

RESEARCH ARTICLE

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Open saccharin-based secondary sulfonamides as potent and selective inhibitors of cancer-related carbonic anhydrase IX and XII isoforms

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ABSTRACT

A large number of novel secondary sulfonamides based on the open saccharin scaffold were synthesized and evaluated as selective inhibitors of four different isoforms of human carbonic anhydrase (hCA I, II, IX and XII, EC 4.2.1.1). They were obtained by reductive ring opening of the newly synthesized *N*-alkylated saccharin derivatives and were shown to be inactive against the two cytosolic off-target hCA I and II ($K_i > 10 \mu\text{M}$). Interestingly, these compounds inhibited hCA IX in the low nanomolar range with K_i s ranging between 20 and 298 nM and were extremely potent inhibitors of hCA XII isoenzyme (K_i s ranging between 4.3 and 432 nM). Since hCA IX and XII are the cancer-related isoforms recently validated as drug targets, these results represent an important goal in the development of new anticancer candidates. Finally, a computational approach has been performed to better correlate the biological data to the binding mode of these inhibitors.

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Cancer-related isoforms; saccharin; secondary sulphonamides; selective carbonic anhydrase inhibitors


Introduction

Carbonic anhydrases are a vast family of metalloenzymes (CAs, EC 4.2.1.1.) strongly involved in regulating cell homeostasis and intracellular pH by catalyzing the reversible hydration of carbonic dioxide to bicarbonate ions¹. Fifteen human isoforms are discovered to date and can be differentiated on the basis of cellular localization, tissue distribution, catalytic activity and affinity to various inhibitors, thus representing validated therapeutic targets for the treatment of a wide range of diseases^{2,3}. In particular, hCA IX and XII were identified as part of the complex machinery that primary and metastatic cancer cell lines use to compensate their dysregulated pH in poorly vascularized microenvironments, like the surroundings of solid tumours^{4–6}. Targeting these cancer-related isoforms has attracted a lot of attention, hence leading to the proliferation of literature on the subject and the development of structurally diverse inhibitors^{7–19}. As a result of this superb effort, a potent hCA IX selective compound (SLC-0111) is currently in Phase I clinical trials for the treatment of solid metastatic tumors, consequently boosting the research for new suitable candidates^{20,21}.

Since the active site of CAs is characterized by the presence of a catalytic zinc ion held in place by three histidine residues, one of the most successful approaches to design novel CAs implies introducing a zinc-binding group (ZBG) in the scaffold of potential inhibitors²². In fact, the most widely prescribed CAs are characterized by the presence of a primary sulfonamide group, which

efficiently engages the positively charged zinc ion upon deprotonation^{23–26}. However, despite primary sulfonamides being preferred "zinc binding group", they are usually associated with promiscuous profiles and lack of selectivity amongst the different isoforms. On the other side, secondary and tertiary sulfonamides have been lately reported in the literature as efficient and selective inhibitors of the cancer-related hCA IX and XII isoforms^{27–31}, although their binding mode remains a subject of investigation³². In a recent publication, Ivanova et al. reported on the spontaneous ring opening of cyclic tertiary sulfonamides under basic (pH=9) crystallization conditions³³. Since hCAs do not possess any peptidase activity, they concluded that the base-catalyzed hydrolysis of the saccharin isothiazolone ring happened before the inhibitor entered the active site, and proved their hypothesis by testing both open and closed analogs against a panel of hCAs (hCA I, hCA II, hCA IX and hCA XII). In particular, these open saccharin derivatives determined an increased inhibition of hCA IX, while retaining high activity against hCA II. Such promising results prompted us to investigate the effects of ring opening on our previously reported *N*-substituted saccharins as selective inhibitors of CA IX and XII^{15,34}. In this paper, we used a reductive ring opening approach to induce the 5-membered isothiazolone ring of saccharin to collapse into its corresponding secondary sulfonamide and benzyl alcohol (Figure 1)³⁵. The rationale behind this choice could be found in the opportunity of generating two new potential anchoring points for the zinc ion, while introducing several

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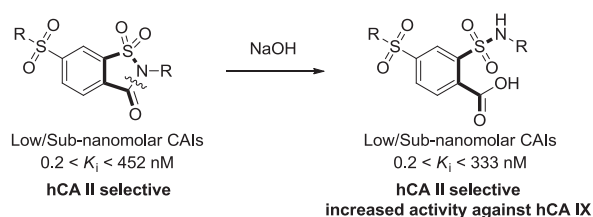
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degrees of freedom to the bonds connecting the two hydrophobic phenyl substituents to the polar core of the molecule. In general, the newly synthesized compounds (**1–21**) proved to be as potent as or slightly less potent than parent inhibitors, while the selectivity for the cancer-related isoforms (hCA IX and XII) over the off-target hCA I and II improved dramatically. In fact, none of the reported compounds inhibited hCA I and II isoforms at concentrations lower than 10 μM , while K_i values spanned from 20 to 298 nM against hCA IX and from 4.3 to 382 nM against hCA XII.

Chemistry

Saccharin (1.0 eq.) was activated using freshly ground anhydrous potassium carbonate and the corresponding salt was then directly reacted with a proper electrophile (2 eq. of substituted benzyl halide or α -haloacetophenone) by stirring the reaction mixture in *N,N*-dimethylformamide at 80 °C overnight (Scheme 1). Following these optimized conditions (polar aprotic solvent), we strictly obtained only the more stable regioisomers (*N*-substituted saccharin derivatives) limiting the Chapman–Mumm thermal rearrangement to the less stable *O*-substituted counterparts³⁶.

Previous work from Ivanova *et al.*³³:



This paper:

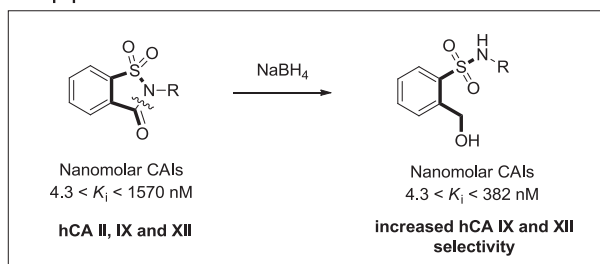


Figure 1. Reductive ring opening approach to the synthesis of novel CAls.

N-substituted saccharin derivatives were then subjected to reductive ring opening with an excess of NaBH_4 in dry methanol at room temperature for 2–8 h to give the corresponding secondary sulfonamide compounds **1–21** in discrete yields following a previously reported procedure with slight modifications³⁵.

Our choice of NaBH_4 as a reducing agent depended on preliminary experiments which suggested that (i) the sulfone group was not affected by these mild reducing conditions leading to the carbonyl group reduction to the alcohol level only with saccharin ring cleavage and that (ii) similar results were obtained for the preparation of *o*-hydroxymethyl-*N*-alkyl-benzamides starting from *N*-alkyl-phthalimides³⁷.

In their IR spectra, we usually registered for the open saccharin derivatives new but expected signals for the OH and NH stretching at 3240 and 3470 cm^{-1} , respectively, and the disappearance of the C=O stretching at 1735 cm^{-1} .

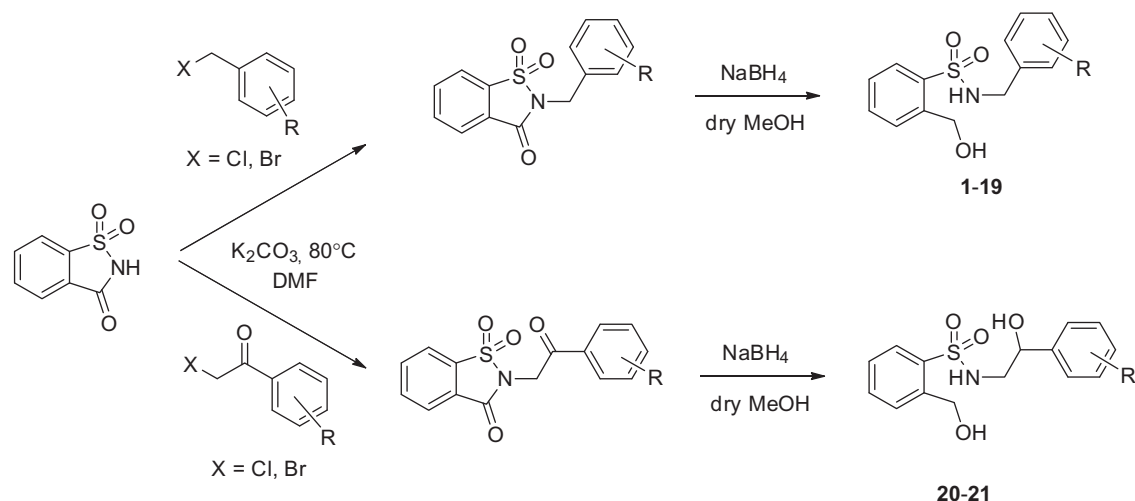
Biological evaluation

All the synthesized compounds 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 (K_i) are summarized in Table 1.

Experimental protocols

General

Solvents and reagents were used as supplied without further purification. Where mixtures of solvents are specified, the stated ratios are volume:volume. Acetazolamide was purchased by Sigma-Aldrich (Italy) and used 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). $^1\text{H-NMR}$ spectra were recorded on a Bruker AV400 (^1H : 400 MHz, ^{13}C : 101 MHz). $^{19}\text{F-NMR}$ 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



Scheme 1. Synthesis and structure of compounds **1–21**. For R substituents see Table 1.

are referenced to an external standard (CF₃COOH, δ = 76.55 ppm). Infrared 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. Where given, systematic compound names are those generated by ChemBioDraw Ultra[®] 12.0 following IUPAC conventions. NMR spectra for some representative compounds could be found in the Supplementary material.

Procedures for the synthesis of the new open saccharin derivatives

2-(Hydroxymethyl)-N-(2-methylbenzyl)benzenesulfonamide (1)

(2-Methylbenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 2 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:4). The title compound was a white solid (52% yield); mp 64–66 °C; IR ν_{\max} 3459 (ν O–H), 3237 (ν N–H), 3065 (ν C_{sp2}–H), 1319 (ν_{as} S=O), 1167 (ν_{s} S=O), 712 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.25 (s, 3H, CH₃), 2.77 (bs, 1H, OH, D₂O exch.), 4.08 (s, 2H, CH₂), 5.01 (s, 2H, CH₂), 5.43 (bs, 1H, NH, D₂O exch.), 7.09–7.19 (m, 4H, CH_{Ar}), 7.47–7.61 (m, 3H, CH_{Ar}), 8.01–8.04 (m, 1H, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 18.7 (CH₃), 45.5 (CH₂), 64.2 (CH₂), 126.2 (Ar), 128.2 (Ar), 128.5 (Ar), 128.9 (Ar), 129.6 (Ar), 129.9 (Ar), 130.5 (Ar), 131.6 (Ar), 133.2 (Ar), 134.1 (Ar), 136.6 (Ar), 138.2 (Ar).

2-(Hydroxymethyl)-N-(2-(trifluoromethyl)benzyl)benzenesulfonamide (2)

(2-Trifluoromethylbenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 4 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product that was purified by column chromatography (EtOAc/*n*-hexane, 1:3). The title compound was a white solid (47% yield); mp 66–68 °C; IR ν_{\max} 3446 (ν O–H), 3214 (ν N–H), 1310 (ν_{as} S=O), 1157 (ν_{s} S=O), 714 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.81 (t, 1H, J = 5.4 Hz, OH, D₂O exch.), 4.17 (d, 2H, J = 6.0 Hz, CH₂), 4.91 (s, 2H, CH₂), 5.83 (m, 1H, NH, D₂O exch.), 7.27 (t, 1H, J = 7.6 Hz, CH_{Ar}), 7.35–7.41 (m, 3H, CH_{Ar}), 7.46–7.50 (m, 3H, CH_{Ar}), 7.89–7.91 (m, 1H, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 43.7 (CH₂), 63.9 (CH₂), 124.2 (C-F, ¹J_{C-F} = 274.8 Hz, CF₃), 125.9 (Ar), 126.0 (Ar), 127.9 (Ar), 128.6 (Ar), 129.7 (Ar), 130.6 (Ar), 131.6 (Ar), 132.3 (Ar), 133.3 (Ar), 135.0 (Ar), 138.0 (Ar), 138.1 (Ar); ¹⁹F-NMR (564.7 MHz, CDCl₃) δ -56.89 (s, CF₃).

2-(Hydroxymethyl)-N-(2-nitrobenzyl)benzenesulfonamide (3)

(2-Nitrobenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 6 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane

(3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give the title compound as a light brown solid (37% yield); mp 103–105 °C; IR ν_{\max} 3498 (ν O–H), 3238 (ν N–H), 2973 (ν C_{sp3}–H), 1522 (ν N–O), 1327 (ν_{as} S=O), 1165 (ν_{s} S=O), 698 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, DMSO-*d*₆) δ 4.34 (d, 2H, J = 5.6 Hz, CH₂), 4.88 (s, 2H, CH₂), 5.45 (bs, 1H, OH, D₂O exch.), 7.39 (t, 1H, J = 7.2 Hz, CH_{Ar}), 7.50 (t, 1H, J = 7.6 Hz, CH_{Ar}), 7.60–7.68 (m, 3H, 3 × CH_{Ar}), 7.74–7.79 (m, 2H, 2 × CH_{Ar}), 7.96 (d, 1H, J = 8.4 Hz, CH_{Ar}), 8.29 (bs, 1H, NH, D₂O exch.); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 43.4 (CH₂), 60.0 (CH₂), 125.1 (Ar), 127.3 (Ar), 128.2 (Ar), 128.5 (Ar), 129.1 (Ar), 130.7 (Ar), 133.0 (Ar), 133.4 (Ar), 134.1 (Ar), 137.0 (Ar), 141.6 (Ar), 148.1 (Ar).

N-(2-bromobenzyl)-2-(hydroxymethyl)benzenesulfonamide (4)

(2-Bromobenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 6 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 2:1). The title compound was a white solid (0.05 g, 11% yield); mp 162–164 °C; IR ν_{\max} 3273 (ν O–H and ν N–H), 2920 (ν C_{sp3}–H), 1402 (ν_{as} S=O), 1065 (ν_{s} S=O), 676 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 3.45 (bs, 1H, OH, D₂O exch.), 4.14 (d, 2H, J = 6.4 Hz, CH₂), 4.93 (s, 2H, CH₂), 6.18 (m, 1H, NH, D₂O exch.), 7.03 (t, 1H, J = 7.6 Hz, CH_{Ar}), 7.15 (t, 1H, J = 7.4 Hz, CH_{Ar}), 7.28 (d, 1H, J = 6.8 Hz, CH_{Ar}), 7.33–7.49 (m, 4H, CH_{Ar}), 7.87 (d, 1H, J = 8.0 Hz, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 47.4 (CH₂), 63.5 (CH₂), 123.4 (Ar), 127.6 (Ar), 128.3 (Ar), 129.4 (Ar), 129.6 (Ar), 130.3 (Ar), 131.3 (Ar), 132.7 (Ar), 133.1 (Ar), 135.6 (Ar), 137.8 (Ar), 138.4 (Ar).

2-(Hydroxymethyl)-N-(3-methylbenzyl)benzenesulfonamide (5)

(3-Methylbenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 7 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:4). The title compound was a white solid (51% yield); mp 79–80 °C; IR ν_{\max} 3431 (ν O–H), 3161 (ν N–H), 2936 (ν C_{sp3}–H), 1316 (ν_{as} S=O), 1152 (ν_{s} S=O), 694 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.27 (s, 3H, CH₃), 2.80 (bs, 1H, OH, D₂O exch.), 4.07 (bs, 2H, CH₂), 4.99 (s, 2H, CH₂), 5.72 (bs, 1H, NH, D₂O exch.), 6.96 (d, 2H, J = 7.6 Hz, CH_{Ar}), 7.04 (d, 1H, J = 8.0 Hz, CH_{Ar}), 7.14 (t, 1H, J = 7.4 Hz, CH_{Ar}), 7.44–7.49 (m, 2H, CH_{Ar}), 7.55–7.59 (m, 1H, CH_{Ar}), 7.98 (d, 1H, 7.8 Hz, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 21.3 (CH₃), 47.5 (CH₂), 63.8 (CH₂), 124.9 (Ar), 128.4 (Ar), 128.5 (Ar), 128.6 (Ar), 128.7 (Ar), 129.8 (Ar), 131.5 (Ar), 133.1 (Ar), 136.1 (Ar), 138.1 (Ar), 138.2 (Ar), 138.3 (Ar).

2-(Hydroxymethyl)-N-(3-(trifluoromethyl)benzyl)benzenesulfonamide (6)

(3-Trifluoromethylbenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 8 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over

sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:3). The title compound was a white solid (71% yield); mp 76–78 °C; IR ν_{\max} 3430 (ν O–H), 3278 (ν N–H), 1317 (ν_{as} S=O), 1160 (ν_{s} S=O), 700 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.98 (t, 1H, J = 4.8 Hz, OH, D₂O exch.), 4.06 (d, 2H, J = 6.4 Hz, CH₂), 4.91 (d, 2H, J = 4.0 Hz, CH₂), 6.07 (m, 1H, NH, D₂O exch.), 7.24–7.37 (m, 6H, CH_{Ar}), 7.42–7.46 (m, 1H, CH_{Ar}), 7.82 (d, 1H, J = 7.6 Hz, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 46.9 (CH₂), 63.9 (CH₂), 123.9 (C–F, ¹J_{C–F} = 273.4 Hz, CF₃), 124.5 (Ar), 124.6 (Ar), 128.6 (Ar), 129.1 (Ar), 129.7 (Ar), 130.7 (Ar), 131.3 (Ar), 131.7 (Ar), 133.3 (Ar), 137.4 (Ar), 137.8 (Ar), 138.0 (Ar); ¹⁹F-NMR (564.7 MHz, CDCl₃) δ -60.09 (s, CF₃).

2-(Hydroxymethyl)-N-(3-nitrobenzyl)benzenesulfonamide (7)

(3-Nitrobenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. Excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 6 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product that was purified by column chromatography (EtOAc/*n*-hexane, 2:1). The title compound was a white solid (37% yield); mp 109–111 °C; IR ν_{\max} 3491 (ν O–H), 3164 (ν N–H), 2958 (ν C_{sp3}–H), 1536 (ν N–O), 1326 (ν_{as} S=O), 1162 (ν_{s} S=O), 695 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, DMSO-*d*₆) δ 4.16 (s, 2H, CH₂), 4.87 (s, 2H, CH₂), 5.47 (bs, 1H, OH, D₂O exch.), 7.33–7.37 (m, 1H, CH_{Ar}), 7.51–7.60 (m, 2H, CH_{Ar}), 7.64 (d, 1H, J = 7.6 Hz, CH_{Ar}), 7.73–7.75 (m, 2H, CH_{Ar}), 8.04 (s, 1H, CH_{Ar}), 8.06 (s, 1H, CH_{Ar}), 8.35 (bs, 1H, NH, D₂O exch.); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 45.4 (CH₂), 59.9 (CH₂), 122.5 (Ar), 122.6 (Ar), 127.1 (Ar), 127.9 (Ar), 128.5 (Ar), 130.2 (Ar), 132.9 (Ar), 134.6 (Ar), 137.2 (Ar), 140.8 (Ar), 141.5 (Ar), 148.1 (Ar).

N-(3-fluorobenzyl)-2-(hydroxymethyl)benzenesulfonamide (8)

(3-Fluorobenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 5 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:1). The title compound was a light yellow oil (67% yield); IR ν_{\max} 3492 (ν O–H), 3283 (ν N–H), 2895 (ν C_{sp3}–H), 1318 (ν_{as} S=O), 1252 (ν C_{sp2}–F), 1175 (ν_{s} S=O), 689 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.84 (bs, 1H, OH, D₂O exch.), 4.05 (s, 2H, CH₂), 4.97 (s, 2H, CH₂), 6.04 (bs, 1H, NH, D₂O exch.), 6.85–6.94 (m, 3H, CH_{Ar}), 7.17–7.20 (m, 1H, CH_{Ar}), 7.39–7.55 (m, 3H, CH_{Ar}), 7.92 (d, 1H, J = 7.6 Hz, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 46.6 (CH₂), 63.6 (CH₂), 114.6 (Ar–F, ²J_{C–F} = 21.1 Hz), 114.8 (Ar–F, ²J_{C–F} = 22.0 Hz), 123.4 (Ar–F, ^{long-range}J_{C–F} = 2.7 Hz), 128.5 (Ar), 129.7 (Ar), 130.1 (Ar–F, ³J_{C–F} = 8.2 Hz), 131.6 (Ar), 133.2 (Ar), 137.9 (Ar), 138.1 (Ar), 139.0 (Ar–F, ³J_{C–F} = 7.3 Hz), 162.7 (Ar–F, ¹J_{C–F} = 247.45 Hz); ¹⁹F-NMR (564.7 MHz, CDCl₃) δ -110.03 (ddd, J_{F–H} = 9.5 Hz, 8.6 Hz, 5.2 Hz, CF).

N-(3-bromobenzyl)-2-(hydroxymethyl)benzenesulfonamide (9)

(3-Bromobenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 5 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane

(3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:1). The title compound was a white solid (70% yield); mp 75–76 °C; IR ν_{\max} 3454 (ν O–H), 3214 (ν N–H), 2955 (ν C_{sp3}–H), 1319 (ν_{as} S=O), 1156 (ν_{s} S=O), 694 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, DMSO-*d*₆) δ 4.00 (d, 2H, J = 6 Hz, CH₂), 4.87 (d, 2H, J = 5.2 Hz, CH₂), 5.42 (bs, 1H, OH, D₂O exch.), 7.20 (bs, 2H, CH_{Ar}), 7.38 (bs, 3H, CH_{Ar}), 7.59–7.63 (m, 1H, CH_{Ar}), 7.75–7.78 (m, 2H, CH_{Ar}), 8.23 (bs, 1H, NH, D₂O exch.); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 45.6 (CH₂), 59.9 (CH₂), 122.0 (Ar), 127.0 (Ar), 127.2 (Ar), 127.9 (Ar), 128.5 (Ar), 130.4 (Ar), 130.7 (Ar), 130.8 (Ar), 132.9 (Ar), 137.2 (Ar), 141.2 (Ar), 141.5 (Ar).

2-(Hydroxymethyl)-N-(4-methylbenzyl)benzenesulfonamide (10)

(4-Methylbenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 6 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:4). The title compound was a white solid (56% yield); mp 100–102 °C; IR ν_{\max} 3437 (ν O–H), 3130 (ν N–H), 2930 (ν C_{sp3}–H), 1310 (ν_{as} S=O), 1151 (ν_{s} S=O), 701 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.31 (s, 3H, CH₃), 2.80 (bs, 1H, OH, D₂O exch.), 4.07 (d, 2H, J = 5.2 Hz, CH₂), 4.99 (s, 2H, CH₂), 5.60 (bs, 1H, NH, D₂O exch.), 7.06 (bs, 4H, CH_{Ar}), 7.44–7.50 (m, 2H, CH_{Ar}), 7.55–7.60 (m, 1H, CH_{Ar}), 8.00 (d, 1H, 7.6 Hz, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 21.1 (CH₃), 47.3 (CH₂), 63.8 (CH₂), 127.9 (Ar), 128.5 (Ar), 129.3 (Ar), 129.9 (Ar), 131.6 (Ar), 133.1 (Ar), 133.2 (Ar), 137.6 (Ar), 138.1 (Ar), 138.2 (Ar).

2-(Hydroxymethyl)-N-(4-(trifluoromethyl)benzyl)benzenesulfonamide (11)

(4-Trifluoromethylbenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 8 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:3). The title compound was a white solid (79% yield); mp 78–80 °C; IR ν_{\max} 3417 (ν O–H), 3161 (ν N–H), 3065 (ν C_{sp2}–H), 1321 (ν_{as} S=O), 1157 (ν_{s} S=O), 688 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 3.03 (bs, 1H, OH, D₂O exch.), 4.15 (d, 2H, J = 6.4 Hz, CH₂), 5.02 (d, 2H, J = 5.2 Hz, CH₂), 6.14 (t, 1H, J = 6.4 Hz, NH, D₂O exch.), 7.32 (d, 2H, J = 8.0 Hz, CH_{Ar}), 7.40–7.49 (m, 4H, CH_{Ar}), 7.54–7.58 (m, 1H, CH_{Ar}), 7.91 (s, 1H, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 46.9 (CH₂), 63.9 (CH₂), 124.0 (C–F, ¹J_{C–F} = 273.1 Hz, CF₃), 125.4 (Ar), 125.5 (Ar), 128.1 (Ar), 128.6 (Ar), 129.7 (Ar), 129.8 (Ar), 130.1 (Ar), 131.7 (Ar), 133.3 (Ar), 137.8 (Ar), 138.0 (Ar), 140.4 (Ar); ¹⁹F-NMR (564.7 MHz, CDCl₃) δ -60.08 (s, CF₃).

N-(4-cyanobenzyl)-2-(hydroxymethyl)benzenesulfonamide (12)

(4-Cyanobenzyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 8 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium

sulfate and concentrated *in vacuo* to give a crude product, which was purified by column chromatography (EtOAc/*n*-hexane, 2:1). The title compound was a white solid (67% yield); mp 93–95 °C; IR ν_{\max} 3481 (ν O–H), 3187 (ν N–H), 2949 (ν C_{sp3}–H), 2235 CN, 1325 (ν_{as} S=O), 1156 (ν_{s} S=O), 703 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, DMSO-*d*₆) δ 4.09 (s, 2H, CH₂), 4.88 (s, 2H, CH₂), 5.46 (bs, 1H, OH, D₂O exch.), 7.35–7.41 (m, 3H, CH_{Ar}), 7.59–7.62 (m, 1H, CH_{Ar}), 7.70–7.77 (m, 4H, CH_{Ar}), 8.33 (bs, 1H, NH, D₂O exch.); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 45.9 (CH₂), 59.9 (CH₂), 110.3 (Ar), 119.3 (CN), 127.2 (Ar), 127.9 (Ar), 128.5 (Ar), 128.7 (Ar), 132.6 (Ar), 132.9 (Ar), 137.1 (Ar), 141.6 (Ar), 144.3 (Ar).

2-(Hydroxymethyl)-N-(4-nitrobenzyl)benzenesulfonamide (13)

(4-Nitrobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 8 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product, which was purified by column chromatography (EtOAc/*n*-hexane, 1:1). The title compound was a yellow solid (57% yield); mp 98–99 °C; IR ν_{\max} 3493 (ν O–H), 3243 (ν N–H), 1515 (ν N–O), 1318 (ν_{as} S=O), 1160 (ν_{s} S=O), 690 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.74 (bs, 1H, OH, D₂O exch.), 4.21 (bs, 2H, CH₂), 5.08 (s, 2H, CH₂), 6.13 (bs, 1H, NH, D₂O exch.), 7.40–7.59 (m, 5H, CH_{Ar}), 7.96–7.97 (m, 1H, CH_{Ar}), 8.11–8.13 (m, 2H, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 46.7 (CH₂), 64.3 (CH₂), 123.7 (Ar), 128.5 (Ar), 128.8 (Ar), 129.8 (Ar), 131.6 (Ar), 133.4 (Ar), 137.6 (Ar), 138.7 (Ar), 144.0 (Ar), 147.2 (Ar).

N-(4-fluorobenzyl)-2-(hydroxymethyl)benzenesulfonamide (14)

(4-Fluorobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 8 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:3). The title compound was a light brown solid (79% yield); mp 99–100 °C; IR ν_{\max} 3439 (ν O–H), 3140 (ν N–H), 1312 (ν_{as} S=O), 1151 (ν_{s} S=O), 696 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 3.18 (bs, 1H, OH, D₂O exch.), 4.04 (d, 2H, J = 6.4 Hz, CH₂), 4.98 (s, 2H, CH₂), 6.00 (bs, 1H, NH, D₂O exch.), 6.88–6.93 (m, 2H, CH_{Ar}), 7.12–7.15 (m, 2H, CH_{Ar}), 7.41–7.57 (m, 3H, CH_{Ar}), 7.93 (d, 1H, J = 8.0 Hz, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 46.7 (CH₂), 63.7 (CH₂), 115.4 (Ar-F, ²J_{C-F} = 21.2 Hz), 128.5 (Ar), 129.6 (Ar), 129.7 (Ar), 131.6 (Ar), 132.0 (Ar), 132.1 (Ar), 133.2 (Ar), 138.0 (Ar), 138.1 (Ar), 135.4 (Ar-F, ¹J_{C-F} = 247.3 Hz); ¹⁹F-NMR (564.7 MHz, CDCl₃) δ -111.71 (tt, J_{F-H} = 8.7 Hz (ortho), 5.3 Hz (meta), CF).

N-(4-chlorobenzyl)-2-(hydroxymethyl)benzenesulfonamide (15)

(4-Chlorobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 7 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:1). The title compound was a white solid (70% yield); mp 103–105 °C;

IR ν_{\max} 3458 (ν O–H), 3236 (ν N–H), 2876 (ν C_{sp3}–H), 1314 (ν_{as} S=O), 1152 (ν_{s} S=O), 690 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.90 (bs, 1H, OH, D₂O exch.), 4.06 (d, 2H, J = 6.0 Hz, CH₂), 5.01 (s, 2H, CH₂), 5.88 (bs, 1H, NH, D₂O exch.), 7.11 (d, 2H, J = 8.4 Hz, CH_{Ar}), 7.21 (d, 2H, J = 8.4 Hz, CH_{Ar}), 7.43–7.49 (m, 2H, CH_{Ar}), 7.56–7.60 (m, 1H, CH_{Ar}), 7.95 (d, 1H, J = 7.6 Hz, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 46.8 (CH₂), 63.9 (CH₂), 128.6 (Ar), 128.7 (Ar), 129.3 (Ar), 129.8 (Ar), 131.6 (Ar), 133.2 (Ar), 133.7 (Ar), 134.8 (Ar), 137.9 (Ar), 138.1 (Ar).

N-(4-bromobenzyl)-2-(hydroxymethyl)benzenesulfonamide (16)

(4-Bromobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 7 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:2). The title compound was a white solid (53% yield); mp 124–125 °C; IR ν_{\max} 3461 (ν O–H), 3234 (ν N–H), 3064 (ν C_{sp2}–H), 1314 (ν_{as} S=O), 1152 (ν_{s} S=O), 690 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.71 (bs, 1H, OH, D₂O exch.), 4.06 (bs, 2H, CH₂), 5.03 (s, 2H, CH₂), 5.78 (bs, 1H, NH, D₂O exch.), 7.06–7.08 (m, 2H, CH_{Ar}), 7.37–7.59 (m, 5H, CH_{Ar}), 7.96–7.97 (m, 1H, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 46.9 (CH₂), 64.1 (CH₂), 121.9 (Ar), 128.6 (Ar), 129.6 (Ar), 129.8 (Ar), 131.7 (Ar), 133.2 (Ar), 137.9 (Ar), 138.3 (Ar).

N-(2,6-difluorobenzyl)-2-(hydroxymethyl)benzenesulfonamide (17)

(2,6-Difluorobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 7 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:1). The title compound was a white solid (67% yield); mp 128–130 °C; IR ν_{\max} 3495 (ν O–H), 3159 (ν N–H), 2958 (ν C_{sp3}–H), 1320 (ν_{as} S=O), 1159 (ν_{s} S=O), 693 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.72 (bs, 1H, OH, D₂O exch.), 4.23 (s, 2H, CH₂), 4.89 (bs, 2H, CH₂), 6.04 (bs, 1H, NH, D₂O exch.), 6.79–6.73 (m, 2H, CH_{Ar}), 7.10–7.14 (m, 1H, CH_{Ar}), 7.26 (s, 1H, CH_{Ar}), 7.33–7.48 (m, 2H, CH_{Ar}), 7.92–7.94 (m, 1H, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 35.1 (CH₂), 63.6 (CH₂), 111.2 (Ar-F, ²J_{C-F} = 24.8 Hz), 128.3 (Ar), 129.7 (Ar), 129.9 (Ar), 130.0 (Ar), 130.1 (Ar), 131.2 (Ar), 133.1 (Ar), 137.8 (Ar), 138.2 (Ar), 161.2 (Ar-F, ¹J_{C-F} = 246.9 Hz); ¹⁹F-NMR (564.7 MHz, CDCl₃) δ -112.08 (t, J_{F-H} = 6.9 Hz, CF).

N-(3,4-dichlorobenzyl)-2-(hydroxymethyl)benzenesulfonamide (18)

(3,4-Chlorobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 7 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 2:1). The title compound was a light brown solid (81% yield); mp 83–84 °C; IR ν_{\max} 3463 (ν O–H), 3192 (ν N–H), 2947 (ν C_{sp3}–H), 1319 (ν_{as} S=O), 1158 (ν_{s} S=O), 690 (δ C_{sp2}–H) cm⁻¹; ¹H-NMR

(400 MHz, CDCl₃) δ 2.88 (bs, 1H, OH, D₂O exch.), 3.96 (d, 2H, $J=6.4$ Hz, CH₂), 4.93 (bs, 2H, CH₂), 6.00 (t, 1H, $J=6.0$ Hz NH, D₂O exch.), 6.94–6.96 (bs, 1H, CH_{Ar}), 7.17–7.22 (m, 2H, CH_{Ar}), 7.33–7.39 (m, 2H, CH_{Ar}), 7.46–7.48 (m, 1H, CH_{Ar}), 7.83 (d, 1H, $J=7.6$ Hz, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 46.3 (CH₂), 64.0 (CH₂), 127.2 (Ar), 128.6 (Ar), 129.7 (Ar), 129.8 (Ar), 130.5 (Ar), 131.7 (Ar), 131.8 (Ar), 132.6 (Ar), 133.3 (Ar), 136.7 (Ar), 137.7 (Ar), 138.1 (Ar).

2-(Hydroxymethyl)-N-(naphthalen-1-ylmethyl)benzenesulfonamide (19)

2-(Naphthalen-1-ylmethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 6 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 \times 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:1). The title compound was a white solid (57% yield); mp 80–81 °C; IR ν_{\max} 3517 (ν O–H), 3347 (ν N–H), 3061 (ν C_{sp²}–H), 1318 (ν_{as} S=O), 1157 (ν_{s} S=O), 690 (δ C_{sp²}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.68 (bs, 1H, OH, D₂O exch.), 4.51 (s, 2H, CH₂), 4.91 (s, 2H, CH₂), 5.69 (bs, 1H, NH, D₂O exch.), 7.29–7.55 (m, 7H, CH_{Ar}), 7.74–7.86 (m, 3H, CH_{Ar}), 8.05 (s, 1H, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 45.6 (CH₂), 63.7 (CH₂), 123.2 (Ar), 125.2 (Ar), 126.0 (Ar), 126.6 (Ar), 127.0 (Ar), 128.4 (Ar), 128.7 (Ar), 129.0 (Ar), 130.0 (Ar), 131.1 (Ar), 131.4 (Ar), 131.5 (Ar), 133.2 (Ar), 133.7 (Ar), 137.6 (Ar), 138.3 (Ar).

N-(2-hydroxy-2-phenylethyl)-2-(hydroxymethyl)benzenesulfonamide (20)

2-(2-Oxo-2-phenylethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 8 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 \times 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 2:1). The title compound was a light yellow oil (49% yield); IR ν_{\max} 3291 (ν O–H and ν N–H), 2923 (ν C_{sp³}–H), 1316 (ν_{as} S=O), 1153 (ν_{s} S=O), 696 (δ C_{sp²}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.94 (bs, 1H, CH₂), 3.15 (bs, 1H, CH₂), 3.42 (bs, 1H, OH, D₂O exch.), 4.56–4.64 (m, 2H, CH₂), 4.91–5.03 (m, 1H, CH), 5.28 (bs, 1H, OH, D₂O exch.), 6.57 (bs, 1H, NH, D₂O exch.), 7.13–7.20 (m, 5H, CH_{Ar}), 7.38–7.47 (m, 3H, CH_{Ar}), 7.91 (d, 1H, $J=6.4$ Hz, CH_{Ar}); ¹³C NMR (101 MHz, CDCl₃) δ 50.4 (CH₂), 62.7 (CH₂), 72.4 (CH), 125.8 (Ar), 127.9 (Ar), 128.2 (Ar), 128.5 (Ar), 129.5 (Ar), 131.3 (Ar), 133.3 (Ar), 137.3 (Ar), 138.6 (Ar), 140.8 (Ar).

N-(2-hydroxy-2-(3-methoxyphenyl)ethyl)-2-(hydroxymethyl)benzenesulfonamide (21)

2-(2-Hydroxy-2-(3-methoxyphenyl)ethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (1.0 eq.) was suspended in 20 mL of anhydrous methanol at room temperature. An excess of NaBH₄ was added portionwise and the reaction monitored by TLC. After 8 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 \times 30 mL). The organic layers were reunited, dried over sodium sulfate, and concentrated *in vacuo* to give a crude product which was purified by column chromatography (EtOAc/*n*-hexane, 1:2). The title compound was a yellow oil (88% yield); IR ν_{\max} 3477 (ν O–H), 3295 (ν N–H), 2935 (ν

C_{sp³}–H), 1317 (ν_{as} S=O), 1160 (ν_{s} S=O), 697 (δ C_{sp²}–H) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 2.89–2.95 (m, 1H, CH₂), 3.13–3.17 (m, 1H, CH₂), 3.64 (s, 3H, OCH₃), 4.41 (bs, 2H, CH₂), 4.58 (bs, 1H, OH), 4.87–4.91 (m, 1H, CH), 4.97 (bs, 1H, OH, D₂O exch.), 6.48 (bs, 1H, NH, D₂O exch.), 6.67–6.71 (m, 3H, CH_{Ar}), 7.07–7.11 (m, 1H, CH_{Ar}), 7.32–7.47 (m, 3H, CH_{Ar}), 7.87 (d, 1H, $J=7.6$ Hz, CH_{Ar}); ¹³C-NMR (101 MHz, CDCl₃) δ 50.4 (CH₂), 55.1 (OCH₃), 60.6 (CH₂), 72.2 (CH), 111.4, (Ar), 113.3 (Ar), 118.1 (Ar), 128.2 (Ar), 129.5 (Ar), 129.6 (Ar), 131.2 (Ar), 133.2 (Ar), 137.3 (Ar), 138.6 (Ar), 142.5 (Ar), 159.5 (ArO).

Enzyme inhibition assays

An applied photophysics stopped-flow instrument has been used for assaying the CA-catalyzed CO₂ 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 NaClO₄ (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO₂ hydration reaction for a period of 10–100 s. The CO₂ 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 each inhibitor (1 μ M) 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, and represent the average from at least three different determinations. All recombinant CA isoforms were obtained in-house as previously reported^{38,39}.

Molecular modeling studies

Preparation of saccharin-based structures

The saccharin-based analogs **1–21** were prepared in 3D with the MOE software package (v2013.08.02, Chemical Computing Group Inc., Montreal, Canada) as previously reported⁴⁰. 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).

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 C α -atoms. The zinc-bound water molecule of hCA II coordinated well to the zinc ions of the other hCAs.

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 thiazolidine ring of acetazolamide in the 1JD0 structure).

Results and discussion

The analysis of the biological data was accomplished comparing the open saccharin-based derivatives with their corresponding parent compounds in order to evaluate if the ring opening enhanced or reduced their biological activity.

All the tested compounds had no affinity for the common off-target hCA I and II isoforms ($K_i > 10000$ nM) compared to their corresponding parent drugs. Moreover, our molecules with a benzyl alcohol group, instead of a carboxylic acid³³, abolished completely this inhibitory activity improving the biological profile of this scaffold.

The inhibition profile of the open saccharin-based derivatives against the two tumor-related hCA IX and XII isoforms also displayed some important changes compared to parent drugs. Among the new open saccharin derivatives reported here, the best activity was obtained toward hCA XII isoform by compounds **1**, **5**, **6**, **10** and **11**, all provided of CH₃ or CF₃ groups. These molecules exhibited a slightly preference for hCA XII respect to hCA IX isoform, although the inhibition of the latter was also in the nanomolar range. Compound **5**, containing a phenyl ring substituted with methyl group in *meta* position, had the highest inhibitory activity against hCA XII ($K_i = 4.3$ nM), but also compound **6**, containing a *meta* trifluoromethyl substituent on phenyl ring, exhibited similar inhibitory activity ($K_i = 4.4$ nM). Comparable profile against hCA XII was observed for compounds **10** ($K_i = 5.7$ nM) and **11** ($K_i = 7.2$ nM), which are *para* substituted regioisomers of **5** and **6**, respectively. Compound **1**, which had a methyl group at *ortho* position of phenyl ring, showed similar inhibitory activity (K_i hCA XII = 4.7 nM) with respect to **5** and **6**. Other compounds with strong selectivity between hCA IX and XII were **9** (K_i hCA IX = 267 nM, K_i hCA XII = 64 nM) with bromine in *meta* position of phenyl ring, **12** (K_i hCA IX = 126 nM, K_i hCA XII = 57 nM) containing a cyano group in *para* position, **15** (K_i hCA IX = 154 nM, K_i hCA XII = 48 nM) which had a *para* chloro-substituted phenyl ring, **17** and **18** with, respectively, 2,6-difluoro and 3,4-dichloro-substituted phenyl rings.

Conversely, some compounds displayed a good selectivity towards hCA IX isoform. Compounds with nitro substituents in *ortho* (**3**), *meta* (**7**) or *para* (**13**) position of the ring had potent inhibitory activity preferentially against this overexpressed isoform in the hypoxic tumoral niche.

For compounds **20** (K_i hCA IX = 224 nM, K_i hCA XII = 64 nM) and **21** (K_i hCA IX = 31 nM, K_i hCA XII = 355 nM), the reaction with NaBH₄ led to the further reduction of exocyclic carbonyl moiety. The presence of this additional group maintained the biological profile with a loss of inhibitory activity against hCA I and II and a preferential selectivity against the cancer-related isoforms.

Collectively, these promising data showed that the reductive ring opening of the saccharin nucleus improved the hCA inhibitory activity with a better selectivity with respect to the off-target isoforms. From the above, we also observed that the inhibition profile was affected positively or negatively by the substitution pattern.

Docking studies into the active site of hCA XII

The open saccharin analogs (compounds **1–21**) were endowed with inhibition values in the nanomolar range against hCA XII isoform (K_i values: 4.3–432 nM, Table 1, approximately 100-fold difference between lowest and highest K_i values). The interaction of compound **6** with the active site of hCA XII was shown as an example (Figure 2). Docking studies indicated that the hydroxymethyl group and one of the sulfonamide oxygen atoms could interact simultaneously with the Zn²⁺-ion, whereas the other sulfonamide oxygen atom was water accessible. This oxygen might also form hydrogen bonds with the backbone of Thr199. The other polar substituents of the molecule were water accessible.

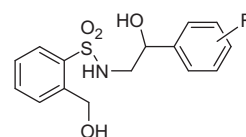
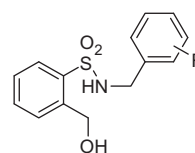
Conclusion

We proposed the design, synthesis, characterization and pharmacological evaluation *in vitro* of several new secondary sulfonamides based on the open saccharin scaffold as selective inhibitors of four different isoforms of human carbonic anhydrase. They were shown to be inactive against the two cytosolic off-target hCA I and II ($K_i > 10$ μM); conversely, all these compounds inhibited hCA IX and XII in the low nanomolar range with K_i s ranging between 4.3 and 432 nM. The analysis of the K_i values showed as the substituent on phenyl moiety that gives the best outcomes relative to inhibition of hCA XII isoform are methyl and trifluoromethyl groups.

Table 1. Inhibitory activity of the open saccharin-based derivatives 1–21 and acetazolamide as a reference drug, against selected hCA isoforms by a stopped-flow CO₂ hydrase assay.

Compound	R	K_i (nM)*			
		hCA I	hCA II	hCA IX	hCA XII
1	2-CH ₃	>10000	>10000	218	4.6
2	2-CF ₃	>10000	>10000	200	54
3	2-NO ₂	>10000	>10000	104	383
4	2-Br	>10000	>10000	113	323
5	3-CH ₃	>10000	>10000	223	4.3
6	3-CF ₃	>10000	>10000	238	4.4
7	3-NO ₂	>10000	>10000	176	382
8	3-F	>10000	>10000	268	247
9	3-Br	>10000	>10000	267	64
10	4-CH ₃	>10000	>10000	120	5.7
11	4-CF ₃	>10000	>10000	253	7.2
12	4-CN	>10000	>10000	126	57
13	4-NO ₂	>10000	>10000	20	54
14	4-F	>10000	>10000	26	63
15	4-Cl	>10000	>10000	154	48
16	4-Br	>10000	>10000	145	432
17	2,6-diF	>10000	>10000	296	45
18	3,4-diCl	>10000	>10000	298	40
19	2,3-Phenylene	>10000	>10000	294	345
20	H	>10000	>10000	224	64
21	3-OCH ₃	>10000	>10000	31	355
AAZ (acetazolamide)		250	12	25	6.0

*Mean from 3 different assays (errors were in the range of ±5–10% of the reported values).



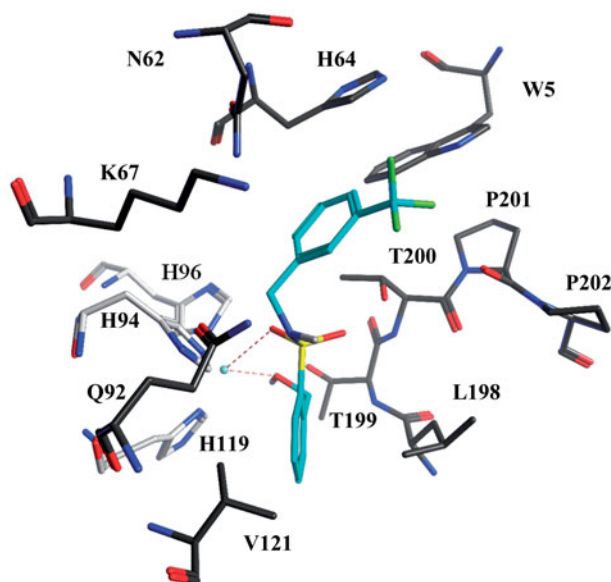


Figure 2. Docked pose of compound 6 in the active site of hCA XII. Hydrogen bonds and interactions to the Zn^{2+} -ion are depicted in red dashed lines. The Zn^{2+} -ion is depicted as a turquoise sphere. The three zinc-binding Histidines (H94, H96 and H119) are depicted in light gray for clarity.

The results were also rationalized by means of docking studies into the active site of hCA XII. Since these two cancer-related hCA isoforms were recently validated as drug targets, these results provided the development of new anticancer candidates.

Declaration of interest

The authors declare no conflict of interest.

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