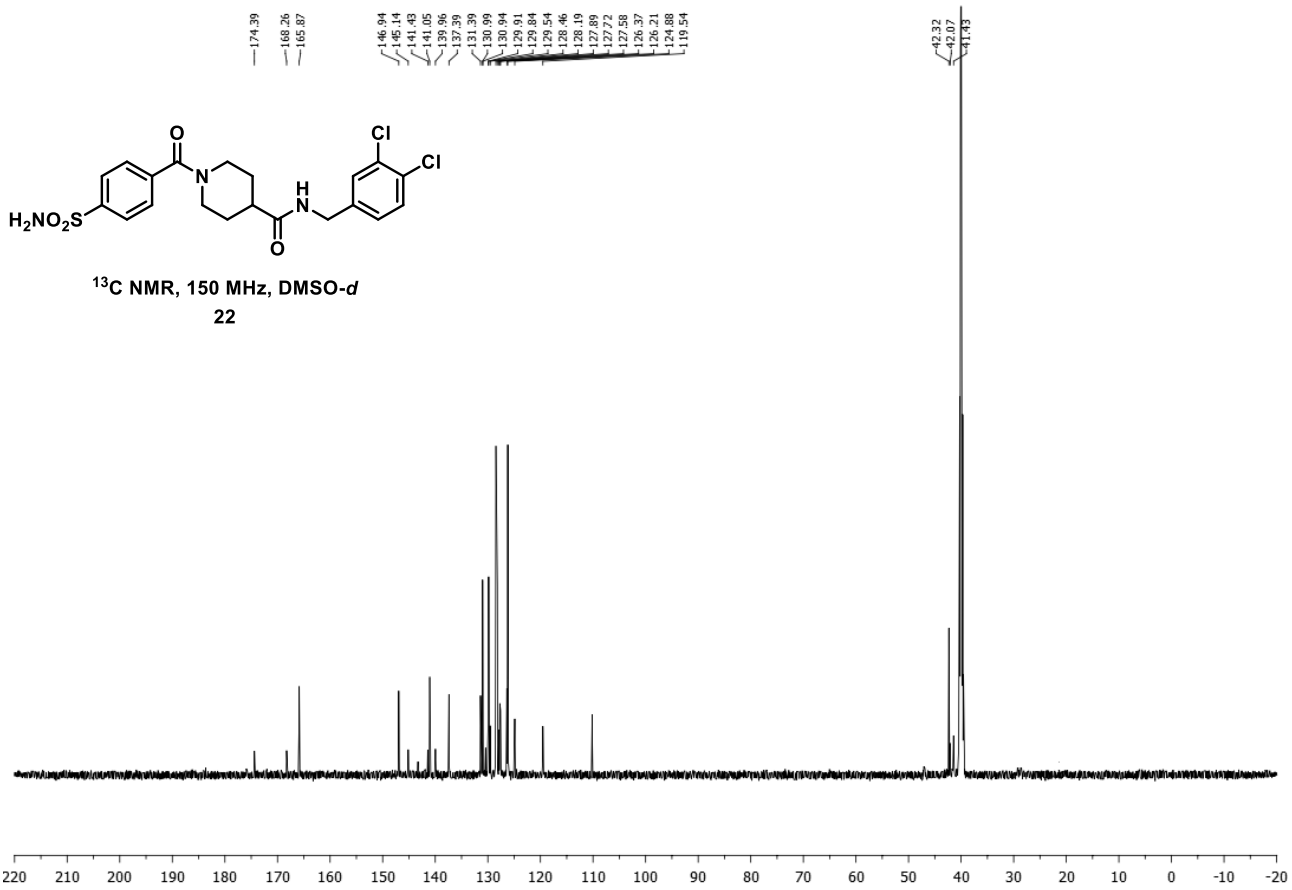
21



^{1}H NMR, 600 MHz, DMSO-*d*

22





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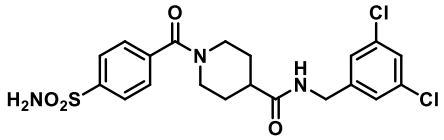

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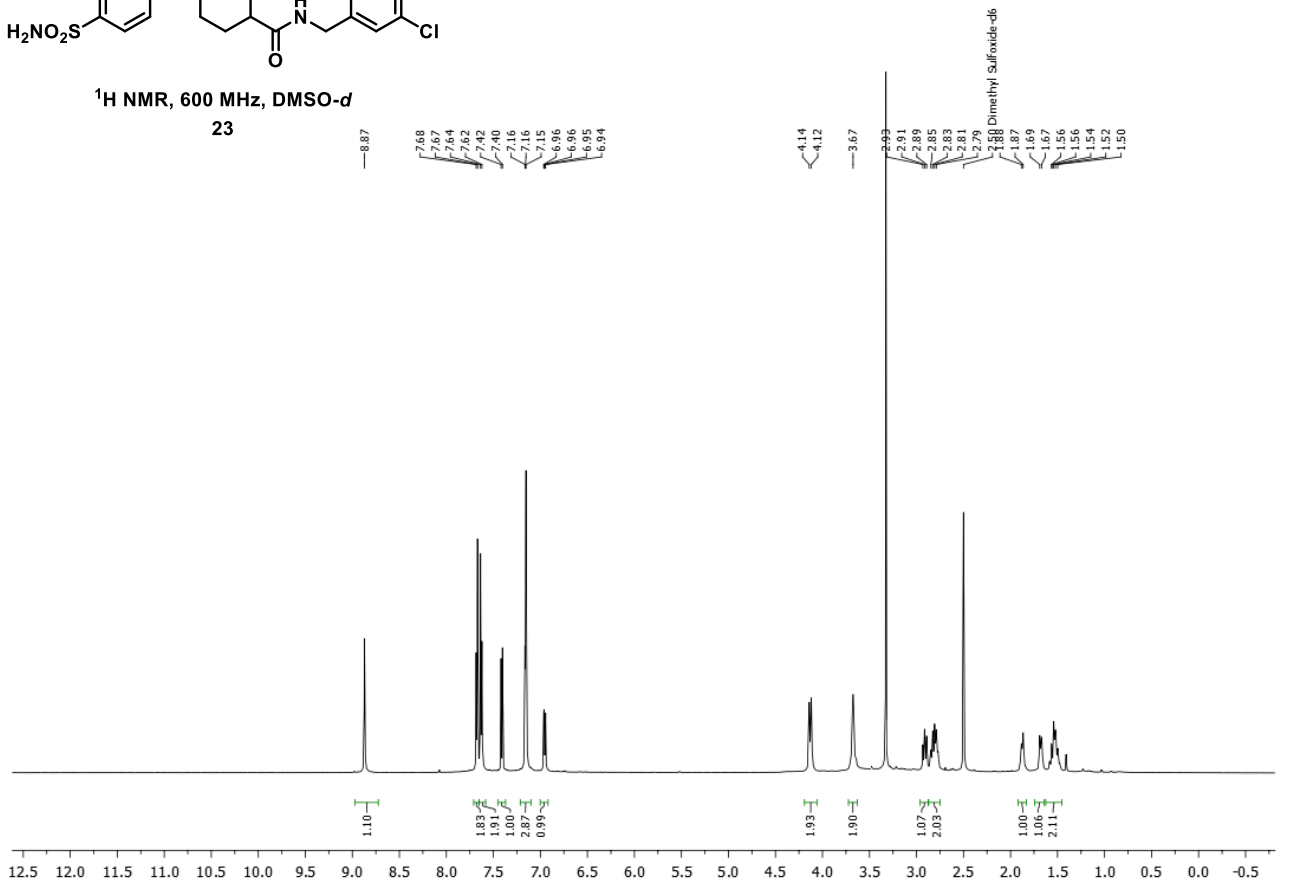

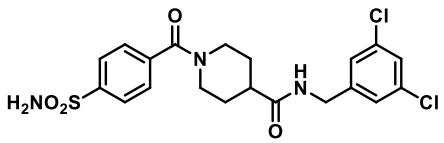

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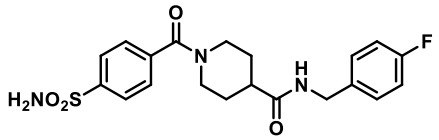

^{1}H NMR, 600 MHz, DMSO-*d*

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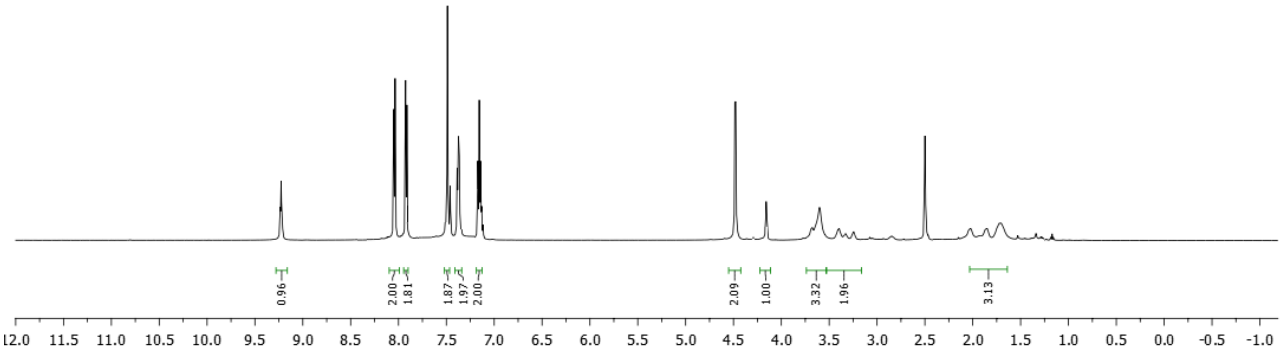

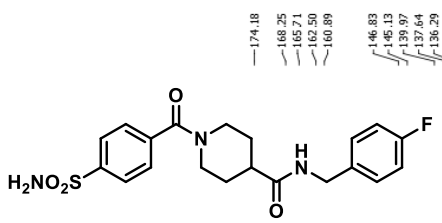

^{13}C NMR, 150 MHz, DMSO-*d*

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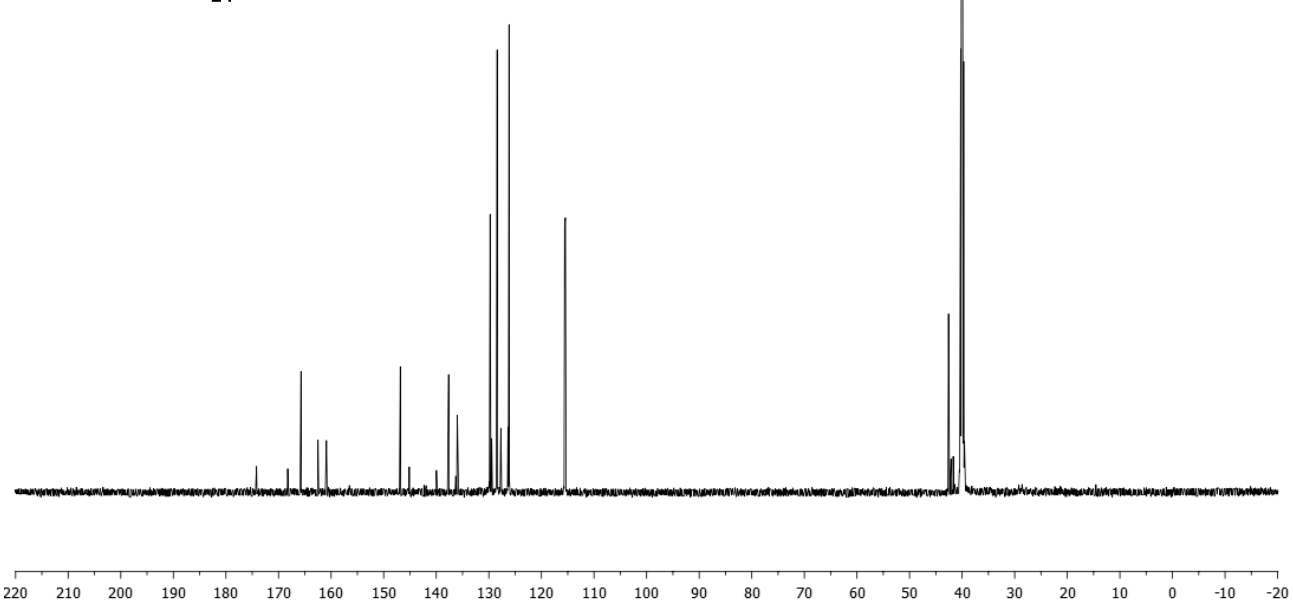

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