Supplementary Material to the ms:

Dithiocarbamates: a new class of carbonic anhydrase inhibitors. Crystallographic and kinetic investigations

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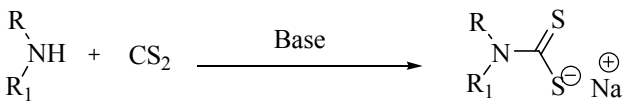
Chemistry. ^{1}H, ^{13}C, DEPT, COSY, HMQC and HMBC spectra were recorded using a Bruker Advance III 400 MHz spectrometer. The chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). For all new compounds DEPT, COSY, HMQC and HMBC were routinely used to definitely assign the signals of ^{1}H and ^{13}C. Infrared spectra were recorded on a Perkin Elmer Spectrum R XI spectrometer as solids on KBr plates. Melting points (m.p.) were measured in open capillary tubes, unless otherwise stated, using a Büchi Melting Point B-540 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} aluminum backed plates. Elution of the plates was carried out using ethyl acetate/*n*-hexane or MeOH/DCM systems. Visualization was achieved with UV light at 254 nm, by dipping into a 0.5 % aqueous potassium permanganate solution, by Hanessian's Stain solution and heating with a hot air gun or by exposure to iodine. All other solvents and chemicals were used as supplied from Aldrich Chemical Co., Acros, Fisher, Alfa Aesar or Lancaster Synthesis. Dimethyl-dithiocarbamate sodium salt **1** and Diethyl-DTC sodium salt **2** are commercially available from Sigma-Aldrich, Milan, Italy. The amines **A-G** used for the preparation of the other DTCs were commercially available (from Sigma-Aldrich, Milan, Italy), as follows: dipropylamine **A** (CAS 142-84-7), dibutylamine **B** (CAS 111-92-2), diisobutylamine **C** (CAS 110-96-3), ethylbutyamine **D** (CAS 13360-63-9), *N*-methylbenzenamine **E** (CAS 100-61-8), *N,N*-benzylmethylamine **F** (CAS 103-67-3), 4-Cyano-4-phenylpiperidine hydrochloride **G** (CAS 51304-58-6). Carbon disulfiode and sodium hydroxide were the highest purity available reagents from the same company.

General procedure for the synthesis of compounds 3-9.[1]

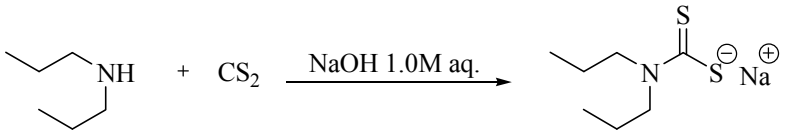


A-G **3-9**

Scheme 1: Preparation of DTCs **1-9** by reaction of amines **A-G** with carbon disulfide in the presence of base (NaOH).

Secondary/primary amines **A-G** (1.0 g, 1.0 eq) were treated with a NaOH (1.0 - 2.2 eq), 4.0 ml of MeOH as co-solvent was used, and the solutions were stirred at 0°C for 20 min (Scheme 1). Then carbon disulfide (1.2 - 2.4 eq) was added dropwise and the mixture was stirred at r.t. until the starting material was consumed (TLC monitoring). The solvents were removed under *vacuo* at r.t. and the residues obtained were dissolved in MeOH, filtered off trough Celite and the filtrate was concentrated *in vacuo* not exceeding 20 °C.

Synthesis of dipropylcarbamodithioate sodium salt **3**

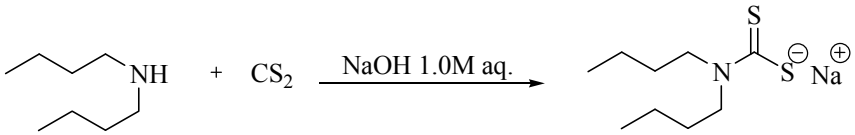


A **3**

Dipropylamine **A** (1.0 g, 1.0 eq) was treated according to the general procedure with 1.0 M aqueous solution of NaOH (1.0 eq) followed by addition of carbon disulfide (1.2 eq). The title compound was obtained as a white semisolid in 87 % yield.

Dipropylcarbamodithioate sodium salt **3**: m.p.112- 114 °C ; ν_{max} (KBr) cm^{-1}, 2961, 2930, 2871, 1635, 1520, 1470, 1198; δ_H (400 MHz, DMSO-d_6) 0.82 (6H, t, *J* 7.6, 2 x CH_3), 1.65 (4H, m, 2 x CH_2), 3.90 (4H, m, 2 x CH_2); δ_C (100 MHz, DMSO-d_6) 12.3 (CH_3), 21.0, 55.2, 213.5 (*C*=S); *m/z* (ESI), 176 $[M-Na]^-$. Data are in agreement with reported data.[2]

Synthesis of dibutylcarbamodithioate sodium salt **4**.



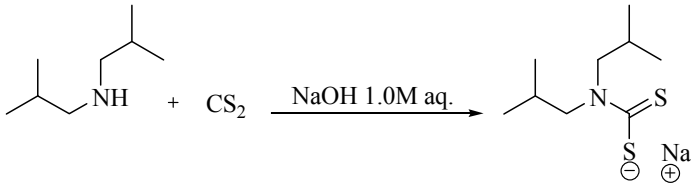
B **4**

Dibutylamine **B** (1.0 g, 1.0 eq) was treated according to the general procedure with 1.0 M aqueous solution of NaOH (1.0 eq) followed by addition of carbon disulfide (1.2 eq). The title compound was obtained as a white solid in 84 % yield.

Dibutylcarbamodithioate sodium salt **4**: semisolid at r.t. ; ν_{max} (KBr) cm^{-1}, 2959, 2935, 2870, 1637, 1520, 1475, 1190; δ_H (400 MHz, DMSO-d_6) 0.91 (6H, t, *J* 8.0, 2 x CH_3), 1.26 (4H, m, 2 x CH_2),

1.62 (4H, m, 2 x CH_2), 3.95 (4H, m, 2 x CH_2); δ_C (100 MHz, DMSO-d_6) 14.9 (*C*H_3), 20.7, 30.0, 53.0, 213.5 (*C*=S); *m/z* (ESI), 204 [M-Na]$^-$.
Data are in agreement with reported data.[3]

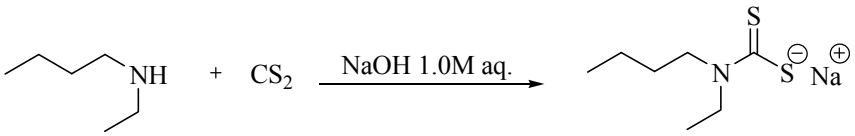
Synthesis of diisobutylcarbodithioic acid sodium salt **5**.



C **%**

Diisobutylamine **C** (1.0 g, 1.0 eq) was treated according to the general procedure described above with 1.0 M aqueous solution of NaOH (1.0 eq) followed by addition of carbon disulfide (1.2 eq). The title compound was obtained as a white solid in 83 % yield.
Diisobutylcarbodithioic acid sodium salt **5**: m.p. 220 °C with dec; ν_{max} (KBr) cm^{-1}, 2961, 2933, 2867, 1640, 1601, 1520, 1480, 1090; δ_H (400 MHz, DMSO-d_6) 0.84 (12H, d, *J* 6.8, 4 x CH_3), 2.43 (4H, m, 2 x C*H*), 3.86 (4H, d, *J* 7.2, 2 x CH_2); δ_C (100 MHz, DMSO-d_6) 21.2, 27.4, 61.5, 215.4 (*C*=S); *m/z* (ESI), 204 [M-Na]$^-$. Data are in agreement with reported data.[4]

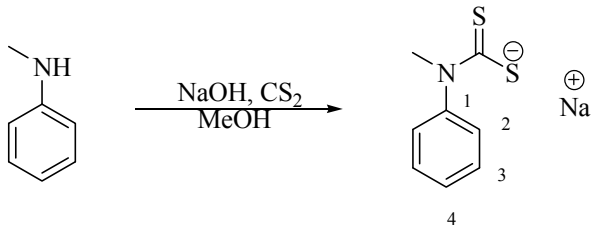
Synthesis of ethylbutylcarbamodithioate sodium salt **6**.



D **6**

Ethylbutyamine **D** (1.0 g, 1.0 eq) was treated according to the general procedure with 1.0 M aqueous solution of NaOH (1.0 eq) followed by addition of carbon disulfide (1.2 eq). The title compound was obtained as a pale yellow semisolid in 98 % yield.
Ethylbutylcarbamodithioate sodium salt **6**: m.p. 71-73 °C; ν_{max} (KBr) cm^{-1}, 2958, 2929, 2860, 1641, 1626, 1520, 1409, 1195; δ_H (400 MHz, DMSO-d_6) 0.92 (3H, t, *J* 8.0, CH_3), 1.12 (3H, t, *J* 8.0, CH_3), 1.27 (2H, m, CH_2), 1.61 (2H, m, CH_2), 3.95 (2H, m, CH_2), 4.03 (2H, q, *J* 8.0, CH_2); δ_C (100 MHz, DMSO-d_6) 13.4 (*C*H_3), 14.9 (*C*H_3), 20.8, 30.1, 47.6, 52.5, 213.3 (*C*=S); *m/z* (ESI), 176 [M-Na]$^-$.

Synthesis of sodium methyl(phenyl)carbamodithioate **7**

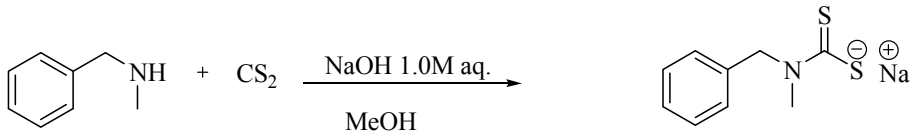


E **7**

N-methylbenzenamine **E** (0.5 g, 1.0 eq) was treated with powdered NaOH (1.0 eq) in MeOH (50 ml) followed by addition of carbon disulfide (1.0 eq) at 0°C. The mixture was warmed to r.t. and stirred for 5h at 40°C then cooled to r.t., filtered through Celite and the solvent removed in vacuo to give a solid that was triturated from diethyl ether to afford the titled compound as a pale yellow solid in 51 % yield.

Sodium methyl(phenyl)carbamodithioate **7:** ν_{max} (KBr) cm^{-1}, 2958, 2890, 1630, 1582, 1520, 1450; δ_H (400 MHz, DMSO-d_6) 3.66 (3H, s, CH_3), 7.17 (3H, m, 2x 2-H, 4-H), 7.29 (2H, dd, *J* 8.3, 7.2, 2x 3-H); δ_C (100 MHz, DMSO-d_6) 46.7, 125.7, 128.3, 129.0, 151.6, 216.9 (*C*=S); *m/z* (ESI), 182 [M-Na]$^-$.

Synthesis of *N,N*-benzylmethylcarbamodithioate sodium salt **8**.

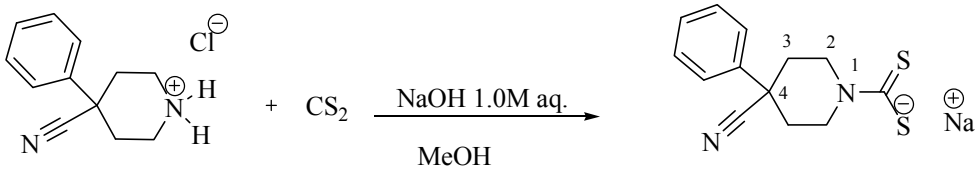


F **8**

N,N-Benzylmethylamine **F** (1.0 g, 1.0 eq) was treated according to the general procedure with 1.0 M aqueous solution of NaOH (1.2 eq) followed by addition of carbon disulfide (2.4 eq). The title compound was obtained as a white solid in 97% yield.

N,N-Benzylmethylcarbamodithioate sodium salt **8**: m.p. 258-260 °C; ν_{max} (KBr) cm^{-1}, 2960, 2930, 1643, 1626, 1520, 1346, 1080; δ_H (400 MHz, DMSO-d_6) 3.32 (3H, s, CH_3), 5.50 (2H, s, CH_2), 7.26 (5H, m, Ar-*H*); δ_C (100 MHz, DMSO-d_6) 41.5 (*C*H$_3$), 58.4 (*C*H$_2$), 127.3, 128.2, 128.9, 140.0 (ipso), 216.1 (*C*=S); *m/z* (ESI), 196 [M-Na]$^-$.

Synthesis of 4-cyano-4-phenylpiperidinecarbamodithiate sodium salt **9**.



G **9**

4-Cyano-4-phenylpiperidine hydrochloride **G** (1.0 g, 1.0 eq) was treated according to the general procedure with 1.0 M aqueous solution of NaOH (2.2 eq) followed by addition of carbon disulfide (1.2 eq). The title compound was obtained as a white solid in 93 % yield.

4-Cyano-4-phenylpiperidinecarbamodithiate sodium salt **9**: m.p. > 320 °C with dec; ν_{max} (KBr) cm^{-1}, 2961, 2891, 1648, 1598, 1520, 1414, 1186; δ_H (400 MHz, DMSO-d_6) 1.89 (2H, td, *J* 12.8, 3.6, 2 x $3H_{ax}$), 2.14 (2H, d, *J* 12.8, 2 x $3H_{eq}$), 3.15 (2H, t, *J* 12.8, 2 x $2H_{ax}$), 6.15 (2H, d, *J* 12.8, 2 x $2H_{eq}$); δ_C (100 MHz, DMSO-d_6) 36.6, 43.3, 51.1, 123.2, 126.5, 129.0, 130.0 (ipso), 141.0, 216.1 (*C*=S); *m/z* (ESI), 261 $[M-Na]^-$.

Co-Crystallization and X-ray data collection of CA II complexes. Co-crystals for each of the two CA II – compound complexes were obtained using the hanging drop vapor diffusion method.[5] Drops of 10 µL (0.3 mM hCA II; 0.7 mM drug; 0.1 % Dimethyl Sulfoxide (DMSO); 0.8 M Sodium Citrate; 50 mM Tris-HCl; pH 8.0) were equilibrated against the precipitant solution (1.6 M sodium citrate; 50 mM Tris-HCl; pH 8.0) at room temperature (~20 ºC), for both the compounds. Crystals were observed after 5 days. Based on visual selection a crystal of both of the CA II - complexes was cryoprotected by quick immersion into 20% glycerol precipitant solution and flash-cooled by exposing to a gaseous stream of nitrogen at 100 K. The X-ray diffraction data was collected using an R-AXIS IV^{++} image plate system on a Rigaku RU-H3R Cu rotating anode operating at 50 kV and 22 mA, using Osmic Varimax HR optics. The detector-crystal distance was set to 80 mm. The oscillation steps were 1° with a 5 min exposure per image. Indexing, integration, and scaling were performed using HKL2000.[6]

Structure determination of CA II drug complexes. Starting phases were calculated from Protein Data Bank (PDB) entry 3KS3[7] with waters removed. Refinement using *Phenix* package,[8] with 5% of the unique reflections selected randomly and excluded from the refinement data set for the purpose of R_{free} calculations,[9] was alternated with manual refitting of the model in *Coot*.[10] The validity of the final model was assessed by *PROCHECK*.[11] Complete refinement statistics and model quality are included in Table S1.

Table S1: Crystallographic data refinement and model quality statistics.

PDB accession number	**3P58**	**3P5L**
Compound	**8**	**9**
Data-collection statistics		
Temperature (K)	100	100
Wavelength (Å)	1.5418	1.5418
Space group	$P2_1$	$P2_1$
Unit-cell parameters (Å,º)	*a* = 42.2 *b* = 41.1 *c* = 71.9 *β* = 104.4	*a* = 42.3 *b* = 41.3 *c* = 72.2 *β* = 104.4
Total theoretical reflections	39387	39014
Total measured reflections	38521	38468
Resolution (Å) [a]R_{sym} (%) I/σ(I) Completeness Redundancy	50.0-1.5 (1.54-1.50)* 6.4 (39.7) 20.2 (3.3) 97.8 (91.7) 4.3 (4.0)	50.0-1.5 (1.55-1.50) 6.7 (37.1) 16.3 (3.2) 98.6 (96.3) 3.4 (3.2)
Final Model Statistics		
[b]R_{cryst} (%) [c]R_{free}(%)	0.150 0.171	0.166 0.190
Residue Nos.	4-261	4-261
[d]No. of protein atoms	2114	2123
No. of compound atoms	12	17
No. of H_2O molecules	291	172
R.M.S.D. bond lengths (Å) bond angles (º)	0.013 1.532	0.012 1.438
Ramachandran statistics (%) Most favored, allowed and outliers	87.9, 12.1, 0.0	87.5, 12.5, 0.0
Average B factors (Å^2) Main-, side-chain, compound, solvent	17.0, 21.5, 20.0, 32.2	14.7, 17.8, 20.7, 25.3

[a]$R_{sym} = \Sigma |I - \langle I \rangle| / \Sigma \langle I \rangle$. [b]$R_{cryst} = (\Sigma ||Fo| - |Fc|| / \Sigma |F_{obs}|) \times 100$. [c]R_{free} is calculated in same manner as R_{cryst}, except that it uses 5% of the reflection data omitted from refinement. [d]Includes alternate conformations. *Values in parenthesis represent highest resolution bin

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