Looking towards the rim of the active site cavity of druggable human Carbonic Anhydrase isoforms

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Content:

1. Experimental Section

1.1.Chemistry

All reagents were used without further purification and bought from common commercial suppliers (Sigma-Aldrich Milan, Italy; Alfa Aesar Karlsruhe, Germany). Melting points were determined on a Buchi B-545 apparatus (BUCHI Labortechnik AG Flawil, Switzerland) and are uncorrected. By combustion analysis (C, H, N) carried out on a Carlo Erba Model 1106-Elemental Analyzer we determined the purity of synthesized compounds; the results confirmed a ≥95% purity. Merck Silica Gel 60 F254 plates were used for analytical TLC (Merck KGaA, Darmstadt, Germany). For detection, iodine vapor and UV light (254 nm) were used. ^{1}H-NMR and ^{13}C-NMR spectra were measured in dimethylsulfoxide-d6 (DMSO-d_6) with a Varian Gemini 300 spectrometer (Varian Inc. Palo Alto, California USA); chemical shifts are expressed in δ (ppm) and coupling constants (*J*) in hertz. All exchangeable protons were confirmed by addition of D_2O. R_f values were determined on TLC plates using a mixture of DCM/MeOH (96/4) as eluent.

General procedures for the synthesis preparation of phenyl(piperazin-1-yl)methanone derivatives *4a-p*

To a stirred solution of 1-Boc-piperazine (1 mmol) in DCM (3 mL), EDIPA (1.5 molar equivalents) and the appropriate benzoyl chloride derivative (**3a-p**) (1 molar equivalent) were added. The reaction mixture was stirred for different reaction times (ranging from 3-4 hours) at room temperature and monitored by TLC until the disappearance of the starting materials. Thus, TFA (8 molar equivalents) was added at 0° C and the resulting mixture was stirred for 3 hours at 25 °C. After the reaction was completed it was cooled into ice and diluted with DCM (2 mL) and 2M K_2CO_3 (2 mL). The mixture was extracted with DCM (3 x 5 mL), the combined organic layers were dried over Na_2SO_4, filtered,

and concentrated under vacuo. The residue was purified by treatment with Et_2O giving the desired known intermediates **4a-p**, some of them are commercially available. The registered CAS numbers for resulting compounds **4a-p** have been already assigned as reported below.

cpd	CAS number
4a	13754-38-6
4b	139516-64-6
4c	179334-10-2
4d	102391-98-0
4e	13754-45-5
4f	100939-90-0
4g	54042-47-6
4h	926202-11-1

cpd	CAS number
4i	1016819-18-3
4j	59939-72-9
4k	100939-88-6
4l	100939-89
4m	94747-49-6
4n	885101-42-8
4o	341529-34-8
4p	72141-41-4

General procedures for the synthesis of 4-(4-aroylpiperazine-1carbonyl)benzenesulfonamide derivatives (5a-s)

To a solution of 4-(aminosulfonyl)benzoic acid (1 mmol) dissolved in dimethylformamide (DMF) (2 mL) N,N,N',N'-tetramethyl-O-(1H-benzotriazol-1-yl)-uraniumhexafluorophosphate (HBTU) (1 molar equivalent) was added. The mixture was stirred at room temperature for 1 h. Then, TEA (2 molar equivalents) and appropriate 4-benzoylpiperazine derivatives **4a-p** (1 molar equivalent) were added. The reaction mixture was left overnight at room temperature and then quenched with H_2O (10 mL) and extracted with EtOAc (3 x 10 mL). The organic phase was washed with saturated NaCl solution, dried with Na_2SO_4 and concentrated until dryness under reduced pressure. The residue was purified by crystallization from Et_2O and EtOH to give the desired final compounds **5a-p** as white powder.

To a suspension of the nitro derivatives **5n-p** (1mmol) and a catalytic amount of Pd/C in EtOH (15 mL), hydrazine hydrate (10 molar equivalents) was slowly added. The reaction was stirred and refluxed (70 °C) under nitrogen atmosphere. Then, the mixture was filtered through celite, the cake was later washed with EtOAc. The solution was evaporated *in vacuo* to give the crude product, then dissolved in EtOAc and washed with H_2O (3 x 10 mL). The organic phase was dried with Na_2SO_4 and concentrated until dryness. The residue was purified by crystallization from Et_2O and EtOH to give the title compounds **5q-s**.

4-(4-Benzoylpiperazine-1-carbonyl)benzenesulfonamide **5a**

Yield: 45%; M.p.: 248-249°C; R_f = 0.24. ^{1}H-NMR (DMSO-d_6): (δ) 3.55-3.93 (m, 8H, CH_2), 7.40-7.47 (m, 5H, ArH), 7.50 (s, 2H, NH_2), 7.62 (d, *J*=8.2, 2H, ArH), 7.89 (d, *J*=8.2, 2H, ArH); ^{13}C-NMR (DMSO-d_6): 42.13, 47.71, 125.64, 126.99, 127.65, 128.33, 129.64, 135.45, 138.62, 144.91, 169.33, 171.66. Anal. for ($C_{18}H_{19}N_3O_4S$): C 57.89%, H 5.13%, N 11.25%; Found: C 57.99%, H 5.23%, N 11.35%.

*4-[4-(2-Fluorobenzoyl)piperazine-1-carbonyl]benzenesulfonamide***, 5b**

Yield: 72%; M.p.: 268-269°C; R_f = 0.12. ^{1}H-NMR (DMSO-d_6): (δ) 3.55-3.76 (m, CH_2, 8H), 7.31-7.44 (m, 4H, ArH), 7.49 (s, 2H, NH_2), 7.57-7.65 (m, 2H, ArH), 7.88-7.95 (m, 2H, ArH). Anal. for ($C_{18}H_{18}FN_3O_4S$): C 55.23%, H 4.64%, N 10.74%; Found: C 55.33%, H 4.74%, N 10.54%.

4-[4-(3-Fluorobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5c**
Yield: 43%; M.p.: 214-215°C; R_f = 0.11. ^{1}H-NMR (DMSO-d_6): (δ) 3.57-3.76 (m, 8H, CH_2), 7.23-7.33 (m, 3H, ArH), 7.49 (s, 2H, NH_2). 7.61-7.64 (m, 3H, ArH), 7.87-7.90 (m, 2H, ArH). Anal. for ($C_{18}H_{18}FN_3O_4S$): C 55.23% , H 4.64% , N 10.74%; Found: C 55.03%, H 4.44%, N 10.54%.

4-[4-(4-Fluorobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5d**
Yield: 42%; M.p.: 212-214°C; R_f = 0.10. ^{1}H-NMR (DMSO-d_6): (δ) 3.03-3.40 (m, 8H, CH_2), 7.26-7.33 (m, 1H, ArH), 7.49 (s, 2H, NH_2), 7.60-7.74 (m, 2H, ArH), 7.83-8.01 (m, 4H, ArH), 8.11-8.14 (m, 1H, ArH) . Anal. for ($C_{18}H_{18}FN_3O_4S$): C 55.23%, H 4.64%, N 10.74% Found: C 55.13%, H 4.54%, N 10.64%.

4-[4-(2-Chlorobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5e**
Yield: 63%; M.p.: 249-250°C; R_f = 0.15. ^{1}H-NMR (DMSO-d_6): (δ) 3.16 (m, 2H), 3.24 (m, 2H), 3.67-3.73 (m, 4H), 7.43 (m, 4H, ArH), 7.50 (s, 2H, NH_2), 7.62 (d, *J*=8.2, 2H, ArH), 7.88 (d, *J*=8.2, 2H, ArH). Anal. for ($C_{18}H_{18}ClN_3O_4S$): C 53.01%, H 4.45%, N 10.30%; Found: C 52.91%, H 4.35%, N 10.20%.

4-[4-(3-Chlorobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5f**
Yield: 63%; M.p.: 193-195°C; R_f = 0.15. ^{1}H-NMR (DMSO-d_6): (δ) 3.27-3.41 (m, 4H), 3.62-3.67 (m, 4H), 7.37-7.43 (m, 2H, ArH), 7.49 (s, 2H, NH_2), 7.62-7.70 (m, 4H, ArH), 7.78-7.90 (m, 2H, ArH). Anal. for ($C_{18}H_{18}ClN_3O_4S$): C 53.01%, H 4.45%, N 10.30%; Found: C 53.11%, H 4.55%, N 10.40%.

4-[4-(4-Chlorobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5g**
Yield: 50%; M.p.: 245-246°C; R_f = 0.14. ^{1}H-NMR (DMSO-d_6): (δ) 3.50-3.75 (m, 8H, CH_2), 7.41-7.48 (m, 2H, ArH), 7.50 (s, 2H, NH_2), 7.52-7.54 (m, 2H, ArH), 7.63 (d, *J*=8.2, 2H, ArH), 7.87 (d, *J*=8.2, 2H, ArH); ^{13}C-NMR (DMSO-d_6): 41.84, 46.94, 125.99, 127.76, 128.70, 129.20, 134.41, 134.55, 138.87, 145.04, 168.20, 168.41. Anal. for ($C_{18}H_{18}ClN_3O_4S$): C 53.01%, H 4.45%, N 10.30%; Found: C 53.21%, H 4.65%, N 10.50%.

4-*[4-(2-Bromobenzoyl)piperazine-1-carbonyl]benzenesulfonamide*, **5h**
Yield: 49%; M.p.: 209-211°C; R_f = 0.19. ^{1}H-NMR (DMSO-d_6): (δ) 3.22-3.40 (m, 4H), 3.69-3.74 (m, 4H), 7.32-7.39 (m, 3H, ArH), 7.49 (s, 2H, NH_2), 7.54-7.69 (m, 3H, ArH), 7.82-7.90 (m, 2H, ArH). Anal. for ($C_{18}H_{18}BrN_3O_4S$): C 47.80%, H 4.01%, N 9.29%; Found: C 47.68%, H 4.21%, N 9.41%.

4-[4-(3-Bromobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5i**
Yield: 86%; M.p.: 193-194°C; R_f = 0.18. ^{1}H-NMR (DMSO-d_6): (δ) 3.52-3.87 (m, 8H, CH_2), 7.38-43 (m, 2H, ArH), 7.50 (s, 2H, NH_2), 7.59-7.68 (m, 4H, ArH), 7.83-7.92 (m, 2H, ArH). Anal. for ($C_{18}H_{18}BrN_3O_4S$): C 47.80%, H 4.01%, N 9.29%; Found: C 47.98%, H 4.11%, N 9.39%.

4-[4-(4-Bromobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5j**
Yield: 47%; M.p.: 222-224°C; R_f = 0.18. ^{1}H-NMR (DMSO-d_6): (δ) 3.51-3.75 (m, 8H, CH_2), 7.43-(m, 2H, ArH), 7.51 (s, 2H, NH_2), 7.53-7.58 (m, 2H, ArH), 7.63-7.70 (m, 2H, ArH), 7.87-7.93 (m, 2H, ArH). Anal. for ($C_{18}H_{18}BrN_3O_4S$): C 47.80%, H 4.01%, N 9.29%; Found: C 47.70%, H 3.91%, N 9.19%.

4-[4-(2-Methoxybenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5k**
Yield: 56%; M.p.: 268-269°C; R_f = 0.13. ^{1}H-NMR (DMSO-*d*$_6$): (δ) 3.24 (m, 4H, CH_2), 3.57 (m, 4H, CH_2), 3.90 (s, 3H, OCH_3), 6.98-7.38 (m, 4H, ArH), 7.50 (s, 2H, NH_2), 7.60-7.65 (m, 2H, ArH). 7.79-7.86 (m, 2H, ArH). Anal. for ($C_{19}H_{21}N_3O_5S$): C 56.56%, H 5.25%, N 10.42%; Found: C 56.36%, H 5.05%, N 10.22%.

4-[4-(3-Methoxybenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5l**
Yield: 43%; M.p.: 144-146°C; R_f = 0.13. ^{1}H-NMR (DMSO-*d*$_6$): (δ) 3.36-3.63 (m, 4H, CH_2), 3.77 (s, 3H, OCH_3), 4.23-4.63 (m, 4H, CH_2), 6.95-7.03 (m, 3H, ArH), 7.45-7.49 (m, 1H, ArH), 7.50 (s, 2H, NH_2), 7.61-7.64 (m, 2H, ArH). 7.87-7.90 (m, 2H, ArH). Anal. for ($C_{19}H_{21}N_3O_5S$): C 56.56%, H 5.25%, N 10.42%; Found: C 56.36%, H 5.05%, N 10.22%.

4-[4-(4-Methoxybenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5m**
Yield: 52%; M.p.: 260-261°C; R_f = 0.12.^{1}H-NMR (DMSO-*d*$_6$): (δ) 3.34-3.47 (m, 4H, CH_2), 3.56-3.68 (m, 4H, CH_2), 3.71 (s, 3H, OCH_3), 6.97-7.01 (m, 2H, ArH), 7.37-7.41 (m, 2H, ArH), 7.50 (s, 2H, NH_2), 7.63-7.65 (d, *J*= 8.2, 2H, ArH). 7.86-7.90 (d, *J*= 8.2, 2H, ArH). Anal. for ($C_{19}H_{21}N_3O_5S$): C 56.56%, H 5.25%, N 10.42%; Found: C 56.46%, H 5.15%, N 10.32%.

4-[4-(2-Nitrobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5n**
Yield: 55%; M.p.: 231-232°C; R_f = 0.16. ^{1}H-NMR (DMSO-*d*$_6$): (δ) 3.37-3.77 (m, 8H, CH_2), 7.48 (m, 2H, ArH), 7.56-7.77 (m, 4H, ArH), 7.83-7.96 (m, 3H, ArH), 8.20-8.25 (m, 1H, ArH). Anal. for ($C_{18}H_{18}N_4O_6S$): C 51.67%, H 4.34%, N 13.39%; Found: C 51.71%, H 4.39%, N 13.44%.

4-[4-(3-Nitrobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5o**
Yield: 75%; M.p.: 243-244°C; R_f =0.18 . ^{1}H-NMR (DMSO-*d*$_6$): (δ) 3.38-3.49 (m, 4H, CH_2), 3.65-3.74 (m, 4H, CH_2), 7.45 (s, 2H, NH_2), 7.61-8.29 (m, 8H, ArH). Anal. for ($C_{18}H_{18}N_4O_6S$): C 51.67%, H 4.34%, N 13.39%; Found: C 51.70%, H 4.28%, N 13.53%.

4-[4-(4-Nitrobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5p**
Yield: 77%; M.p.: 272-273°C; R_f = 0.18. ^{1}H-NMR (DMSO-*d*$_6$): (δ) 3.30-3.40 (m, 4H, CH_2), 3.64-3.74 (m, 4H, CH_2), 7.46 (m, 2H, ArH), 7.61-8.29 (m, 8H, ArH). Anal. for ($C_{18}H_{18}N_4O_6S$): C 51.67%, H 4.34%, N 13.39%; Found: C 51.77%, H 4.44%, N 13.49%.

4-[4-(2-Aminobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5q**
Yield: 99%; m.p.: 231-232°C; R_f = 0.10. ^{1}H-NMR (DMSO-*d*$_6$): (δ) 3.36-3.76 (m, 8H, CH_2), 5.20 (bs, 2H, NH_2), 6.56 (t, *J*=7.6, *J*=7.4, 1H, ArH), 6.70 (d, *J*=8.1, 1H, ArH), 7.01 (d, *J*=7.6, 1H, ArH), 7.08 (t, *J*= 8.1, *J*=7.4, 1H, ArH), 7.49 (s, 2H, NH_2), 7.60 (d, *J*=8.0, 2H, ArH), 7.88 (d, *J*=8.0, 2H, ArH). Anal. for ($C_{18}H_{20}N_4O_4S$): C 55.66%, H 5.19%, N 14.42%; Found: C 55.63%, H 5.16%, N 14.39%.

4-[4-(3-Aminobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5r**
Yield: 87%; m.p.: 220-223°C; R_f =0.13. ^{1}H-NMR (DMSO-*d*$_6$): (δ) 3.40-3.66 (m, 8H, CH_2), 5.26 (bs, 2H, NH_2), 6.50 (d, *J*=7.5, 1H, ArH,), 6.57 (s, 1H, ArH), 6.62 (d, *J*= 7.5, 1H, ArH,), 7.07 (t, 1H, ArH,

J= 7.5), 7.48 (s, 2H, NH_2), 7.62 (d, *J*=8.2, 2H, ArH), 7.89 (d, *J*=8.2, 2H, ArH). Anal. for ($C_{18}H_{20}N_4O_4S$): C 55.66%, H 5.19%, N 14.42%; Found: C 55.63%, H 5.16%, N 14.39%.

4-[4-(4-Aminobenzoyl)piperazine-1-carbonyl]benzenesulfonamide, **5s**
Yield: 50%; m.p.: 229-230°C; R_f = 0.14. ^{1}H-NMR (DMSO-d_6): (δ) 3.49-3.65 (m, 8H, CH_2), 5.53 (bs, 2H, NH_2), 6.52 (d, *J*=8.4, 2H, ArH), 7.13 (d, *J*=8.4, 2H, ArH), 7.46 (s, 2H, NH_2), 7.59 (d, *J*=8.5, 2H, ArH), 7.86 (d, *J*=8.5, 2H, ArH). Anal. for ($C_{18}H_{20}N_4O_4S$): C 55.66%, H 5.19%, N 14.42%; Found: C 55.62%, H 5.15%, N 14.38%.

1.2 CA Inhibition Assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 – 20 mM Hepes (pH 7.5) or Tris (pH 8.3) as buffers, and 20 mM Na_2SO_4 or 20 mM $NaClO_4$ (for maintaining constant the 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 have been 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 (10 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to 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, as reported earlier, and represent the mean from at least three different determinations. CA isoforms were recombinant ones obtained as reported earlier by this group.[1-3]

1.3 X-ray crystallographic studies

Crystals of the hCA II/**5a** adduct were obtained by co-crystallization. In detail, the complex was prepared by adding a 5 fold excess of the inhibitor to a 10 mg/mL protein solution. The mixture was equilibrated for 1 h at room temperature and used for the crystallization experiments. Drops were prepared by mixing 1 μl of hCA II/**5a** solution with 1 μl of precipitant solution containing 1.2 M sodium citrate, 0.1 M Tris–HCl pH 8.5 and further equilibrated over a well containing 1 mL of precipitant buffer. Crystals appeared in the drops within 2 days and grew to a maximum dimension of 0.3x0.3x0.2 mm^3 in about one week.

X-ray diffraction data were collected at the Elettra Synchrotron Light Source (Trieste) by using one single crystal. Prior to cryogenic cooling, the crystal was transferred into precipitant solution with the addition of 15% (v/v) glycerol. Diffraction data were indexed, integrated and scaled using the HKL2000 software package.[4] Data collection statistics are reported in Table S1.

The initial phases of the structure were calculated using the atomic coordinates of the native hCA II with waters removed.[5] The structure was refined using the program REFMAC5.8[6] in CCP4i[7], whereas model building and map inspections were performed with the program O.[8] Inhibitor topologies and parameters were generated using SKETCHER.[9] Several rounds of restrained refinement and anisotropic temperature factor refinement alternated with manual rebuilding were necessary to reduce the crystallographic Rwork/Rfree values to 0.135/0.146. Refinement statistics are summarized in

Table S1. The stereochemical quality of the model was finally checked using Procheck[10] and Whatcheck[11] programs.
Coordinates and structure factors have been deposited in the Protein Data Bank (accession code 6XXT).

1.4 Docking studies

The crystal structures of hCA XII in complex with the inhibitor acetazolamide was retrieved from the RCSB Protein Data Bank (PDB code 1JD0)[12] The protein and ligands were prepared by Vega[13] and used to setup docking simulation by Gold Suite 5.7.1[14]
The region of interest used by Gold program was defined in order to contain residues within 10 Å from the original position of the ligand in the X-ray structure. A scaffold constraint (penalty = 5.0) was used to restrict the solutions in which the sulfonamide moiety was able to coordinate the metal within the catalytic binding site. ChemPLP was chosen as fitness function and the standard default settings were used in all calculations. Ligands were submitted to 100 genetic algorithm runs and the "allow early termination" command was deactivated. Results differing by less than 0.75 Å in ligand-all atom RMSD, were clustered together. The best GOLD-calculated conformation was considered both for analysis and representation.

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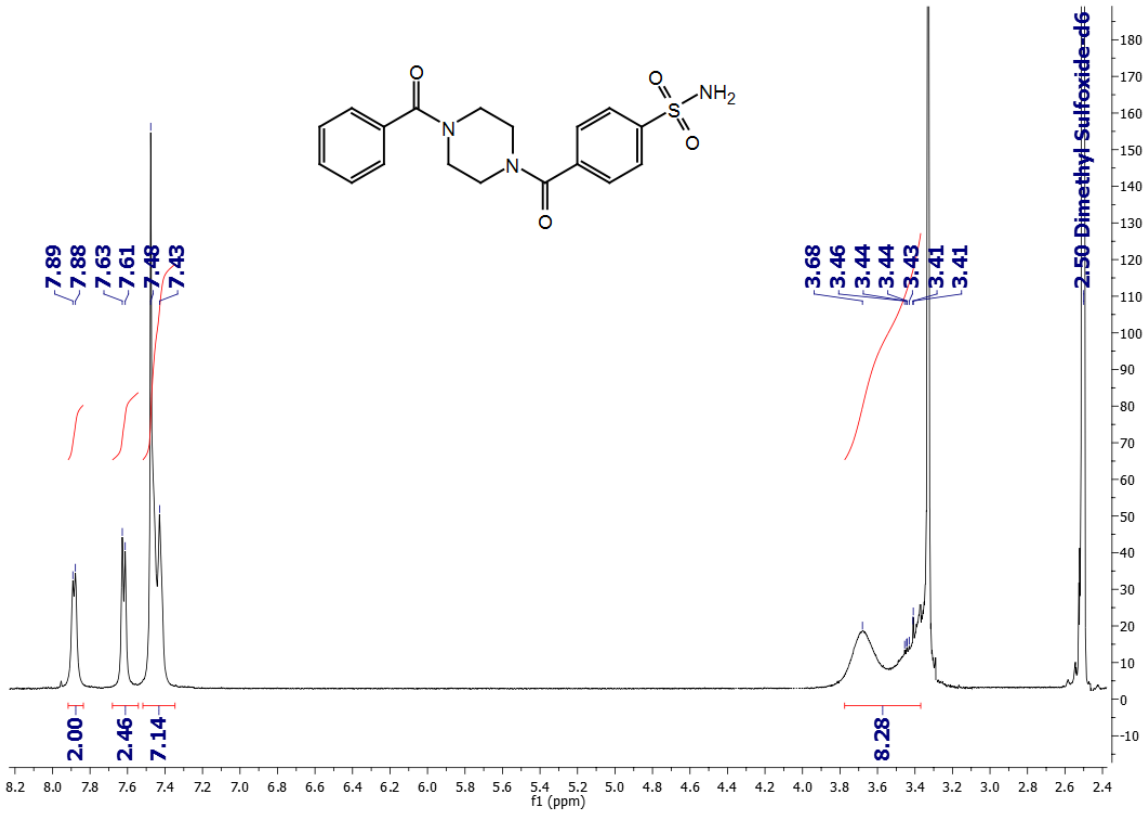
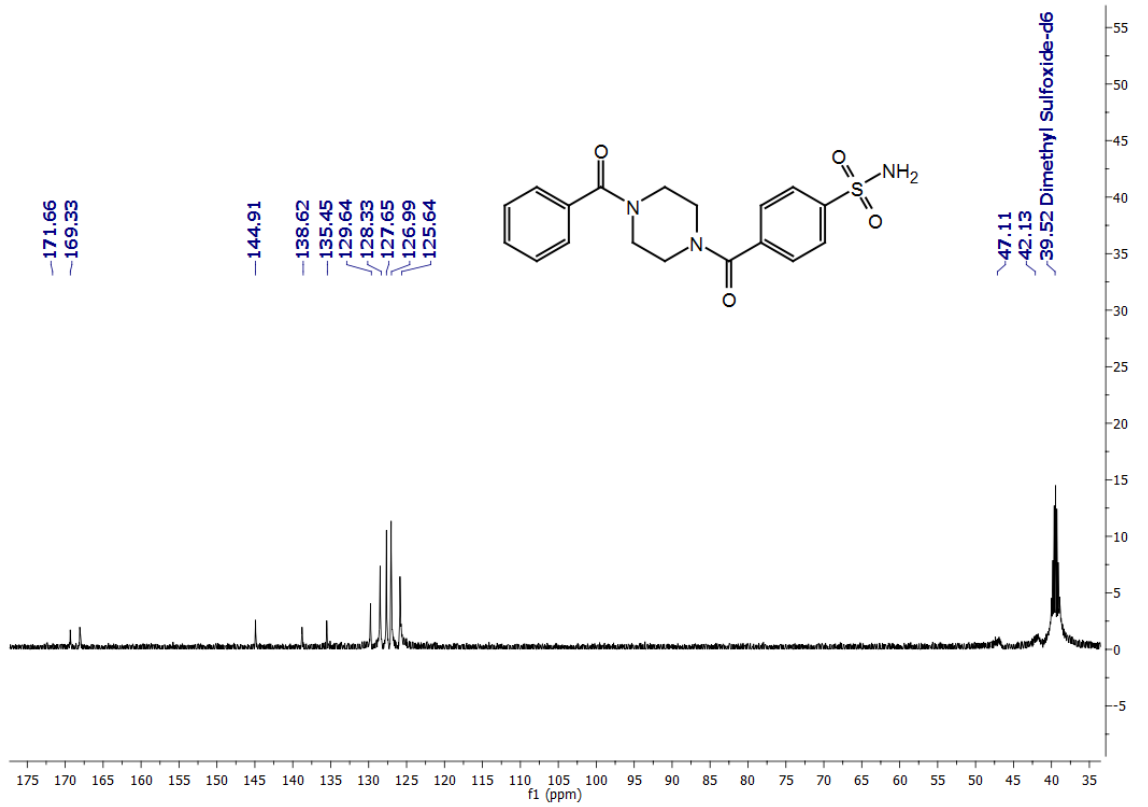
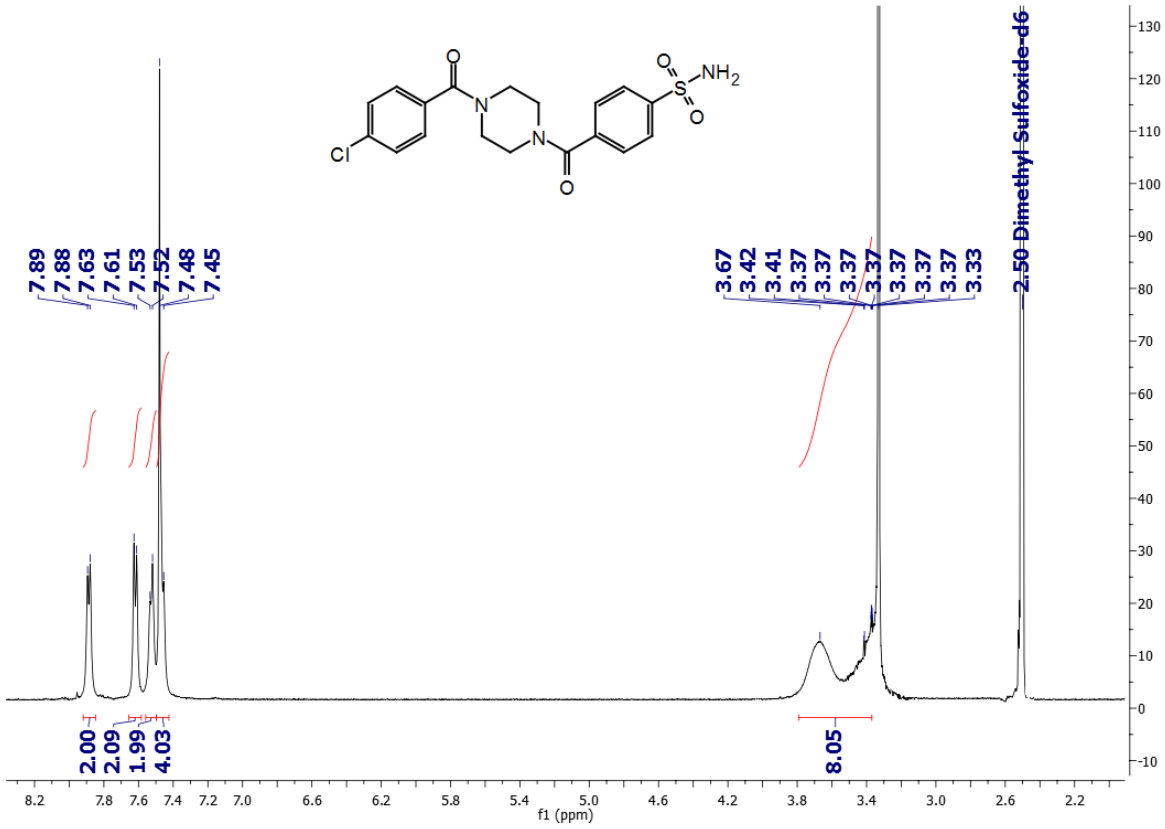
2. *Selected representative* ^{1}H and ^{13}C NMR spectra



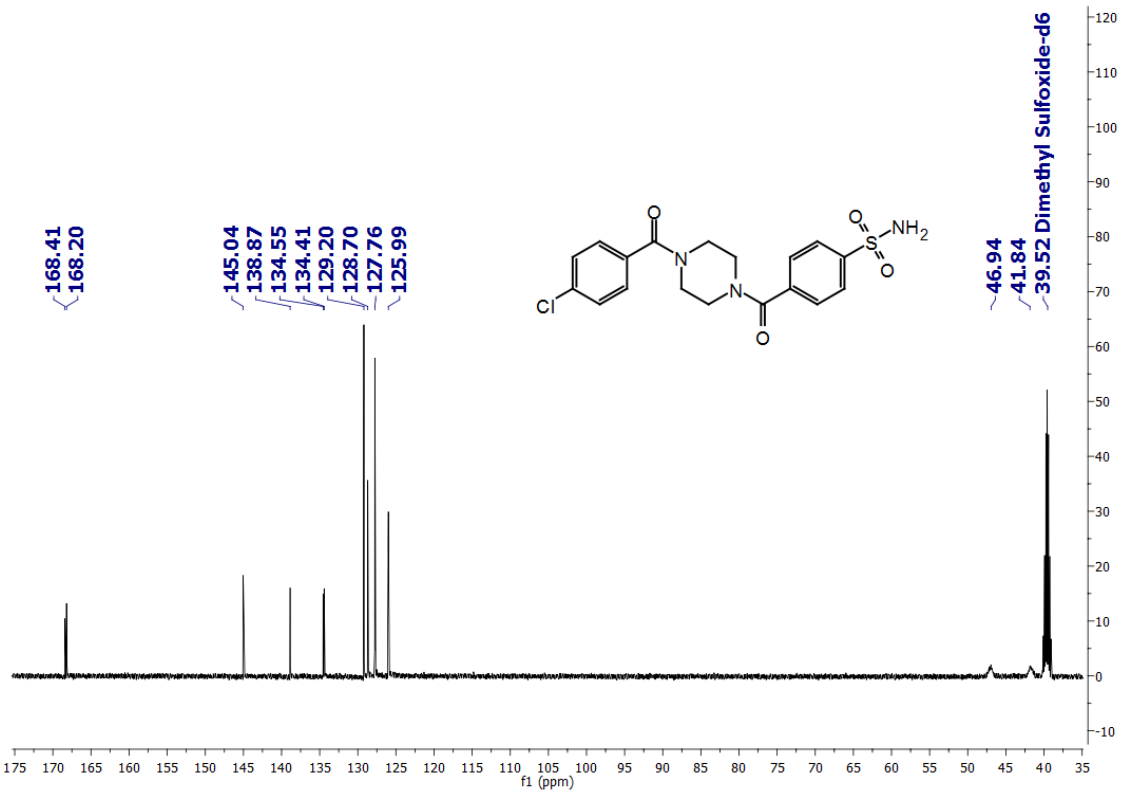
^{1}H-NMR (DMSO-d_6) spectrum of *4-[4-(4-Aminobenzoyl)piperazine-1-carbonyl]benzenesulfonamide* ***(5a)***



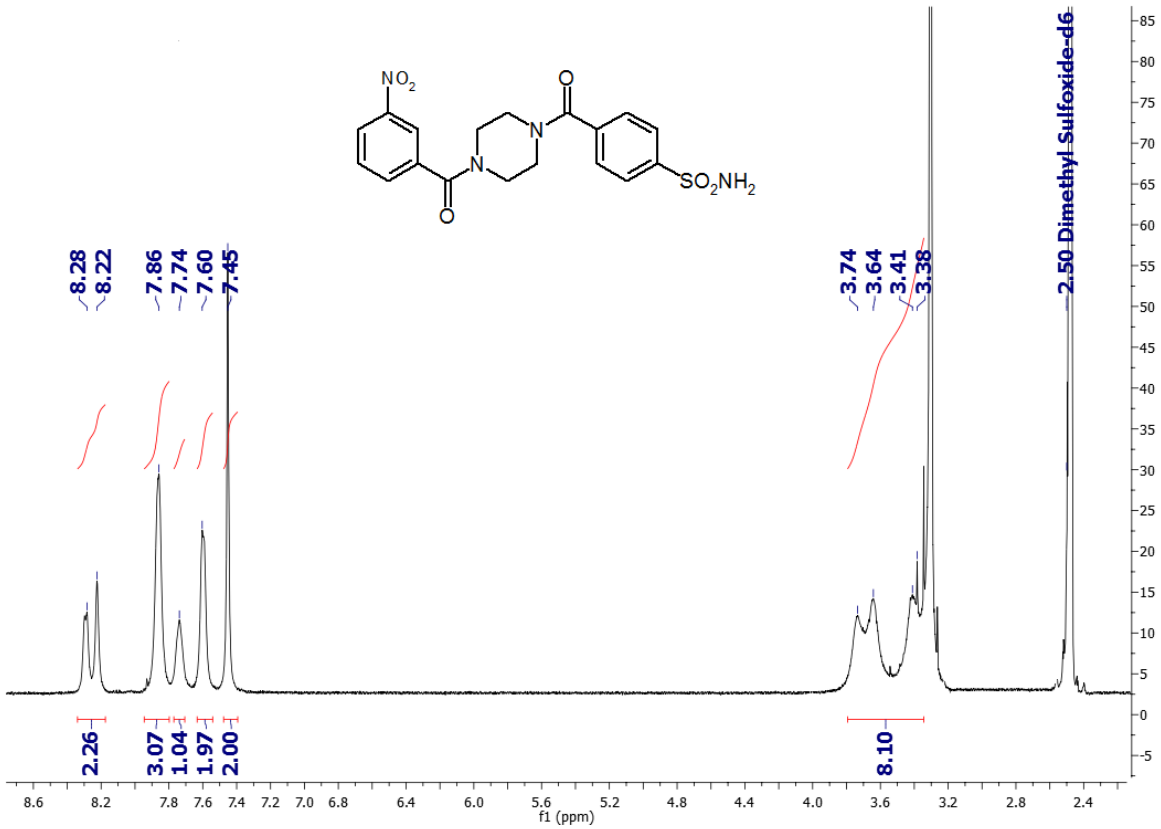
^{13}C-NMR (DMSO-d_6) spectrum of *4-[4-(4-Aminobenzoyl)piperazine-1-carbonyl]benzenesulfonamide* ***(5a)***



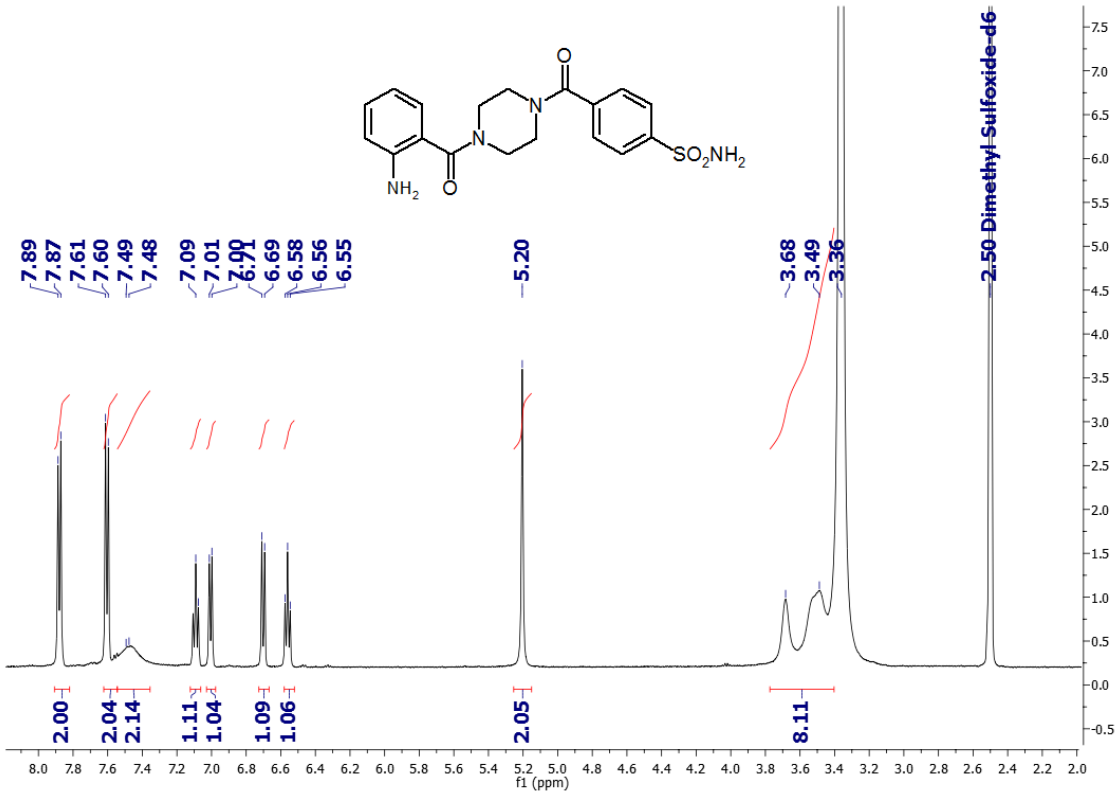
^{1}H-NMR (DMSO-d_6) spectrum of 4-[4-(4-Chlorobenzoyl)piperazine-1-carbonyl]benzenesulfonamide ***(5g)***



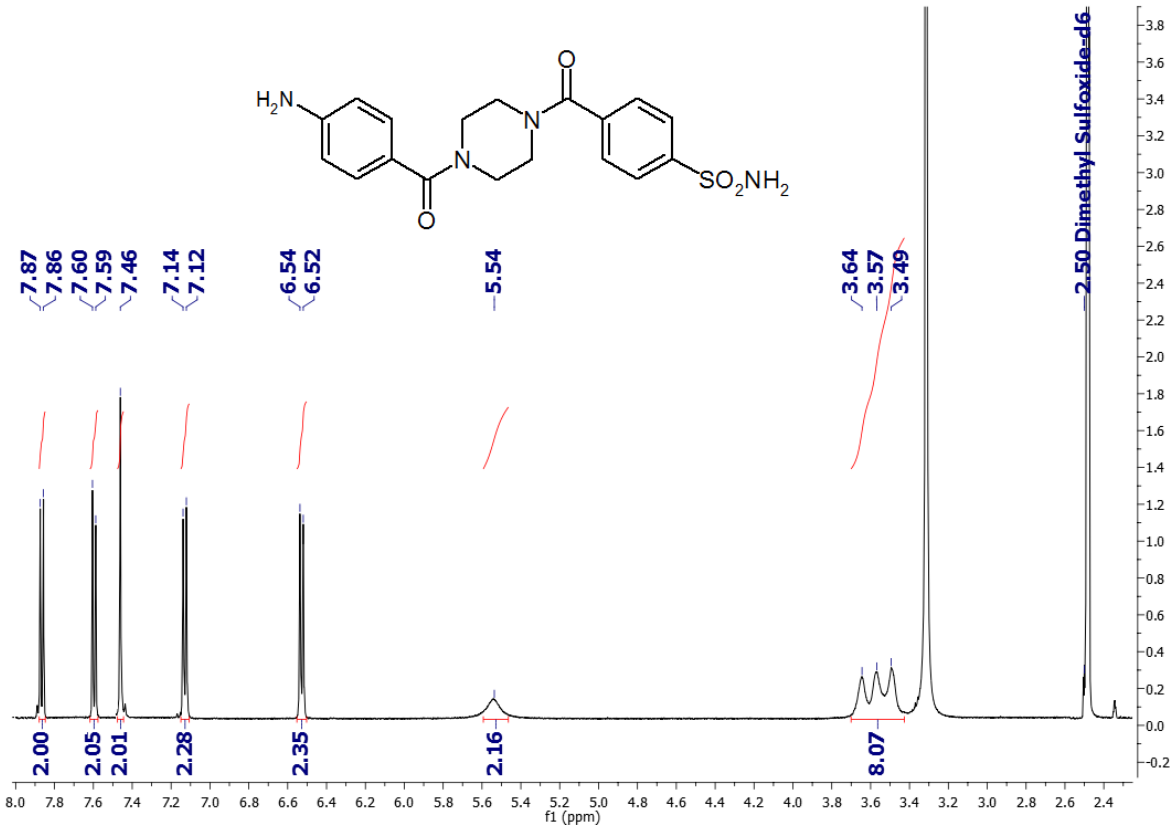
^{13}C-NMR (DMSO-d_6) spectrum of 4-[4-(4-Chlorobenzoyl)piperazine-1-carbonyl]benzenesulfonamide ***(5g)***



^{1}H-NMR (DMSO-d_6) spectrum of *4-[4-(3-Nitrobenzoyl)piperazine-1-carbonyl]benzenesulfonamide* (***5o***)



^{1}H-NMR (DMSO-d_6) spectrum of *4-[4-(2-Aminobenzoyl)piperazine-1-carbonyl]benzenesulfonamide* (***5q***)



^{1}H-NMR (DMSO-d_6) spectrum of *4-[4-(4-Aminobenzoyl)piperazine-1-carbonyl]benzenesulfonamide* ***(5s)***

3. Table S1

Data collection and refinement statistics for hCA II/**5a** complex

	hCA II/5a
Cell parameters	
Space group	$P2_1$
Cell dimensions (Å, °)	a= 42.3
	b= 41.5
	c= 72.1
	β= 104.3
Number of independent molecules	1
Data collection statistics	
Wavelength (Å)	1.0
Resolution limits (Å)	35.7-1.05
Total reflections	652807
Unique reflections	112517
Redundancy	5.8
Completeness (%)	99.2 (86.9)
R-merge*	0.072 (0.277)
Rmeas§	0.078 (0.323)
Rpim¶	0.031 (0.159)
<I>/<σ(I)>	22.1 (4.3)
Refinement statistics	
Resolution limits (Å)	35.7-1.05
R-work** (%)	13.5
R-free** (%)	14.6
r.m.s.d. from ideal geometry:	
Bond lengths (Å)	0.008
Bond angles (°)	1.5
Number of protein atoms	2129
Number of inhibitor atoms	26
Number of water molecules	271
Average B factor (Å^2)	
All atoms	16.03
Protein atoms	10.54
Inhibitor atoms	13.49
Waters	21.36
PDB accession code	6XXT

*R-merge = $\Sigma_{hkl}\Sigma_i|I_i(hkl)-\langle I(hkl)\rangle|/\ \Sigma_{hkl}\Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of an observation and $\langle I(hkl)\rangle$ is the mean value for its unique reflection; summations are over all reflections; §Rmeas = $\Sigma_{hkl}\{N(hkl)/[N(hkl)-1]\}^{1/2}x\Sigma_i|I_i(hkl)-\langle I(hkl)\rangle|/\ \Sigma_{hkl}\Sigma_i I_i(hkl)$; ¶Rpim= $\Sigma_{hkl}\{1/[N(hkl)-1]\}^{1/2}x\Sigma_i|I_i(hkl)-\langle I(hkl)\rangle|/\ \Sigma_{hkl}\Sigma_i I_i(hkl)$; **R-work = $\Sigma_{hkl}||Fo(hkl)| - |Fc(hkl)||/\Sigma_{hkl}|Fo(hkl)|$ calculated for the working set of reflections. R-free is calculated as for R-work, but from data of the test set that was not used for refinement (Test Set Size (%) = 1.0). Values in parentheses refer to the highest resolution shell (1.07-1.05 Å).