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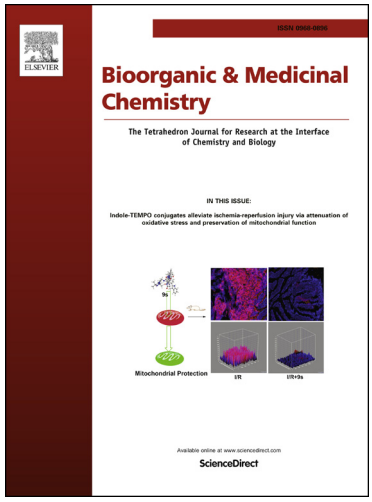

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N-Substituted and ring opened saccharin derivatives selectively inhibit transmembrane, tumor-associated carbonic anhydrases IX and XII

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Abstract. A series of *N*-substituted saccharins incorporating aryl, alkyl and alkynyl moieties, as well as some ring opened derivatives were prepared and investigated as inhibitors of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1). The widespread cytosolic isoforms CA I and II were not inhibited by these sulfonamides whereas transmembrane, tumor-associated ones were effectively inhibited, with K_Is in the range of 22.1 – 481 nM for CA IX and of 3.9 – 245 nM for hCA XII. Although the inhibition mechanism of these tertiary/secondary sulfonamides is unknown for the moment, the good efficacy and especially selectivity for the inhibition of the tumor-associated over the cytosolic, widespread isoforms, make these derivatives of considerable interest as enzyme inhibitors with various pharmacologic applications.

Keywords: carbonic anhydrase; inhibitor; saccharin; secondary/tertiary sulfonamide; isoform selectivity

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1. Introduction

The carbonic anhydrases (CAs, EC 4.2.1.1) catalyze CO_2 hydration to bicarbonate and protons and the corresponding reverse reaction between bicarbonate and protons, being among the most effective catalysts known in Nature.[1,2] Since these small molecules/ions are involved in many physiologic processes connected to pH regulation, general homeostasis of cell/organisms, electrolyte secretion, ion transport, metabolic pathways involving among others gluconeogenesis, urea and lipid biosynthesis, as well as tumorigenesis, their production through the CA-catalyzed reaction leads to pharmacologic responses which have been exploited for decades.[1-3] Indeed, the primary sulfonamides, the most investigated class of CA inhibitors (CAIs) has applications as diuretics, antiglaucoma, anticonvulsant and antiobesity drugs, whereas in the last period anticancer/antimetastatic properties of these pharmacologic agents were also investigated in detail.[1-3]

Unlike the primary sulfonamides, the secondary/tertiary ones, although much investigated from the synthetic viewpoints,[4-7] have found less pharmacologic applications, although we and Klebe's group reported the interesting CA inhibitory effects of saccharin **1**,[8] an acylated secondary sulfonamide widely used as a sweetening agent.

Considering the fact that CAs are widespread enzymes not only in humans, but in most organisms on earth, with seven distinct genetic families known to date,[9-11] a large number of novel applications of their inhibitors and activators were investigated in the last decade, being shown that CAIs have a great potential in pathologies for which their role was not recognized till recently, such as neuropathic pain,[12] arthritis,[13] and cerebral ischemia.[14] In fact, several CA isoforms of the 12 catalytically active known so far in humans,[1,9,10] have recently been validated for these new pharmacologic applications. Thus, the development of isoform-selective CAIs is a challenging task, which has been successfully addressed through a variety of methods in the last period, starting with structure-based drug design of sulfonamide inhibitors and ending with the discovery of totally new chemotypes with such properties, among which the coumarins,[15] dithiocarbamates,[16] polyamines,[17] etc. Many of them show diverse inhibition mechanisms apart Zn(II) ion coordination, as shown schematically in Fig. 1. Among such new chemotypes with efficient CA inhibitory properties and isoform-selective inhibition profiles, the secondary/tertiary

sulfonamides were reported only several years ago.[18] As a particular example of such derivatives are also the saccharins,[2a,3] of which many examples have been investigated in detail by this and Carradori's group.[3a,b] Here we extend these earlier studies,[3a] and report a novel series of *N*-substituted saccharins and the corresponding opened ring compounds, which show interesting, isoform-selective CA inhibitory properties.

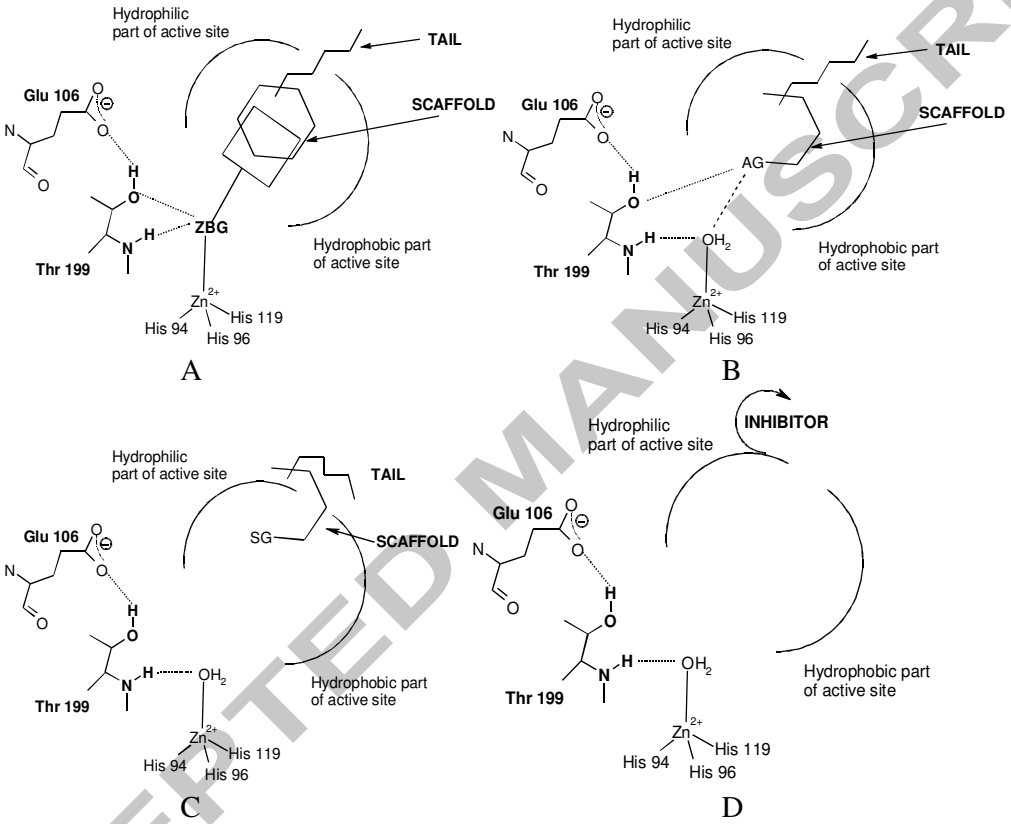


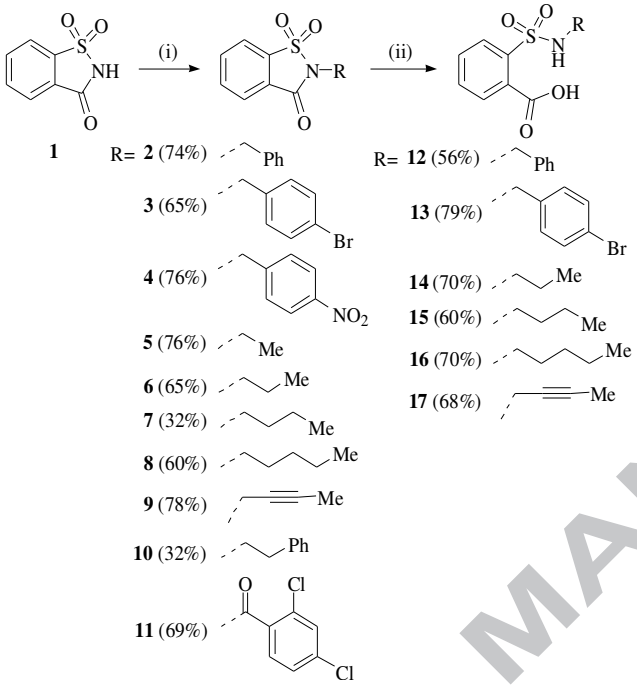
Fig. 1. CA inhibition mechanisms: A. Zinc-binders; B. Compounds which anchor to the metal ion coordinated water/hydroxide ion; C. Occlusion of the active site entrance. D. Out of the active site binding.[1,9]

2. Results and Discussions

2.1. Chemistry

Continuing our drug design of saccharin-type CAIs,[3] the synthesis of *N*-substituted saccharin derivatives (**2**–**11**) was performed according to the general procedure presented in Scheme 1. Considering the fact that several such derivatives were reported earlier (e.g., **3** and **11**) and that they showed an interesting biological activity,

we decided to increase the number of compounds bearing a similar substitution pattern as **3** and **11**, including both aromatic R moieties with a diverse substitution pattern, as well as aliphatic ones, which were less investigated to date for N-substituted saccharin CAIs.[2,3a]



Scheme 1. Reagents and conditions: (i) 1) K_2CO_3, H_2O, 2) R-Br, DMF, 90 ºC, 16h; (ii) NaOH, THF/MeOH/water (2:1:2), RT, 16h.

The procedure involves preparation of saccharin potassium salt by reaction saccharin (**1**) (1 equiv) with potassium carbonate (0.5 equiv) in water with following replacement of solvent and reaction with appropriate benzyl or alkyl bromide. *N*-benzyl (**2**-**4**) and alkyl (**5**-**10**) derivatives were mostly obtained in good yields. *N*-benzoyl derivative **11** was obtained by reaction of saccharin potassium salt prepared us above with corresponding benzoyl chloride in 69% yield. For the synthesis of derivatives of open-saccharin (**12**-**17**) we applied previous developed strategy, where corresponding saccharin derivative was hydrolyzed by treatment with sodium hydroxide with following acidic work-up. The desired carboxylic acids (**12**-**17**) were obtained in moderate to good yields.

2.2. Carbonic anhydrase inhibition

We assessed the CA inhibitory activity of compounds **1** - **17**, using the clinical drug acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide, **AAZ**) as positive control, for the inhibition of four CA isoforms of medical importance, hCA I and II (cytosolic, widely distributed enzymes) as well as hCA IX and XII (transmembrane, tumor-associated enzymes) – Table 1.

Table 1. CA inhibition data against isoforms hCA I, II, IX and XII with compounds **1-17** and acetazolamide (**AAZ**) as standard, by a stopped-flow CO_2 hydrase assay.[19]

Compound	K_I (nM)*			
	hCA I	hCA II	hCA IX	hCA XII
1 (SAC)	18,540	5950	103	633
2	>10,000	>10,000	89.2	18.7
3 [3a]	>10,000	>10,000	22.1	4.3
4[3a]	324	>10,000	1169	29.0
5	>10,000	>10,000	236	45.1
6	>10,000	>10,000	209	37.9
7	>10,000	>10,000	164	36.5
8	>10,000	>10,000	102	34.0
9	>10,000	>10,000	563	43.7
10	>10,000	>10,000	76.1	10.4
11[3a]	1480	>10,000	191	3.9
12	>10,000	>10,000	342	125
13	>10,000	>10,000	277	86.4
14	>10,000	>10,000	481	245
15	>10,000	>10,000	436	227
16	>10,000	>10,000	392	151
17	>10,000	>10,000	250	74.6
AAZ	250	12	25	5.6

*Errors in the range of ±5 % of the reported values, from three different assays. Compounds **3, 4** and **11** were already reported in ref. [3a]

Data of Table 1 show the following structure-activity relationship (SAR) for the inhibition of CAs with sulfonamides **2-17** investigated here:

(i) The cytosolic isoforms hCA I and II are not inhibited by the new sulfonamides up to 10 μM concentration of inhibitor in the assay system, a feature which has already been observed for other saccharin derivatives investigated earlier.[3] In fact, only two compounds show a weak hCA I inhibitory action, **4** and **11**, with K_Is in the range of 324-1480 nM (Table 1). This behavior of poor inhibitory action against these widespread cytosolic enzymes is a favorable feature for compounds for which isoform-selectivity is searched for, such as for example derivatives which should target the tumor-associated isoforms hCA IX and XII.[1,9-11]

(ii) Indeed, hCA IX was rather efficiently inhibited by most secondary/tertiary sulfonamides **2-17**, with K_Is in the range of 22.1 – 1169 nM (Table 1). It should be observed that saccharin itself is a medium potency CA IX inhibitor, with an inhibition constant of 103 nM,[8] whereas acetazolamide **AAZ** is roughly 4-time a better inhibitor compared to **1**, with a K_I of 25 nM. Compounds **2, 3** and **10** reported here were the best hCA IX inhibitors, with K_Is in the range of 22.1 – 89.2 nM. They incorporate benzyl, 4-bromobenzyl and phenethyl R moieties. It is interesting to note that a bromine in para (as in **3**) leads to a 4-times increase of the CA IX inhibitory action compared to the unsubstituted benzyl derivative **2**, whereas the replacement of the bromine by a nitro moiety greatly reduces inhibitory action (as in **4**, which is 53 times a weaker CA IX inhibitor compared to **3**). The remaining compounds, irrespective of the nature of R (aromatic, aliphatic or unsaturated) showed a behavior of medium-weak CA IX inhibitor, with inhibition constants in the range of 103 – 481 nM (Table 1). The "opened" saccharins (compounds **12-17**) showed a rather modest activity as hCA IX inhibitors (Table 1).

(iii) hCA XII was on the other hand better inhibited by the investigated sulfonamides compared to hCA IX, with K_Is in the range of 3.9 – 245 nM. All these derivatives were much better inhibitors compared to saccharin **1** (K_I of 633 nM against this isoform). Derivatives **3** and **11** were the best inhibitors (K_Is in the range of 3.9 – 4.3 nM) and they incorporate 4-bromobenzyl and 2,4-dichlorobenzoyl moieties as R group. Other efficient hCA XII inhibitors were **2**, and **4-10**, which had K_Is in the range of 10.4 – 45.1 nM. It may be observed that they incorporate a variety of R groups, among which benzyl, 4-nitrobenzyl, as well as aliphatic and unsaturated moieties with 2-5 carbon atoms in the chain. The least effective hCA XII inhibitors

were **14** and **15**, which are "opened" saccharins incorporating a secondary sulfonamide and a COOH moiety.

3. Conclusions

Continuing our research of isoform-selective CAIs belonging to the secondary/tertiary sulfonamide chemotype, we investigated here a series of *N*-substituted saccharins incorporating aryl, alkyl and alkynyl moieties, as well as some ring opened derivatives, all of which were prepared from saccharin. The widespread cytosolic isoforms CA I and II were not inhibited by these sulfonamides whereas transmembrane, tumor-associated ones were effectively inhibited, with K_Is in the range of 22.1 – 481 nM for CA IX and of 3.9 – 245 nM for hCA XII. Although the inhibition mechanism of these tertiary/secondary sulfonamides is unknown for the moment, the good efficacy and especially selectivity for the inhibition of the tumor-associated over the cytosolic, widespread isoforms, make these derivatives of considerable interest as enzyme inhibitors with various pharmacologic applications.

4. Experimental part

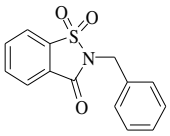
4.1. Chemistry

Reagents, starting materials and solvents were obtained from commercial sources and used as received. Thin-layer chromatography was performed on silica gel, spots were visualized with UV light (254 and 365 nm). Melting points were determined on an OptiMelt automated melting point system. NMR spectra were recorded on Varian Mercury (400 MHz) spectrometer with chemical shifts values (δ) in ppm relative to TMS using the residual DMSO-d_6 signal (^{1}H 2.50; ^{13}C 39.52) as an internal standard. In the IR spectra of the new compounds the strong SO_2 vibrations at 1170 and 1340 cm^{-1} were present, together with the CON band at 1745 cm^{-1} (data not shown)

General procedure for the synthesis of 2-alkyl-1,2-benzothiazol-3(2H)-one 1,1-dioxides (**2-10**)

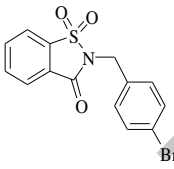
Saccharin (**1**) (1 equiv) was suspended in H_2O (20 mL), potassium carbonate (0.5 equiv) was slowly added, and solvent was driven off in vacuum. The dry residue was suspended in DMF (10 mL) and corresponding bromide (1 equiv) was added. The

reaction mixture was stirred at 90 ºC for 16 h. After cooling to room temperature CH_2Cl_2 (20 mL), H_2O (20 mL), and 2 M HCl (20 mL) were added, and it was extracted with CH_2Cl_2 (3×20 mL). Organic layer was washed with brine (30 mL) and dried over Na_2SO_4. CH_2Cl_2 was driven off in vacuum. Addition of H_2O to the remaining DMF solution resulted in precipitate formation, which were collected and dried.



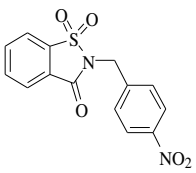
2-Benzyl-1,2-benzothiazol-3(*2H*)-one 1,1-dioxide (2)[4]

Compound **2** was prepared according to the general procedure from saccharin (**1**) (0.50 g, 2.73 mmol), potassium carbonate (0.19 g, 1.37 mmol) and benzyl bromide (0.32 mL, 2.73 mmol) as white solid (0.55 g, 74%). Mp 123-124 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 8.36-8.32 (m, 1H), 8.14-8.11 (m, 1H), 8.10-7.99 (m, 2H), 7.45-7.40 (m, 2H), 7.39-7.28 (m, 3H), 4.92 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 158.6, 136.9, 135.9, 135.3, 135.1, 128.5, 127.9, 127.8, 126.2, 125.2, 121.6, 41.7.



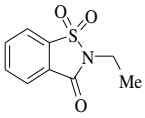
2-(4-Bromobenzyl)-1,2-benzothiazol-3(*2H*)-one 1,1-dioxide (3) [3a]

Compound **3** was prepared according to the general procedure from saccharin (**1**) (0.20 g, 1.09 mmol), potassium carbonate (0.08 g, 0.55 mmol) and 4-bromo benzyl bromide (0.27 g, 1.09 mmol) as white solid (0.25 g, 65%). Mp 168-169 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 8.36-8.33 (m, 1H), 8.14-8.10 (m, 1H), 8.07 (td, 1H, J = 7.6, 1.2 Hz), 8.01 (td, 1H, J = 7.6, 1.2 Hz), 7.59-7.54 (m, 2H), 7.41-7.37 (m, 2H), 4.91 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 158.6, 136.8, 136.0, 135.4, 134.7, 131.4, 130.1, 126.2, 125.2, 121.7, 121.0, 41.0.



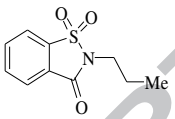
2-(4-Nitrobenzyl)-1,2-benzothiazol-3(*2H*)-one 1,1-dioxide (4) [3a]

Compound **4** was prepared according to the general procedure from saccharin (**1**) (0.50 g, 2.73 mmol), potassium carbonate (0.19 g, 1.37 mmol) and 4-nitrobenzyl bromide (0.59 g, 2.73 mmol) as white solid (0.66 g, 76%). Mp 190-191 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 8.39-8.35 (m, 1H), 8.26-8.21 (m, 2H), 8.16-8.13 (m, 1H), 8.09 (td, 1H, J = 7.6, 1.2 Hz), 8.03 (dt, 1H, J = 7.6, 1.2 Hz), 7.72-7.67 (m, 2H), 5.10 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 158.7, 147.1, 142.9, 136.8, 136.0, 135.4, 128.9, 126.2, 125.3, 123.7, 121.8, 40.9.



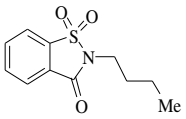
2-Ethyl-1,2-benzothiazol-3(*2H*)-one 1,1-dioxide (5) [5]

Compound **5** was prepared according to the general procedure from saccharin (**1**) (0.50 g, 2.73 mmol), potassium carbonate (0.19 g, 1.37 mmol) and ethyl bromide (0.20 mL, 2.73 mmol) as white solid (0.36 g, 63%). Mp 104-105 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 8.32-8.27 (m, 1H), 8.13-8.08 (m, 1H), 8.08-8.02 (m, 1H), 8.01-7.97 (m, 1H), 3.77 (q, 2H, J = 7.2 Hz), 1.31 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 158.2, 136.9, 135.7, 135.1, 126.4, 124.9, 121.4, 33.8, 13.7.



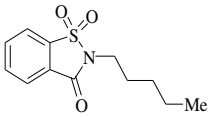
2-Propyl-1,2-benzothiazol-3(*2H*)-one 1,1-dioxide (6) [4]

Compound **6** was prepared according to the general procedure from saccharin (**1**) (0.50 g, 2.73 mmol), potassium carbonate (0.19 g, 1.37 mmol) and *n*-propyl bromide (0.25 mL, 2.73 mmol) as white solid (0.40 g, 65%). Mp 87-88 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 8.32-8.29 (m, 1H), 8.13-8.09 (m, 1H), 8.05 (td, 1H, J = 7.6, 1.3 Hz), 8.00 (td, 1H, J = 7.6, 1.3 Hz), 3.68 (t, 2H, J = 7.3 Hz), 1.75 (sextet, 2H, J = 7.3 Hz), 0.94 (t, 3H, J = 7.3 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 158.6, 136.8, 135.7, 135.2, 126.3, 125.0, 121.5, 40.3, 21.3, 11.0.



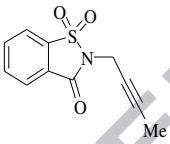
2-Butyl-1,2-benzothiazol-3(*2H*)-one 1,1-dioxide (7) [6]

Compound **7** was prepared according to the general procedure from saccharin (**1**) (0.50 g, 2.73 mmol), potassium carbonate (0.19 g, 1.37 mmol) and butyl bromide (0.29 mL, 2.73 mmol). Additional purification by column chromatography (silica gel, hexane/EtOAc 4:1) afforded **7** as colorless oil (0.21 g, 32%). ^{1}H NMR (400 MHz, DMSO-d_6) δ= 8.28-8.25 (m, 1H), 8.09-8.05 (m, 1H), 8.03 (td, 1H, J = 7.5, 1.2 Hz), 7.97 (td, 1H, J = 7.5, 1.2 Hz), 3.69 (t, 2H, J = 7.3 Hz), 1.74-1.65 (m, 2H), 1.40-1.29 (m, 2H), 0.89 (t, 3H, J = 7.3 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 158.6, 136.8, 135.7, 135.1, 126.3, 125.0, 121.4, 38.4, 29.9, 19.3, 13.3.



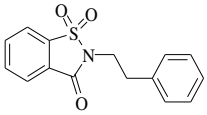
2-Pentyl-1,2-benzothiazol-3(*2H*)-one 1,1-dioxide (8) [6]

Compound **8** was prepared according to the general procedure from saccharin (**1**) (0.30 g, 1.64 mmol), potassium carbonate (0.11 g, 0.82 mmol) and pentyl bromide (0.20 mL, 1.64 mmol) as white solid (0.25 g, 60%). Mp 62-63 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 8.32-8.28 (m, 1H), 8.12-8.09 (m, 1H), 8.05 (td, 1H, J = 7.5, 1.2 Hz), 8.00 (td, 1H, J = 7.5, 1.2 Hz), 3.70 (t, 2H, J = 7.3 Hz), 1.78-1.68 (m, 2H), 1.36-1.28 (m, 4H), 0.90-0.83 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 158.6, 136.8, 135.7, 135.2, 126.3, 125.0, 121.5, 38.6, 28.2, 27.5, 21.5, 13.7.



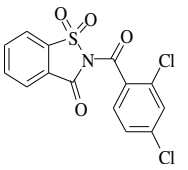
2-(But-2-yn-1-yl)-1,2-benzothiazol-3(*2H*)-one 1,1-dioxide (9)

Compound **9** was prepared according to the general procedure from saccharin (**1**) (0.30 g, 1.64 mmol), potassium carbonate (0.11 g, 0.82 mmol) and 1-bromo-2-butyne (0.14 mL, 1.64 mmol) as light brown solid (0.30 g, 78%). Mp 127-128 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 8.34-8.31 (m, 1H), 8.15-8.12 (m, 1H), 8.07 (td, 1H, J = 7.6, 1.2 Hz), 8.01 (td, 1H, J = 7.6, 1.2 Hz), 4.52 (q, 2H, J = 2.5 Hz), 1.80 (t, 3H, J = 2.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 157.9, 137.0, 136.0, 135.3, 126.0, 125.2, 121.6, 80.3, 72.4, 27.9, 3.0.



2-(2-Phenylethyl)-1,2-benzothiazol-3(*2H*)-one 1,1-dioxide (10)

Compound **10** was prepared according to the general procedure from saccharin (**1**) (0.30 g, 1.64 mmol), potassium carbonate (0.11 g, 0.82 mmol) and phenylethyl bromide (0.22 mL, 1.64 mmol) as white solid (0.15 g, 32%). Mp 139-140 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 8.33-8.30 (m, 1H), 8.09-8.03 (m, 2H), 7.99 (td, 1H, J = 7.5, 1.1 Hz), 7.33-7.27 (m, 4H), 7.25-7.19 (m, 1H), 3.98-3.92 (m, 2H), 3.07-3.02 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 158.3, 137.6, 136.8, 135.8, 135.2, 128.7, 128.4, 126.6, 126.2, 125.0, 121.5, 39.7, 33.9.

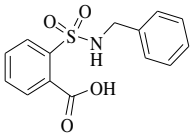


2-(2,4-Dichlorobenzoyl)-1,2-benzothiazol-3(2H)-one 1,1-dioxide (11) [3a]

Saccharin (**1**) (0.30 g, 1.64 mmol) was suspended in acetone (5 mL), reaction mixture was cooled to 0 ºC, triethylamine (0.25 mL, 1.80 mmol) was added followed by 2,4-dichlorobenzoyl chloride (0.25 mL, 1.80 mmol). It was allowed to reach room temperature and stirred at room temperature for 16 h. Reaction mixture was poured onto ice, precipitate were collected and washed with water. Recrystallization from DCM/MeOH afforded **11** (0.40 g, 69%) as white solid. Mp 221-222 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 8.46 (dt, 1H, J = 7.8, 0.8 Hz), 8.22-8.14 (m, 2H), 8.11-8.06 (m, 1H), 7.84 (d, 1H, J = 2.0 Hz), 7.78 (d, 1H, J = 8.3 Hz), 7.63 (dd, 1H, J = 8.3, 2.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 163.2, 156.1, 137.7, 137.1, 136.7, 136.0, 132.1, 130.8, 130.6, 129.2, 127.9, 126.5, 124.3, 121.9.

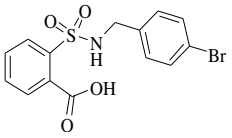
General procedure for the synthesis of 2-(alkylsulfamoyl)benzoic acids (**12-17**)

The appropriate 1,2-benzothiazol-3(*2H*)-one 1,1-dioxide (1 equiv) was dissolved in a mixture of THF/MeOH/water (2:1:2). NaOH (5 equiv) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 16 h, acidified with HCl (conc.), the solvent was evaporated. The crude product was re-crystallized from water and dried in vacuum.



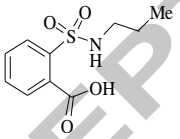
2-(Benzylsulfamoyl)benzoic acid (12) [7]

Compound **12** was prepared according to the general procedure from compound **2** (0.20 g, 0.73 mmol) and NaOH (0.15 g, 3.66 mmol) as white solid (0.12 g, 56%). Mp 143-144 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 7.85-7.82 (m, 1H), 7.72 (t, 1H, J = 6.4 Hz), 7.68-7.59 (m, 3H), 7.28-7.17 (m, 5H), 4.10 (d, 2H, J = 6.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 169.0, 138.0, 137.7, 133.0, 132.4, 130.6, 128.9, 128.3, 128.2, 127.6, 127.1, 46.2.



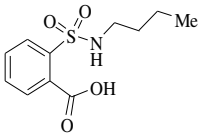
2-[(4-Bromobenzyl)sulfamoyl]benzoic acid (13)

Compound **13** was prepared according to the general procedure from compound **3** (0.12 g, 0.34 mmol) and NaOH (0.07 g, 1.70 mmol) as white solid (0.10 g, 79%). Mp 181-182 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 7.86-7.79 (m, 2H), 7.70-7.58 (m, 3H), 7.45-7.41 (m, 2H), 7.22-7.17 (m, 2H), 4.08 (d, 2H, J = 5.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 169.0, 137.9, 137.4, 133.1, 132.4, 131.0, 130.6, 129.8, 128.9, 128.3, 120.2, 45.4.



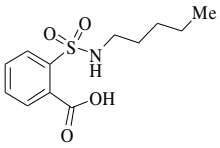
2-(Propylsulfamoyl)benzoic acid (14)

Compound **14** was prepared according to the general procedure from compound **6** (0.25 g, 1.11 mmol) and NaOH (0.22 g, 5.55 mmol) as white solid (0.19 g, 70%). Mp 106-107 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 7.92-7.86 (m, 1H), 7.72-7.65 (m, 3H), 7.11 (t, 1H, J = 6.0 Hz), 2.84-2.77 (m, 2H), 1.38 (sextet, 2H, J = 7.4 Hz), 0.77 (t, 3H, J = 7.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 169.0, 137.8, 133.2, 132.4, 130.7, 129.0, 128.3, 44.5, 22.5, 11.0.



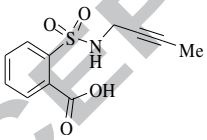
2-(Butylsulfamoyl)benzoic acid (15)

Compound **15** was prepared according to the general procedure from compound **7** (0.20 g, 0.84 mmol) and NaOH (0.17 g, 4.20 mmol) as white solid (0.13 g, 60%). Mp 112-113 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 7.93-7.86 (m, 1H), 7.72-7.65 (m, 3H), 7.09 (t, 1H, J = 6.0 Hz), 2.87-2.80 (m, 2H), 1.39-1.30 (m, 2H), 1.26-1.15 (m, 2H), 0.77 (t, 3H, J = 7.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 158.6, 136.8, 135.7, 135.2, 126.3, 125.0, 121.5, 38.4, 29.9, 19.3, 13.3.



2-(Pentylsulfamoyl)benzoic acid (16)

Compound **10** was prepared according to the general procedure from compound **8** (0.16 g, 0.63 mmol) and NaOH (0.13 g, 3.16 mmol) as white solid (0.12 g, 70%). Mp 133-134 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 7.91-7.86 (m, 1H), 7.73-7.64 (m, 3H), 7.09 (t, 1H, J = 5.8 Hz), 2.87-2.80 (m, 2H), 1.40-1.32 (m, 2H), 1.21-1.13 (m, 4H), 0.81-0.75 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 169.0, 137.8, 133.2, 132.4, 130.6, 129.0, 128.2, 42.6, 28.6, 28.1, 21.5, 13.7.



2-(But-2-yn-1-yl-sulfamoyl)benzoic acid (17)

Compound **10** was prepared according to the general procedure from compound **9** (0.15 g, 0.64 mmol) and NaOH (0.13 g, 3.20 mmol) as brown solid (0.11 g, 68%). Mp 145-146 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 7.96-7.91 (m, 1H), 7.74-7.68 (m, 3H), 7.38 (t, 1H, J = 6.1 Hz), 3.77-3.72 (m, 2H), 1.49 (t, 3H, J = 2.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ= 168.9, 138.3, 132.8, 132.5, 130.6, 129.2, 128.8, 80.1, 74.5, 32.6, 2.8.

CA inhibition assay

An SX.18MV-R Applied Photophysics (Oxford, UK) stopped-flow instrument has been used to assay the catalytic/inhibition of various CA isozymes.[19] Phenol Red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.4) as buffer, 0.1 M Na_2SO_4 or $NaClO_4$ (for maintaining constant the ionic strength; these anions are not inhibitory in the used concentration),[20] following the CA-catalyzed CO_2 hydration reaction for a period of 5-10 s. Saturated CO_2 solutions in water at 25 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 10 mM (in DMSO-water 1:1, v/v) and dilutions up to 1 nM done with the assay buffer mentioned above. At least 7 different inhibitor concentrations have been used for measuring the inhibition constant. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. Triplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. The inhibition constants were obtained by non-linear least-squares methods using the Cheng-Prusoff equation, as reported earlier,[20] and represent the mean from at least three different determinations. All CA isozymes used here were recombinant proteins obtained as reported earlier by our group.[21-25]

Acknowledgments. This work was financed in part by two EU FP7 projects, InnovaBalt and Dynano.

References

1. a) Supuran CT, Nat Rev Drug Discov. 2008; 7: 168; b) Neri D, Supuran CT, Nat Rev Drug Discov. 2011; 10: 767; c) S. Durdagi, D. Vullo, P. Pan, N. Kähkönen, J.A. Määttä, V.P. Hytönen, A. Scozzafava, S. Parkkila, C.T. Supuran, J Med Chem. 2012; 55: 5529.

2. a) J. Ivanova, J. Leitans, M. Tanc, A. Kazaks, R. Zalubovskis, C.T. Supuran, K. Tars, Chem. Commun. 2015; 51: 7108; b) G. De Simone, V. Alterio, C.T. Supuran, Expert Opin Drug Discov. 2013; 8: 793; c) E. Masini, F. Carta, A. Scozzafava, C.T. Supuran, Expert Opin Ther Pat. 23 (2013) 705; d) Supuran CT. Bioorg Med Chem Lett. 2010; 20: 3467; e) Supuran CT. J Enzyme Inhib Med Chem. 2013; 28: 229; f) K. D'Ambrosio, S. Carradori, S.M. Monti, M. Buonanno, D. Secci, D. Vullo, C.T. Supuran, G. De Simone, Chem Commun. 2015; 51: 302.

3. a) S. Carradori, D, Secci, C. De Monte, A. Mollica, M. Ceruso, A. Akdemir, A.P. Sobolev, R. Codispoti, F. De Cosmi, P. Guglielmi, C.T. Supuran, Bioorg. Med. Chem. 2016; 24: 1095; b) D'Ascenzio M, Guglielmi P, Carradori S, Secci D, Florio R, Mollica A, Ceruso M, Akdemir A, Sobolev AP, Supuran CT. J Enzyme Inhib Med Chem. 2017; 32: 51; c) Alterio V, Tanc M, Ivanova J, Zalubovskis R, Vozny I, Monti SM, Di Fiore A, De Simone G, Supuran CT. Org Biomol Chem. 2015; 13: 4064; d) Mahon BP, Hendon AM, Driscoll JM, Rankin GM, Poulsen SA, Supuran CT, McKenna R. Bioorg Med Chem. 2015; 23: 849; e) Moeker J, Peat TS, Bornaghi LF, Vullo D, Supuran CT, Poulsen SA. J Med Chem. 2014; 57: 3522; f) D'Ascenzio M, Carradori S, De Monte C, Secci D, Ceruso M, Supuran CT. Bioorg Med Chem. 2014; 22: 1821; g) Coviello V, Marchi B, Sartini S, Quattrini L, Marini AM, Simorini F, Taliani S, Salerno S, Orlandi P, Fioravanti A, Desidero TD, Vullo D, Da Settimo F, Supuran CT, Bocci G, La Motta C. J Med Chem. 2016; 59: 6547.

4. Robinson RI, Fryatt R, Wilson C, Woodward S, Eur J Org Chem. 2006; 6: 4483.

5. Wrobel J, Dietrich A, Woolson SA, et al. J Med Chem 1992; 35: 4613.

6. Chapman, Jr JM, Cocolas GH, Hall IH. J Med Chem 1983; 26: 243.

7. Barta TE, Becker DP, Bedell LJ, et al. Bioorg Med Chem Lett. 2000; 10: 2815.

8. Köhler K, Hillebrecht A, Schulze Wischeler J, et al. Angew Chem Int Ed Engl. 2007; 46: 7697.

9. a) Supuran CT, Biochem J. 2016; 473: 2023; b) Supuran CT, J Enzyme Inhib Med Chem. 2016; 31: 345; c) Supuran CT, Bioorg Med Chem. 2013; 21: 1377; d) Supuran

CT, Expert Opin Drug Discov. 2017; 12: 61; e) Alterio V, Di Fiore A, D'Ambrosio K, et al, Chem Rev. 2012; 112: 4421; f) Supuran CT, Pathogens 2016; 5: E44.

10. a) Supuran CT, J Enzyme Inhib Med Chem. 2012; 27: 759; b) Supuran CT, Expert Opin Ther Pat. 2013; 23: 677; c) J.Y. Winum, C.T. Supuran, J Enzyme Inhib Med Chem. 30 (2015) 321; d) V. De Luca, S. Del Prete, C.T. Supuran, C. Capasso, J Enzyme Inhib Med Chem. 30 (2015) 277; e) C.L. Lomelino, C.T. Supuran, R. McKenna, Int J Mol Sci. 17 (2016) E1150.

11. a) C.T. Supuran, M.A. Ilies, A. Scozzafava, Eur J Med Chem. 33 (1998) 739; b) C.T. Supuran, Curr Pharm Des. 2008, 14, 641; c) F. Carta, C.T. Supuran, Expert Opin Ther Pat. 23 (2013) 681; d) C. Temperini, A. Cecchi, A, Scozzafava, C.T. Supuran, Org Biomol Chem. 6 (2008) 2499.

12. a) Carta F, Di Cesare Mannelli L, Pinard M, Ghelardini C, Scozzafava A, McKenna R, Supuran CT. Bioorg Med Chem. 2015; 23: 1828; b) Supuran CT, Expert Rev Neurother. 2016; 16: 961.

13. a) Bua S, Di Cesare Mannelli L, Vullo D, Ghelardini C, Bartolucci G, Scozzafava A, Supuran CT, Carta F. J Med Chem. 2017; 60: 1159; b) Margheri F, Ceruso M, Carta F, Laurenzana A, Maggi L, Lazzeri S, Simonini G, Annunziato F, Del Rosso M, Supuran CT, Cimaz R. J Enzyme Inhib Med Chem. 2016; 31(sup4): 60.

14. L. Di Cesare Mannelli, L. Micheli, F. Carta, A. Cozzi, C. Ghelardini, C.T. Supuran, J Enzyme Inhib Med Chem. 31 (2016) 894.

15. a) Maresca A, Temperini C, Vu H, Pham NB, Poulsen SA, Scozzafava A, Quinn RJ, Supuran CT. J Am Chem Soc. 2009; 131: 3057; b) Maresca A, Temperini C, Pochet L, Masereel B, Scozzafava A, Supuran CT. J Med Chem. 2010; 53: 335.

16. a) Carta F, Aggarwal M, Maresca A, Scozzafava A, McKenna R, Masini E, Supuran CT. J Med Chem. 2012; 55: 1721; b) Carta F, Aggarwal M, Maresca A, Scozzafava A, McKenna R, Supuran CT. Chem Commun (Camb). 2012; 48: 1868.

17. a) Carta F, Temperini C, Innocenti A, Scozzafava A, Kaila K, Supuran CT. J Med Chem. 2010; 53: 5511; b) Scozzafava A, Supuran CT, Carta F. Molecules. 2016; 21: E1726.

18. a) Alp C, Maresca A, Alp NA, Gültekin MS, Ekinci D, Scozzafava A, Supuran CT. J Enzyme Inhib Med Chem. 2013; 28: 294; b) Rosatelli E, Carotti A, Ceruso M, Supuran CT, Gioiello A. Bioorg Med Chem Lett. 2014; 24: 3422; c) Petrou A, Geronikaki A, Terzi E, Guler OO, Tuccinardi T, Supuran CT. J Enzyme Inhib Med Chem. 2016; 31: 1306; d) Villalba ML, Palestro P, Ceruso M, Gonzalez Funes JL,

Talevi A, Bruno Blanch L, Supuran CT, Gavernet L. Bioorg Med Chem. 2016; 24: 894.

19. Khalifah RG. J. Biol. Chem. 1971**,** 246, 2561.

20. Leitans, J.; Sprudza, A.; Tanc, M.; Vozny, I.; Zalubovskis, R.; Tars, K.; Supuran, C.T. *Bioorg. Med. Chem.* **2013**, *21*, 5130.

21. a) Korkmaz, N.; Obaidi, O.A.; Senturk, M.; Astley, D.; Ekinci, D.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* 2015, *30*, 75; b) Abdel-Aziz, A.A.M.; El-Azab; A.S., Ekinci, D.; Şentürk, M.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. 2015, *30*, 81; c) Abdel-Aziz, A.A.M.; El-Azab, A.S.; Ekinci, D.; Şentürk, M.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* 2015, *30*, 81; d) Akdemir, A; De Monte, C.; Carradori, S.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* 2015, *30*, 114; e) Leitans, J.; Kazaks, A.; Balode, A.; Ivanova, J.; Zalubovskis, R.; Supuran, C.T.; Tars, K. *J. Med. Chem.* 2015, *58*, 9004; f) Del Prete S, Vullo D, Fisher GM, et al. *Bioorg Med Chem Lett*. 2014; 24: 4389.

22. a) Sahin, A.; Isik, S.; Arslan, O.; Supuran, C.T.; Ozensoy Guler, O. *J. Enzyme Inhib. Med. Chem*. 2015, *30*, 224; b) Bozdag, M.; Isik, S.; Beyaztas, S.; Arslan, O.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. 2015, *30*, 240; c) De Luca, V.; Del Prete, S.; Supuran, C.T.; Capasso, C. *J. Enzyme Inhib. Med. Chem*. 2015, *30*, 277; d) Vullo, D.; Milos, M.; Galic, B.; Scozzafava, A.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* 2015, *30*, 341; e) Del Prete, S.; Vullo, D.; De Luca, V.; AlOthman, Z.; Supuran, C.T.; Capasso, C. *J. Enzyme Inhib. Med. Chem*. 2015, *30*, 366; f) Riafrecha, L.E.; Vullo, D.; Supuran, C.T.; Colinas, P.A. *J. Enzyme Inhib. Med. Chem*. 2015, *30*, 857.

23. a) Göçer, H.; Akincioğlu, A.S.; Gülçin, G.I.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. 2015, *30*, 316; b) Ceruso, M.; Bragagni, M.; AlOthman, Z.; Osman, S.M.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem*. 2015, *30*, 430; c) Zolfaghari Emameh, R.; Syrjänen, L.; Barker, H.; Supuran, C.T.; Parkkila, S. *J. Enzyme Inhib. Med. Chem.* 2015, *30*, 505; d) Alafeefy, A.M.; Ceruso, M.; Al-Tamimi, A.M.S.; Del Prete, S.; Supuran, C.T.; C. Capasso, *J. Enzyme Inhib. Med. Chem*. 2015, *30*, 592; e) Scozzafava, A.; Passaponti, M.; Supuran, C.T.; Gülçin, I. *J. Enzyme. Med. Chem.* 2015, *30*, 586; f) Le Darz, A.; Mingot, A.; Bouazza, F.; Castelli, U.; Tanc Karam, O.M.; Supuran, C.T.; Thibaudeau, S. *J. Enzyme Inhib. Med. Chem*. 2015, *30*, 737.

24. a) D. Vullo, J. Voipio, A. Innocenti, C. Rivera, H. Ranki, A. Scozzafava, K. Kaila, C.T. Supuran, Bioorg. Med. Chem. Lett. 15 (2005) 971; b) I. Nishimori, D. Vullo, A. Innocenti, A. Scozzafava, A. Mastrolorenzo, C.T. Supuran, J. Med. Chem. 48 (2005)

7860; c) A. Scozzafava, L. Menabuoni, F. Mincione, C.T. Supuran, J. Med. Chem. 45 (2002) 1466; d) C.T. Supuran, M.A. Ilies, A. Scozzafava, Eur. J. Med. Chem. 33 (1998) 739.

25. a) C.T. Supuran, M. Barboiu, C. Luca, E. Pop, M.E. Brewster, A. Dinculescu, Eur. J. Med. Chem. 31 (1996) 597; b) F. Carta, M. Aggarwal, A. Maresca, A. Scozzafava, R. McKenna, E. Masini, C.T. Supuran, J. Med. Chem. 55 (2012) 1721; c) L. Puccetti, Fasolis G., D. Vullo, Z.H. Chohan, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. Lett. 15 (2005) 3096; d) C.T. Supuran, A. Nicolae, A. Popescu, Eur. J. Med. Chem. 31 (1996) 431; e) S. Bilginer, E. Unluer, H.I. Gul, E. Mete, S. Isik, D. Vullo, S. Beyaztas, O. Ozensoy-Guler, C. Capasso, C.T. Supuran, J Enzyme Inhib Med Chem. 29 (2014) 495.

N-Substituted and ring opened saccharin derivatives selectively inhibit transmembrane, tumor-associated carbonic anhydrases IX and XII

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