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Cyclic Secondary Sulfonamides: Unusually Good Inhibitors of Cancer-Related Carbonic Anhydrase Enzymes

Janina Moeker,[†] Thomas S. Peat,[‡] Laurent F. Bornaghi,[†] Daniela Vullo,[§] Claudiu T. Supuran,*[,§] and Sally-Ann Poulsen*[,†]

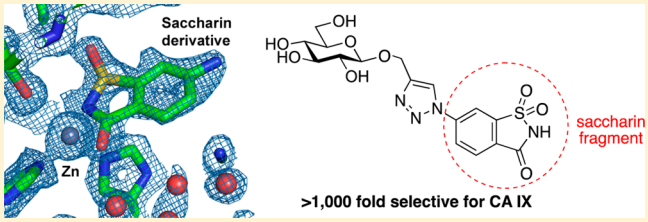
[†]Eskitis Institute for Drug Discovery, Griffith University, Nathan, Queensland 4111, Australia

[‡]CSIRO Biosciences Program, 343 Royal Parade, Parkville, Victoria 3052, Australia

[§]Polo Scientifico, Laboratorio di Chimica Bioinorganica, Rm. 188, Università degli Studi di Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy

Supporting Information

ABSTRACT: Carbonic anhydrase IX (CA IX) is a target for hypoxic cancer therapies, and the discovery of CA IX selective ligands is imperative for the development of these agents. Primary sulfonamides are broad specificity inhibitors of CA enzymes, while secondary sulfonamides are generally poor CA inhibitors. However, saccharin, a cyclic secondary sulfonamide, has unusually good inhibition of CA IX (K_i = 103 nM). In this study, we demonstrate that the affinity and selectivity of saccharin for CA IX can be further modulated when linked to hydrophobic or hydrophilic substituents. The hydrophilic glycoconjugate derivative (**12**) showed improved inhibition of CA IX (K_i = 49.5 nM) and extremely poor inhibition of the predominant off-target CAs (K_i > 50 000 nM) compared to saccharin. This >1000-fold selectivity for CA IX over off-target CAs is unprecedented for classical primary sulfonamide CA inhibitors. Our study highlights the potential of cyclic secondary sulfonamides to be exploited for the discovery of potent, cancer-selective CA inhibitors.



INTRODUCTION

Saccharin (1,2-benzisothiazol-3-one-1,1-dioxide) has been widely used as calorie-free sugar substitute for more than a century[1] and features as a substructure in a number of bioactive compounds, including leukocyte elastase inhibitors and 5-HT_{1A} agonists (Figure 1).[2] Our interest in saccharin stems from its ability to inhibit carbonic anhydrase enzymes (CAs), specifically CA IX (Table 1).[3] CAs catalyze the interconversion of CO_2 and H_2O with HCO_3^- and a H^+ to perform a pivotal role in pH regulation.[4] While CA IX expression is lacking in many healthy cells (the stomach and GI tract are exceptions), the expression is upregulated in a wide selection of hypoxic tumors as an aid to survival, proliferation, and metastasis in the otherwise hostile tumor microenvironment that results from increased metabolism.[5] There is substantial interest in assessing the impact of disrupting cancer cell pH homeostasis as a therapy, and central to this challenge is the need for selective and efficacious inhibitors of CA IX.[6] It is widely reported that primary sulfonamides (RSO_2NH_2) are excellent inhibitors of CAs, and in the greater than 200 protein X-ray crystal structures of this compound class bound to CA II, all have the sulfonamide anion (RSO_2NH^-) coordinated to the CA active site zinc cation.[7] In contrast, secondary sulfonamides (RSO_2NHR') are generally very poor CA inhibitors;[8] however, saccharin as a cyclic secondary sulfonamide is the premier exception to this general finding. To generate CA isozyme selectivity is challenging with primary sulfonamide compounds, and the unusual SAR of saccharin, with an impressive selectivity profile toward CA IX compared to nearly all other CA isozymes (with the exception of CA VII, Table 1), is of considerable interest. Although CA VII is strongly inhibited by saccharin, its localization in the CNS likely limits the capacity for saccharin to encounter CA VII in vivo, owing to predicted poor blood–brain barrier permeability of acidic molecules (pK_a of saccharin

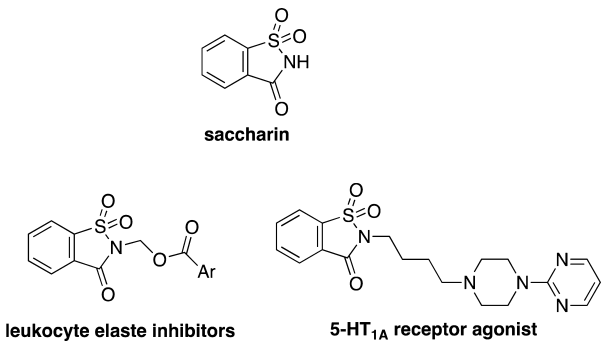


Figure 1. Structure of saccharin and bioactive structures comprising an *N*-alkylated saccharin substructure.

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Table 1. Inhibition and CA IX Selectivity Data of Human CA Isozymes with Saccharin[3a]

CA isozyme	I	II	IV	VA	VB	VI	VII	IX	XII	XIV
K_i (nM)	18 540	5950	7920	10 060	7210	935	10	103	633	773
CA IX selectivity[a]	180	57.8	76.9	97.7	70	9.1	0.1	1.0	6.1	7.1

[a]CA IX selectivity is determined by the ratio of K_i values for CA isozyme relative to CA IX.

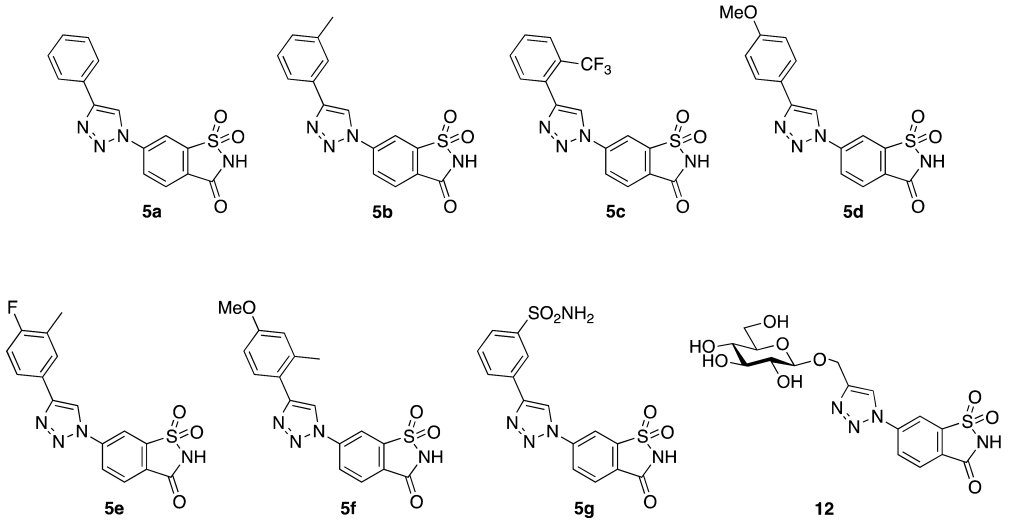

Figure 2. Target saccharin–triazole analogues for investigation as CA IX inhibitors.

is 1.3).[9] The structure of human CA II (hCA II) bound to saccharin was reported by Klebe and colleagues in 2007 (PDB entry 2Q38); this structure shows that saccharin utilizes both the sulfonamide and lactam carbonyl groups to form favorable interactions with hCA II.[3a] Like primary sulfonamides, saccharin is likely deprotonated with its anionic nitrogen coordinated to the CA active site zinc. The amino acid sequence alignment of CA isozymes indicates that several amino acids are likely responsible for the altered CA selectivity profile of saccharin compared to that for simple primary sulfonamides.[3a] We reasoned that improving CA IX binding and selectivity may result from the addition of substituents to the benzene ring of saccharin, which is oriented away from the CA IX active site zinc. In addition to providing insight on SAR, our findings demonstrate the potential of the cyclic secondary sulfonamide motif for the development of effective agents targeting CA IX.

Saccharin is a rule-of-three compliant fragment,[10] that is, molecular weight <300 Da (=183 Da), cLog P ≤ 3 (=0.45), hydrogen bond donors ≤3 (=1), hydrogen bond acceptors ≤3 (=3), and rotatable bonds ≤3 (=0). Ligand efficiency (LE) is the relationship between a compound's potency and its molecular size, and there are several definitions that represent this ratio; here we will use LE = pK_i/MW (MW in kDa).[11] Optimizing potency while keeping MW low is important as larger ligands have inherent drawbacks in terms of most physicochemical properties (e.g., bioavailability, stability, and solubility) that are relevant to drug discovery. Perola's early analysis of 60 lead/drug pairs revealed that 90% of drug leads had LE > 12.4, while 90% of drugs had LE > 14.7.[11b] The LE of saccharin binding to CA IX is 38.2; this far exceeds the drug lead and drug thresholds, signifying that the saccharin fragment is a promising starting point for developing CA IX inhibitors. The compact size of the saccharin fragment within the open CA active site architecture (PDB entry 2Q38) allows space for saccharin to be further grown to add interactions with either the hydrophobic half or the hydrophilic half of the CA active site. Our interest in saccharin stems from its unusual ability to inhibit CA enzymes. Here we report the design, synthesis, and SAR for a panel of saccharin analogues to assess if it is possible to exploit the atypical selectivity profile of saccharin to improve targeting of CA IX over off-target CA isozymes.

RESULTS AND DISCUSSION

There are several synthetically distinct methods employed to obtain the saccharin core, with functionalization at either the nitrogen or the carbonyl oxygen readily accomplished either during or post- synthesis, while functionalization elsewhere is more challenging. As we were looking to synthesize analogues of saccharin functionalized on the phenyl ring, we hoped to develop a straightforward synthesis that would allow this. Cu(I)-catalyzed azide alkyne cycloaddition (CuAAC) has contributed to a resurgence in the application of azides in the synthesis of more complex molecules across a range of chemistry disciplines.[9−11] Azidosaccharins are not known compounds; however, 6-aminosaccharin (**3**) is a known compound,[12] and we approached the synthesis of 6-azidosaccharin (**4**) by employing the common functional group interconversion of anilines to phenyl azides via a diazonium intermediate. 6-Aminosaccharin **3** was synthesized from *p*-nitrotoluene (**1**) in three steps using published procedures,[12] and **3** was then treated with *t*-butyl nitrite (1.5 equiv) and trimethylsilyl azide (1.2 equiv) to synthesize 6-azidosaccharin **4** in 46% yield. The attempted reaction of azide **4** with 4-ethynylbenzenesulfonamide (**g**) and six commercially available phenyl acetylenes (**a**–**f**): phenyl acetylene (**a**), 1-ethynyl-3-methylbenzene (**b**), 1-ethynyl-2-(trifluoromethyl)benzene (**c**), 1-ethynyl-4-methoxybenzene (**d**), 4-ethynyl-1-fluoro-2-methylbenzene (**e**), 1-ethynyl-4-methoxy-2-methylbenzene (**f**); under typical CuAAC conditions (0.2 equiv of $CuSO_4{\cdot}5H_2O$, 0.4 equiv of sodium ascorbate, *t*BuOH/water 1:1, 40 °C), failed to give the target 1,4-disubstituted 1,2,3-triazole (Figure 2) in

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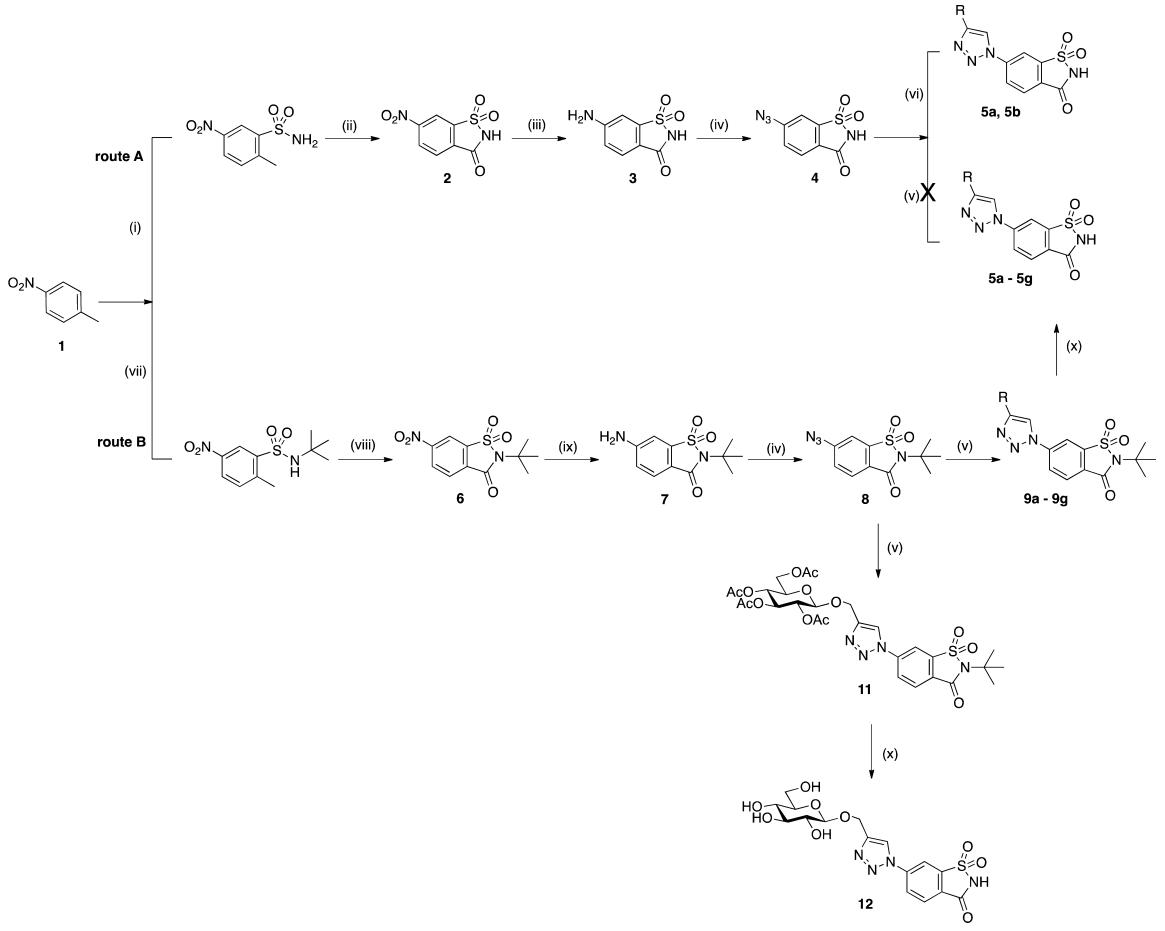
Scheme 1[a]



[a]Reagents and conditions: (i) (a) 5.0 equiv of $ClSO_3H$ acid, 60 °C, 48 h; (b) NH_4OH, 50 °C, 2 h, 35%; (ii) 45.0 equiv of CrO_3, H_2SO_4, rt, 24 h, 40%; (iii) H_2, 10% Pd/C, MeOH, rt, 4 h, 80%; (iv) 1.5 equiv *t*-butyl nitrite, 1.2 equiv of trimethylsilyl azide, acetonitrile, 0 °C to rt, 16 h, 46% (**4**), 88% (**8**); (v) 1.0 equiv of alkyne (**a**−**g**, **10**), 0.2 equiv of $CuSO_4{\cdot}5H_2O$, 0.4 equiv of sodium ascorbate, *t*BuOH/water 1:1, 40 °C, 24 h, no product (**5a**−**5g**), 34−90% (**9a**−**9g**), 89% (**11**); (vi) 1.0 equiv of alkyne (**a** or **b**), 1.0 equiv of $CuSO_4{\cdot}5H_2O$, 2.0 equiv of sodium ascorbate, *t*BuOH/water 1:1, 60 °C, 24 h, 43% (**5a**), 33% (**5b**); (vii) (a) 10.0 equiv of $ClSO_3H$, −20 °C, 1 h then rt, 4 h; (b) *t*$BuNH_2$/Et_3N, 0 °C, 2 h, then rt, 6 h, 52%; (viii) 8.0 equiv of H_5IO_6, 5 mol % of CrO_3, 7.0 equiv of Ac_2O, acetonitrile, reflux, 5−10 h, 48%; (ix) H_2, 10% Pd/C, EtOAc, rt, 4 h, 73%; (x) TFA, rt, 1−6 days, 23−89% (**5a**−**5g**), 34% (**12**).

useful yield (Scheme 1, route A). Metal complexes of saccharin have been described wherein the NH hydrogen is lost (pK_a of this hydrogen is 1.3),[9] and the resulting anion coordinates to the metal(II) cation.[13] Specifically, a crystal structure of a 2:1 saccharin/Cu(II) complex has been reported,[14] and related complexes in solution may account for the poor conversion of alkynes **a**−**g** and azide **4** to triazole products under the standard CuAAC reaction conditions with catalytic source of Cu(I). Next, with 1.0 equiv of $CuSO_4{\cdot}5H_2O$, 2.0 equiv of sodium ascorbate, and a higher reaction temperature of 60 °C, we were able to demonstrate conversion to triazoles; however, both the 1,4-disubstituted and 1,5-disubstituted 1,2,3-triazoles were formed. Workup of these reactions required challenging chromatography to separate regioisomers, and this was further hampered by the increased amount of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate used for the reaction. Nevertheless, we were able to prepare 1,4-disubstituted triazoles **5a** and **5b** using this method, and isolated yields were 43 and 33%, respectively.

With the intention to improve our synthetic methodology, we explored the use of a NH-protected 6-azidosaccharin as an alternate approach to the target compounds **5a**−**5g**. This NH-protected compound should allow standard CuAAC conditions to be used to form the triazole link, with subsequent removal of the NH protecting group from the purified saccharin−triazole product. The synthesis of the saccharin heterocycle may be achieved by the direct oxidation of 2-methylbenzenesulfonamide using the H_5IO_6−CrO_3 as the oxidation system. Xu and colleagues investigated these oxidation conditions with a number of *N*-alkyl-*o*-toluenesulfonamides and were able to show that oxidation of *N*-*t*-butyl-2-methyl-5-nitrobenzenesulfonamide with H_5IO_6−CrO_3 in acetonitrile gave the corresponding *N*-*t*-butyl saccharin compound (*N*-*t*-butyl-6-nitro-1,2-benzisothiazole-3-one-1,1-dioxide, **6**) in 88% yield (62% yield after recrystallization). This good yield and straightforward purification encouraged us to utilize the *t*-butyl moiety as a protecting group strategy, with removal of the group under acidic conditions as a final step in the synthesis of target compounds.[15] The synthetic route using this NH-*t*-butyl-protected azidosaccharin approach is depicted in Scheme 1 (route B). The synthesis of **5a**−**5g** from compound **6** comprises four steps, while compound **6** is synthesized from *p*-nitrotoluene in two steps.[16] Compound **6** was reduced with H_2, 10% Pd/C to give the new compound, 6-amino-2-*N*-*t*-butyl saccharin/*N*-*t*-butyl-6-amino-1,2-benzisothiazole-3-one-1,1-di-

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Table 2. Inhibition of human CA Isozymes I, II, IX, and XII with saccharin and saccharin analogues 2, 3, 4, 5a−5g and 12 and selectivity ratio data for cancer-associated CA IX and CA XII

		K_i (nM)[a]				selectivity ratio[b]			
compd	cLogP	CA I	CA II	CA IX	CA XII	I/IX	II/IX	I/XII	II/XII
saccharin	+0.45	18540 ± 62	5950 ± 24	103 ± 5	633 ± 24	180	57.8	29.3	9.4
2	+0.39	>50000	>50000	95.8 ± 4	15600 ± 49	>522	>522	>3.2	>3.2
3	−0.66	>50000	>50000	610 ± 30	2750 ± 121	>82	>82	>18.2	>18.2
4	+0.76	4930 ± 230	8058 ± 326	466 ± 18	665 ± 27	10.6	17.3	7.4	12.1
5a	+2.19	>50000	9315 ± 318	484 ± 14	86.2 ± 3	>103	19.2	>580	108.1
5b	+2.70	>50000	8106 ± 119	89.7 ± 5	57.3 ± 3	>557	90.4	>873	141.5
5c	+3.06	8025 ± 372	>50000	443 ± 16	747 ± 28	18.1	>113	10.7	>67
5d	+2.03	5320 ± 212	>50000	341 ± 16	866 ± 38	15.6	>147	6.1	>58
5e	+2.84	>50000	4870 ± 206	47.8 ± 2	81.2 ± 4	>1046	101.9	>616	60.0
5f	+2.54	8745 ± 314	>50000	95.7 ± 5	201 ± 9	91.4	>522	43.5	>249
5g	+0.79	354 ± 13	87.3 ± 2	8.6 ± 0.4	9.5 ± 0.2	41.2	10.2	37.3	9.2
12	−2.30	>50000	>50000	49.5 ± 1	572 ± 15	>1010	>1010	>87	>87

[a]Errors in the range of ±5% of the reported value, from three determinations. [b]Selectivity is determined by the ratio of K_i values for CA isozyme relative to CA IX and XII.

oxide (**7**). The amino function of **7** was next converted into the azido group using *t*-butyl nitrite and trimethylsilyl azide to give the new compound *N*-*t*-butyl-6-azido-1,2-benzisothiazole-3-one-1,1-dioxide (**8**) in 82% yield. The CuAAC reaction of **8** with alkynes **a**−**g** under standard conditions (0.2 equiv of $CuSO_4 \cdot 5H_2O$ and 0.4 equiv of sodium ascorbate, *t*BuOH/water 1:1, 40 °C) gave triazoles **9a**−**9g** in yields ranging from 34 to 90%. The *N*-*t*-butyl group of compounds **9a**−**9g** was removed in refluxing trifluoroacetic acid[15] with variable isolated yields (23−89%). Long reaction times (between 4 and 7 days) were needed for this final reaction to complete; however, the relative ease of workup compared to the initial approach (route A) compensated for the extended reaction times.

In the past few years, our group has synthesized a large collection of glycoconjugate CA inhibitors, wherein the hydrophilic carbohydrate moiety is employed to reduce the inhibitor's Log P.[17] The calculated Log P (cLog P) parameter generally provides a good correlation with experimental permeability data,[18] and molecules with cLogP values between 1 and 3 typically have good passive membrane permeability properties while those with cLogP values of <0 are more likely to have a low capacity for penetrating cell membranes. Herein, we have designed and synthesized the saccharin glycoconjugate **12** (Figure 2), in order to compare the CA inhibition profile with less hydrophilic compounds **5a**−**5g**. The cLogP for the saccharin glycoconjugate **12** is −2.30, compared to values of +2.09 to +3.06 for **5a**−**5f** (Table 2). This structure−property relationship for **12** is consistent with very poor passive membrane permeability, a useful property for selective targeting of cell surface CA IX. The synthesis of **12** is shown in Scheme 1. Reaction of the peracetylated *O*-propynyl glucoside **10** with the *t*-butyl-protected 6-azidosaccharin **8** gave the protected glycoconjugate **11** in 89% yield. The treatment of **11** with refluxing TFA conveniently removed both the sugar acetate protecting groups and the NH-*t*-butyl protecting group to give the target hydrophilic saccharin analogue **12** in 34% yield from **11**. Selected compounds of this study have been submitted to the Compounds Australia (formerly Queensland Compound Library) Open Compound Collection (Academic) (see http://www.griffith.edu.au/science-aviation/queensland-compound-library).

CA Inhibition. One goal of this study was to generate compounds that would enable us to assess if it was possible to exploit the atypical selectivity profile of saccharin to improve targeting of CA IX over the two major off-target CA isozymes (I and II). In addition to CA IX expression, CA XII expression is upregulated in a wide selection of hypoxic tumors, so it is of general interest to also monitor for inhibition of CA XII to provide a comprehensive understanding of any individual compound's potential as a cancer therapeutic. The CA inhibition data to block the interconversion of CO_2 with HCO_3^- and a H^+, for the saccharin−triazole analogues **5a**−**5g** and **12**, the simple saccharin analogues **2**, **3**, and **4**, as well as saccharin itself as a reference compound, were measured for CA I, II, IX, and XII, results are presented in Table 2. The selectivity ratios for CA IX and CA XII inhibition over the off-target CA I and CA II are presented in Table 2.

Saccharin, the reference compound for SAR, has a K_i of 103 nM for CA IX and is 180-fold selective over CA I and 58-fold selective over CA II, while for the other cancer-associated CA, CA XII, the K_i of saccharin is 633 nM, much weaker inhibition than for CA IX. Saccharin thus has better CA IX selectivity than CA XII selectivity (29- and 9-fold for CA I and II, respectively). Noticeably the simple nitro- and aminosaccharin derivatives, compounds **2** and **3**, as well as the hydrophilic glycoconjugate **12** had extremely poor inhibition of CA I and II (>50 000 nM) while **2** and **12** were equal or better inhibitors of CA IX than saccharin, with K_i values of 95.8 and 49.5 nM, respectively. This SAR is ideal as it leads to very high selectivity for CA IX over both off-target CAs, >522-fold for **2** and >1010-fold for **12**. This selectivity is substantially higher than for the reported primary benzenesulfonamide class of CA inhibitors, signifying that compounds **2** and **12** are excellent candidates for follow-up cell-based studies. For the phenyltriazole-substituted saccharin compounds **5a**−**5f**, the SAR was related to the substitution on the phenyl ring, compounds typically displayed very weak inhibition of off-target CA I and II. For CA I, inhibition was very poor with K_i > 50 000 nM for **5a**, **5b**, and **5e**, while for CA II the K_i values were similarly weak (>50 000 nM) for **5c** and **5d**. For **5a**−**5e**, the inhibition of the other off-target CA, although greater, was still weak with K_i values ranging from 4870 to 9315 nM. For CA IX, a methyl substituent on the phenyl ring gave the best inhibition, with K_i values below 100 nM for **5b**, **5e**, and **5f**. Similarly for CA XII, the 3-methyl substitution of **5b** and **5e** also gave K_i values below 100 nM. Compound **5g** comprises a primary sulfonamide group in

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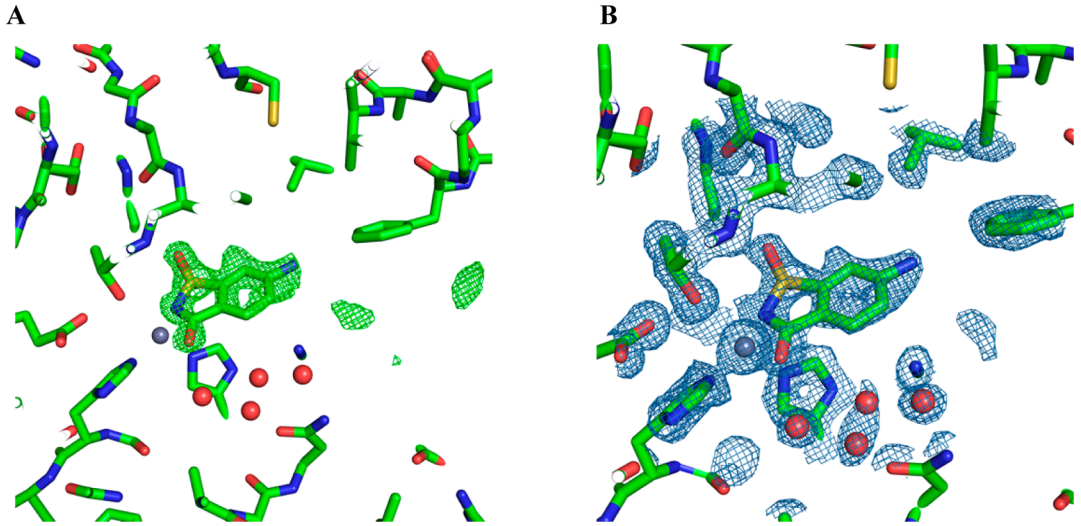

Figure 3. (A) Difference density map with the density set to 2.5σ following the removal of the ligand from the refined model. (B) Standard density map contoured at 1.2σ. The saccharin moiety is shown in both figures, and the labeled zinc atom is shown as a gray sphere. Density maps were generated using the FFT algorithm in the CCP4 suite 6.3.[20] The figures were prepared using PyMol.[21] Coordinates and structure factors for CA II/**5b** have been deposited with the PDB (accession code 4cq0).

addition to the secondary sulfonamide group of the saccharin fragment. It is expected that the primary sulfonamide group of **5g** will dominate the recognition interaction with the CAs, and inhibition data support this assumption as **5g** is the most potent CA inhibitor at all isozymes tested. When comparing compound **5g** with **5a**, the difference in structure is a primary sulfonamide moiety versus a hydrogen atom, respectively; this compound pair thus allows the impact of the primary sulfonamide−CA IX interaction to be disconnected in part from the saccharin−CA IX interaction. Compound **5g** (K_i = 8.6 nM at CA IX) has 56-fold greater activity than for **5a** (K_i = 484 nM at CA IX). Our findings present the first report that to the best of our knowledge has investigated the SAR of the saccharin fragment against cancer-relevant CA IX, with a single earlier study reporting data only at off-target CA I and CA II.[19]

Structure of hCA II/Saccharin Ligand Complex. To obtain insight into ligand−protein interactions at the atomic level, we next determined the protein X-ray crystal structure of hCA II in complex with compound **5b**. Almost all of the residues in and around the active site are conserved among hCA II, IX, and XII; this includes His94, His96, and His119 as part of the zinc binding triad and Leu197, Thr198, Thr199, Val121, Leu140, Val142, and Val206. Near the active site entrance, there are three residues that differ between CA II, CA IX, and CA XII: Ile91, Phe130, and Asn67. Asn67 is located away from where the saccharin derivatives are directed, while Ile91 (Leu in CA IX, Thr in CA XII) and Phe130 (Val in CA IX, Ala in CA XII) are located where they are likely to form interactions with the different substituents on the saccharin derivatives. There was clear density for the compound adjacent to the zinc atom in the active site after the initial rounds of refinement. The model refined well against the data, giving a R_{work} of 16.8% and R_{free} of 20.2%. The average B factors were 11.4 Å^2 for the protein, 21.2 Å^2 for the solvent, and 14.9 Å^2 for the compound. The data were 98.5% complete throughout the resolution range of 70 to 1.45 Å. As can be seen in Figure 3, both the difference density map (A) and the standard electron density map (B) show density for the saccharin moiety of **5b**, but little to no density beyond this ring system. As the data are complete and high resolution, we hypothesize that this is due to disorder in this latter part of the compound (i.e., multiple potential conformations beyond the saccharin ring).

CONCLUSION

Saccharin as a cyclic secondary sulfonamide is an exception to the general finding that secondary sulfonamides are poor inhibitors of CA enzymes. Selective binding to CA IX is an important undertaking for targeting hypoxic tumors and is challenging with classical primary sulfonamide CA inhibitors. Saccharin and some of the saccharin derivatives reported herein (e.g., **2** and **12**) have very impressive selectivity toward CA IX compared to the major off-target CA isozymes. This finding is of substantial interest and highlights the potential of cyclic secondary sulfonamides to be exploited for the discovery of potent, cancer-selective CA inhibitors.

EXPERIMENTAL SECTION

Compound Synthesis. All reagents were purchased from commercial suppliers. All reactions were monitored by TLC. TLC plates were visualized with UV fluorescence (λ = 254 nm). Silica gel flash chromatography was performed using silica gel 60 Å (230−400 mesh). ^{1}H NMR data were acquired at 500 MHz and ^{13}C NMR at 125 MHz at 30 °C. For ^{1}H and ^{13}C NMR acquired in DMSO-d_6, chemical shifts (δ) are reported in parts per million relative to the solvent residual peak: proton (δ 2.50 ppm) and carbon (δ 39.5 ppm) signals, respectively. Assignments for ^{1}H NMR were confirmed by ^{1}H−^{1}H gCOSY, while assignments for ^{13}C NMR were confirmed by ^{1}H−^{13}C HSQC. Multiplicity is indicated as follows: s (singlet); d (doublet); t (triplet); m (multiplet); dd (doublet of doublet); b (broad). Coupling constants are reported in hertz (Hz). Melting points are uncorrected. High- and low-resolution electrospray ionization mass spectra were acquired using electrospray as the ionization technique in positive ion and/or negative ion modes as stated. All MS analysis samples were prepared as solutions in MeOH. Purity of all compounds was ≥95% as determined by HPLC with UV detection. Compounds **2**, **3**, **6**, and **10** were synthesized using previously reported methods.[12,16,17c,22] ^{1}H NMR data for compounds were in agreement with literature.

Synthesis of 6-(4-Aryl-1*H*-1,2,3-triazol-1-yl)-1,2-benziso-thiazole-3-one-1,1-dioxides. General Procedure 1. A mixture of azide (**4**, 1.0 equiv) and alkyne (**a** and **b**, 1.0 equiv) was suspended in a *tert*-butyl alcohol/distilled water mixture (1:1, 0.2−0.5 M final concentration). A solution of sodium ascorbate (2.0 equiv) in water,

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followed by a solution of $CuSO_4 \cdot 5H_2O$ (1.0 equiv) in water, was successively added. The bright yellow heterogeneous mixture was stirred vigorously at 60 °C for 16 h. The solvent was removed under reduced pressure, and the resulting residue was purified by flash chromatography as described below. The 1,4-regioselectivity of the products was verified by characteristic ^1H and ^{13}C NMR chemical shift values for the triazole H-4 proton and C-4 carbon of the 1,4-disubstituted triazole.[23]

Synthesis of *N*-*t*-Butyl-6-(4-aryl-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxides. General Procedure 2. A mixture of azide (**8**, 1.0 equiv) and alkyne (**a**−**g**, **10**, 1.0 equiv) was suspended in THF. A solution of sodium ascorbate (0.4 equiv) in water, followed by a solution of $CuSO_4 \cdot 5H_2O$ (0.2 equiv) in water, was successively added. The bright yellow heterogeneous mixture was stirred vigorously at 40 °C until TLC indicated reaction was complete; typically, 16 h was sufficient. The solvent was removed under reduced pressure, and the resulting residue was purified by flash chromatography (adsorbed on silica from an acetone solution) and eluted with hexane/EtOAc as described below.

Removal of the *N*-*t*-Butyl Group of 9a−9g, 11. General Procedure 3. A solution of *N*-*t*-butyl-protected compound (**9a**−**9g**, **11**) in TFA was refluxed for 1−6 days as needed; the TFA was removed under reduced pressure, the remaining solid was suspended in EtOAc, and the insoluble components were removed by filtration. The solvent was removed under reduced pressure, and the resulting residue was purified by flash chromatography as described below.

6-Azido-1,2-benzisothiazole-3-one-1,1-dioxide (4). To a suspension of 6-amino-1,2-benzisothiazole-3-one-1,1-dioxide (**3**, 3.64 g, 18.4 mmol, 1.0 equiv)[12] in anhydrous acetonitrile (35 mL) at 0 °C were added *t*-butyl nitrite (3.2 mL, 1.5 equiv) and azidotrimethylsilane (2.9 mL, 1.2 equiv) by dropwise addition. The reaction mixture was stirred at 0 °C for 1 h and warmed to room temperature for 16 h after which TLC (DCM/MeOH, 4:1) showed reaction was complete. The acetonitrile was removed under reduced pressure, and the remaining residue was purified by column chromatography (solid addition from MeOH, DCM/MeOH, 8:1) to give the product as a pale yellow solid (46% yield). R_f = 0.29 (DCM/MeOH, 4:1). ^1H NMR (500 MHz, DMSO-d_6): δ = 7.59 (d, J = 8.0 Hz, 1H, H-4), 7.36 (d, J = 1.8 Hz, 1H, H-7), 7.27 (dd, J = 8.0, 1.8 Hz, 1H, H-5), assignments were confirmed by $^1H-^1H$ gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 160.6 (C═O), 147.2 (C-3a), 141.6 (C-6), 126.3 (C-7a), 125.0 (C-4), 123.9 (C-5), 112.0 (C-7), assignments were confirmed by $^1H-^{13}C$ gHSQC. LRMS (ESI^-): m/z = 223 $[M-H]^-$. HRMS (ESI): calcd for $C_7H_3N_4O_3S^-$ 222.9931, found 222.9952.

6-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (5a). (1) The title compound **5a** was prepared from phenyl acetylene **a** (27 mg, 0.27 mmol) according to General Procedure 1. Purification of the crude product by flash chromatography (EtOAc/hexane 7:1) afforded **5a** (43%) as a pale yellow solid. R_f = 0.11 (EtOAc/hexane 7:1). Mp = 258−260 °C. ^1H NMR (400 MHz, DMSO-d_6): δ = 9.50 (s, 1H, $H_{triazole}$), 8.26 (s, 1H, H-7), 8.25−8.21 (m, 1H, H-5), 7.95 (d, J = 8.0 Hz, 2H, H-2′, H-6′), 7.82 (d, J = 8.1 Hz, 1H, H-4), 7.52 (t, J = 7.7 Hz, 2H, H-3′, H-5′), 7.40 (t, J = 7.3 Hz, 1H, H-4′), assignments were confirmed by $^1H-^1H$ gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 166.6 (C═O), 147.5 ($C_{triazole}$), 147.1 (C-3a), 138.4 (C-7a), 134.3 (C-6), 130.0 (C-1′), 129.0 (C-3′, C-5′), 128.3 (C-4′), 125.3 (C-2′, C-6′), 124.2 (C-4), 123.1 (C-5), 120.1 ($CH_{triazole}$), 110.6 (C-7), assignments were confirmed by $^1H-^{13}C$ gHSQC. LRMS (ESI^-): m/z 325 $[M-H]^-$. HRMS (ESI): calcd for $C_{15}H_9N_4O_3S^-$ 325.0401, found 325.0414. (2) The title compound **5a** was prepared from **9a** (80 mg, 0.2 mmol) according to General Procedure 3 in 24 h to give **5a** (56%) as a pale yellow solid. The characterization data for **5a** prepared from **9a** are consistent with preparation by General Procedure 1 (above).

6-(4-[3′-Methylphenyl]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (5b). (1) The title compound **5b** was prepared from 1-ethynyl-3-methylbenzene (**b**) (35 μL, 0.27 mmol) according to General Procedure 1. Purification of the crude product by flash chromatography (EtOAc/hexane 7:1) afforded **5b** (33%) as a pale yellow solid. R_f = 0.39 (DCM/MeOH 4:1). Mp = 265−267 °C (decomp.). ^1H NMR (400 MHz, DMSO-d_6): δ = 9.48 (s, 1H, $H_{triazole}$), 8.26 (d, J = 1.6 Hz, 1H, H-7), 8.23 (dd, J = 8.1, 1.9 Hz, 1H, H-5), 7.82 (d, J = 8.1 Hz, 1H, H-4), 7.79 (bs, 1H, H-2′), 7.74 (d, J = 7.7 Hz, 1H, H-6′), 7.40 (t, J = 7.6 Hz, 1H, H-5′), 7.21 (d, J = 7.4 Hz, 1H, H-4′), 2.40 (s, 3H, CH_3), assignments were confirmed by $^1H-^1H$ gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 166.6 (C═O), 147.6 ($C_{triazole}$), 147.1 (C-3a), 138.4 (C-7a), 138.2 (C-3′), 134.3 (C-6), 129.9 (C-1′), 129.0 (C-4′), 128.9 (C-5′), 125.9 (C-2′), 124.2 (C-4), 123.0 (C-5), 122.5 (C-6′), 120.0 ($CH_{triazole}$), 110.5 (C-7), 21.1 (CH_3), assignments were confirmed by $^1H-^{13}C$ gHSQC. LRMS (ESI^-): m/z 339 $[M-H]^-$. HRMS (ESI): calcd for $C_{16}H_{11}N_4O_3S^-$ 339.0557, found 339.0569. (2) The title compound **5b** was prepared from **9b** (50 mg, 0.13 mmol) according to General Procedure 3 in 4 days to give **5b** (89%) as a pale yellow solid. The characterization data for **5b** prepared from **9b** are consistent with preparation by General Procedure 1 (above).

***N*-*t*-Butyl-6-amino-1,2-benzisothiazole-3-one-1,1-dioxide (7).** The title compound (7) was synthesized by reduction of *N*-*t*-butyl-6-nitro-1,2-benzisothiazole-3-one-1,1-dioxide (**6**) (500 mg, 1.76 mmol) in MeOH (6 mL) with H_2 (325 mg, 1 atm) and Pd−C (10%) as catalyst, rt, 16 h. The crude reaction mixture was filtered through Celite, and the solvent was removed under reduced pressure to give **7** as a beige solid in 90% yield. The compound was used in the next step without further purification. R_f = 0.26 (EtOAc/hexane 1:2). Mp = 224−226 °C. ^1H NMR (500 MHz, DMSO-d_6): δ = 7.60 (d, J = 8.4 Hz, 1H, H-4), 6.94−6.88 (m, 2H, H-5, H-7), 6.74 (s, 2H, NH_2), 1.63 (s, 9H, 3 × CH_3), assignments were confirmed by $^1H-^1H$ gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 160.2 (C═O), 155.4 (C-3a), 139.8 (C-7a), 126.0 (C-4), 118.1 (C-5), 111.7 (C-6), 102.0 (C-7), 59.4 ($C(CH_3)_3$), 27.5 (3 × CH_3), assignments were confirmed by $^1H-^{13}C$ gHSQC. LRMS (ESI^+): m/z = 277 $[M + Na]^+$. HRMS (ESI): calcd for $C_{11}H_{14}N_2O_3SNa^+$ 277.0617, found 277.0618.

***N*-*t*-Butyl-6-azido-1,2-benzisothiazole-3-one-1,1-dioxide (8).** To a suspension of *N*-*t*-butyl-6-amino-1,2-benzisothiazole-3-one-1,1-dioxide (**7**, 240 mg, 0.94 mmol, 1.0 equiv) in anhydrous acetonitrile (2.6 mL) at 0 °C were added *t*-butyl nitrite (168 μL, 1.5 equiv) and azidotrimethylsilane (150 μL, 1.2 equiv) by dropwise addition. The reaction mixture was stirred at 0 °C for 1 h and warmed to room temperature for 16 h after which TLC (EtOAc/hexane, 1:2) showed reaction was complete. The acetonitrile was removed under reduced pressure, and the remaining residue was purified by column chromatography (solid addition from EtOAc, EtOAc/hexane, 1:4) to give the product as a pale yellow solid (216 mg, 46% yield). R_f = 0.67 (EtOAc/hexane, 1:2). Mp = 173−175 °C. ^1H NMR (500 MHz, DMSO-d_6): δ = 8.03 (d, J = 1.9 Hz, 1H, H-7), 7.99 (d, J = 8.3 Hz, 1H, H-4), 7.60 (dd, J = 8.3, 2.0 Hz, 1H, H-5), 1.68 (s, 9H, 3 × CH_3), assignments were confirmed by $^1H-^1H$ gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 158.9 (C═O), 147.5 (C-3a), 138.8 (C-7a), 126.2 (C-4), 125.5 (C-6), 122.0 (C-5), 111.6 (C-7), 60.5 ($C(CH_3)_3$), 27.3 (3 × CH_3), assignments were confirmed by $^1H-^{13}C$ gHSQC. LRMS (ESI^+): m/z = 303 $[M + Na]^+$. HRMS (ESI): calcd for $C_{11}H_{12}N_4O_3SNa^+$ 303.0522, found 303.0519.

***N*-*t*-Butyl-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (9a).** The title compound **9a** was prepared from phenyl acetylene (**a**) (20 μL, 0.178 mmol) according to the General Procedure 2. Purification of the crude product by flash chromatography (EtOAc/hexane 1:4) afforded **9a** (90%) as a pale yellow solid. R_f = 0.58 (EtOAc/hexane, 1:2). Mp = 212−214 °C. ^1H NMR (500 MHz, DMSO-d_6): δ = 9.51 (s, 1H, $H_{triazole}$), 8.77 (d, J = 1.5 Hz, 1H, H-7), 8.56 (dd, J = 8.4, 1.9 Hz, 1H, H-5), 8.25 (d, J = 8.4 Hz, 1H, H-4), 8.00−7.84 (m, 2H, H-3′, H-5′), 7.52 (t, J = 7.7 Hz, 2H, H-2′, H-6′), 7.41 (t, J = 7.4 Hz, 1H, H-4′), 1.71 (s, 9H, 3 × CH_3), assignments were confirmed by $^1H-^1H$ gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 159.0 (C═O), 148.1 ($C_{triazole}$), 141.6 (C-3a), 138.8 (C-7a), 129.8 (C-6), 129.4 (C-3′, H-5′), 128.9 (C-4′), 127.6 (C-4), 126.3 (C-5), 125.63 (C-2′, H-6′), 125.57 (C-1′), 120.4 ($CH_{triazole}$), 112.0 (C-7), 61.2 ($C(CH_3)_3$), 27.5 (3 × CH_3), assignments were confirmed by $^1H-^{13}C$ gHSQC. LRMS (ESI^+): m/z = 383 $[M + Na]^+$. HRMS (ESI): calcd for $C_{19}H_{19}N_4O_3S^+$ 383.1172, found 383.1171.

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***N*-*t*-Butyl-6-(4-[3′-methyl(phenyl)]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (9b).** The title compound **9b** was prepared from 1-ethynyl-3-methylbenzene (**b**) (69 μL, 0.54 mmol) according to the General Procedure 2. Purification of the crude product by flash chromatography (EtOAc/hexane 1:4) afforded **9b** (111 mg, 52%) as a white solid R_f = 0.50 (EtOAc/hexane, 1:2). Mp = 175−177 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.57 (s, 1H, $H_{triazole}$), 8.80 (d, *J* = 1.6 Hz, 1H, H-7), 8.58 (dd, *J* = 8.4, 1.8 Hz, 1H, H-5), 8.27 (d, *J* = 8.4 Hz, 1H, H-4), 7.77 (bs, 1H, H-2′), 7.73 (d, *J* = 7.7 Hz, 1H, H-6′), 7.41 (t, *J* = 7.6 Hz, 1H, H-5′), 7.23 (d, *J* = 7.6 Hz, 1H, H-4′), 2.40 (s, 3H, CH_3), 1.73 (s, 9H, 3 × CH_3 (*t*Bu)), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 158.7 (C═O), 147.9 ($C_{triazole}$), 141.4 (C-3a), 138.6 (C-7a), 138.3 (C-3′), 129.6 (C-6), 129.2 (C-1′), 129.0 (C-4′), 126.8 (C-5′), 125.9 (C-2′), 125.6 (C-4), 125.3 (C-5), 122.5 (C-6′), 120.2 ($CH_{triazole}$), 111.7 (C-7), 60.9 ($C(CH_3)_3$), 27.3 (3 × CH_3 (*t*Bu)), 21.04 (CH_3), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): *m*/*z* = 397 [M + H]$^+$. HRMS (ESI): calcd for $C_{20}H_{21}N_4O_3S^+$ 397.1329, found 397.1346.

***N*-*t*-Butyl-6-(4-[2′-(trifluoromethyl)phenyl]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (9c).** The title compound **9c** was prepared from 1-ethynyl-2-(trifluoromethyl)benzene (**c**) (57 μL, 0.41 mmol) according to General Procedure 2. Purification of the crude product by flash chromatography (EtOAc/hexane 1:6) afforded **9c** (158 mg, 86%) as a white solid. R_f = 0.43 (EtOAc/hexane, 1:6). Mp = 184−186 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.31 (s, 1H, $H_{triazole}$), 8.92 (d, *J* = 1.8 Hz, 1H, H-7), 8.63 (dd, *J* = 8.4, 1.9 Hz, 1H, H-5), 8.27 (d, *J* = 8.4 Hz, 1H, H-4), 7.94 (d, *J* = 7.8 Hz, 1H, H-3′), 7.88−7.79 (m, 2H, H-6′, H-5′), 7.73 (t, *J* = 7.4 Hz, 1H, H-4′), 1.73 (s, 9H, 3 × CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 158.7 (C═O), 145.3 ($C_{triazole}$), 141.2 (C-3a), 138.6 (C-7a), 132.8 (C-5′), 132.1 (C-6′), 129.6 (C-4′), 128.8 (C-1′), 127.0 (q, *J* = 31 Hz, CF_3), 126.78 (C-4), 126.5 (q, *J* = 5.5 Hz, C-2′), 126.0 (C-5), 125.5 (C-6), 124.9 ($CH_{triazole}$), 122.8 (C-3′), 112.2 (C-7), 60.9 ($C(CH_3)_3$), 27.8 (3 × CH_3), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): *m*/*z* = 451 [M + H]$^+$. HRMS (ESI): calcd for $C_{20}H_{18}N_4O_3F_3S^+$ 451.1046, found 451.1056.

***N*-*t*-Butyl-6-(4-[4′-methoxy(phenyl)]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (9d).** The title compound **9d** was prepared from 1-ethynyl-4-methoxybenzene (**d**) (46 μL, 0.36 mmol) according to General Procedure 2. Purification of the crude product by flash chromatography (EtOAc/hexane 1:6) afforded **9d** (50 mg, 34%) as a pale yellow solid. R_f = 0.50 (EtOAc/hexane, 1:6). Mp = 214−216 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.48 (s, 1H, $H_{triazole}$), 8.79 (d, *J* = 1.8 Hz, 1H, H-7), 8.57 (dd, *J* = 8.4, 1.9 Hz, 1H, H-5), 8.27 (d, *J* = 8.4 Hz, 1H, H-4), 7.87 (d, *J* = 8.8 Hz, 2H, H-3′, H-5′), 7.10 (d, *J* = 8.8 Hz, 2H, H-2′, H-6′), 3.82 (s, 3H, OCH_3), 1.73 (s, 9H, 3 × CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 159.6 (C-4′), 158.7 (C═O), 147.8 ($C_{triazole}$), 141.4 (C-3a), 138.6 (C-7a), 126.79 (C-4), 126.76 (C-3′, C-5′), 125.6 (C-5), 125.2 (C-6), 122.1 (C-1′), 119.2 ($CH_{triazole}$), 114.6 (C-2′, C-6′), 111.6 (C-7), 60.9 ($C(CH_3)_3$), 55.2 (OCH_3), 27.3 (3 × CH_3), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): *m*/*z* = 413 [M + H]$^+$. HRMS (ESI): calcd for $C_{20}H_{21}N_4O_4S^+$ 413.1278, found 413.1293.

***N*-*t*-Butyl-6-(4-[4′-fluoro-3′-methyl(phenyl)]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (9e).** The title compound **9e** was prepared from 4-ethynyl-1-fluoro-2-methylbenzene (**e**) (71 μL, 0.54 mmol) according to General Procedure 2. Purification of the crude product by flash chromatography (EtOAc/hexane 1:6) afforded **9e** (186 mg, 84%) as a pale yellow solid. R_f = 0.50 (EtOAc/hexane, 1:2). Mp = 205−207 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.55 (s, 1H, $H_{triazole}$), 8.78 (d, *J* = 1.7 Hz, 1H, H-7), 8.56 (dd, *J* = 8.4, 1.5 Hz, 1H, H-5), 8.27 (d, *J* = 8.4 Hz, 1H, H-4), 7.86 (d, $J_{F,H}$ = 7.3 Hz, 1H, H-2′), 7.81−7.71 (m, 1H, H-6′), 7.32−7.28 (m, 1H, H-5′), 2.33 (bs, 3H, CH_3), 1.73 (s, 9H, 3 × CH_3 (*t*Bu)), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 160.7 (d, *J* = 244.8 Hz, C-4′), 158.7 (C═O), 147.1 ($C_{triazole}$), 141.3 (C-3a), 138.6 (C-7a), 128.6 (d, *J* = 5.3 Hz, C-2′), 126.8 (C-4), 125.9 (d, *J* = 3.4 Hz, C-1′), 125.6 (C-5), 125.3 (C-6), 125.1 (d, *J* = 17.8 Hz, C-3′), 124.8 (d, *J* = 8.4 Hz, C-6′), 120.1 ($CH_{triazole}$), 115.8 (d, *J* = 22.8 Hz, C-5′), 111.7 (C-7), 60.9 ($C(CH_3)_3$), 27.3 (3 × CH_3 (*t*Bu)), 14.2 (d, *J* = 3.2 Hz, CH_3), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): *m*/*z* = 415 [M + H]$^+$. HRMS (ESI): calcd for $C_{20}H_{20}FN_4O_3S^+$ 415.1235, found 415.1254.

***N*-*t*-Butyl-6-(4-[4′-methoxy-2′-methyl(phenyl)]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (9f).** The title compound **9f** was prepared from 1-ethynyl-4-methoxy-2-methylbenzene (**f**) (78 μL, 0.54 mmol) according to General Procedure 2. Purification of the crude product by flash chromatography (EtOAc/hexane 1:7) afforded **9f** (120 mg, 53%) as a pale yellow solid. R_f = 0.27 (EtOAc/hexane, 1:2). Mp = 182−184 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.22 (s, 1H, $H_{triazole}$), 8.90 (d, *J* = 1.8 Hz, 1H, H-7), 8.63 (dd, *J* = 8.4, 1.8 Hz, 1H, H-5), 8.26 (d, *J* = 8.4 Hz, 1H, H-4), 7.78 (d, *J* = 8.4 Hz, 1H, H-6′), 7.00−6.86 (m, 2H, H-3′, H-5′), 3.81 (s, 3H, OCH_3), 2.52 (s, 3H, CH_3), 1.73 (s, 9H, 3 × CH_3(*t*Bu)), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 159.2 (C-4′), 158.7 (C═O), 147.1($C_{triazole}$), 141.5 (C-3a), 138.6 (C-7a), 137.1 (C-5′), 129.7 (C-4), 126.7 (C-3′), 125.6 (C-5), 125.1 (C-6), 121.5 (C-1′), 120.9 ($CH_{triazole}$), 116.2 (C-6′), 111.8 (C-2′), 111.7 (C-7), 60.8 ($C(CH_3)_3$), 55.1 (OCH_3), 27.3 (3 × CH_3(*t*Bu)), 21.3 (CH_3), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): *m*/*z* = 427 [M + H]$^+$. HRMS (ESI): calcd for $C_{21}H_{23}N_4O_4S^+$ 427.1435, found 427.1442.

***N*-*t*-Butyl-6-(4-[3′-sulfonamido(phenyl)]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (9g).** The title compound **9g** was prepared from 3-ethynyl benzenesulfonamide (**g**) (97 mg, 0.54 mmol) according to General Procedure 2. Purification of the crude product by flash chromatography (EtOAc/hexane 1:4) afforded **9g** (99 mg, 40%) as a pale yellow solid. R_f = 0.14 (EtOAc/hexane, 1:2). Mp = 200−202 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.75 (s, 1H, $H_{triazole}$), 8.84 (s, 1H, H-7), 8.61 (d, *J* = 8.3 Hz, 1H, H-5), 8.43 (s, 1H, H-2′), 8.29 (d, *J* = 8.3 Hz, 1H, H-4), 8.13 (d, *J* = 7.8 Hz, 1H, H-6′), 7.87 (d, *J* = 7.9 Hz, 1H, H-4′), 7.75 (t, *J* = 7.7 Hz, 1H, H-5′), 7.48 (s, 2H, SO_2NH_2), 1.73 (s, 9H, 3 × CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 158.7 (C═O), 146.6 ($C_{triazole}$), 145.1 (C-3′), 141.3 (C-3a), 138.6 (C-7a), 130.4 (C-5′), 130.0 (C-1′), 128.4 (C-6′), 126.8 (C-4), 125.8 (C-5), 125.6 (C-4′), 125.5 (C-6), 122.3 (C-2′), 121.2 ($CH_{triazole}$), 111.9 (C-7), 60.9 ($C(CH_3)_3$), 27.3 (3 × CH_3), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): *m*/*z* = 462 [M + H]$^+$. HRMS (ESI): calcd for $C_{19}H_{19}N_5O_5S_2Na_1$ 484.0720, found 484.0733.

6-(4-[2′-Trifluoromethyl(phenyl)]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (5c). The title compound **5c** was prepared from **9c** (75 mg, 0.167 mmol) according to General Procedure 3 in 4 days. Purification of the crude product by flash chromatography (EtOAc/hexane 1:2) afforded **5c** (15 mg, 23%) as a pale yellow solid. R_f = 0.12 (EtOAc/hexane, 1:2). Mp = 276−278 °C (decomp.). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.22 (s, 1H, $H_{triazole}$), 8.45 (bs, 1H, H-7), 8.33 (d, *J* = 8.1 Hz, 1H, H-5), 7.95−7.89 (m, 2H, H-4, H-6′), 7.86−7.78 (m, 2H, H-4′, H-5′), 7.77−7.66 (m, 1H, H-3′), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 166.9 (C═O), 147.1 ($C_{triazole}$), 145.2 (C-7a), 138.5 (C-3a), 132.9 (C-5′), 132.4 (C-6′), 129.6 (C-4′), 128.9 (C-1′), 127.3 (C-6), 127.1 (CF_3), 126.6 (C-2′), 124.7 (C-5), 123.8 (C-4), 123.0 ($CH_{triazole}$), 122.7 (C-3′), 111.3 (C-7), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^-$): *m*/*z* = 393 [M − H]$^-$. HRMS: calcd for $C_{16}H_{10}F_3N_4O_3S^-$ 393.0274, found 393.0288.

6-(4-[4′-Methoxy(phenyl)]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (5d). The title compound **5d** was prepared from **9d** (42 mg, 0.102 mmol) according to General Procedure 3 in 1 day. Purification of the crude product by flash chromatography (DCM/MeOH 19:1 to 15:1) afforded **5d** (21 mg, 58%) as a pale yellow solid. R_f = 0.14 (DCM/MeOH 19:1). Mp = 280−282 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.39 (s, 1H, $H_{triazole}$), 8.24 (d, *J* = 1.5 Hz, 1H, H-7), 8.21 (dd, *J* = 8.1, 1.8 Hz, 1H, H-5), 7.87 (d, *J* = 8.7 Hz, 2H, H-3′, H-5′), 7.81 (d, *J* = 8.1 Hz, 1H, H-4), 7.08 (d, *J* = 8.8 Hz, 2H, H-2′, H-6′), 3.82 (s, 3H, OCH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz,

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DMSO-d_6): δ = 166.6 (C═O), 159.4 (C-4′), 147.4 ($C_{triazole}$), 147.1 (C-7a), 138.5 (C-3a), 134.2 (C-6), 126.7 (C-3′, C-5′), 124.1 (C-4), 122.9 (C-5), 122.6 (C-1′), 119.0 ($CH_{triazole}$), 114.5 (C-2′, C-6′), 110.4 (C-7), 55.2 (OCH_3), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^-$): m/z = 355 [M − H]$^-$. HRMS (ESI): calcd for $C_{16}H_{11}N_4O_4S^-$ 355.0507, found 355.0504.

6-(4-[4′-Fluoro-3′-methyl(phenyl)]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (5e). The title compound **5e** was prepared from **9e** (50 mg, 0.120 mmol) according to General Procedure 3 in 4 days. Purification of the crude product by flash chromatography (DCM/MeOH 15:1) afforded **5e** (21 mg, 49%) as a pale yellow solid. R_f = 0.14 (DCM/MeOH 15:1). Mp = >300 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.48 (s, 1H, $H_{triazole}$), 8.25 (d, J = 1.8 Hz, 1H, H-7), 8.22 (dd, J = 8.0, 1.8 Hz, 1H, H-5), 7.89 (dd, J = 7.4, 1.5 Hz, 1H, H-2′), 7.82 (d, J = 8.1 Hz, 1H, H-4), 7.81−7.77 (m, 1H, H-6′), 7.32−7.27 (m, 1H, H-5′), 2.33 (d, J = 1.1 Hz, 3H, CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 166.6 (C-4′), 159.6 (C═O), 147.1 ($C_{triazole}$), 146.7 (C-3a), 138.4 (C-7a), 134.3 (C-6), 128.6 (d, J = 5.2 Hz, C-2′), 126.3 (d, J = 3.2 Hz, C-1′), 124.95 (d, J = 16.9 Hz, C-3′), 124.75 (d, J = 8.2 Hz, C-6′), 124.2 (C-5), 123.0 (C-4), 119.9 ($CH_{triazole}$), 115.7 (d, J = 3.2 Hz, C-5′), 110.5 (C-7), 14.2 (d, J = 3.2 Hz, CH_3), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^-$): m/z = 357 [M − H]$^-$. HRMS (ESI): calcd for $C_{16}H_{10}FN_4O_3S^-$ 357.0463, found 357.0454.

6-(4-[4′-Methoxy-2′-methyl(phenyl)]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (5f). The title compound **5f** was prepared as a pale yellow solid (15 mg, 33%) from **9f** (53 mg, 0.124 mmol) according to General Procedure 3 in 4 days. R_f = 0.20 (DCM/MeOH 4:1). Mp = 275−277 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.17 (s, 1H, $H_{triazole}$), 8.64−8.55 (m, 1H, H-7), 8.46−8.39 (m, 1H, H-5), 8.05−7.98 (m, 1H, H-4), 7.77 (d, J = 8.3 Hz, 1H, H-5′), 6.95−6.89 (m, 1H, H-3′, H-6′), 3.81 (s, 3H, OCH_3), 2.52 (s, 3H, CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 159.2 (C-4′), 158.5 (C═O), 146.9 ($C_{triazole}$), 139.8 (C-3a), 137.1 (C-7a), 134.9 (C-4), 129.8 (C-2′), 125.3 (C-5), 124.1 (C-6), 121.72 (C-1′), 121.69 (C-5′), 120.9 ($CH_{triazole}$), 116.2 (C-6′), 111.7 (C-3′), 111.5 (C-7), 55.1 (OCH_3), 21.3 (CH_3), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^-$): m/z = 369 [M − H]$^-$. HRMS (ESI): calcd for $C_{17}H_{13}N_4O_4S^-$ 369.0663, found 369.0674.

6-(4-[3′-Sulfonamido(phenyl)]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (5g). The title compound **5g** was prepared as a pale yellow solid (35 mg, 79%) from **9g** (50 mg, 0.108 mmol) according to General Procedure 3 in 1 day. R_f = 0.10 (DCM/MeOH 4:1). Mp = >300 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.69 (s, 1H, $H_{triazole}$), 8.51 (d, J = 1.9 Hz, 1H, H-7), 8.44 (d, J = 1.8 Hz, 1H, H-2′), 8.39 (dd, J = 8.2, 1.9 Hz, 1H, H-5), 8.18−8.09 (m, 1H, H-4′), 8.01 (d, J = 8.2 Hz, 1H, H-4), 7.89−7.83 (m, 1H, H-6′), 7.74 (t, J = 7.7 Hz, 1H, H-5′), 7.46 (s, 2H, SO_2NH_2), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 146.4 (C-3′), 145.1 ($C_{triazole}$), 144.6 (C-7a), 139.6 (C-3a), 130.6 (C-6), 129.9 (C-2′), 128.4 (C-4′), 125.5 (C-6′), 125.4 (C-5′), 124.2 (C-4), 122.3 (C-5), 121.0 (C-1′), 111.5 ($CH_{triazole}$), 109.5 (C-7), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^-$): m/z = 404 [M − H]$^-$. HRMS (ESI): calcd for $C_{15}H_{10}N_5O_5S_2^-$ 404.0129, found 404.0148.

***N*-*t*-Butyl-6-(4-[2′,3′,4′,6′-tetra-*O*-acetyl-*β*-D-glucopyranoside-*O*-methylene]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (11).** The title compound **11** was prepared from **10** (138 mg, 0.36 mmol) according to General Procedure 2. Purification of the crude product by flash chromatography (hexane/EtOAc 2:1 to 1:1) afforded **11** (211 mg, 89%) as a pale yellow solid. R_f = 0.45 (hexane/EtOAc 1:2). Mp = 90−92 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.06 (s, 1H, $H_{triazole}$), 8.80 (d, J = 1.8 Hz, 1H, H-7), 8.54 (dd, J = 8.4, 1.9 Hz, 1H, H-5), 8.25 (d, J = 8.4 Hz, 1H, H-4), 5.27 (t, J = 9.6 Hz, 1H, H-3′), 4.99−4.88 (m, 3H, H-1′, H-2′, CH of CH_2), 4.83−4.76 (m, 2H, H-4′, CH of CH_2), 4.21 (dd, J = 12.4, 4.8 Hz, 1H, H-6′), 4.08 (d, J = 12.1, 2.3 Hz, 1H, H-6″), 4.06−4.02 (m, 1H, H-5′), 2.02, 1.99, 1.94, 1.93 (4 × s, 4 × 3H, 4 × OCH_3, 1.72 (s, 9H, 3 × CH_3 (*t*Bu)) assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 170.0, 169.5, 169.2, 169.0 (4 × C═O(Ac)), 158.7 (C═O(Sac)), 144.6 ($C_{triazole}$), 141.3 (C-3a), 138.6 (C-7a), 126.8 (C-5), 125.9 (C-4), 125.4 (C-6), 123.5 ($CH_{triazole}$), 112.0 (C-7), 98.5 (C-1′), 72.1 (C-5′), 70.8 (C-3′), 70.7 (C-2′), 68.1 (C-4′), 61.6 (CH_2), 61.4 (C-6′), 60.9 ($C(CH_3)_3$), 27.3 (3 × CH_3(*t*Bu)), 20.5, 20.3, 20.3, 20.2 (4 × OCH_3), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): m/z = 667 [M + H]$^+$, 689 [M + Na]$^+$. HRMS (ESI): calcd for $C_{28}H_{35}N_4O_{13}S^+$ 667.1916, found 667.1903.

6-(4-[*β*-D-Glucopyranoside-*O*-methylene]-1*H*-1,2,3-triazol-1-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (12). The title compound **12** was prepared as a pale white solid (13 mg, 34%) from **11** (50 mg, 0.075 mmol) according to General Procedure 3 in 6 days. R_f = 0.45 (acetonitrile/water 9:1). Mp = 90−92 °C. ^{1}H NMR (500 MHz, DMSO-d_6, 1% D_2O): δ = 9.04 (s, 1H, $H_{triazole}$), 8.49 (s, 1H, H-7), 8.34 (d, J = 8.4 Hz, 1H, H-5), 8.02 (d, J = 8.2 Hz, 1H, H-4), 4.95 (d, J = 12.5 Hz, 1H, CH of CH_2), 4.77 (d, J = 12.5 Hz, 1H, CH of CH_2), 4.34 (d, J = 7.8 Hz, 1H, H-1′), 3.73 (d, J = 10.5 Hz, 1H, H-6′), 3.48 (dd, J = 11.8, 6.1 Hz, 1H, H-6″), 3.21−3.13 (m, 2H, H-2′, H-5′), 3.10−3.05 (m, 1H, H-3′), 3.02 (t, J = 8.4 Hz, 1H, H-4′), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6): δ =162.9 (C═O), 145.5 ($C_{triazole}$), 143.8 (C-3a), 140.0 (C-7a), 130.1 (C-4), 125.6 (C-5), 124.5 (C-6), 123.1 ($CH_{triazole}$), 111.6 (C-7), 102.3 (C-1′), 77.0 (C-3′), 76.6 (C-2′), 73.4 (C-5′), 70.1 (C-4′), 61.3 (CH_2), 61.2 (C-6′), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^-$): m/z = 441 [M − H]$^-$. HRMS (ESI): calcd for $C_{16}H_{17}N_4O_9S^-$ 441.0722, found 441.0739.

Protein X-ray Crystallography. hCA II was concentrated down to ∼14 mg mL^{-1} and set up in SD-2 plates (Molecular Dimensions) using a Phoenix crystallization robot (ARI) with the following ratio of protein plus reservoir plus compound: 210 nL + 140 nL + 70 nL. The compound was dissolved in neat DMSO, and the plate was incubated at 20 °C. The reservoir condition consisted of 2.9 M ammonium sulfate with 0.1 M Tris buffer at pH 8.5. Additional dry compound was added to the crystallization drop after crystals had formed and several days before data were collected. Then, 360 frames of one degree oscillation were taken at the MX-2 beamline of the Australian Synchrotron. The data were indexed using XDS[24] and scaled using SCALA.[25] Molecular replacement was done using Phaser[26] using 3ML2 as the initial starting model. The model was manually rebuilt using Coot[27] and refined using Refmac.[28] The compound was placed in density using the program Afitt (OpenEye Scientific Software) and further refined using Refmac.[28]

Protein Expression and Purification. BL21-DE3 cells were transformed with 1 μL of the CA II-containing plasmid pACA, and the cells were plated out onto a YT-Amp plate and left at 37 °C overnight. The next day a single colony was selected and grown up in 5 mL of 2xYT overnight to form a starter culture. One liter of 2xYT was started with 2 mL of overnight culture, and the cells were induced with 1 mM IPTG at OD595 = 0.68. The cells were allowed to grow for another 3.5 h, and then the cells were spun down, washed with 20 mM Tris pH 7.5 and 100 mM NaCl, and spun again. The cell pellet was frozen at −80 °C. The cell pellet was brought up in 30 mL (of 50 mL total) buffer A that had one protease inhibitor tablet (Roche), 20 μL of 50 mg mL^{-1} lysozyme, and 20 μL benzoase added and used to lyse the cells with a sonicator. The sample was on ice; the sonicator was used in 15 s bursts with 15 s of rest between bursts, and the setting was at 80 to 100% for a total of 11 min of sonication. The 20 mL "extra" buffer A was added when extra cooling was needed throughout the procedure. When done, the sample was centrifuged at 4000 rpm for 25 min at 4 °C to remove cell debris. Next, 45 mL of the sample was loaded onto a Sepharose S 5 mL column and washed with buffer A for 10 column volumes. The sample was then eluted from the column with buffer B in a linear gradient; fractions were collected and analyzed by SDS-PAGE gel. Fractions containing the majority of the protein of interest were combined (∼4 mL total). Next, a preparative gel filtration column, GE Sepharose16/60, was used, and the sample was run in 2 × 2 mL lots onto this column. Collected fractions were analyzed by SDS-PAGE. The pure hCA II fractions were combined, concentrated using an Amicon Ultra 10 kDa concentration device to

14 mg mL^{-1} (as measured on a NanoDrop spectrophotometer) in GF buffer, and subsequently flash frozen in 100 μL aliquots in liquid N_2 and transferred to −80 °C. Buffers: (buffer A) 20 mM MES pH 6.5, 10 mM NaCl, 0.1 mM $ZnSO_4$, 0.4 mM DTT; (buffer B) 100 mM Tris pH 8.0, 10 mM NaCl, 0.1 mM $ZnSO_4$, 0.4 mM DTT; (GF buffer) 30 mM Tris pH 8.0, 20 mM NaCl, 0.1 mM $ZnSO_4$, 1 mM DTT.

CA Inhibition Assay. An Applied Photophysics stopped-flow instrument was used for assaying the CA-catalyzed CO_2 hydration activity.[29] IC_{50} values were obtained from dose−response curves working at seven different concentrations of test compound, by fitting the curves using PRISM (www.graphpad.com) and nonlinear least-squares methods; values represent the mean of at least three different determinations as described by us previously.[30] The inhibition constants (K_i) were then derived by using the Cheng-Prusoff equation[31] as follows: $K_i = IC_{50}/(1 + [S]/K_m)$, where $[S]$ represents the CO_2 concentration at which the measurement was carried out, and K_m is the concentration of substrate at which the enzyme activity is at half-maximal. All enzymes used were recombinant, produced in *Escherichia coli* as reported earlier.[32] The concentrations of enzymes used in the assay were as follows: hCA I, 10.4 nM; hCA II, 8.3 nM; hCA IX, 8.0 nM; and hCA XII, 12.4 nM.

■ ASSOCIATED CONTENT

Supporting Information

^1H and ^{13}C NMR spectra of compounds **4**, **7**, **8**, **5a**−**5g**, **9a**−**9g**, **11**, and **12**. Data collection and structure refinement statistics of **5b**/hCA II crystal structure. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*Telephone: +39 055 457 3729. E-mail: claudiu.supuran@unifi.it.

*Telephone: +61 7 3735 7825. E-mail: s.poulsen@griffith.edu.au.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

1,3-DCR, 1,3-dipolar cycloaddition reaction; CuAAC, copper-catalyzed azide alkyne cycloaddition; CA, carbonic anhydrase; K_i, inhibition constant

■ REFERENCES

(1) (a) Larsen, J. C. Artificial sweeteners. *Nutrafoods* **2012**, *11*, 3−9. (b) Weihrauch, M. R.; Diehl, V. Artificial sweeteners—do they bear a carcinogenic risk? *Ann. Oncol.* **2004**, *15*, 1460−1465.

(2) (a) Blanchet, J.; Macklin, T.; Ang, P.; Metallinos, C.; Snieckus, V. Directed ortho metalation−cross coupling strategies. N-Cumyl arylsulfonamides. Facile deprotection and expedient route to 7- and 4,7-substituted saccharins. *J. Org. Chem.* **2007**, *72*, 3199−3206. (b) Jakopin, Z.; Dolenc, M. S. Advances in the chemistry of saccharins: from synthetic novelties towards biologically active compounds. *Curr. Med. Chem.* **2010**, *17*, 651−671.

(3) (a) Kohler, K.; Hillebrecht, A.; Wischeler, J. S.; Innocenti, A.; Heine, A.; Supuran, C. T.; Klebe, G. Saccharin inhibits carbonic anhydrases: possible explanation for its unpleasant metallic aftertaste. *Angew. Chem., Int. Ed.* **2007**, *46*, 7697−7699. (b) Wilson, J. M.; Tanko, Q.; Wendland, M. M.; Meany, J. E.; Nedved, J. F.; Pocker, Y. The inhibition of bovine carbonic anhydrase by saccharin and 2- and 4-carbobenzoxybenzene sulfonamide. *Physiol. Chem. Phys. Med. NMR* **1998**, *30*, 149−162.

(4) Svastova, E.; Hulikova, A.; Rafajova, M.; Zatovicova, M.; Gibadulinova, A.; Casini, A.; Cecchi, A.; Scozzafava, A.; Supuran, C. T.; Pastorek, J.; Pastoreková, S. Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH. *FEBS Lett.* **2004**, *577*, 439−445.

(5) (a) Swietach, P.; Vaughan-Jones, R.; Harris, A. Regulation of tumor pH and the role of carbonic anhydrase 9. *Cancer Metastasis Rev.* **2007**, *26*, 299−310. (b) Chiche, J.; Ilc, K.; Laferriere, J.; Trottier, E.; Dayan, F.; Mazure, N. M.; Brahimi-Horn, M. C.; Pouyssegur, J. Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. *Cancer Res.* **2009**, *69*, 358−368. (c) Swietach, P.; Vaughan-Jones, R.; Harris, A.; Hulikova, A. The chemistry, physiology and pathology of pH in cancer. *Philos. Trans R Soc., B* **2014**, *369*, 1638.

(6) (a) Neri, D.; Supuran, C. T. Interfering with pH regulation in tumours as a therapeutic strategy. *Nat. Rev. Drug Discovery* **2011**, *10*, 767−777. (b) Poulsen, S. Carbonic anhydrase inhibition as a cancer therapy: a review of patent literature, 2007−2009. *Expert Opin. Ther. Pat.* **2010**, *20*, 795−806.

(7) Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? *Chem. Rev.* **2012**, *112*, 4421−4468.

(8) Di Fiore, A.; Maresca, A.; Alterio, V.; Supuran, C. T.; De Simone, G. Carbonic anhydrase inhibitors: X-ray crystallographic studies for the binding of N-substituted benzenesulfonamides to human isoform II. *Chem. Commun.* **2011**, *47*, 11636−11638.

(9) Pitman, I. H.; Dawn, H.; Higuchi, T.; Hussain, A. A. Prediction of chlorine potentials of N-chlorinated organic molecules. *J. Chem. Soc. B* **1969**, 1230−1232.

(10) Congreve, M.; Carr, R.; Murray, C. J.; Jhoti, H. A 'rule of three' for fragment-based lead discovery? *Drug Discovery Today* **2003**, *8*, 876−877.

(11) (a) Hopkins, A. L.; Groom, C. R.; Alexander, A. Ligand efficiency: a useful metric for lead selection. *Drug Discuss. Today* **2004**, *9*, 430−431. (b) Perola, E. An analysis of the binding efficiencies of drugs and their leads in successful drug discoveryprograms. *J. Med. Chem.* **2010**, *53*, 2986−2997.

(12) Kamogawa, H.; Yamamoto, S.; Nanasawa, M. Syntheses of saccharin and cyclamate derivatives bearing polymerizable vinyl group. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3824−3827.

(13) Seddigi, Z. K.; Banu, A.; Hossain, G. M. G. Synthesis and crystal structure of mercury complex of saccharin with pyridine [$Hg(sac)_2(py)_2$]. *Arab J. Sci. Eng.* **2007**, *32*, 181−187.

(14) Ahmed, K. J.; Habib, A.; Haider, S. Z.; Malik, K. M. A.; Hurthouse, M. B. The preparatation and X-ray crystal structure of a saccharin complex of copper(II). *Inorg. Chim. Acta* **1981**, *56*, L37.

(15) Yeung, K.-S.; Meanwell, N. A.; Li, Y.; Gao, Q. A facile construction of 4-hydroxymethylbenzoisothiazolone-1,1-dioxide. *Tetrahedron Lett.* **1998**, *39*, 1483−1486.

(16) Xu, L.; Shu, H.; Liu, Y.; Zhang, S.; Trudell, M. L. Oxidative cyclization of *N*-alkyl-*o*-methyl-arenesulfonamides to biologically important saccharin derivatives. *Tetrahedron* **2006**, *62*, 7902−7910.

(17) (a) Carroux, C. J.; Rankin, G. M.; Moeker, J.; Bornaghi, L. F.; Katneni, K.; Morizzi, J.; Charman, S. A.; Vullo, D.; Supuran, C. T.; Poulsen, S.-A. A prodrug approach toward cancer-related carbonic anhydrase inhibition. *J. Med. Chem.* **2013**, *56*, 9623−9634. (b) Moeker, J.; Teruya, K.; Rossit, S.; Wilkinson, B. L.; Lopez, M.; Bornaghi, L. F.; Innocenti, A.; Supuran, C. T.; Poulsen, S.-A. Design and synthesis of thiourea compounds that inhibit transmembrane anchored carbonic anhydrases. *Bioorg. Med. Chem.* **2012**, *20*, 2392−2404. (c) Wilkinson,

B. L.; Bornaghi, L. F.; Houston, T. A.; Innocenti, A.; Supuran, C. T.; Poulsen, S.-A. A novel class of carbonic anhydrase inhibitors: glycoconjugate benzene sulfonamides prepared by "click-tailing". *J. Med. Chem.* **2006**, *49*, 6539−6548. (d) Lopez, M.; Salmon, A. J.; Supuran, C. T.; Poulsen, S.-A. Carbonic anhydrase inhibitors developed through "click tailing". *Curr. Pharm. Des.* **2010**, *16*, 3277−3287. (e) Morris, J. C.; Chiche, J.; Grellier, C.; Lopez, M.; Bornaghi, L. F.; Maresca, A.; Supuran, C. T.; Pouyssegur, J.; Poulsen, S.-A. Targeting hypoxic tumor cell viability with carbohydrate-based carbonic anhydrase IX and XII inhibitors. *J. Med. Chem.* **2011**, *54*, 6905−6918.

(18) Kerns, E. H.; Di, L. *Drug-like Properties: Concepts, Structure Design and Methods: From ADME to Toxicity Optimization*. Academic Press: London, 2008.

(19) Sonmez, F.; Bilen, C.; Sumersan, S.; Gencer, N.; Isik, S.; Arslan, O.; Kucukislamoglu, M. In vitro inhibition effect and structure−activity relationships of some saccharin derivatives on erythrocyte carbonic anhydrase I and II. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 118−123.

(20) Winn, M. D.; Ballard, C. C.; Cowlan, K. D.; Dodson, E. J.; Emsly, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G. W.; McCoy, A.; McNicholas, S. J.; Murshudov, G. N.; Pannu, N. S.; Potterton, E. A.; Powell, H. R.; Read, R. J.; Vagin, A.; Wilson, K. S. Overview of the CCP4 suite and current developments. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2011**, *67*, 235−242.

(21) The PyMOL Molecular Graphics System, version 1.3, Schrödinger, LLC; http://www.pymol.org.

(22) Wilkinson, B.; Bornaghi, L.; Houston, T.; Innocenti, A.; Vullo, D.; Supuran, C. T.; Poulsen, S.-A. Inhibition of membrane-associated carbonic anhydrase isozymes IX, XII and XIV with a library of glycoconjugate benzenesulfonamides. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 987−992.

(23) Holzer, W. On the application of NOE difference spectroscopy for spectral and structural assignments with substituted 1,2,3-triazoles. *Tetrahedron* **1991**, *47*, 9783−9792.

(24) Kabsch, W. XDS. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2010**, *66*, 125−132.

(25) Evans, P. Scaling and assessment of data quality. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2006**, *62*, 72−82.

(26) McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. Phaser crystallographic software. *J. Appl. Crystallogr.* **2007**, *40*, 658−674.

(27) Emsley, P.; Cowton, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2004**, *60*, 2126−2132.

(28) Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1997**, *53*, 240−255.

(29) Khalifah, R. G. The carbon dioxide hydration activity of carbonic anhydrase. *J. Biol. Chem.* **1971**, *246*, 2561−2573.

(30) Lopez, M.; Drillaud, N.; Bornaghi, L. F.; Poulsen, S.-A. Synthesis of *S*-glycosyl primary sulfonamides. *J. Org. Chem.* **2009**, *74*, 2811−2816.

(31) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC50) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099.

(32) (a) Winum, J.-Y.; Vullo, D.; Casini, A.; Montero, J.-L.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: Inhibition of cytosolic isozymes I and II and the membrane-bound, tumor associated isozyme IX with sulfamates also acting as steroid sulfatase inhibitors. *J. Med. Chem.* **2003**, *46*, 2197−2204. (b) Vullo, D.; Innocenti, A.; Nishimori, I.; Pastorek, J.; Scozzafava, A.; Pastoreková, S.; Supuran, C. T. Carbonic anhydrase inhibitors. Inhibition of the transmembrane isozyme XII with sulfonamides—a new target for the design of antitumor and antiglaucoma drugs? *Bioorg. Med. Chem. Lett.* **2005**, *15*, 963−969.