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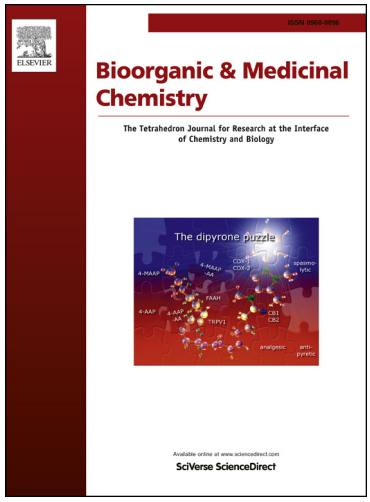

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PII: S0968-0896(16)30038-4
DOI: http://dx.doi.org/10.1016/j.bmc.2016.01.038
Reference: BMC 12781

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 19 November 2015
Revised Date: 18 January 2016
Accepted Date: 19 January 2016

Please cite this article as: Carradori, S., Secci, D., De Monte, C., Mollica, A., Ceruso, M., Akdemir, A., Sobolev, A.P., Codispoti, R., De Cosmi, F., Guglielmi, P., Supuran, C.T., A novel library of saccharin and acesulfame derivatives as potent and selective inhibitors of carbonic anhydrase IX and XII isoforms, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.01.038

A novel library of saccharin and acesulfame derivatives as potent and selective inhibitors of carbonic anhydrase IX and XII isoforms

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Abstract

Small libraries of *N*-substituted saccharin and *N*-/*O*-substituted acesulfame derivatives were synthesized and tested as atypical and selective inhibitors of four different isoforms of human carbonic anhydrase (hCA I, II, IX and XII, EC 4.2.1.1). Most of them inhibited hCA XII in the low nanomolar range, hCA IX with K_Is ranging between 19 and 2482 nM, whereas they were poorly active against hCA II (K_Is > 10 μM) and hCA I (K_Is ranging between 318 nM to 50 μM). Since hCA I and II are ubiquitous off-target isoforms, whereas the cancer-related isoforms hCA IX and XII were recently validated as drug targets, these results represent an encouraging achievement in the development of new anticancer candidates. Moreover, the lack of a classical zinc binding group in the structure of these inhibitors opens innovative, yet unexplored scenarios for different mechanisms of inhibition that could explain the high inhibitory selectivity. A computational approach has been carried out to further rationalize the biological data and to characterize the binding mode of some of these inhibitors.

Keywords: saccharin, acesulfame, carbonic anhydrase inhibitor, cancer-related isoforms, *N*/*O*-substitution.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are important metalloenzymes involved in many cellular and physiological processes which require bicarbonate as substrate such as electrolyte secretion, pH homoeostasis, respiration, and biosynthetic pathways including lipogenesis, glucogenesis, and ureagenesis, as well as tumorigenicity.[1,2] Human CAs (hCAs) exist in fifteen isoforms and differ in their cellular localization (cytosol, mitochondria or cell membrane), sensibility to inhibitors and catalytic activity. CAs are well established therapeutic targets to treat a wide range of disorders through the use of inhibitors or activators,[3-5] as well as recognized tools for drug delivery purposes.[6,7]

Classical CA inhibitors (CAIs) usually act by complexing the metal ion (Zn^{+2}) in the enzyme active site such as primary sulfonamides and their bioisosters, inorganic anions, thiophenols, hydroxamates and carboxylic acids.[8] In this context, the emerging "non-zinc binding inhibitors" have deeply increased over the last few years, especially in the quest of potent and selective anti-cancer agents (targeting the tumor-associated isoforms hCA IX and XII).[9-13] Our recent studies, focused on the synthesis of saccharin- and acesulfame-based compounds,[14,15] allowed identifying a series of cyclic tertiary sulfonamides and their bioisosteric sulfamates acting as selective hCA IX/XII inhibitors. The peculiar isoform selectivity displayed by these compounds was attributed to a newly hypothesized mode of inhibition similar to other non-zinc binding inhibitors (e.g., probenecid derivatives).[16–18] As a consequence, this new class of CAIs might be considered as an innovative tool in the design of selective CAIs for further applications in medicinal chemistry.[19-21]

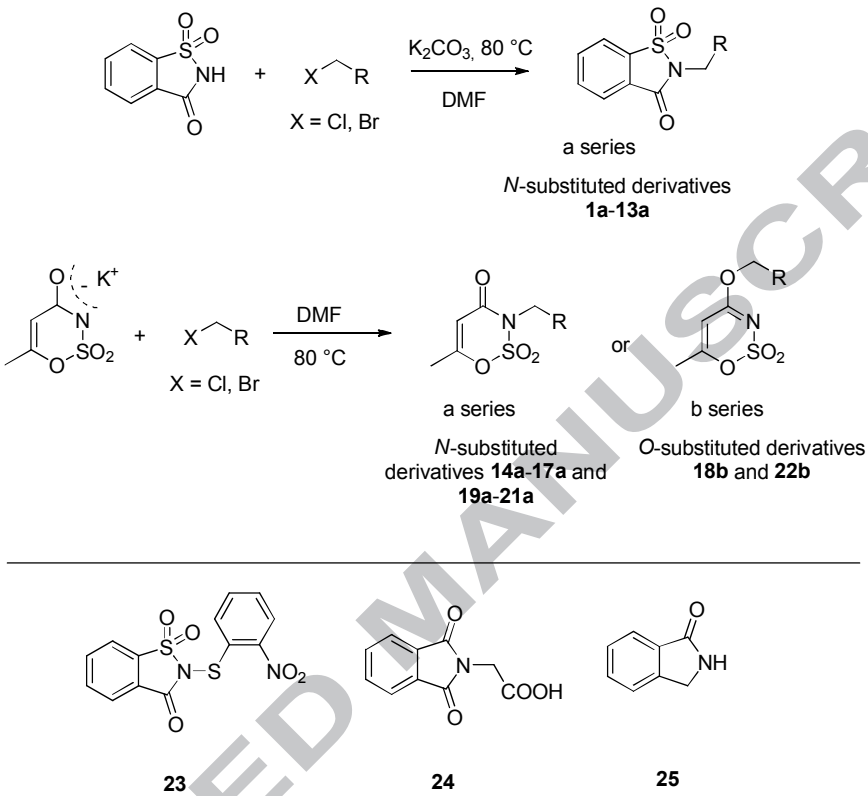
Pursuing our efforts for the development of innovative treatment of cancer,[22-25] in our previous reports we investigated several *N*/*O*-substituted compounds exploring the chemical space around these positions. In particular, among the reported derivatives we co-crystallized one of them with hCA II demonstrating the interactions within the active site.[26] In order to complete structure-activity relationships (SARs) within these scaffolds, we designed and synthesized new derivatives characterized by different substituents (CH_3, CF_3, CN, F, Cl, Br, NO_2, OCH_3, Ph) at the ortho, meta

and para positions of the benzylic or benzoylic portion keeping constant the saccharin/acesulfame skeleton.[27] In addition, we studied the change in the biological activity of bioisosteric replacement of the methylene bridge (in the *N*-benzylic saccharin derivatives) with a sulfur atom and the substitution of the SO_2 group of the saccharin with C=O (phthalimide) and CH_2 (isoindolinone), respectively. This approach allowed us to obtain promising candidates not only potent and selective inhibitors of the cancer-related hCA IX and hCA XII, but also showing appropriate physico-chemical properties (water solubility, balanced hydro/liposolubility) for the development of potential drugs.

2. Chemistry

N-substituted saccharin derivatives (**1a**-**13a**) were synthesized according to the general procedure reported in Scheme 1.[28] Saccharin (1.0 eq.) was deprotonated using freshly grinded anhydrous potassium carbonate and the corresponding salt was then reacted with a proper electrophile (2 eq.) by stirring the reaction mixture in *N*,*N*-dimethylformamide at 80 °C overnight. Under these conditions (polar aprotic solvent) and if the reaction reached thermal equilibrium, we observed the formation of the more stable isomer (*N*-substituted saccharin) limiting the Chapman-Mumm thermal rearrangement to the less stable *O*-substituted derivative.[29] Conversely, the reaction between potassium acesulfame and the electrophile reagents in *N*,*N*-dimethylformamide gave the corresponding *N*-substituted derivatives (**a** series) or *O*-substituted derivatives (**b** series) (compounds **14a-22b**, Scheme 1). The formation of a mixture of amide and imidate compounds or the generation of only one of them usually was influenced by the thermodynamic stability of the products and by the steric hindrance of the electrophile reactant. In fact, the formation of the *O*-substituted structure could compete with the more thermodynamically preferred *N*-substituted one whether the C=N bond was contained in an aromatic or unsaturated nucleus and, furthermore, the attack at the oxygen atom could also occur for steric effects.[30] These considerations could explain the formation of the derivatives belonging to **a** series and/or **b** series starting from the ambident nucleophile (potassium acesulfame or saccharin). Moreover, compounds **23-25** (Scheme 1) were

purchased and used in the biological assays without further purification in order to explore the structure-activity relationship within the saccharin scaffold.

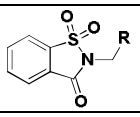


Scheme 1. Synthesis and structure of compounds **1**-**25.**

3. Biological evaluation

All the synthesized and purchased compounds of **a** and **b series** were tested to evaluate their inhibitory activity towards the ubiquitous off-target isoforms, hCA I and II, and the cancer-related ones, hCA IX and XII by a stopped-flow, CO_2 hydrase assay method and their CA inhibition data are summarized in Tables 1-3.

Table 1. Inhibitory activity of saccharin-based derivatives **1a**-**13a**, against selected hCA isoforms by a stopped-flow CO_2 hydrase assay.



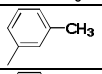
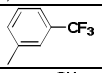
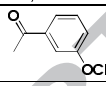
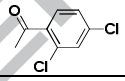
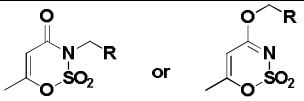
Compound	R	K_i (nM)			
		hCA I	hCA II	hCA IX	hCA XII
1a		1368	> 10000	221	16
2a		3579	> 10000	616	15
3a		1503	> 10000	184	26
4a		> 10000	> 10000	19	17
5a		> 10000	> 10000	234	5.1
6a		1347	> 10000	51	4.7
7a		2479	> 10000	539	41
8a		2361	> 10000	19	4.4
9a		> 10000	> 10000	22	4.3
10a		324	> 10000	1169	29
11a		1342	> 10000	1570	6.0
12a		1106	> 10000	23	41
13a		1480	> 10000	191	3.9
AAZ (acetazolamide)		250	12	25	6.0

Table 2. Inhibitory activity of acesulfame-based derivatives **14a**-**22b**, against selected hCA isoforms by a stopped-flow CO_2 hydrase assay.



a series: *N*-alkylated derivatives b series: *O*-alkylated derivatives

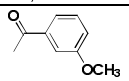
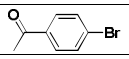
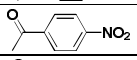
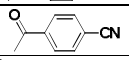
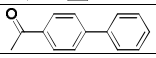
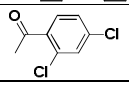
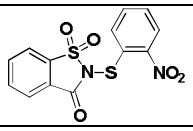
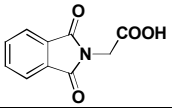
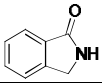
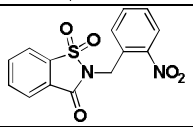
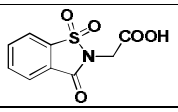
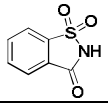
Compound	R	K_i (nM)			
		hCA I	hCA II	hCA IX	hCA XII
14a		> 10000	> 10000	261	4.9
15a		> 10000	> 10000	239	4.5
16a		> 10000	> 10000	210	4.3
17a		> 10000	> 10000	161	5.6
18b		9717	> 10000	196	3.6
19a		2047	1749	2482	3.9
20a		318	140	27	4.1
21a		> 10000	> 10000	26	4.0
22b		> 10000	> 10000	265	5.5
potassium acesulfame		> 20000	> 20000	2410	> 20000

Table 3. Inhibitory activity of saccharin analogues **23**-**27**, against selected hCA isoforms by a stopped-flow CO_2 hydrase assay.

Compound	R	K_i (nM)			
		hCA I	hCA II	hCA IX	hCA XII
23		> 10000	> 10000	18	4.0
24		> 10000	> 10000	223	4.3
25		> 10000	> 10000	174	4.1
26[15]		> 50000	41	91	28
27[26]		110000	78000	310	42
Saccharin		18540	5950	103	633

4. Results and discussion

4.1. New N-substituted saccharin derivatives (**1a-13a**).

All the tested compounds had no affinity for the common off-target hCA II isoform (K_I > 10000 nM), and many of them were more active against the tumor-related ones with respect to the starting compound saccharin. Compounds **4a**, **5a** and **9a** displayed their effect exclusively against the tumor-associated isoenzymes hCA IX and hCA XII (both transmembrane), without affecting either hCA I or hCA II (both cytosolic). While compound **4a**, containing a phenyl ring substituted in the *meta* position, possessed a similar effect against hCA IX and XII (K_I = 19 nM and K_I = 17 nM, respectively), compounds **5a** and **9a**, which had a substituent at C4 of the phenyl ring, were more effective against hCA XII, with a K_I of 5.1 and 4.3 nM, respectively. Compound **8a**, bearing a phenyl moiety with a chlorine at C4, was weakly active against hCA I (K_I = 2361 nM), but it was more selective towards hCA XII at very low nanomolar concentration (K_I = 4.4 nM). Similarly, the

acetophenone derivative **13a**, containing two chlorine atoms at C2 and C4 of the aromatic ring, was weakly active towards hCA I (K_I = 1480 nM) and more selective for hCA XII (K_I = 3.9 nM).

4.2. New N- and O-substituted acesulfame derivatives (**14a-22b**).

This scaffold consists of compounds divided into two groups (**a** series and **b** series), according to the *N*- or *O*- functionalization. Most of the compounds had more affinity for hCA IX and XII than that registered with potassium acesulfame. Compounds **14a**, **15a** and **16a**, bearing a phenyl ring substituted at C4 with a halogen or a nitro group, had no effect towards the two off-target isoforms and displayed the best biological activity selectively against the tumor-associated hCA XII (K_I = 4.9, 4.5 and 4.3 nM, respectively). With regard to the acetophenone derivatives, they all inhibited the cancer-related hCA XII at very low nanomolar concentrations. Compounds **19a** and **20a** were not selective, while the *N*-substituted derivatives **17a**, **21a** and the *O*-substituted derivatives **18b** and **22b** were not active against the two off-target enzymes and more effective towards hCA XII with respect to hCA IX.

Compound **23** allowed us to explore the biological change after the substitution of the methylene bridge with a sulphur atom (see for comparison our previously synthesized compound **26**). This new derivative improved the inhibitory activity and selectivity against hCA IX and XII and lost its interaction with hCA II. Among the other purchased compounds, both compound **25** (molecular simplification of the saccharin skeleton by deletion of the SO_2 moiety) and the *N*-alkylated phthalimide **24** (bioisosteric replacement of the SO_2 group with a C=O, see for comparison our previously synthesized compound **27**), which were analogues of saccharin but without the cyclic sulfonamide moiety, were also active only towards the tumor-associated isoforms, without affecting the other isoenzymes. These *N*-substituted saccharin derivatives and *N*- and *O*-substituted acesulfame derivatives have proven to be promising lead compounds for further studies in the discovery of new inhibitors of the tumor-associated hCA isoforms.

5. Docking studies into the active site of hCA XII

All saccharin and acesulfame analogues (compounds **1a**–**25**) inhibited hCA XII in the nanomolar range and show approximately 11-fold difference between the lowest and highest values (K_I values: 3.6-41 nM, Tables 1-3). Docking studies indicated that the sulfonamide oxygen atoms could form interactions with the Zn^{2+}-ion and the backbone of Thr199 (and occasionally also Thr200). The *para* substituted bromophenyl group of saccharin-based compound **9a** pointed either towards Asn62, Ser65 and Lys67 or towards the solvent, while the carbonyl group was oriented towards the solvent in both poses (Figure 1A). The carbonyl group of acesulfame-based compound **18b** formed a hydrogen bond with either Asn62 or Gln92, while the *para* substituted bromophenyl group pointed towards the solvent (Figure 1B).

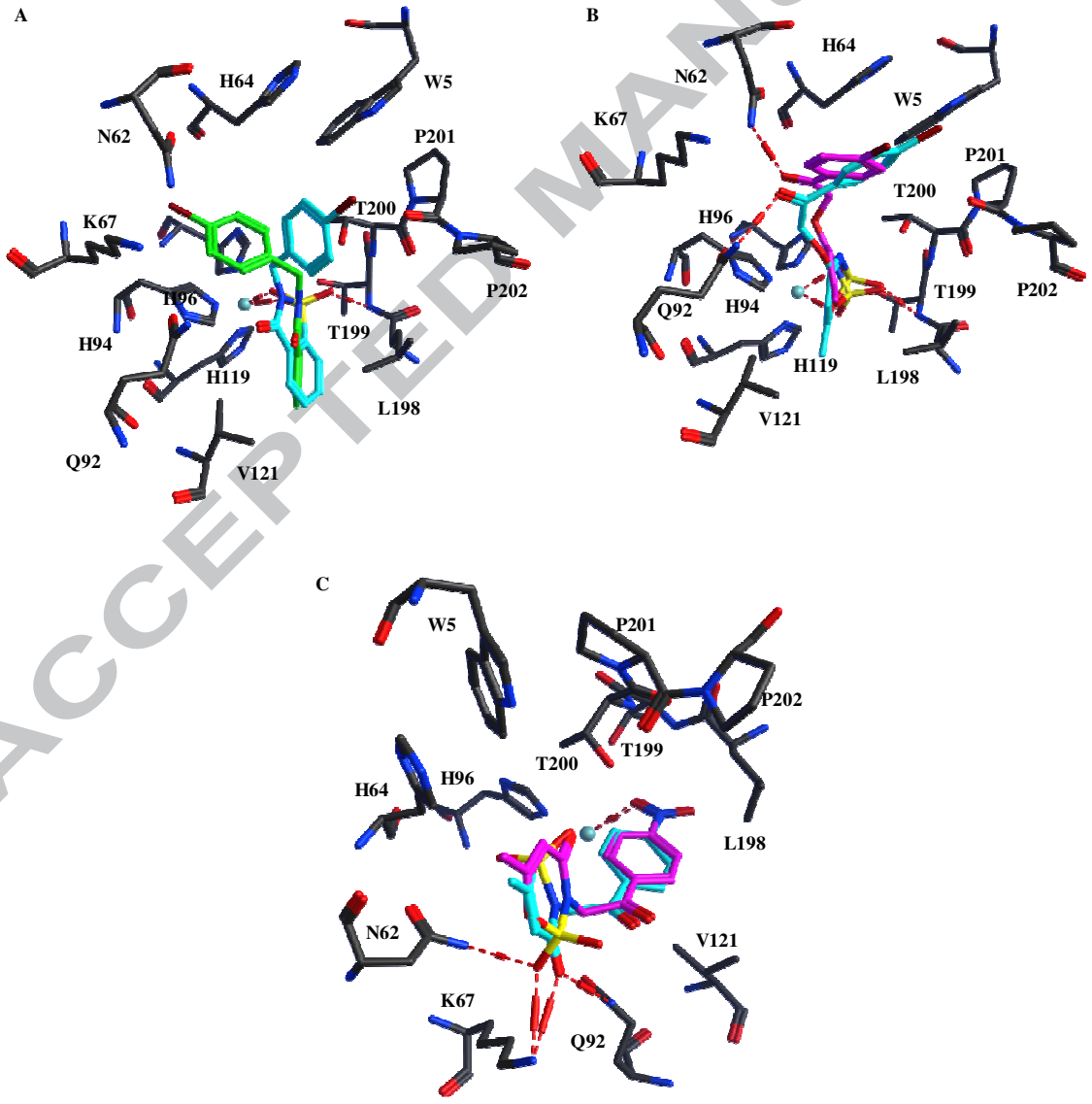


Figure 1. Docked poses of compounds **9a** (**A**), **18b** (**B**) and **19a** (**C**) in the active site of hCA XII. Hydrogen bonds and interactions to the Zn^{2+}-ion are depicted in red dashed lines. The Zn^{2+}-ion is depicted as a turquoise sphere.

The two docked poses observed for acesulfame-based compound **19a** were different from the other saccharin analogues and showed interactions between the nitro group and the Zn^{2+}-ion (Figure 1C). In the first pose, one of the sulfonamide oxygen atoms interacted with Asn62 and Lys67, while the other sulfonamide oxygen and the carbonyl groups were water accessible. In the other docked pose, the carbonyl group, embedded in the six-ring, interacted with Lys67 and Gln92. Docking studies were not able to rationalize the difference between the K_i values measured for saccharin and compound **25** (Table 3), as both compounds formed a direct interaction with the Zn^{2+}-ion and saccharin had additional hydrogen bonds. Probably, long ranged electrostatic interactions and entropy were responsible for this unexpected effect.

6. Docking studies into the active sites of hCA I, II and IX

Docking studies into hCA IX indicated that none of the compounds can directly interact with the Zn^{2+}-ion. Lys67 and Thr91 in hCA XII were replaced by Gln67 and Leu9, respectively, in hCA IX, while the conserved Gln92 had a different conformation. Most likely Gln67 in hCA IX prevented the ligands from interacting with the Zn^{2+}-ion in hCA IX due to steric clashes with the ligand. In addition, the cationic and flexible Lys67 could adopt a different conformation and approach the ligands to form cation-π interactions with the aromatic groups (Figure 2).

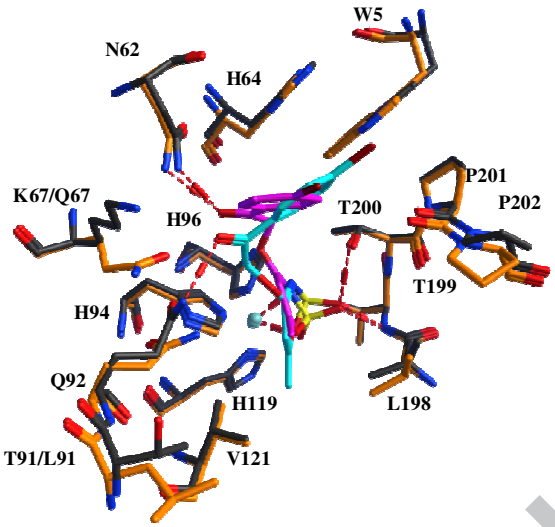


Figure 2. An overlay of the hCA IX active site (carbon atoms represented as light brown sticks) upon the docked poses of acesulfame-based compound **18b** (magenta and turquoise sticks) in the active site of hCA XII (carbon atoms represented as grey sticks). Hydrogen bonds and interactions to the Zn^{2+}-ion are depicted in red dashed lines. The Zn^{2+}-ion is depicted as a turquoise sphere.

Docking studies of hCA I and II showed that the ligands can form direct interactions with the Zn^{2+}-ion of both enzymes. However, enzyme inhibition assays showed in general high K_I values for these derivatives. We assume that this might be caused by long-ranged electrostatic interactions, enzyme flexibility upon ligand binding (induced-fit) and entropic effects (such as the inability of the vicinal Asn67 and Gln92 of hCA II to form hydrogen bonds with water or the ligand).

7. Conclusion

A large library of *N*-substituted saccharin derivatives and *N*- and *O*-substituted acesulfame derivatives were synthesized and tested against four human carbonic anhydrases: hCA I and II, the most common off-targets in the discovery of antitumor drugs, and hCA IX and XII, the cancer-related isoforms. Most of the compounds were not active towards hCA I and II, while they were very effective against hCA IX and XII and, in particular, they all inhibited hCA XII in the low nanomolar range. These large series of cyclic tertiary sulfonamide and sulfamate compounds could

represent an important starting point for the development of new antitumor agents based on the inhibition of the cancer-related isoforms of human carbonic anhydrase overexpressed in hypoxic tumors.[31]

8. Experimental protocols

8.1. General

Solvents were used as supplied without further purification. Where mixtures of solvents are specified, the stated ratios are volume:volume. Unless otherwise indicated, all aqueous solutions used were saturated. Saccharin, potassium acesulfame and acetazolamide were purchased by Sigma-Aldrich (Italy) and used in the syntheses and in the biological assays without further purification. All synthesized compounds have been fully characterized by analytical and spectral data. Column chromatography was carried out using Sigma-Aldrich® silica gel (high purity grade, pore size 60 Å, 200-425 mesh particle size). Analytical thin-layer chromatography was carried out on Sigma-Aldrich® silica gel on TLA aluminum foils with fluorescent indicator. Visualization was carried out under UV irradiation (254 nm). ^{1}H-NMR spectra were recorded on a Bruker AV400 (^{1}H: 400 MHz, ^{13}C: 101 MHz). ^{19}F NMR spectra were recorded on a Bruker AVANCE 600 spectrometer (^{19}F: 564.7 MHz). Chemical shifts are quoted in ppm, based on appearance rather than interpretation, and are referenced to the residual non deuterated solvent peak. In the case of ^{19}F, chemical shifts are referenced to external standard (CF_3COOH, δ -76.55 ppm). Infra-red spectra were recorded on a Bruker Tensor 27 FTIR spectrometer equipped with an attenuated total reflectance attachment with internal calibration. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). All melting points were measured on a Stuart® melting point apparatus SMP1 and are uncorrected. Temperatures are reported in °C. Elemental analyses were performed on a Perkin Elmer series II, 2400 CHN analyzer. Where given, systematic compound names are those generated by ChemBioDraw Ultra® 12.0 following IUPAC conventions.

8.2. Procedures for the synthesis of saccharin derivatives

8.2.1. 2-(2-Methylbenzyl)-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (1a)

1.51 g of anhydrous potassium carbonate (1 eq.) were added to a stirring solution of saccharin (2.0 g, 1.0 eq.) in 20 mL of *N*,*N*-dimethylformamide at 80 °C. 2-Methylbenzyl bromide (2.01 g, 1 eq.) was added and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the resulting suspension was filtered. Purification *via* column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:2) gave the title compound as a white solid (72% yield); mp 153-155 °C; IR ν_{max} 3073 (ν C_{sp2}-H), 1726 (ν C=O), 1335 (ν_{as} S=O), 1244 (ν C-N), 1181 (ν_s S=O), 754 (δ C_{sp2}-H), 676 (δ C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.50 (3H, s, CH_3), 4.98 (2H, s, CH_2), 7.21-7.25 (m, 3H, CH_{Ar}), 7.44 (d, J = 7.2 Hz, 1H, CH_{Ar}), 7.83-7.91 (m, 2H, CH_{Ar}), 7.95 (d, J = 6.8 Hz, 1H, CH_{Ar}), 8.10 (d, J = 8.0 Hz, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 19.3 (CH_3), 40.6 (CH_2), 121.0 (Ar), 125.3 (Ar), 126.3 (Ar), 127.3 (Ar), 128.3 (Ar), 128.7 (Ar), 130.5 (Ar), 132.1 (Ar), 134.4 (Ar), 134.9 (Ar), 136.3 (Ar), 137.9 (Ar), 159.0 (C=O). Anal. Calcd for $C_{15}H_{13}NO_3S$: C, 62.70; H, 4.56; N, 4.87. Found: C, 62.52 ; H, 4.81; N, 5.06.

8.2.2. 2-(2-Trifluoromethylbenzyl)-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (2a)

1.51 g of anhydrous potassium carbonate (1 eq.) were added to a stirring solution of saccharin (2.0 g, 1.0 eq.) in 20 mL of *N*,*N*-dimethylformamide at 80 °C. 2-Trifluoromethylbenzyl bromide (2.63 g, 1 eq.) was added and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the resulting suspension was filtered. Purification *via* column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:2) gave the title compound as a white solid (60% yield); mp 142-143 °C; IR ν_{max} 3095 (ν C_{sp2}-H), 1733 (ν C=O), 1336 (ν_{as} S=O), 1241 (ν C-N), 1171 (ν_s S=O), 749 (δ C_{sp2}-H), 676 (δ C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.20 (2H, s, CH_2), 7.41-7.45 (t, 1H, CH_{Ar}), 7.51-7.57 (m, 2H, CH_{Ar}), 7.72 (d, J = 8.0 Hz, 1H, CH_{Ar}), 7.87-7.95 (m, 3H, CH_{Ar}), 8.13 (d, J = 7.6 Hz, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 39.0 (CH_2), 121.2 (Ar), 122.9 (Ar), 125.5 (Ar), 126.2 (Ar), 127.0 (Ar), 128.0 (Ar), 128.3 (Ar), 132.3 (Ar), 133.0 (Ar), 134.6 (Ar), 135.1 (Ar), 137.9 (Ar), 159.1 (C=O). ^{19}F-NMR (564.7 MHz, $CDCl_3$) δ -57.34 (s, CF_3). Anal. Calcd for $C_{15}H_{10}F_3NO_3S$: C, 52.79; H, 2.95; N, 4.10. Found: C, 52.55 ; H, 3.17; N, 3.91.

8.2.3. 2-(3-Methylbenzyl)-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (3a)

1.51 g of anhydrous potassium carbonate (1 eq.) were added to a stirring solution of saccharin (2.0 g, 1.0 eq.) in 20 mL of *N*,*N*-dimethylformamide at 80 °C. 3-Methylbenzyl bromide (2.01 g, 1 eq.) was added and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the resulting suspension was filtered. Purification *via* column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:2) gave the title compound as a white solid (72% yield); mp 94-96 °C; IR ν_{max} 3065 (ν C_{sp2}-H), 1729 (ν C=O), 1329 (ν_{as} S=O), 1260 (ν C-N), 1178 (ν_s S=O), 748 (δ C_{sp2}-H), 676 (δ C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.37 (3H, s, CH_3), 4.90 (2H, s, CH_2), 7.14 (d, J = 7.6 Hz, 1H, CH_{Ar}), 7.24-7.33 (m, 3H, CH_{Ar}), 7.82-7.88 (m, 2H, CH_{Ar}), 7.95 (d, J = 7.2 Hz, 1H, CH_{Ar}), 8.08 (d, J = 8.0 Hz, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 21.4 (CH_3), 42.7 (CH_2), 121.0 (Ar), 125.2 (Ar), 125.8 (Ar), 127.3 (Ar), 128.6 (Ar), 129.1 (Ar), 129.4 (Ar), 134.3 (Ar), 134.4 (Ar), 134.8 (Ar), 137.8 (Ar), 138.4 (Ar), 158.9 (C=O). Anal. Calcd for $C_{15}H_{13}NO_3S$: C, 62.70; H, 4.56; N, 4.87. Found: C, 62.99; H, 4.28; N, 4.69.

8.2.4. 2-(3-Trifluoromethylbenzyl)-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (4a)

1.51 g of anhydrous potassium carbonate (1 eq.) were added to a stirring solution of saccharin (2.0 g, 1.0 eq.) in 20 mL of *N*,*N*-dimethylformamide at 80 °C. 3-Trifluoromethylbenzyl bromide (2.63 g, 1 eq.) was added and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the resulting suspension was filtered. Purification *via* column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:2) gave the title compound as a white solid (79% yield); mp 128-130 °C; IR ν_{max} 3092 (ν C_{sp2}-H), 1720 (ν C=O), 1332 (ν_{as} S=O), 1263 (ν C-N), 1175 (ν_s S=O), 752 (δ C_{sp2}-H), 680 (δ C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.97 (2H, s, CH_2), 7.49-7.52 (m, 1H, CH_{Ar}), 7.60 (d, J = 8.0 Hz, 1H, CH_{Ar}), 7.72 (d, J = 7.6 Hz, 1H, CH_{Ar}), 7.79 (s, 1H, CH_{Ar}), 7.84-7.97 (m, 3H, CH_{Ar}), 8.09 (d, J = 7.6 Hz, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 42.1 (CH_2), 121.1 (Ar), 125.3 (Ar), 125.4 (Ar), 125.6 (Ar), 127.1 (Ar), 129.3 (Ar), 131.0 (Ar), 132.1 (Ar), 134.5 (Ar), 135.0 (Ar), 135.5 (Ar), 137.7 (Ar), 158.9 (C=O). ^{19}F-NMR (564.7 MHz, $CDCl_3$) δ -60.08 (s, CF_3). Anal. Calcd for $C_{15}H_{10}F_3NO_3S$: C, 52.79; H, 2.95; N, 4.10. Found: C, 52.98; H, 2.76; N, 4.32.

8.2.5. 2-(4-Methylbenzyl)-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (5a)

1.51 g of anhydrous potassium carbonate (1 eq.) were added to a stirring solution of saccharin (2.0 g, 1.0 eq.) in 20 mL of *N*,*N*-dimethylformamide at 80 °C. 4-Methylbenzyl bromide (2.01 g, 1 eq.) was added and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the resulting suspension was filtered. Purification *via* column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:2) gave the title compound as a white solid (67% yield); mp 111-113 °C; IR ν_{max} 2920 (ν C_{sp3}-H), 1732 (ν C=O), 1329 (ν_{as} S=O), 1257 (ν C-N), 1174 (ν_s S=O), 747 (δ C_{sp2}-H), 674 (δ C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.35 (3H, s, CH_3), 4.89 (2H, s, CH_2), 7.18 (d, J = 8.0 Hz, 2H, CH_{Ar}), 7.42 (d, J = 8.0 Hz, 2H, CH_{Ar}), 7.81-7.88 (m, 3H, CH_{Ar}), 8.06 (d, J = 8.0 Hz, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 21.2 (CH_3), 42.5 (CH_2), 121.0 (Ar), 125.2 (Ar), 127.4 (Ar), 128.8 (Ar), 129.4 (Ar), 131.5 (Ar), 134.3 (Ar), 134.7 (Ar), 137.8 (Ar), 138.1 (Ar), 158.9 (C=O). Anal. Calcd for $C_{15}H_{13}NO_3S$: C, 62.70; H, 4.56; N, 4.87. Found: C, 62.57; H, 4.34; N, 4.58.

8.2.6. 2-(4-Trifluoromethylbenzyl)-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (6a)

1.51 g of anhydrous potassium carbonate (1 eq.) were added to a stirring solution of saccharin (2.0 g, 1.0 eq.) in 20 mL of *N*,*N*-dimethylformamide at 80 °C. 4-Trifluoromethylbenzyl bromide (2.63 g, 1 eq.) was added and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the resulting suspension was filtered. Purification *via* column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:2) gave the title compound as a white solid (57% yield); mp 119-121 °C; IR ν_{max} 2963 (ν C_{sp3}-H), 1741 (ν C=O), 1319 (ν_{as} S=O), 1264 (ν C-N), 1167 (ν_s S=O), 750 (δ C_{sp2}-H), 679 (δ C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.97 (2H, s, CH_2), 7.64 (bs, 4H, CH_{Ar}), 7.84-7.98 (m, 3H, CH_{Ar}), 8.08 (d, J = 8.0 Hz, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 42.0 (CH_2), 121.2 (Ar), 125.4 (Ar), 125.6 (Ar), 125.7 (Ar), 127.1 (Ar), 129.0 (Ar), 134.5 (Ar), 135.0 (Ar), 137.7 (Ar), 138.4 (Ar), 158.9 (C=O). ^{19}F-NMR (564.7 MHz, $CDCl_3$) δ -60.12 (s, CF_3). Anal. Calcd for $C_{15}H_{10}F_3NO_3S$: C, 52.79; H, 2.95; N, 4.10. Found: C, 53.04; H, 2.78; N, 3.95.

8.2.7. 2-(4-Fluorobenzyl)-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (7a)

Anhydrous potassium carbonate (1.1 eq.) was added to a stirring solution of saccharin (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. Then 4-fluorobenzyl bromide (1.5 eq.) was added and the

reaction mixture was stirred at 80 °C for 72 h. The mixture was poured on ice and the resulting suspension was filtered and washed with water and petroleum ether. Purification by column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:3) gave compound **7a** as a white powder (89% yield); mp 125-126 °C; IR ν_{max} 1726 (ν C=O), 1335 (ν_{as} S=O), 1295 (ν C-N), 1180 (ν_{s} S=O), 1158 (ν C_{sp2}-F), 838 (δ C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 4.92 (s, 2H, CH_2), 7.18 (t, J = 8.8 Hz, 2H, CH_{Ar}), 7.47-7.50 (m, 2H, CH_{Ar}), 7.97-8.11 (m, 3H, CH_{Ar}), 8.31 (d, J = 7.6 Hz, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 41.4 (*C*H_2), 115.6 (Ar), 115.9 (Ar), 122.1 (Ar), 125.6 (Ar), 126.7 (Ar), 130.6 (Ar), 130.7 (Ar), 131.8 (Ar), 135.7 (Ar), 136.3 (Ar), 137.3 (Ar), 159.1 (*C*=O), 162.2 (d, J_{C-F} = 244.9 Hz, Ar). ^{19}F-NMR (564.7 MHz, $CDCl_3$) δ -111.04 (tt, J_{F-H} = 8.6 Hz (ortho), 5.2 Hz (meta), CF). Anal. Calcd for $C_{14}H_{10}FNO_3S$: C, 57.72; H, 3.46; N, 4.81. Found: C, 57.99; H, 3.67; N, 4.55.

8.2.8. 2-(4-Chlorobenzyl)-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (8a)

Anhydrous potassium carbonate (1.1 eq.) was added to a stirring solution of saccharin (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. Then 4-chlorobenzyl bromide (1.1 eq.) was added and the reaction mixture was stirred at 80 °C for 48 h. The mixture was poured on ice and the resulting suspension was filtered and washed with water, petroleum ether and diethyl ether. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **8a** as a white powder (89% yield); mp 153-155 °C; IR ν_{max} 3092 (ν C_{sp2}-H), 1726 (ν C=O), 1331 (ν_{as} S=O), 1307 (ν C-N), 1197 (ν_{s} S=O), 749 (δ C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 4.93 (s, 2H, CH_2), 7.41-7.45 (m, 4H, CH_{Ar}), 7.98-8.08 (m, 3H, CH_{Ar}), 8.30-8.32 (m, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 41.4 (*C*H_2), 122.1 (Ar), 125.6 (Ar), 126.7 (Ar), 128.9 (Ar), 130.3 (Ar), 133.0 (Ar), 134.6 (Ar), 135.6 (Ar), 136.3 (Ar), 137.2 (Ar), 159.1 (*C*=O). Anal. Calcd for $C_{14}H_{10}ClNO_3S$: C, 54.64; H, 3.28; N, 4.55. Found: C, 54.90; H, 3.47; N, 4.27.

8.2.9. 2-(4-Bromobenzyl)-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (9a)

Anhydrous potassium carbonate (1.1 eq.) was added to a stirring solution of saccharin (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. Then 4-bromobenzyl bromide (1.5 eq.) was added and the

reaction mixture was stirred at 80 °C for 72 h. The mixture was poured on ice and the resulting suspension was filtered and washed with water and petroleum ether. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **9a** as a white powder (89% yield); mp 158-160 °C; IR ν_{max} 1725 (ν C=O), 1332 (ν_{as} S=O), 1303 (ν C-N), 1180 (ν_s S=O or ν C-Br), 861 (δ C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.87 (s, 2H, CH_2), 7.40 (d, J = 8.4 Hz, 2H, CH_{Ar}), 7.49 (d, J = 8.4 Hz, 2H, CH_{Ar}), 7.81-7.95 (m, 3H, CH_{Ar}), 8.06 (d, J = 7.2 Hz, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 42.0 (*C*H_2), 121.1 (Ar), 122.4 (Ar), 125.3 (Ar), 127.1 (Ar), 130.5 (Ar), 131.9 (Ar), 133.6 (Ar), 134.5 (Ar), 134.9 (Ar), 137.6 (Ar), 158.9 (*C*=O). Anal. Calcd for $C_{14}H_{10}BrNO_3S$: C, 47.74; H, 2.86; N, 3.98. Found: C, 47.92; H, 3.01; N, 4.15.

8.2.10. 2-(4-Nitrobenzyl)-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (10a)

Anhydrous potassium carbonate (1.1 eq.) was added to a stirring solution of saccharin (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. Then 4-nitrobenzyl bromide (1.1 eq.) was added and the reaction mixture was stirred at 80 °C for 24 h. The mixture was poured on ice and the resulting suspension was filtered and washed with water and *n*-hexane. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **10a** as a yellow powder (91% yield); mp 178-180 °C; IR ν_{max} 3092 (ν C_{sp2}-H), 1727 (ν C=O), 1516 (ν_{as} N-O), 1338 (ν_{as} S=O), 1299 (ν_s N-O), 1248 (ν C-N), 1197 (ν_s S=O), 755 (δ C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.10 (s, 2H, CH_2), 7.69-7.71 (m, 2H, CH_{Ar}), 8.03-8.36 (m, 6H, CH_{Ar}); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 41.3 (*C*H_2), 122.2 (Ar), 124.1 (Ar), 125.7 (Ar), 126.7 (Ar), 129.3 (Ar), 135.8 (Ar), 136.4 (Ar), 137.2 (Ar), 143.3 (Ar), 147.5 (Ar), 159.1 (*C*=O). Anal. Calcd for $C_{14}H_{10}N_2O_5S$: C, 52.83; H, 3.17; N, 8.80. Found: C, 52.56; H, 3.41; N, 9.04.

8.2.11. 2-[2-(3-Methoxyphenyl)-2-oxoethyl]-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (11a)

Anhydrous potassium carbonate (1.1 eq.) was added to a stirring solution of saccharin (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. Then, newly synthesized 2-bromo-3'-methoxyacetophenone[32] (1.1 eq.) was added and the reaction mixture was stirred at 80 °C for 72 h. The mixture was poured on ice and the resulting suspension was filtered and washed with water and petroleum ether.

Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **11a** as a yellow powder (58% yield); mp 167-169 °C; IR ν_{max} 1736 (ν C=O), 1694 (ν C=O), 1328 (ν_{as} S=O), 1258 (ν C-N), 1182 (ν_s S=O), 864 (δ C_{sp2}-H) 752 (δ_o C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.86 (s, 3H, OCH_3), 5.14 (s, 2H, CH_2), 7.18-7.20 (m, 1H, CH_{Ar}), 7.41-7.58 (m, 3H, CH_{Ar}), 7.87-7.96 (m, 3H, CH_{Ar}), 8.11 (s, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 44.6 (*C*H_2), 55.5 (O*C*H_3), 112.4 (Ar), 120.7 (Ar), 120.9 (Ar), 121.2 (Ar), 125.5 (Ar), 127.3 (Ar), 129.9 (Ar), 134.5 (Ar), 134.9 (Ar), 135.3 (Ar), 137.9 (Ar), 159.1 (Ar), 160.0 (*C*=O), 188.7 (*C*=O). Anal. Calcd for $C_{16}H_{13}NO_5S$: C, 58.00; H, 3.95; N, 4.23. Found: C, 58.22; H, 4.11; N, 4.02.

8.2.12. 2-[2-(4-Fluorophenyl)-2-oxoethyl]-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (12a)

Anhydrous potassium carbonate (1.1 eq.) was added to a stirring solution of saccharin (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. Then 2-chloro-4'-fluoroacetophenone (1.1 eq.) was added and the reaction mixture was stirred at 80 °C for 72 h. The mixture was poured on ice and extracted with chloroform (3 x 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:3) gave compound **12a** as a light yellow powder (75% yield); mp 166-167 °C; IR ν_{max} 1736 (ν C=O), 1692 (ν C=O), 1329 (ν_{as} S=O), 1305 (ν C-N), 1181 (ν_s S=O), 1156 (ν C_{sp2}-F), 746 (δ_o C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.14 (s, 2H, CH_2), 7.19-7.23 (m, 2H, CH_{Ar}), 7.86-7.94 (m, 2H, CH_{Ar}), 7.97-7.99 (m, 1H, CH_{Ar}), 8.04-8.12 (m, 3H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 44.3 (*C*H_2), 116.2 (Ar), 116.4 (Ar), 121.2 (Ar), 125.5 (Ar), 127.2 (Ar), 130.6 (Ar), 130.9 (Ar), 131.0 (Ar), 134.5 (Ar), 135.0 (Ar), 137.9 (Ar), 159.1 (*C*=O), 166.3 (d, J_{C-F} = 257.85 Hz, Ar), 187.4 (*C*=O). ^{19}F-NMR (564.7 MHz, DMSO-d_6) δ -96.66 (tt, J_{F-H} = 8.8 Hz (ortho), 5.5 Hz (meta), CF). Anal. Calcd for $C_{15}H_{10}FNO_4S$: C, 56.42; H, 3.16; N, 4.39. Found: C, 56.21; H, 2.91; N, 4.12.

8.2.13. 2-[2-(2,4-Dichlorophenyl)-2-oxoethyl]-1,2-benzothiazol-3(2*H*)-one 1,1-dioxide (13a)

Anhydrous potassium carbonate (1.1 eq.) was added to a stirring solution of saccharin (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. Then 2,2',4'-trichloroacetophenone (1.1 eq.) was added and the reaction mixture was stirred at 80 °C for 72 h. The mixture was poured on ice and the resulting

suspension was filtered and washed with water, *n*-hexane and petroleum ether. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **13a** as a yellow powder (92% yield); mp 117-119 °C; IR ν_{max} 3082 (ν C_{sp2}-H), 1736 (ν C=O), 1719 (ν C=O), 1332 (ν_{as} S=O), 1316 (ν C-N), 1184 (ν_{s} S=O), 883 (δ C_{sp2}-H), 817 (δ C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.36 (s, 2H, CH_2), 7.62-7.64 (m, 1H, CH_{Ar}), 7.76-7.77 (m, 1H, CH_{Ar}), 7.95-8.15 (m, 4H, CH_{Ar}), 8.35 (s, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 46.9 (*C*H_2), 122.2 (Ar), 125.7 (Ar), 126.5 (Ar), 128.1 (Ar), 131.0 (Ar), 132.2 (Ar), 132.8 (Ar), 133.5 (Ar), 135.8 (Ar), 136.5 (Ar), 137.7 (Ar), 138.1 (Ar), 159.1 (*C*=O), 191.4 (*C*=O). Anal. Calcd for $C_{15}H_9Cl_2NO_4S$: C, 48.66; H, 2.45; N, 3.78. Found: C, 48.24; H, 2.21; N, 4.01.

8.3. Procedures for the synthesis of acesulfame derivatives

8.3.1. 3-(4-Fluorobenzyl)-6-methyl-1,2,3-oxathiazin-4(3*H*)-one 2,2-dioxide (14a)

4-Fluorobenzyl bromide (1.5 eq.) was added to a stirring solution of potassium acesulfame (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 72 h, poured on ice and extracted with dichloromethane (3 x 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **14a** as a yellow oil (45% yield); IR ν_{max} 3089 (ν C_{sp2}-H), 1697 (ν C=O), 1397 (ν_{as} S=O), 1306 (ν C-N), 1198 (ν_{s} S=O), 1158 (ν C_{sp2}-F), 924 (ν S-O), 839 (δ_{p} C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.18 (s, 3H, CH_3), 4.96 (s, 2H, CH_2), 5.83 (s, 1H, CH=), 7.00-7.05 (m, 2H, CH_{Ar}), 7.41-7.44 (m, 2H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 19.5 (*C*H_3), 45.4 (*C*H_2), 104.4 (*C*H=), 115.5 (Ar), 115.7 (Ar), 130.8 (Ar), 130.9 (Ar), 161.1 (d, J_{C-F} = 196.3, Ar), 161.4 (CH_3*C*=), 163.9 (*C*=O). ^{19}F-NMR (564.7 MHz, $CDCl_3$) δ -110.63 (tt, J_{F-H} = 8.6 Hz (ortho), 5.2 Hz (meta), CF). Anal. Calcd for $C_{11}H_{10}FNO_4S$: C, 48.70; H, 3.72; N, 5.16. Found: C, 48.94; H, 3.97; N, 4.90.

8.3.2. 3-(4-Chlorobenzyl)-6-methyl-1,2,3-oxathiazin-4(3*H*)-one 2,2-dioxide (15a)

4-Chlorobenzyl bromide (1.1 eq.) was added to a stirring solution of potassium acesulfame (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 72 h, poured on

ice and extracted with dichloromethane (3 x 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **15a** as a white powder (45% yield); mp 54-56 °C; IR ν_{max} 3107 (ν C_{sp2}-H), 1700 (ν C=O), 1398 (ν_{as} S=O), 1320 (ν C-N), 1199 (ν_s S=O), 922 (ν S-O), 795 (δ_p C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, Methanol-d_4) δ 2.14 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 5.95 (s, 1H, CH=), 7.29-7.33 (m, 4H, CH_{Ar}); ^{13}C-NMR (101 MHz, Methanol-d_4) δ 18.3 (*C*H_3), 45.0 (*C*H_2), 103.8 (*C*H=), 128.5 (Ar), 129.9 (Ar), 133.8 (Ar), 134.0 (Ar), 160.3 (CH_3*C*=), 162.9 (*C*=O). Anal. Calcd for $C_{11}H_{10}ClNO_4S$: C, 45.92; H, 3.50; N, 4.87. Found: C, 46.11; H, 3.77; N, 4.68.

8.3.3. 6-Methyl-3-(4-nitrobenzyl)-1,2,3-oxathiazin-4(3*H*)-one 2,2-dioxide (16a)

4-Nitrobenzyl bromide (1.1 eq.) was added to a stirring solution of potassium acesulfame (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 72 h and poured on ice. The resulting suspension was filtered and washed with water, *n*-hexane and petroleum ether to give compound **16a** as a light yellow powder (85% yield); mp 81-83 °C; IR ν_{max} 3087 (ν C_{sp2}-H), 1698 (ν C=O), 1549 (ν_{as} N-O), 1390 (ν_{as} S=O), 1349 (ν_s N-O), 1306 (ν C-N), 1200 (ν_s S=O), 926 (ν S-O), 855 (δ_p C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.24 (s, 3H, CH_3), 5.06 (s, 2H, CH_2), 5.88 (s, 1H, CH=), 7.56 (d, J = 7.6 Hz, 2H, CH_{Ar}), 8.16 (d, J = 8.0 Hz, 2H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 19.7 (*C*H_3), 45.2 (*C*H_2), 104.3 (*C*H=), 123.9 (Ar), 129.4 (Ar), 141.9 (Ar), 147.8 (Ar), 160.0 (CH_3*C*=), 162.5 (*C*=O). Anal. Calcd for $C_{11}H_{10}N_2O_6S$: C, 44.29; H, 3.38; N, 9.39. Found: C, 44.02; H, 3.67; N, 9.58.

8.3.4. 3-[2-(3-Methoxyphenyl)-2-oxoethyl]-6-methyl-1,2,3-oxathiazin-4(3*H*)-one 2,2-dioxide (17a)

Newly synthesized 2-bromo-3'-methoxyacetophenone[32] (1.1 eq.) was added to a stirring solution of potassium acesulfame (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 48 h, poured on ice and extracted with dichloromethane (3 x 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **17a** as a

yellow powder (53% yield); mp 95-99 °C; IR ν_{max} 2961 (ν C_{sp3}-H), 1714 (ν C=O), 1696 (ν C=O), 1320 (ν_{as} S=O), 1200 (ν_{s} S=O), 930 (ν S-O), 861 (δ_{m} C_{sp2}-H), 782 (δ_{m} C_{sp2}-H), 679 (δ_{m} C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.23 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 5.24 (s, 2H, CH_2), 5.89 (s, 1H, CH=), 7.15 (s, 1H, CH_{Ar}), 7.38-7.50 (m, 3H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 19.7 (*C*H_3), 49.0 (O*C*H_3), 55.4 (*C*H_2), 104.3 (*C*H=), 112.3 (Ar), 120.6 (Ar), 120.8 (Ar), 130.0 (Ar), 135.2 (Ar), 159.9 (Ar + CH_3*C*=), 162.6 (*C*=O), 189.7 (*C*=O). Anal. Calcd for $C_{13}H_{13}NO_6S$: C, 50.16; H, 4.21; N, 4.50. Found: C, 49.91; H, 4.03; N, 4.72.

8.3.5. 1-(4-Bromophenyl)-2-[(6-methyl-2,2-dioxido-1,2,3-oxathiazin-4-yl)oxy]ethanone (18b)

Newly synthesized 2-bromo-4'-bromoacetophenone[32] (1.1 eq.) was added to a stirring solution of potassium acesulfame (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 24 h, poured on ice and extracted with chloroform (3 x 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **18b** as a yellow powder (84% yield); mp 125-127 °C; IR ν_{max} 3080 (ν C_{sp2}-H), 1688 (ν C=O), 1651 (ν C=N), 1327 (ν_{as} S=O), 1199 (ν_{s} S=O), 1175 (ν C-O-C), 931 (ν S-O), 812 (δ_{p} C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 3H, CH_3), 5.22 (s, 2H, CH_2), 5.92 (s, 1H, CH=), 7.65-7.67 (m, 2H, CH_{Ar}), 7.82-7.84 (m, 2H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 19.8 (*C*H_3), 48.7 (*C*H_2), 104.3 (*C*H=), 129.6 (Ar), 132.3 (Ar), 132.6 (Ar), 159.9 (Ar), 162.6 (CH_3*C*= + *C*=N), 189.0 (*C*=O). Anal. Calcd for $C_{12}H_{10}BrNO_5S$: C, 40.02; H, 2.80; N, 3.89. Found: C, 39.84; H, 3.01; N, 4.05.

8.3.6. 6-Methyl-3-[2-(4-nitrophenyl)-2-oxoethyl]-1,2,3-oxathiazin-4(3*H*)-one 2,2-dioxide (19a)

Newly synthesized 2-bromo-4'-nitroacetophenone[32] (1.1 eq.) was added to a stirring solution of potassium acesulfame (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 24 h and poured on ice. The resulting suspension was filtered and washed with water and petroleum ether. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **19a** as a brown powder (44% yield); mp 133-135 °C; IR ν_{max} 3071 (ν C_{sp2}-H), 1707 (ν C=O), 1684 (ν C=O), 1524 (ν_{as} N-O), 1348 (ν_{s} N-O), 1333 (ν_{as}

S=O), 1201 (ν_s S=O), 929 (ν S-O), 857 (δ_p C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.33 (s, 3H, CH_3), 5.60 (s, 2H, CH_2), 6.37 (s, 1H, CH=), 8.31-8.39 (m, 4H, CH_{Ar}); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 19.5 (*C*H_3), 49.7 (*C*H_2), 104.1 (*C*H=), 124.4 (Ar), 130.3 (Ar), 138.7 (Ar), 150.9 (Ar), 160.1 (CH_3*C*=), 163.8 (*C*=O), 191.11 (*C*=O). Anal. Calcd for $C_{12}H_{10}N_2O_7S$: C, 44.17; H, 3.09; N, 8.59. Found: C, 43.94; H, 3.31; N, 8.22.

8.3.7. 4-[(6-Methyl-2,2-dioxido-4-oxo-1,2,3-oxathiazin-3(4*H*)-yl)acetyl]benzonitrile (20a)

Newly synthesized 2-bromo-4'-cyanoacetophenone[33] (1.1 eq.) was added to a stirring solution of potassium acesulfame (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 24 h, poured on ice and filtered. Purification by column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:3) gave compound **20a** as a yellow powder (70% yield); mp 165-166 °C; IR ν_{max} 3077 (ν C_{sp2}-H), 2248 (ν CN), 1732 (ν C=O), 1687 (ν C=O), 1321 (ν_{as} S=O), 1199 (ν_s S=O), 918 (ν S-O), 825 (δ_p C_{sp2}-H), 775 (δ_o C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.31 (s, 3H, CH_3), 5.58 (s, 2H, CH_2), 6.36 (s, 1H, CH=), 8.20-8.28 (m, 4H, CH_{Ar}); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 19.4 (*C*H_3), 48.9 (*C*H_2), 104.2 (*C*H=), 123.3 (*C*N), 131.4 (Ar), 138.9 (Ar), 141.2 (Ar), 149.1 (Ar), 160.3 (CH_3*C*=), 163.7 (*C*=O), 191.5 (*C*=O). Anal. Calcd for $C_{13}H_{10}N_2O_5S$: C, 50.98; H, 3.29; N, 9.15. Found: C, 51.19; H, 3.42; N, 8.92.

8.3.8. 3-[2-(Biphenyl-4-yl)-2-oxoethyl]-6-methyl-1,2,3-oxathiazin-4(3*H*)-one 2,2-dioxide (21a)

Newly synthesized 2-bromo-4'-phenylacetophenone[33] (1.1 eq.) was added to a stirring solution of potassium acesulfame (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 48 h, poured on ice and filtered. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **21a** as a grey powder (77% yield); mp 170-172 °C; IR ν_{max} 3085 (ν C_{sp2}-H), 1731 (ν C=O), 1684 (ν C=O), 1328 (ν_{as} S=O), 1197 (ν_s S=O), 929 (ν S-O), 829 (δ_p C_{sp2}-H), 778 (δ_o C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.32 (s, 3H, CH_3), 5.54 (s, 2H, CH_2), 6.29 (s, 1H, CH=), 8.13-8.35 (m, 9H, CH_{Ar}); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 20.0 (*C*H_3), 48.2 (*C*H_2), 104.9 (*C*H=), 122.8 (Ar), 123.6 (Ar), 123.9 (Ar), 125.1 (Ar),

125.6 (Ar), 132.8 (Ar), 133.9 (Ar), 148.9 (Ar), 161.8 (CH_3C=), 160.2 (*C*=O), 190.9 (*C*=O). Anal. Calcd for $C_{18}H_{15}NO_5S$: C, 60.49; H, 4.23; N, 3.92. Found: C, 60.21; H, 4.45; N, 4.11.

8.3.9. 1-(2,4-Dichlorophenyl)-2-[(6-methyl-2,2-dioxido-1,2,3-oxathiazin-4-yl)oxy]ethanone (22b)

2,2',4'-Trichloroacetophenone (1.1 eq.) was added to a stirring solution of potassium acesulfame (1.0 eq.) in 10 mL of *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 24 h, poured on ice and extracted with dichloromethane (3 x 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compound **22b** as an orange powder (83% yield); mp 90-94 °C; IR ν_{max} 3080 (ν C_{sp2}-H), 1737 (ν C=O), 1647 (ν C=N), 1369 (ν_{as} S=O), 1259 (ν C-O-C), 1198 (ν_s S=O), 921 (ν S-O), 827 (δ_p C_{sp2}-H), 777 (δ_o C_{sp2}-H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.22 (s, 3H, CH_3), 5.45 (s, 2H, CH_2), 5.94 (s, 1H, CH=), 7.34-7.35 (m, 1H, CH_{Ar}), 7.43 (s, 1H, CH_{Ar}), 7.61-7.63 (m, 1H, CH_{Ar}); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 20.6 (CH_3), 71.0 (CH_2), 95.2 (*C*H=), 127.9 (Ar), 130.6 (Ar), 131.6 (Ar), 132.8 (Ar), 133.2 (Ar), 139.3 (Ar), 168.8 (CH_3C=), 169.9 (*C*=N), 191.5 (*C*=O). Anal. Calcd for $C_{12}H_9Cl_2NO_5S$: C, 41.16; H, 2.59; N, 4.00. Found: C, 40.91; H, 2.78; N, 4.25.

8.4. Enzyme inhibition assays

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity. Phenol red (0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5, for α-CAs) as buffer, and 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 (1 μM) were prepared in distilled-deionized water and dilutions up to

0.1 nM were done thereafter with the assay buffer. 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 or for the eventual active site mediated hydrolysis of the inhibitor. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation,[34] and represent the average from at least three different determinations. All recombinant CA isoforms were obtained in-house as previously reported.[35]

8.5. Molecular modelling studies

8.5.1. Preparation of saccharin- and acesulfame-based structures

The saccharin- and acesulfame-based analogues **1**-**25** were prepared in 3D with the MOE software package (v2013.08.02, Chemical Computing Group Inc., Montreal, Canada) as previously reported.[36] All possible structural isomers of compounds were constructed. Strong acids were deprotonated and strong bases were protonated. Finally, the ligands were energy minimized using a steepest-descent protocol (MMFF94x force field).

8.5.2. Preparation of hCA crystal structures for docking studies

The structures of hCA I (PDB: 3LXE, 1.90 Å), hCA II (PDB: 4E3D, 1.60 Å), hCA IX (PDB: 3IAI, 2.20 Å) and hCA XII (PDB: 1JD0, 1.50 Å) were obtained from the protein databank (PDB). The protein atoms, the active site zinc ions and the zinc-bound water molecule of hCA II were retained and all other atoms were omitted. The remaining structure was protonated using the MOE software package and subsequently the obtained structure was energy-minimized (AMBER99 force field). Finally, the obtained protein models were superposed on the hCA I structure using the backbone Cα-atoms. The zinc-bound water molecule of hCA II coordinated well to the zinc ions of the other hCAs.

8.5.3. Docking studies

The GOLD Suite software package (v5.2, CCDC, Cambridge, UK) and the GoldScore scoring function were used to dock the derivatives into the hCA structures with and without the zinc-bound water molecule (25 dockings per ligand). The binding pocket was defined as all residues within 13

Å of a centroid (x: -17.071, y: 35.081, 43.681; corresponding approximately to the position of the thiadiazole ring of acetazolamide in the 1JD0 structure).

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was financed by two FP7 EU projects (Metoxia and Dynano) to CTS.

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Graphical Abstract

A novel library of saccharin and acesulfame derivatives as potent and selective inhibitors of carbonic anhydrase IX and XII isoforms

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