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Structural Insights into Carbonic Anhydrase IX Isoform Specificity of Carbohydrate-Based Sulfamates

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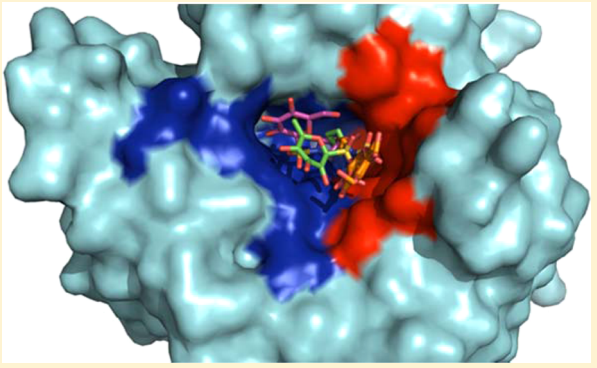
Supporting Information

ABSTRACT: Carbonic anhydrase IX (CA IX) is an extracellular transmembrane homodimeric zinc metalloenzyme that has been validated as a prognostic marker and therapeutic target for several types of aggressive cancers. CA IX shares a close homology with other CA isoforms, making the design of CA IX isoform selective inhibitors challenging. In this paper, we describe the development of a new class of CA IX inhibitors that comprise a sulfamate as the zinc binding group, a variable linker, and a carbohydrate "tail" moiety. Seven compounds inhibited CA IX with low nM K_i values of 1−2 nM and also exhibited permeability profiles to preferentially target the binding of extracellular CA IX over cytosolic CAs. The crystal structures of two of these compounds in complex with a CA IX-mimic (a variant of CA II, with active site residues that mimic CA IX) and one compound in complex with CA II have been determined to 1.7 Å resolution or better and demonstrate a selective mechanism of binding between the hydrophilic and hydrophobic pockets of CA IX versus CA II. These compounds present promising candidates for anti-CA IX drugs and the treatment for several aggressive cancer types.



INTRODUCTION

A characteristic feature of many solid tumors and hypoxia-treated cancer cells in culture is the expression of carbonic anhydrase IX (CA IX),[1] an extracellular facing enzyme that catalyzes the interconversion of CO_2 and H_2O to HCO_3^- and a H^+. CA IX works in concert with cell membrane transporters to regulate the pH of tumor cells, thereby enabling them to manage the acid load due to the metabolic transition known as the Warburg effect.[2] The action of CA IX maintains the intracellular pH (pH_i) of solid tumors within the tight range needed for cell survival and growth, while at the same time the extracellular pH (pH_e) becomes more acidic, promoting tumor growth and metastasis.[3] The expression of CA IX in a broad range of solid tumor types is associated with a poor patient prognosis, and more recently the enzyme has been established as a therapeutic target for several aggressive cancers.[2a,c,4] Because the CA IX expression profile in healthy cells is restricted to a few tissues (stomach and GI tract), there is substantial interest in investigating CA IX as a diagnostic and novel drug target for cancer management.[2,4,5]

A diverse collection of aromatic and heterocyclic compounds with primary sulfonamide or sulfamate zinc binding functional groups (ZBGs) are known with CA inhibition activity. The ZBG of these compounds bind with the CA active site in an invariant manner, however, interactions of the remainder of the compound with the CA active site are variable and this has allowed a range of CA binding configurations and CA isozyme selectivity profiles. Our group recently established synthetic methodology to incorporate either the primary sulfonamide or primary sulfamate ZBGs directly onto monosaccharide scaffolds. When compared to conventional CA inhibitors, these novel glycoconjugates have poor cell membrane passive diffusion characteristics, a property that leads to the selective inhibition of CA IX owing to the extracellular facing active site of this enzyme.[6] Glycosidic inhibitors of carbonic anhydrase IX comprising a classical aromatic sulfonamide pharmacophore have been reviewed previously.[7] This alternate inhibitor design has delivered improved selectivity for CA IX when compared to optimizing CA IX efficacy alone. In this study, we extend our focus on preparing sulfamate-based glycoconjugate CA inhibitors to modulate selective CA IX inhibition through the

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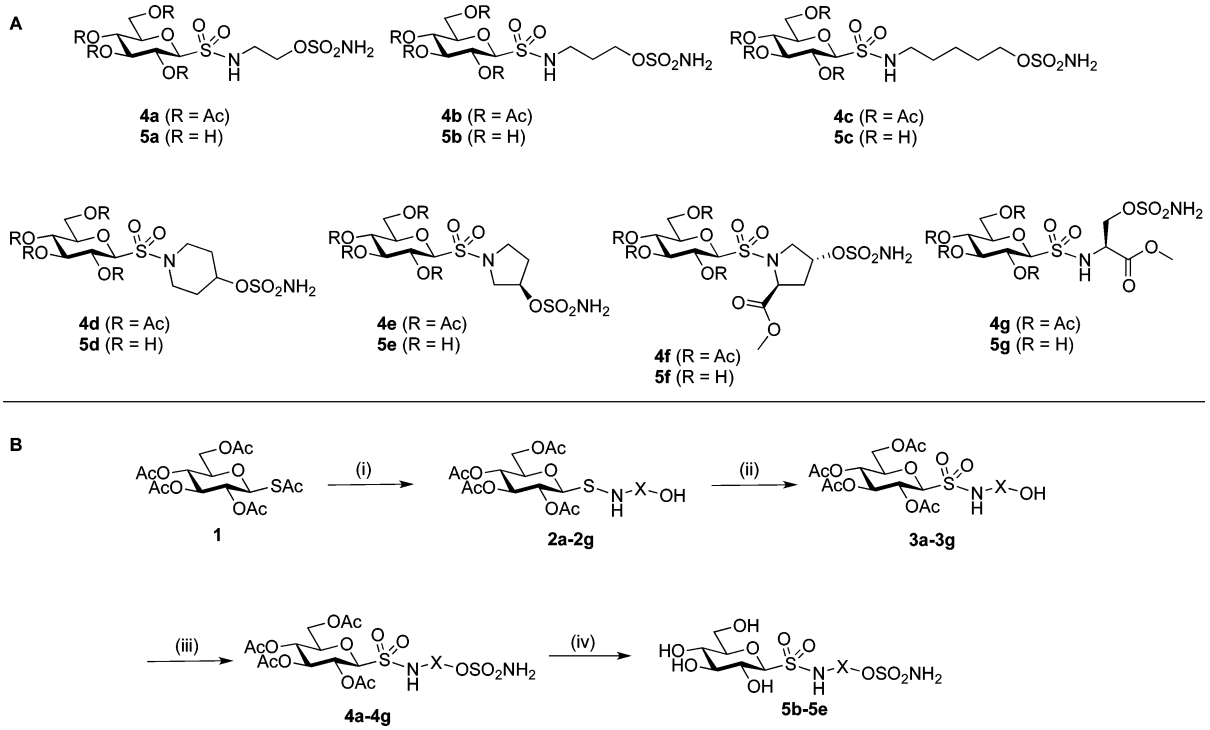
Scheme 1. (A) Carbohydrate-Based Sulfamate Target Compounds with a Variable Linker Region 4a−4g and 5a−5g; (B) Synthetic Approach Towards Target Carbohydrate:Sulfamate Compounds Showing Variable Linker Region as 'X'[a]



[a]Reagents and conditions. (i) (a) 2.5 equiv $BrCH(CO_2Et)_2$, MeOH, rt, 20 min, (b) 3.0 equiv amino alcohol **a**−**g**, rt, 1 h; (ii) 6.0 equiv *m*CPBA, CH_2Cl_2, rt, 1−5 h; (iii) $ClSO_2NCO$, HCO_2H, DMA 0 °C → rt, 3 h; (iv) NaOH, MeOH, 0 °C to rt, 2−4 h; then Amberlite IR120-H^+.

combination of structure−activity and structure−property relationships. We then examined the interactions of selected compounds when binding to wild-type CA II and a CA IX-mimic. The use of the CA IX-mimic is a powerful and relatively new structural tool in the field of CA drug discovery. This CA IX-mimic construct was engineered by site-directed mutagenesis of residues in the active site of CA II to residues unique to CA IX, such that the active site of the CA IX-mimic is analogous to wild-type CA IX.[6] The advantage of the CA IX-mimic is that it extremely stable and readily crystallizes, allowing, for the first time, direct structural insight into CA II versus CA IX inhibitor binding with a range of novel small molecules.[8] The CA II to CA IX-mimic mutations are A65S, N67Q, E69T, I91L, F131V, K170E, and L204A.[8a] Here we present the first study reporting structural insights of sulfamate small molecules binding to the catalytic site of CA IX via use of the CA IX-mimic.[6,7]

RESULTS AND DISCUSSION

Compound Design and Synthesis. The primary goal of this study was to move away from the classical aromatic sulfonamide CA inhibitor structure toward novel chemical entities with an alternate pharmacophore for CA binding. We designed and synthesized a series of carbohydrate-based sulfamate compounds to build upon our earlier success with this approach to CA IX inhibitor design, Scheme 1.[9] Sulfamates have been shown to feature in the structures of compounds with important medicinal applications.[10] The glucose moiety is common to all target compounds, and the glucose moiety is bridged via a sulfonamide functionality to a variable linker region (X) that terminates with the primary sulfamate ZBG, Scheme 1.[6e,11] These compounds are novel, and sulfamates of this level of diversity have not been investigated for CA inhibition previously. The origin of the linker region diversity is the selected panel of primary (**a**−**c**) and secondary (**d**−**g**) amino alcohols that were chosen to probe potential CA active site interactions through altering the linker length, steric bulk, and stereochemistry close to the ZBG. In particular, it is hoped that these compounds will form different interactions with CA II and CA IX, exploiting their seven key amino acid active site residue differences, and providing a structure-based starting point to guide future inhibitor design.

The synthetic approach toward the acylated carbohydrate-based sulfamates **4a**−**4g** proceeded in three steps commencing from 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose[6e,9a] **1** and a panel of seven amino alcohols, **a**−**g**, Scheme 1. Reaction of **1** with **a**−**g** gave the corresponding sulfonamides terminating in a primary or secondary alcohol group, **2a**−**2g**, which were either purified (**2f** and **2g**) or immediately oxidized (**2a**−**2e**) to give the sulfonamide-bridged glycoconjugates **3a**−**3g**. Next, sulfamoylation of the terminal alcohol functionality of **3a**−**3g** using sulfamoyl chloride (generated in situ from formic acid and chlorosulfonylisocyanate) gave the target sulfamates **4a**−**4g** in yields of 44−79%. Lastly, the fully deprotected compounds **5a**−**5g** were prepared by deacetylation with standard Zemplén conditions.[12] The fully deprotected sulfamates **5b**−**5e** were synthesized in almost quantitative yields from **4b**−**4e**, however, glycoconjugates **5a**, **5f**, and **5g** contained byproducts that could not be separated. Using either milder basic deacetylation conditions with ammonia[13] or acidic deacetylation conditions (AcCl[14] or TsOH[15] as catalysts) did not give **5a**, **5f**, and **5g**.

CA Inhibition. The aim of this study was to generate glycoconjugate sulfamates for targeting cancer-associated CA

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Table 1. Inhibition, Permeability, and Isozyme Selectivity Ratio Data for Human CA Isozymes I, II, IX, and XII with Compounds 4a−4g and 5b−5e

		K_i (nM)[b]				selectivity ratio[c]			
compd	cLogP[a]	CA I	CA II	CA IX	CA XII	I/IX	II/IX	I/XII	II/XII
4a	−0.76	1050	94	215	9	5	<1	117	10
4b	−0.51	1350	525	215	94	6	2	14	6
4c	−0.39	350	10	2	9	175	5	39	1
4d	−0.93	2400	265	2	60	1200	133	40	4
4e	−0.30	1550	110	2	8	775	55	194	14
4f	−0.01	9500	725	2	1	4750	363	9500	725
4g	−0.89	>20000	190	115	85	>174	2	>235	2
5b	−2.65	9000	5	2	1	4500	3	9000	5
5c	−2.53	2400	6400	2	21	1200	3200	114	305
5d	−3.07	>20000	>20000	11	85	>1800	>1800	>235	>235
5e	−2.44	2400	185	2	34	1200	93	71	5

[a]Calculated using ChemDraw Ultra 12. [b]Errors in the range of ±5% of the reported value, from three determinations. [c]Selectivity is determined by the ratio of K_is for CA isozyme relative to CA IX and XII.

IX. Both CA IX and CA XII have an extracellular facing active site with expression upregulated in a wide selection of hypoxic tumors, however, CA XII has more widespread constitutive expression than CA IX, so it is of general interest to evaluate new compounds for inhibition of both CA IX and CA XII enzymatic activity so as to provide a better understanding of the compounds potential as a cancer therapeutic. The CA inhibition data to block the interconversion of CO_2 and H_2O yielding HCO_3^- and a H^+ for the 11 high purity sulfamates **4a−4g** and **5b−5e** was measured for CA I, CA II, CA IX, and CA XII, and results are presented in Table 1. The selectivity ratios for CA IX and CA XII inhibition over the intracellular and physiologically dominant CA I and CA II are also presented in Table 1.

All compounds showed weakest inhibition at CA I compared to CA II, CA IX, and CA XII. This weaker CA I inhibition, typically with K_is in the micromolar range, is a common observation for reported primary sulfonamide and sulfamate compounds. As CA I is considered an off-target CA isozyme, this weak CA I inhibition is a desirable attribute for the compounds of this study. For CA II, CA IX, and CA XII, the SAR is more variable and more interesting in terms of the goal to deliver improved inhibitors targeting hypoxic tumors. The inhibition profile for all compounds depended on both the linker as well as the acetylation status of the glucose moiety. The former affects the positioning of the ligand relative to the active site residues, while the later impacts on the steric bulk as well as hydrogen bonding capacity of the "tail" moiety. At CA II the K_i values exhibited a broad range from 5 nM for **5b** to >20000 nM for **5d**. Compounds with CA IX efficacy and selectivity as well as compounds with good efficacy at both CA II and CA IX would be useful probe compounds for establishing the potential of CA inhibitors in treating hypoxic tumors or impeding metastasis.

Of the compounds with acetylated glucose "tail" moieties, **4c**, **4d**, **4e**, and **4f**, were shown to be excellent CA IX inhibitors, with K_i values of ∼2 nM, while the remaining acetylated compounds **4a**, **4b**, and **4g** were ∼50−100-fold poorer CA IX inhibitors (K_is of 215, 215, and 115 nM, respectively). Compounds **4a−4c** have the sulfamate ZBG attached to a linear ethyl, propyl, or butyl linker, respectively, while **4d**, **4e**, and **4f** have a six (**4d**) or five (**4e** and **4f**) membered piperidine or pyrrolidine ring system, respectively, directly attached to the sulfamate moiety. Compound **4g** is an analogue of **4b**, however, the propyl linker has a substituent that results in a T-shaped ligand close to the ZBG. The SAR at CA IX for the acetylated compounds reveals several trends: First, the steric bulk of the ring system that separates the glucosyl sulfonamide "tail" from the ZBG is preferred over the short linear alkyl chains (ethyl and propyl) as linker, however, for **4c**, where the linker is a linear butyl group, this trend is broken and this compound has a comparable K_i to the cyclic systems (K_i = 2 nM). This indicates that both the five-membered or six-membered ring and the butyl chain position the glycosyl sulfonamide moiety to form favorable interactions with CA IX active site residues that is not possible with the shorter linked **4a** and **4b** or branched compound **4g**. The deacetylated compounds **5b−5e** are all good inhibitors of CA IX (K_is 2−11 nM). When comparing each acetylated inhibitor with its deacetylated counterpart, the **4b**/**5b** pair of compounds stand out (**4b** K_i = 215 nM, **5b** K_i = 2 nM), and this ∼100-fold difference in inhibition shows that **4b** has potential as a produg for **5b** and the removal of the acetyl masking groups from **4b** would lead to reinstatement of the CA IX binding 100-fold on formation of **5b**. Our group has previously demonstrated the in vitro metabolic stability, plasma stability, and plasma protein binding characteristics important for prodrugs for the acetyl group when presented on a glucose scaffold.[16] The inhibition of CA XII, the other tumor-associated CA, revealed two noteworthy compounds with subnanomolar K_i values, compounds **4f** (K_i = 1 nM) and **5b** (K_i = <1 nM). Compounds with this activity level are relatively uncommon. Both **4f** and **5b** are also very potent CA IX inhibitors, with with K_is of 2 nM. These compounds may be valuable probes for cells in which CA IX inhibition leads to upregulation of CA XII expression.[5a,17] The remaining sulfamates have CA XII K_i values of 8−94 nM. Compound **4b** showed good selectivity for CA XII over CA I and CA II, while compound **5b** had good selectivity for CA XII over CA I but was equipotent at CA II. In summary, the qualitative SAR analysis of these novel sulfamates has revealed several compounds with valuable inhibition activity and selectivity for CA IX. Lastly, the cLogP values for all compounds are less than zero (Table 1), indicating that these compounds are likely to have limited membrane permeability, also improving the targeting of the extracellular facing CA IX active site over intracellular CAs.

Crystallographic Studies. X-ray crystallography was used to analyze the mode of binding of the CA IX selective sulfamoylpyrrolidinyl compound, **5e**, in complex with CA II

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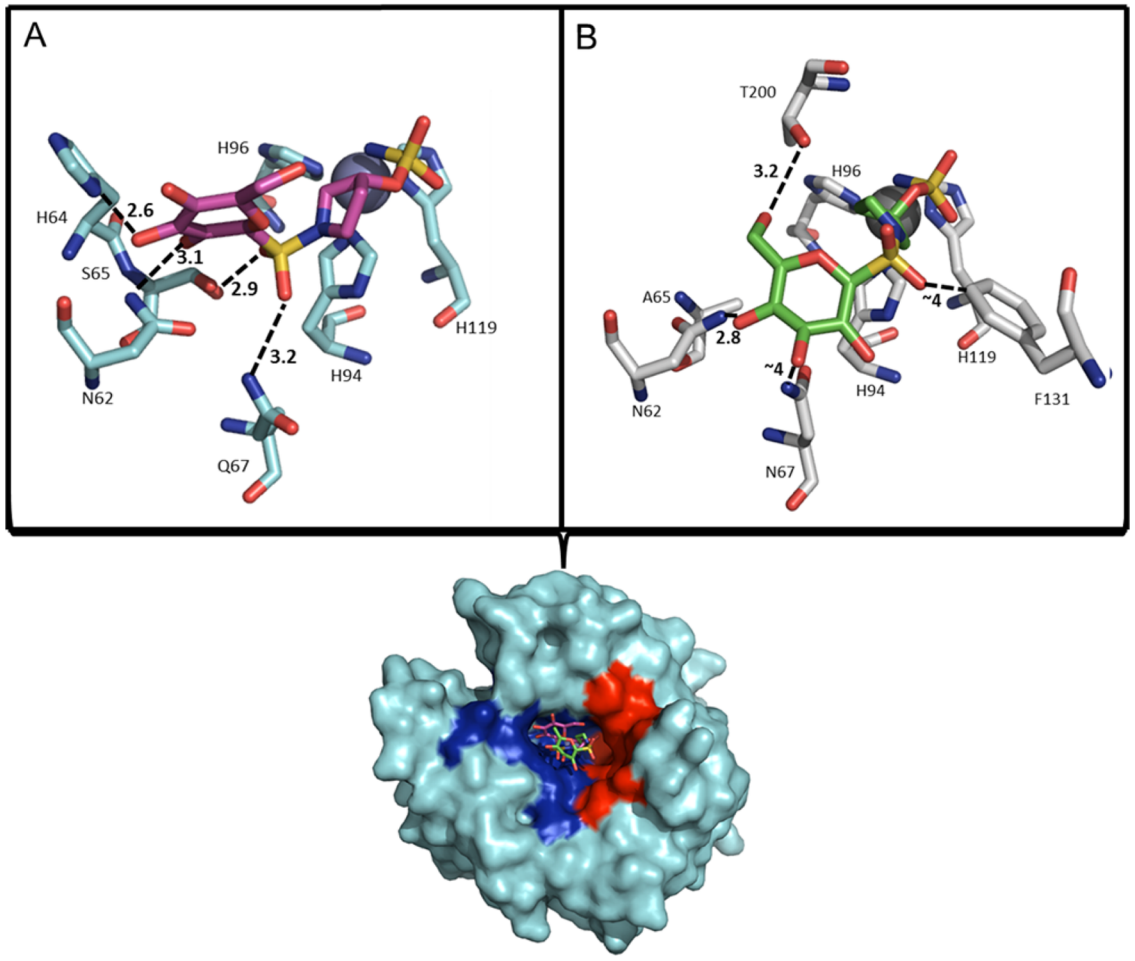

Figure 1. (A) CA IX-mimic (cyan) and **5e** (magenta). (B) CA II (gray) and **5e** (green) as they correlate to a surface representation depicting the location of **5e**. Highlighted hydrophobic (red) and hydrophilic (blue) residues. Specific interactions and hydrogen-bond distances (Å) are shown. Figure was made using PyMol.[16] Residues are as labeled (CA II numbering).

(CA II K_i = 185 nM) and the CA IX-mimic (CA IX K_i = 2 nM) to gain insight in to the ∼90-fold selectivity of **5e** for CA IX. The structural analysis of the CA IX-mimic in complex with **5e** (Figure 1A) reveals that **5e** binds with the primary sulfamate group interacting directly with the catalytic zinc. Furthermore, binding of **5e** induces complete displacement of the ordered water network of the CA active site, stabilization of residue H64 (which facilitates proton transfer during catalysis), and ordering of N-terminal residues that are not typically observed in crystal structures of CA II.[8,9] The "tail" region of **5e**, comprising the sulfonamide-bridged glucose moiety, interacts primarily with the hydrophilic pocket of the enzyme (Figure 1; surface rendition). Specifically, interactions between the nitrogen of the imidazole ring of H64 and the amine of N62 form predicted stabilizing hydrogen bonds with the 3′ or 2′ hydroxyl of the glucose moiety, respectively, and interactions between the carbonyl of Ser65 and the amine of Q67 with one each of the oxygen atoms of the sulfonamide-bridging moiety. The distances between the nitrogen of the imidazole ring of H64 and the 3′ hydroxyl of the glucose moiety (2.6 Å) imply a strong hydrogen bond interaction. Potential van der Waals interactions also contribute to stabilizing the "tail" region of **5e** within the hydrophilic pocket of the CA IX-mimic. The side chain of S65 exists as a dual conformer that shows a minor fluctuation in bond distance between the carbonyl of S65 and the 2′ hydroxyl of the sugar moiety. Stabilization of this interaction may induce stronger binding events between ligands and CA IX. The interactions involving S65 and Q67 most likely result in the significant increase in binding events observed in CA IX-mimic over CA II. Furthermore, the replacement of F131 with a valine between CA II and CA IX, respectively, may induce favorable entry of larger ligands such as **5e** into the active site of CA IX and thus contribute to an increase in affinity.

The structural analysis of the CA II_**5e** complex (Figure 1B) reveals the expected primary sulfamate interaction with the zinc as observed in the structure of the CA IX-mimic_**5e** complex. The CA II_**5e** complex also shows complete displacement of the ordered water network of the CA active site, ordering of the N-terminal residues and stabilization of the imidazole group of H64. In addition the "tail" region of **5e** interacts primarily with the hydrophilic pocket (Figure 1; surface rendition) of the CA II active site, however with fewer interactions than the CA IX-mimic. Primary interactions occur between the amine of N62, and the carbonyl of T200, forming potential H-bonds (2.8 and 3.2 Å, respectively) with the 4′ and 2′ hydroxyl groups of the glucose moiety of **5e**, respectively. A weaker interaction is predicted to occur between the amine of N67 and the 3′ hydroxyl of **5e** (bond distance of ∼4 Å). Additional weak hydrophobic interactions are observed between the oxygen of the sulfonamide bridge and the benzyl ring of F131. A structural overlay of the CAII_**5e** and CA IX-mimic_**5e**

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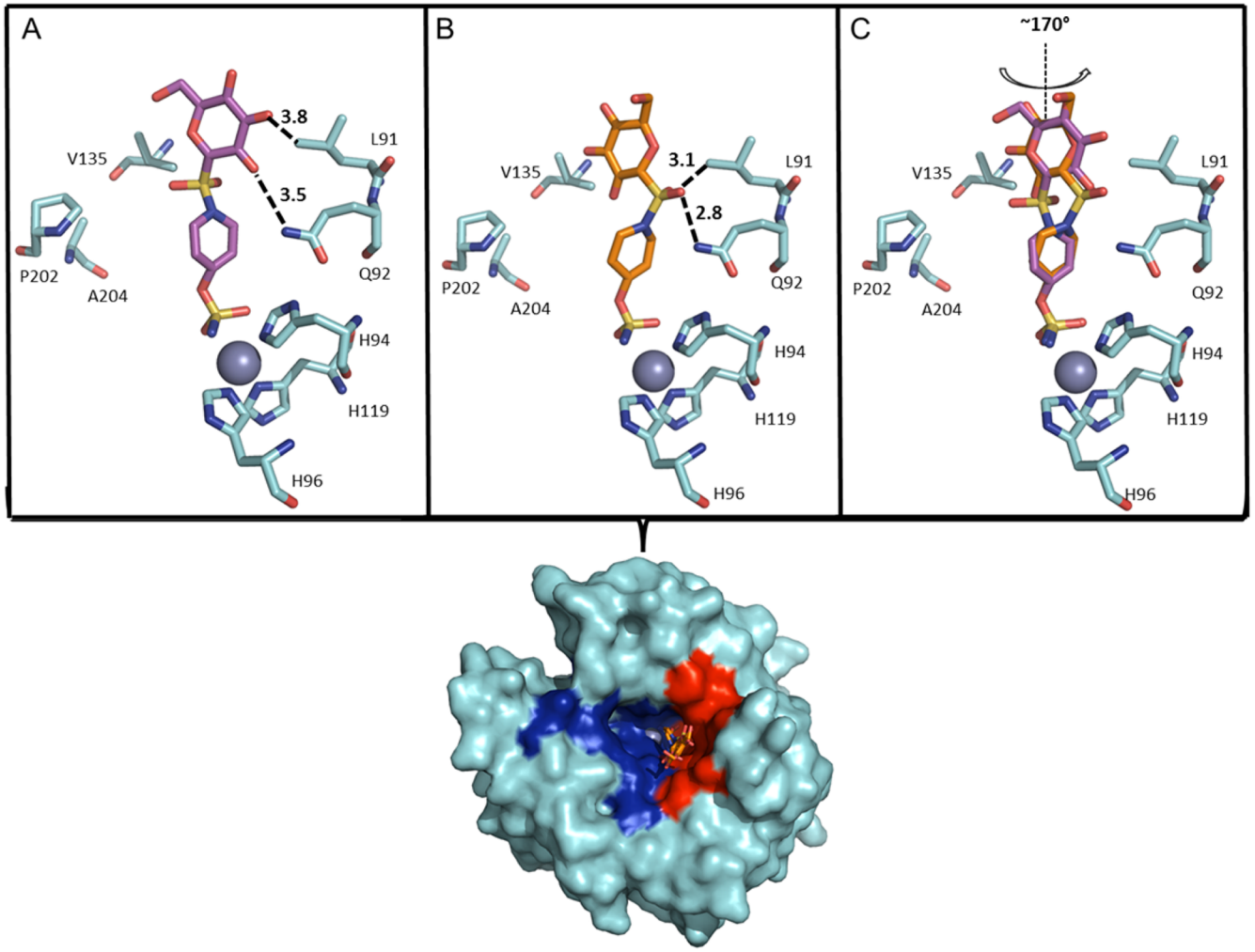

Figure 2. (A) CA IX-mimic (cyan) and **5d**. (A) Conformation 1 (purple) and (B) conformation 2 (orange) as they correlate to a surface representation depicting the location of **5d**. Highlighted hydrophobic (red) and hydrophilic (blue) residues. Specific interactions and hydrogen bond distances (Å) are shown. (C) An overlay of each conformation of **5d**. Note: there is a 170° rotation observed between sulfonamide bridges that distinguishes the two conformers. Figure was made using PyMol.[16] Residues are as labeled (CA II numbering).

structures (rmsd = 0.11 Å) reveals distinct differences in binding mode of the "tail" of **5e** (not shown). The sugar moiety of **5e** bound in the CA IX-mimic active site has greater interactions with residues in the hydrophilic cleft, most likely due to the A65S and N67Q residue differences between CA II and the CA IX-mimic. The addition of these interactions most likely contributes to the increased affinity the ligand has for CA IX. Both structures are stabilized by interactions observed between the amine of Q92 and the sulfonamide bridge (not shown).

We next turned our attention to investigate the mode of binding of the sulfamoylpiperidinyl compound, **5d**, in complex with the CA IX-mimic. Compound **5d** has >3 orders of magnitude selectivity for CA IX (K_i = 11 nM) over CA II (K_i = >20000 nM). Similar to the sulfamoylpyrrolidinyl compound **5e**, compound **5d** binds to the CA IX-mimic active site with the primary sulfamate group interacting directly with the zinc and displacing the zinc bound hydroxyl and ordered water network, however, unlike **5e**, there is no observable N-terminal ordering or stabilization of the H64. Interestingly, the electron density maps indicate that **5d** binds in two different conformations in the active site of CA IX-mimic (Figure 2C). In both conformations, the orientation of the sulfamate interacting with the zinc is conserved, but there is an observable ∼170° rotation at the S−N bond of the sulfonamide bridging moiety resulting in the $-NH-SO_2-$ group of each of the two conformers of **5d** pointing in the opposite directions (Figure 2C). This leads to two sets of different interactions observed between the "tail" region of **5d** with interfacing residues of the CA IX-mimic. We propose that conformation 1 is stabilized by a weak hydrogen bond (3.5 Å) between the amine of Q92 and the 3′ hydroxyl of the glucose moiety (Figure 2A). Alternatively, conformation 2 appears to be stabilized through an interaction of a bridging sulfonamide oxygen and the same amine of Q92, most likely forming a stronger hydrogen bond (2.8 Å) (Figure 2B). In addition, both conformations appear to be stabilized by weak van der Waals interactions within the hydrophobic cleft of the CA IX-mimic active site (Figure 2; surface rendition), specifically with L91.

Interestingly, this observation appears in contrast to the binding events of **5e**, which interacts more closely with the hydrophilic residues of the active site. This explains the difference between binding observed by our inhibition data. Compound **5e**, despite showing a ∼90-fold difference in inhibition between CA IX and CA II, still inhibits CA II with nanomolar affinity. This is due to the conserved regions of the

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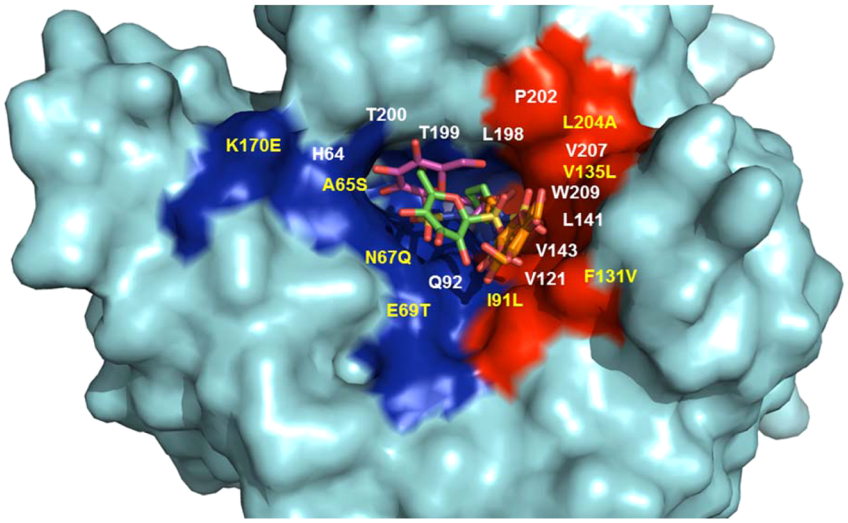

Figure 3. Surface representation depicting the location of **5e** (in both CA II and CA IX-mimic) and **5d** (in CA IX-mimic, only). Highlighted hydrophobic (red) and hydrophilic (blue) residues. Relative location of interfacing residues are labeled. Residues that differ between CA II and CA IX (and CAIX mimic) (yellow) and that are conserved (white). Figure was made using PyMol.[16]

hydrophilic pockets of CA II and CA IX, allowing for relatively high binding to occur between each isoform (Figure 3). Specifically, residues N62 and Q92 are conserved between CA II and CA IX and also interact directly with compound **5e**, hence observable nanomolar inhibition between both isoforms. In contrast, however, the hydrophobic pocket of CA IX versus CA II shows higher variability (Figure 3). This is indicative of the binding observed by compound **5d** where there is a much larger difference (>1000-fold) between CA II and CA IX. A similar but less pronounced relationship (>100-fold) was observed for **4d**, the per-*O*-acetylated analogue of **5d**. Specifically, the substitutions of I91L and F131V contribute highly to the difference in binding. L91 has been classified as a key residue that makes up one of the "selective pockets" observed in the CA active site.[18] As shown in the CA IX-mimic, this residue interacts directly with compound **5d**, most likely contributing to the increased inhibition. As mentioned previously, the residue at position 131 seems to act as "steric-blocker" such that it impedes bulky compounds entering the CA active site. The absence of a phenylalanine at position 131 in CA IX allows compounds, such as **5d**, to readily enter the active site and interact with the hydrophobic pocket. Figure 3 summarizes the overall modes of binding between both compound **5e** and **5d** and their generic interactions with both the hydrophilic and hydrophobic pockets. In addition, variable residues are highlighted within each pocket. It should be noted that attempts were made to complex **5d** with wild-type CA II in order to obtain structural data. However, due to the aforementioned attributes, we were unsuccessful in attempts to bind **5d** to CA II for structural comparisons.

CONCLUSION

In summary, CA IX inhibitors have a role to play in balancing pH to support cancer therapy by limiting the survival and metastasis of hypoxic tumors. Specifically, we present the design, synthesis, biological evaluation, and structural study of carbohydrate-based sulfamates of the motif [sulfamate]-[variable linker]-[sugar] as CA IX inhibitors. Seven of the novel sulfamates of this study were shown to be excellent CA IX inhibitors with K_i values of 1.9−2.4 nM, including the acetylated glucose "tail" moieties **4c**, **4d**, **4e**, and **4f** and the deacetylated compounds **5b**, **5c**, and **5e**. Of these, several had 2−3 orders of magnitude selectivity for the inhibition of CA IX over CA I and CA II and so may be useful in vivo in a setting where off-target CAs are abundant. The use of the CA IX-mimic has provided important structural information that may be used to improve the potency and selectivity of these compounds. Our structural analysis indicates that there exist two distinct modes of binding between CA IX and CA II of compound **5e**, however, in both cases, this compound interacts with the hydrophilic pocket of the enzyme. As this pocket is generally conserved between CA II and CA IX, it may account for the nanomolar binding affinities between both enzymes. In contrast, compound **5d**, which showed a differential inhibition profile between CA II and CA IX, binds to the CA IX active via interactions with the hydrophobic pocket. This region in the CA active site contains more variability between residues of CA II versus CA IX. As a result, this region has been termed as one of the "selective pockets" in the CA active site. Overall, these compounds provide promise in terms of targeting the extracellular active site of CA IX. We anticipate that our findings will bring the field a step closer to valuable compounds capable of rising to the challenge of targeting CA IX therapeutically for the treatment of several cancers.

EXPERIMENTAL SECTION

General Methods. All starting materials, reagents, and solvents were purchased from commercial suppliers. 1-*S*-Acetyl-2,3,4,6-tetra-*O*-acetyl-1-thio-*β*-D-glucopyranose (**1**) was prepared as described previously.[19] TLC plates were visualized with UV light, ninhydrin stain (1 g of ninhydrin in 100 mL of EtOH containing 3% (v/v) acetic acid), and/or orcinol stain (1 g of orcinol monohydrate in a mixture of EtOH:H_2O:H_2SO_4 72.5:22.5:5 mL). Silica gel flash chromatography was performed using silica gel 60 Å (230−400 mesh). ^{1}H NMR were acquired at 500 MHz and ^{13}C NMR at 125 MHz at 30 °C. ^{1}H and ^{13}C NMR acquired in DMSO-d_6 are reported in ppm relative to residual solvent 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), ddd (doublet of doublet of doublet), br (broad). Coupling constants are reported in

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hertz (Hz). Melting points for nonhygroscopic compounds were measured and 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. Purity of all compounds was ≥95%.

Synthesis Methods. *General Procedure 1: Synthesis of Sulfenamide Linked Glycoconjugates.* Diethyl bromomalonate (2.5 equiv) was added to a solution of 1-*S*-acetyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (**1**) (1 equiv) in anhydrous MeOH. The reaction was stirred at rt under nitrogen for 20 min, then the desired amino alcohol (**a**−**g**) (3.0 equiv) added. The reaction was stirred at rt under nitrogen until complete as evidenced by TLC (hexane/EtOAc), typically 1 h. The solvent was removed and the residue dissolved in CH_2Cl_2 and washed with brine (×3). The combined aqueous fractions were back extracted with CH_2Cl_2 (×3). The combined organic fractions were dried over $MgSO_4$, filtered, and the solvent removed to give the crude sulfenamides **2a**−**2g**. The amino acid methyl ester derived sulfenamides **2f** and **2g** were purified with flash chromatography (hexane/EtOAc), while sulfenamides **2a**−**2e** had low stability and were used immediately in the next reaction without further purification or characterization.

General Procedure 2: Oxidation of Sulfenamide Linked Glycoconjugates. To a solution of the sulfenamide derivative (1.0 equiv) in CH_2Cl_2 at 0 °C was added *meta*-chloroperoxybenzoic acid (6.0 equiv) in CH_2Cl_2 dropwise over ∼20 min. The reaction was warmed to rt and left until full conversion of the starting material, as evidenced by TLC (hexane/EtOAc), typically 1−5 h. The reaction mixture was diluted with CH_2Cl_2 and quenched with $NaHCO_{3(satd)}$. The organic fraction was separated and washed with $NaHCO_{3(satd)}$ (×1) and brine (×2). The aqueous fractions were back extracted with CH_2Cl_2 (×2). The combined organic fractions were dried over $MgSO_4$, filtered, and concentrated to give the expected sulfonamides. The crude products were purified using flash chromatography (hexane/EtOAc) to give sulfonamides **3a**−**3g**.

General Procedure 3: Conversion of Sulfonamide Linked Glycoconjugates to Sulfamates. The glycoconjugate precursor (1.0 equiv) was dried under vacuum and solubilized in anhydrous *N*,*N*-dimethylacetamide (15 equiv) under argon. Sulfamoyl chloride (3.8 equiv) was prepared from formic acid and chlorosufonyl isocyanate as described previously.[9a,20] The precursor solution was added slowly to the flask containing sulfamoyl chloride, and the mixture was stirred for 10 min, then warmed to room temperature and stirred for 1 h. The crude reaction mixture was diluted in EtOAc, washed with brine (×3), and the aqueous fractions back extracted with EtOAc (×2). The combined organic fractions were dried over $MgSO_4$, filtered, and concentrated. The crude products were purified using flash chromatography (hexane/EtOAc) to give sulfamates **4a**−**4g**.

General Procedure 4: Deacetylation of Glycoconjugates. The deacylated glycoconjugates were prepared by treating a solution of the per-*O*-acetylated glucose sulfamate precursor (1.0 equiv) in methanolic sodium methoxide in methanol (25%) at rt. Complete deacetylation was observed after ∼2−4 h (TLC). The reaction mixture was neutralized with Amberlite IR-120 [H^+], filtered, and the resin washed with MeOH (×3). The methanol was evaporated under reduced pressure and the residue redissolved in water and lyophilized to afford fully deprotected glycoconjugates **5b**−**5e**. Glycoconjugates **5a**, **5f**, and **5g** could not be purified sufficiently for characterization.

*Methyl N-[(2,3,4,6-Tetra-O-acetyl-β-D-glucosyl)thio]-L-4′-hydroxyprolinate (**2f**).* The title compound was synthesized from L-4-hydroxyproline methyl ester (**f**) using general procedure 1 and isolated as a white solid (809 mg, 1.59 mmol, 65%). R_f 0.12 (hexane/EtOAc 1/1); mp 80−82 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.28 (t, *J* = 9.4 Hz, 1H, H-3), 5.05 (d, *J* = 4.0 Hz, 1H, OH), 5.02 (d, *J* = 10.4 Hz, 1H, H-1), 4.88 (t, *J* = 9.8 Hz, 1H, H-4), 4.74 (dd, *J* = 10.4, 9.3 Hz, 1H, H-2), 4.22−4.16 (m, 1H, H-4′), 4.14 (dd, *J* = 12.3, 5.4 Hz, 1H, H-6a), 4.03 (dd, *J* = 12.5, 2.5 Hz, 1H, H-6b), 3.98−3.91 (m, 2H, H-5, H-2′), 3.62 (s, 3H, OCH_3), 3.43 (dd, *J* = 10.0, 5.1 Hz, 1H, H-3′a), 3.06 (dd, *J* = 9.9, 3.5 Hz, 1H, H-3′b), 2.07−2.01 (m, 4H, H-5′a, 1 × CH_3), 1.99 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 1.98−1.92 (m, 4H, H-5′b, 1 × CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.2 (CO_2CH_3), 170.0, 169.5, 169.2, 169.0 (4 × $OCOCH_3$), 86.5 (C-1), 74.4 (C-5), 73.1 (C-3), 68.9 (C-4, C-4′), 68.0 (C-2), 67.9 (C-2′), 66.4 (C-5′), 65.0 (C-3′), 61.9 (C-6), 51.7 (CO_2CH_3), 20.4, 20.3, 20.28, 20.25 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C HSQC. LRMS (ESI+): *m*/*z* 508.1 [M + H]$^+$, 530.1 [M + Na]$^+$. HRMS: calcd for $C_{20}H_{29}NO_{12}SNa$ [M + Na]$^+$ 530.1303, found 530.1321.

*Methyl N-[(2,3,4,6-Tetra-O-acetyl-β-D-glucosyl)thio]-L-serinate (**2g**).* The title compound was synthesized from L-serine methyl ester (**g**) according to general procedure 1 and isolated as colorless syrup (322 mg, 670 mmol, 77%). Characterization data for **2g** is consistent with literature values.[21]

*N-2′-Hydroxyethyl-S-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl)sulfonamide (**3a**).* Sulfonamide **3a** was prepared from crude *N*-2′-hydroxyethyl-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosyl)sulfenamide (**2a**) according to general procedure 2. The title compound was obtained as a white solid (333 mg, 731 μmol, 30% over two steps). R_f 0.24 (hexane/EtOAc 1/2); mp 121−123 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.46 (t, *J* = 5.9 Hz, 1H, NH), 5.38 (t, *J* = 9.4 Hz, 1H, H-3), 5.19 (t, *J* = 9.5 Hz, 1H, H-2), 5.01−4.82 (m, 2H, H-1, H-4), 4.68 (t, *J* = 5.5 Hz, 1H, OH), 4.23−4.12 (m, 2H, H-5, H-6a), 4.08−4.00 (m, 1H, H-6b), 3.45−3.41 (m, 2H, CH_2OH), 3.08−3.04 (m, 2H, NCH_2), 2.02, 2.00, 1.95, 1.95 (4 × s, 4 × 3H, 4 × CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.5, 169.2, 168.6 (4 × $OCOCH_3$), 85.9 (C-1), 74.4 (C-5), 72.8 (C-3), 67.64 (C-2), 67.62 (C-4), 61.8 (C-6), 60.5 (CH_2OH), 45.5 (NCH_2), 20.44, 20.42, 20.3, 20.2 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C HSQC. LRMS (ESI+): *m*/*z* 478.1 [M + Na]$^+$. HRMS: calcd for $C_{16}H_{25}NO_{12}SNa$ [M + Na]$^+$ 478.0990, found 478.1005.

*N-3′-Hydroxypropyl-S-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl)sulfonamide (**3b**).* Sulfonamide **3b** was prepared from crude *N*-3′-hydroxypropyl-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosyl)sulfenamide (**2b**) according to general procedure 2. The title compound was obtained as a white solid (130 mg, 277 μmol, 39% over two steps). R_f 0.18 (hexane/EtOAc 1/2); mp 105−107 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.48 (t, *J* = 5.7 Hz, 1H, NH), 5.39 (t, *J* = 9.4 Hz, 1H, H-3), 5.18 (t, *J* = 9.5 Hz, 1H, H-2), 4.96−4.87 (m, 2H, H-1, H-4), 4.42 (t, *J* = 5.1 Hz, 1H, OH), 4.21−4.14 (m, 2H, H-5, H-6a), 4.07−4.00 (m, 1H, H-6b), 3.43 (dt, *J* = 6.9, 5.6 Hz, 2H, CH_2OH), 3.08−3.04 (m, 2H, NCH_2), 2.02, 1.99, 1.95, 1.94 (4 × s, 4 × 3H, 4 × CH_3), 1.63−1.57 (m, 2H, CH_2), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.1, 169.6, 169.3, 168.7 (4 × $OCOCH_3$), 85.5 (C-1), 74.5 (C-5), 72.8 (C-3), 67.65, 67.64 (C-2, C-4), 61.8 (C-6), 58.1 (CH_2OH), 40.4 ($NHCH_2$), 33.0 (C-2′), 20.48, 20.46, 20.36, 20.3 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C HSQC. LRMS (ESI+): *m*/*z* 492.1 [M + Na]$^+$. HRMS: calcd for $C_{17}H_{27}NO_{12}SNa$ [M + Na]$^+$ 492.1146, found 492.1154.

*N-5′-Hydroxypentyl-S-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl)sulfonamide (**3c**).* Sulfonamide **3c** was prepared from crude *N*-5′-hydroxypentyl-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosyl)sulfenamide (**2c**) according to general procedure 2. The title compound was obtained as a white solid (235 mg, 471 μmol, 19% over two steps). R_f 0.28 (hexane/EtOAc 1/2); mp 104−106 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.52 (t, *J* = 5.7 Hz, 1H, NH), 5.39 (t, *J* = 9.5 Hz, 1H, H-3), 5.18 (t, *J* = 9.5 Hz, 1H, H-2), 4.97−4.83 (m, 2H, H-1, H-4), 4.32 (t, *J* = 5.1 Hz, 1H, OH), 4.22−4.12 (m, 2H, H-5, H-6a), 4.09−3.98 (m, 1H, H-6b), 3.40−3.37 (m, 2H, CH_2OH), 3.06−2.91 (m, 2H, NCH_2), 2.01, 1.99, 1.95 1.94 (4 × s, 4 × 3H, 4 × CH_3), 1.46−1.38 (m, 4H, CH_2-2′, CH_2-4′), 1.35−1.21 (m, 2H, CH_2-3′), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.9, 169.5, 169.2, 168.6 (4 × $OCOCH_3$), 85.6 (C-1), 74.4 (C-5), 72.8 (C-3), 67.6 (C-4 and C-2), 61.8 (C-6), 60.6 (CH_2OH), 43.1 (NCH_2), 32.0, 29.7 (C-2′, C-4′), 22.6 (C-3′), 20.4, 20.4, 20.3, 20.2 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C HSQC. LRMS (ESI+): *m*/*z* 520.1 [M + Na]$^+$. HRMS: calcd for $C_{19}H31NO_{12}SNa$ [M + Na]$^+$ 520.1459, found 520.1480.

*N-4′-Hydroxypiperidinyl-S-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl)sulfonamide (**3d**).* Sulfonamide **3d** was prepared

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from crude *N*-4′-hydroxypiperidyinyl-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosyl)sulfenamide (**2d**) according to general procedure 2. The title compound was obtained as a white solid (182 mg, 367 μmol, 15% over two steps). R_f 0.20 (hexane/EtOAc 1/1); mp 156−158 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.36 (t, J = 9.4 Hz, 1H, H-3), 5.20 (t, J = 9.5 Hz, 1H, H-2), 5.13 (d, J = 9.8 Hz, 1H, H-1), 4.93 (t, J = 9.6 Hz, 1H, H-4), 4.79 (d, J = 4.2 Hz, 1H, OH), 4.22−4.15 (m, 1H, H-5), 4.14−4.10 (m, 2H, H-6a/b), 3.69−3.62 (m, 1H, C*H*OH), 3.55−3.45 (m, 2H, NCH_2), 3.17−3.05 (m, 2H, NCH_2), 2.05, 2.01, 1.97, 1.96 (4 × s, 4 × 3H, 4 × CH_3), 1.78−1.70 (m, 2H, CH_2), 1.47−1.36 (m, 2H, CH_2), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.5, 169.2, 168.6 (4 × $OCOCH_3$), 85.6 (C-1), 74.6 (C-5), 72.6 (C-3), 67.6 (C-2), 67.1 (C-4), 64.5 (CHOH), 61.7 (C-6), 43.7, 43.5 (2 × NCH_2), 33.9, 33.8 (2 × CH_2), 20.5, 20.4, 20.3, 20.2 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C HSQC. LRMS (ESI+): m/z 518.1 [M + Na$^+$]. HRMS: Calcd for $C_{19}H_{29}NO_{12}SNa$ [M + Na]$^+$ 518.1303, found 518.1298.

*N-3′- R-Hydroxypyrrolidinyl-S-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl)sulfonamide (**3e**).* Sulfonamide **3e** was prepared from crude *N*-3′-*R*-hydroxypiperidyinyl-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosyl) sulfenamide (**2e**) according to general procedure 2. The title compound was obtained as a white solid (334 mg, 694 μmol, 38% over two steps). R_f 0.58 (hexane/EtOAc 1/2); mp 218−220 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.32 (t, J = 9.0 Hz, 1H, H-3), 5.27−5.18 (m, 2H, H-1, H-2), 5.07 (d, J = 3.7 Hz, 1H, OH), 4.92 (t, J = 9.3 Hz, 1H, H-4), 4.35−4.27 (m, 1H, C*H*OH), 4.17−4.07 (m, 3H, H-5, H-6a/b), 3.53−3.46 (m, 1H, CH_2-2′a), 3.46−3.37 (m, 2H, CH_2-5′), 3.19 (dd, J = 10.4, 2.7, 1H, CH_2-2′b), 2.02, 1.99 (2 × s, 2 × 3H, 2 × CH_3), 1.98−1.93 (m, 7H, CH_2-4′a, 2 × CH_3), 1.80−1.74 (m, 1H, CH_2-4′b), assignments were confirmed by ^{1}H−^{13}C HSQC. ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.5, 169.2, 168.5 (4 × $OCOCH_3$), 85.1 (C-1), 74.6 (C-5), 72.7 (C-3), 69.3 (C-2), 67.5 (C-4), 67.2 (CHOH), 61.7 (C-6), 55.5 (C-2′), 46.9 (C-5′), 33.8 (C-4′), 20.44, 20.37, 20.3, 20.2 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C HSQC. LRMS (ESI+): m/z 504.1 [M + Na]$^+$. HRMS: calcd for $C_{18}H_{27}NO_{12}SNa$ [M + Na]$^+$ 504.1146, found 504.1163.

*Methyl N-[(2,3,4,6-Tetra-O-acetyl-β-D-glucosyl)sulfonyl]-L-4′-hydroxyprolinate (**3f**).* The title compound was synthesized from **2f** according to general procedure 2 and isolated as a white solid (197 mg, 365 μmol, 25%). R_f 0.34 (hexane/EtOAc 1/2); mp 165−167 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.42−5.26 (m, 3H, H-2, H-3, OH), 5.23 (d, J = 9.7 Hz, 1H, H-1), 4.95 (t, J = 9.6 Hz, 1H, H-4), 4.51 (dd, J = 8.6, 5.6 Hz, 1H, H-2′), 4.35−4.31 (m, 1H, C*H*OH), 4.18 (dd, J = 12.6, 5.6 Hz, 1H, H-6a), 4.16−4.03 (m, 2H, H-5, H-6b), 3.66 (s, 3H, OCH_3), 3.56 (dd, J = 10.1, 3.8 Hz, 1H, CH_2-5′a), 3.49 (dd, J = 10.2, 4.9 Hz, 1H, CH_2-5′b), 2.19 (ddd, J = 13.2, 8.7, 5.0 Hz, 1H, CH_2-3′a), 2.09−2.04 (m, 1H, CH_2-3′b), 2.04, 1.99, 1.95, 1.94 (4 × s, 4 × 3H, 4 × CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.3 (CO_2CH_3), 170.0, 169.5, 169.1, 168.6 (4 × $OCOCH_3$), 87.0 (C-1), 74.5 (C-5), 72.7 (C-3), 68.3 (C-4), 67.3 (C-2), 67.1 (CHOH), 61.6 (C-6), 59.2 (C-2′), 56.3 (C-5′), 52.1 (CO_2CH_3), 40.0 (C-3′, under DMSO), 20.4, 20.4, 20.3, 20.2 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C HSQC. LRMS (ESI+): m/z 562.1 [M + Na$^+$]. HRMS: calcd for $C_{20}H_{29}NO_{14}SNa$ [M + Na]$^+$ 562.1201, found 562.1215.

*Methyl N-[(2,3,4,6-Tetra-O-acetyl-β-D-glucosyl)sulfonyl]-L-serinate (**3g**).* The title compound was synthesized from **2g** according to general procedure 2 and isolated as a white solid (148 mg, 288 mmol, 69%). Characterization data for **3g** is consistent with literature values.[21]

*N-2′-Sulfamoylethyl-S-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl)sulfonamide (**4a**).* The title compound was synthesized from **3a** according to general procedure 3 and isolated as a white solid (96 mg, 179 μmol, 44%). R_f 0.36 (hexane/EtOAc 1/2); mp 139−141 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.85 (t, J = 5.9 Hz, 1H, NH), 7.51 (s, 2H, OSO_2NH_2), 5.37 (t, J = 9.4 Hz, 1H, H-3), 5.21 (t, J = 9.5 Hz, 1H, H-2), 4.97−4.90 (m, 2H, H-1, H-4), 4.23−4.11 (m, 2H, H-5, H-6a), 4.10−3.98 (m, 3H, H-6b, CH_2OSO$_2$NH$_2$), ∼ 3.33 (m, 2H, NCH_2 under water signal), 2.02, 1.99, 1.96, 1.95 (4 × s, 4 × 3H, 4 × CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.5, 169.2, 168.6 (4 × $OCOCH_3$), 86.2 (C-1), 74.6 (C-5), 72.8 (C-3), 67.9 (C-2), 67.5 (C-4), 67.6 ($CH_2OSO_2NH_2$), 61.7 (C-6), 42.2 (NCH_2), 20.5, 20.4, 20.3, 20.2 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C HSQC. LRMS (ESI$^+$): m/z 557 [M + Na]$^+$. HRMS: calcd for $C_{16}H_{26}N_2O_{14}S_2Na$ [M + Na]$^+$ 557.0718, found 557.0743.

*N-3′-Sulfamoylpropyl-S-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl)sulfonamide (**4b**).* The title compound was synthesized from **3b** according to general procedure 3 and isolated as a white solid (94 mg, 172 μmol, 68%). R_f 0.38 (hexane/EtOAc 1/2); mp 140−142 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.68 (t, J = 5.8 Hz, 1H, NH), 7.43 (s, 2H, OSO_2NH_2), 5.39 (t, J = 9.4 Hz, 1H, H-3), 5.20 (t, J = 9.3 Hz, 1H, H-2), 5.04−4.87 (m, 2H, H-1, H-4), 4.26−4.14 (m, 2H, H-5, H-6a), 4.11−4.01 (m, 3H, H-6b, CH_2OSO$_2$NH$_2$), 3.12−3.09 (m, 2H, NCH_2), 2.03, 2.00, 1.97, 1.95 (4 × s, 4 × 3H, 4 × CH_3), 1.87−1.81 (m, 2H, CH_2), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.1, 169.6, 169.3, 168.6 (4 × $OCOCH_3$), 85.6 (C-1), 74.5 (C-5), 72.8 (C-3), 67.58, 67.56 (C-2, C-4), 66.6 ($CH_2OSO_2NH_2$), 61.8 (C-6), 40.0 (NCH_2, under DMSO), 29.5 (C-2′), 20.5, 20.4, 20.34, 20.26 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): m/z 571 [M + Na]$^+$. HRMS: calcd for $C_{17}H_{28}N_2O_{14}S_2Na$ [M + Na]$^+$ 571.0874, found 571.0891.

*N-5′-Sulfamoylpentyl-S-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl)sulfonamide (**4c**).* The title compound was synthesized from **3c** according to general procedure 3 and isolated as a white solid (171 mg, 296 μmol, 73%). R_f 0.24 (hexane/EtOAc 1/2); mp 114−116 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.57 (t, J = 5.7 Hz, 1H, NH), 7.38 (s, 2H, OSO_2NH_2), 5.39 (t, J = 9.4 Hz, 1H, H-3), 5.19 (t, J = 9.5 Hz, 1H, H-2), 4.95−4.89 (m, 2H, H-1, H-4), 4.25−4.14 (m, 2H, H-5, H-6a), 4.09−3.97 (m, 3H, H-6b, CH_2OSO$_2$NH$_2$), 3.02−2.98 (m, 2H, NCH_2), 2.02, 2.00, 1.96, 1.95 (4 × s, 4 × 3H, 4 × CH_3), 1.69−1.59 (m, 2H, CH_2-4′), 1.51−1.46 (m, 2H, CH_2-2′), 1.42−1.30 (m, 2H, CH_2-3′), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.9, 169.5, 169.2, 168.6 (4 × $OCOCH_3$), 85.6 (C-1), 74.4 (C-5), 72.8 (C-3), 68.9 (C-4), 67.6 (C-2), 61.7 ($CH_2OSO_2NH_2$), 59.7 (C-6), 42.8 (NCH_2), 29.3 (C-2′), 27.9 (C-4′), 22.2 (C-3′), 20.5, 20.4, 20.3, 20.2 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): m/z 599 [M + Na]$^+$. HRMS: calcd for $C_{19}H_{32}N_2O_{14}S_2Na$ [M + Na]$^+$ 599.1187, found 599.1200.

*N-4′-Sulfamoylpiperidinyl-S-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl)sulfonamide (**4d**).* The title compound was synthesized from **3d** according to general procedure 3 and isolated as a white solid (84 mg, 146 μmol, 52%). R_f 0.34 (hexane/EtOAc 1/2); mp 180−182 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.49 (s, 2H, OSO_2NH_2), 5.36 (t, J = 9.2 Hz, 1H, H-3), 5.22 (t, J = 9.3 Hz, 1H, H-2), 5.17 (d, J = 9.7 Hz, 1H, H-1), 4.95 (t, J = 9.7 Hz, 1H, H-4), 4.61 (dt, J = 7.7, 3.7 Hz, 1H, C*H*OSO$_2$NH$_2$), 4.24−4.17 (m, 1H, H-5), 4.13 (d, J = 4.1 Hz, 2H, H-6a/b), 3.51−3.40 (m, 2H, NCH_2), ∼ 3.30 (m, 2H, NCH_2, under H_2O), 2.05, 2.01 (2 × s, 2 × 3H, 2 × CH_3), 1.99−1.93 (m, 8H, 2 × CH_3, CH_2), 1.77 (ddt, J = 12.7, 8.1, 3.7 Hz, 2H, CH_2), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.5, 169.2, 168.6 (4 × $OCOCH_3$), 85.6 (C-1), 74.72 ($CHOSO_2NH_2$), 74.69 (C-5), 72.6 (C-3), 67.5 (C-4), 67.0 (C-2), 61.7 (C-6), 43.01, 42.98 (2 × NCH_2) 31.0 (2 × CH_2), 20.5, 20.34, 20.32, 20.2 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): m/z 597.0 [M + Na]$^+$. HRMS: calcd for $C_{19}H_{30}N_2O_{14}S_2Na$ [M + Na]$^+$ 597.1031, found 597.1038.

*N-3′-R-Sulfamoylpyrrolidinyl-S-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl)sulfonamide (**4e**).* The title compound was synthesized from **3e** according to general procedure 3 and isolated as a white solid (177 mg, 317 μmol, 61%). R_f 0.43 (hexane/EtOAc 1/3); mp 140−142 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.62 (s, 2H, OSO_2NH_2), 5.36−5.30 (m, 1H, H-3), 5.29−5.21 (m, 2H, H-1, H-2), 5.07 (dt, J = 5.2, 2.7 Hz, 1H, C*H*OSO$_2$NH$_2$), 5.00−4.93 (m, 1H, H-4), 4.14 (d, J = 3.9 Hz, 3H, H-5, H-6a/b), 3.67 (dd, J = 11.4, 4.6 Hz, 1H, CH_2-2′a), 3.61 (dt, J = 9.0, 3.1 Hz, 1H, CH_2-4′a), 3.55 (dd, J = 11.4, 1.1 Hz, 1H, CH_2-2′b), 3.38 (td, J = 9.7, 6.9 Hz, 1H, CH_2-4′b), 2.28−

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Table 2. X-ray Crystallography Statistics for Data Processing and Refinement of Ligand Bound CAIX-Mimic and CA II Crystal Structures

sample	CAIX-mimic_**5e**	CAIX-mimic_**5d**	CAII_**5e**
PDB accession no.	4R5A	4R59	4R5B
space group	$P2_1$		
cell dimensions (Å; deg)	a = 42 ± 0.4, b = 42 ± 0.4, c = 72 ± 0.3; β = 104 ± 0.4		
resolution (Å)	20.0−1.64	19.9−1.74	19.86−1.50
total reflections	29753	35999	37836
R_{sym}[a] (%)	6.2 (31.0)	7.0 (60.0)	6.0 (41.5)
$I/I\sigma$	15.35 (3.7)	18.74 (1.39)	12.78 (2.87)
completeness (%)	93.2 (87.9)	92.0 (92.3)	93.2 (96.5)
R_{cryst}[b] (%)	15.5 (19.9)	15.7 (28.2)	15.7 (27.0)
R_{free}[c] (%)	18.5 (24.5)	20.5 (34.4)	18.5 (26.9)
no. of protein atoms	2124	2111	2106
no. of water molecules	242	211	233
no. of ligand molecules	24	50[e]	24
Ramachandran stats (%): favored, allowed, outliers	95.8, 3.44, 0.76	96.5, 3.5, 0.0	96.5, 2.69, 0.77
av B factors (Å^2): main-chain, side-chain, solvent, ligand[d]	16.7, 21.3, 22.4, 29.8	16.5, 20.9, 36.4,28.0	21.1, 25.4, 32.6, 32.5

[a] $R_{sym} = (\sum|I - \langle I\rangle|/\sum\langle I\rangle) \times 100$. [b] $R_{cryst} = (\sum|F_o - F_c|/\sum|F_o|) \times 100$ [c] R_{free} is calculated in the same way as R_{cryst}, except it is for data omitted from refinement (5% of reflections for all data sets). [d] Values in parentheses correspond to the highest resolution shell. [e] Total ligand atoms for both conformations of **5d**, hence one conformation contains 25 atoms.

2.12 (m, 2H, CH_2-5′), 2.03, 2.00, 1.97, 1.95 (4 × s, 4 × 3H, 4 × CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.9, 169.5, 169.1, 168.5 (4 × $OCOCH_3$), 85.3 (C-1), 78.7 (C-5), 74.7 ($CHOSO_2NH_2$), 72.6 (C-3), 67.3 (C-2), 67.1 (C-4), 61.5 (C-6), 53.3 (C-2′), 46.6 (C-5′), 31.5 (C-4′), 20.4, 20.34, 20.29, 20.2 (4 × CH_3) (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): m/z 583 [M + Na]$^+$. HRMS: calcd for $C_{18}H_{28}N_2O_{14}S_2Na$ [M + Na]$^+$ 583.0874, found 583.0897.

*Methyl N-[(2,3,4,6-Tetra-O-acetyl-β-D-glucosyl)sulfonyl]-L-4′-sulfamoylprolinate (**4f**).* The title compound was synthesized from **3f** according to general procedure 3 and isolated as a white solid (210 mg, 339 μmol, 61%). R_f 0.31 (hexane/EtOAc 1/2); mp 166−168 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.68 (s, 2H, OSO_2NH_2), 5.36−5.22 (m, 3H, H-1, H-2, H-3), 5.07−5.03 (m, 1H, $CHOSO_2NH_2$), 4.99 (t, J = 9.5 Hz, 1H, H-4), 4.54 (dd, J = 8.6, 5.2 Hz, 1H, H-2′), 4.20−4.09 (m, 3H, H-5, H-6a/b), 3.79 (d, J = 4.8 Hz, 2H, CH_2-5′), 3.69 (s, 3H, OCH_3), 2.60 (ddd, J = 13.9, 8.7, 5.6 Hz, 1H, CH_2-3′a), 2.38−2.33 (m, 1H, CH_2-3′b), 2.03, 1.99, 1.95 (3 × s, 2 × 3H, 1× 6H, 4 × CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.5 (CO_2CH_3), 170.0, 169.5, 169.1, 168.6 (4 × $OCOCH_3$), 86.5 (C-1), 76.4 ($CHOSO_2NH_2$), 74.7 (C-5), 72.6 (C-3), 67.2 (C-4), 66.9 (C-2), 61.4 (C-6), 58.5 (C-2′), 53.8 (C-5′), 52.4 (OCH_3), 35.9 (C-3′), 20.4, 20.32, 20.28, 20.2 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): m/z 641.0 [M + Na]$^+$. HRMS: calcd for $C_{20}H_{30}N_2O_{16}S_2Na$ [M + Na]$^+$ 641.0929, found 641.0957.

*Methyl N-[(2,3,4,6-Tetra-O-acetyl-β-D-glucosyl)sulfonyl]-L-sulfamoylserinate (**4g**).* The title compound was synthesized from **3g** according to general procedure 3 and isolated as a white solid (110 mg, 186 μmol, 79%). R_f 0.35 (hexane/EtOAc 1/2); mp 116−118 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.59 (d, J = 8.6 Hz, 1H, NH), 7.62 (s, 2H, OSO_2NH_2), 5.38 (t, J = 9.4 Hz, 1H, H-3), 5.20 (t, J = 9.5 Hz, 1H, H-2), 5.02−4.85 (m, 2H, H-1, H-4), 4.38−4.34 (m, 1H, CH), 4.24−4.16 (m, 3H, H-6a, $CH_2OSO_2NH_2$), 4.11 (ddd, J = 10.0, 4.4, 2.2 Hz, 1H, H-5), 3.98 (dd, J = 12.6, 2.3 Hz, 1H, H-6b), 3.72 (s, 3H, OCH_3), 2.02, 1.99, 1.96, 1.95 (4 × s, 4 × 3H, 4 × CH_3), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0 (CO_2CH_3), 169.5, 169.1, 168.8, 168.6 (4 × $OCOCH_3$), 86.5 (C-1), 74.5 (C-5), 72.7 (C-3), 68.6 ($CH_2OSO_2NH_2$), 67.33 (C-4), 67.25 (C-2), 61.3 (C-6), 55.5 (CH), 52.6 (OCH_3), 20.42, 20.38, 20.3, 20.2 (4 × $OCOCH_3$), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): m/z 615 [M + Na]$^+$. HRMS: calcd for $C_{18}H_{28}N_2O_{16}S_2Na$ [M + Na]$^+$ 615.0772, found 615.0789.

*N-3′-Sulfamoylpropyl-S-(1-thio-β-D-glucopyranosyl)sulfonamide (**5b**).* The title compound was synthesized from **4b** according to general procedure 4 and isolated as a white highly hygroscopic solid (47 mg, 123 μmol, 96%). R_f 0.47 (MeCN/H_2O 9/1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.41 (s, 2H, OSO_2NH_2), 7.01 (t, J = 5.9 Hz, 1H, NH), 5.15−5.09 (m, 2H, OH-2, OH-3), 5.04 (d, J = 5.4 Hz, 1H, OH-4), 4.48 (t, J = 6.1 Hz, 1H, OH-6), 4.21 (d, J = 9.4 Hz, 1H, H-1), 4.07 (t, J = 6.4 Hz, 2H, $CH_2OSO_2NH_2$), 3.69 (ddd, J = 12.3, 6.9, 2.4 Hz, 1H, H-6a), 3.48−3.40 (m, 1H, H-2), 3.30−3.22 (m, 3H, H-3, H-5, H-6b), 3.13−3.02 (m, 3H, H-4, NCH_2), 1.89−1.77 (m, 2H, CH_2), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 89.0 (C-1), 81.1 (C-5), 77.5 (C-2), 70.5 ($CH_2OSO_2NH_2$), 69.7 (C-3), 66.7 (C-4), 61.1 (C-6), 40.0 (NCH_2, under DMSO), 29.4 (C-2′), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): m/z 403 [M + Na]$^+$. HRMS: calcd for $C_9H_{20}N_2O_{10}S_2Na$ [M + Na]$^+$ 403.0452, found 403.0450.

*N-5′-Sulfamoylpentyl-S-(1-thio-β-D-glucopyranosyl)sulfonamide (**5c**).* The title compound was synthesized from **4c** according to general procedure 4 and isolated as a white highly hygroscopic solid (51 mg, 125 μmol, 96%). R_f 0.53 (MeCN/H_2O 9/1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.38 (s, 2H, OSO_2NH_2), 6.89 (t, J = 5.9 Hz, 1H, NH), 5.12−5.08 (m, 2H, OH-2, OH-3), 5.04 (d, J = 5.4 Hz, 1H, OH-4), 4.45 (t, J = 6.7 Hz, 1H, OH-6), 4.18 (d, J = 9.4 Hz, 1H, H-1), 4.01 (t, J = 6.5 Hz, 2H, $CH_2OSO_2NH_2$), 3.68 (ddd, J = 12.2, 6.6, 2.0 Hz, 1H, H-6a), 3.52−3.39 (m, 2H, H-2, H-6b), 3.28−3.21 (m, 2H, H-3, H-5), 3.05 (td, J = 9.2, 5.2 Hz, 1H, H-4), 3.01−2.96 (m, 2H, NCH_2), 1.66−1.60 (m, 2H, CH_2-4′), 1.51−1.42 (m, 2H, CH_2-2′), 1.39−1.33 (m, 2H, CH_2-3′), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 89.0 (C-1), 81.0 (C-5), 77.4 (C-2), 70.5 ($CH_2OSO_2NH_2$), 69.7 (C-3), 68.9 (C-4), 61.1 (C-6), 42.6 (NCH_2), 29.2, 27.9 (C-2′, C-4′), 22.2 (C-3′), assignments were confirmed by ^{1}H−^{13}C gHSQC. LRMS (ESI$^+$): m/z 431 [M + Na]$^+$. HRMS: calcd for $C_{11}H_{24}N_2O_{10}S_2Na$ [M + Na]$^+$ 431.0765, found 431.0766.

*N-4′-Sulfamoylpiperidinyl-S-(1-thio-β-D-glucopyranosyl)-sulfonamide (**5d**).* The title compound was synthesized from **4d** according to general procedure 3 and isolated as a white highly hygroscopic solid (39 mg, 97 μmol, 95%). R_f 0.50 (MeCN/H_2O 9/1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.47 (s, 2H, OSO_2NH_2), 5.28−5.21 (m, 1H, OH-2), 5.13 (d, J = 5.3 Hz, 1H, OH-3), 5.05 (d, J = 5.3 Hz, 1H, OH-4), 4.58−4.53 (m, OH-6, $CHOSO_2NH_2$), 4.39 (d, J = 9.4 Hz, 1H, H-1), 3.78−3.67 (m, 1H, H-6a), 3.60−3.40 (m, 5H, H-2, H-3, H-6b, NCH_2), 3.26−3.16 (m, 3H, H-5, NCH_2), 3.09 (td, J = 9.6, 5.6 Hz, 1H, H-4), 2.02−1.99 (m, 2H, CH_2), 1.74−1.68 (m, 2H, CH_2), assignments were confirmed by ^{1}H−^{1}H gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 90.2 (C-1), 81.3 (C-5), 77.6, 75.5 (C-2, C-3), 70.3 ($CHOSO_2NH_2$), 69.3 (C-4), 60.8 (C-6), 43.4, 42.6 (2 × NCH_2), 31.2,

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31.1 (2 × CH_2), assignments were confirmed by $^1H-^{13}C$ gHSQC. LRMS (ESI^+): m/z 429 $[M + Na]^+$. HRMS: calcd for $C_{11}H_{22}N_2O_{10}S_2Na$ $[M + Na]^+$ 429.0608, found 429.0613.

*N-3′-R-Sulfamoylpyrrolidinyl-S-(1-thio-β-D-glucopyranosyl)-sulfonamide (**5e**).* The title compound was synthesized from **4e** according to general procedure 4 and isolated as a white highly hygroscopic solid (68 mg, 173 μmol, 97%). R_f 0.56 (MeCN/H_2O 9/1). ^1H NMR (500 MHz, DMSO-d_6) δ 7.58 (s, 2H, OSO_2NH_2), 5.26 (d, J = 6.1, 1H, OH-2), 5.12 (d, J = 5.4, 1H, OH-3), 5.08−5.00 (m, 2H, OH-4, C*H*OSO_2NH_2), 4.56 (t, J = 5.3 Hz, 1H, OH-6), 4.53 (d, J = 9.5 Hz, 1H, H-1), 3.76−3.68 (m, 2H, H-6a/b), 3.69−3.63 (m, 1H, H-3), 3.51−3.46 (m, 2H, H-2, H-5), 3.44−3.40 (m, 1H, H-4), 3.34−3.27 (m, 2H, CH_2-5′), 3.25−3.21 (m, 1H, CH_2-2′a), 3.09−3. 05 (m, 1H, CH_2-2′b), 2.36−2.34 (m, 1H, CH_2-4′a), 2.09−2.01 (m, 1H, CH_2-4′b), assignments were confirmed by $^1H-^1H$ gCOSY. ^{13}C NMR (125 MHz, DMSO-d_6) δ 89.3 (C-1), 81.3 (C-5), 79.1 (C-2), 77.6 ($CHOSO_2NH_2$), 70.3 (C-3), 69.4 (C-4), 60.9 (C-6), 52.7 (C-2′), 46.7 (C-5′), 31.4 (C-4′), assignments were confirmed by $^1H-^{13}C$ gHSQC. LRMS (ESI^+): m/z 415 $[M + Na]^+$. HRMS: calcd for $C_{10}H_{20}N_2O_{10}S_2Na$ $[M + Na]^+$ 415.0452, found 415.0449.

Protein Expression, Purification, and CA IX-Mimic Design. Wild-type CA II and CA IX-mimic were expressed and purified using BL21DE3 competent cells as described by Pinard et al.[7] The CA IX-mimic used for this study was designed and engineered previous by Genis et al.[6] (containing two active site mutations) and reconstructed by Pinard et al.[7] (to contain seven active site mutations). The mimic utilized the well-studied and crystallizable CA II, with seven point mutations in the active site that creates a chimeric CA IX active site that can be used for structural analysis. Active site mutations in the CA IX-mimic include: A65S, N67Q, E69T, I91L, F131V, K170E, and L204A. Purity of each enzyme was checked by SDS-PAGE. Concentrations were determined by UV/vis spectroscopy and measured at 43 and 55 mg/mL for CA IX-mimic and CA II, respectively.

X-ray Crystallography. Purified CA II and CA IX-mimic were crystallized in 1.6 M Na-citrate, 50 mM Tris, pH 7.8, using hanging drop vapor diffusion.[8a,22] Wells formed crystals for both enzymes were observed after 5 days. Stock solutions of each compound were made using deionized water and to a final concentration of ∼50 mM for each ligand. Crystals were than soaked with desired compound solution 24 h prior to data collection. Diffraction data was collected "in-house" using an RU-H3R rotating Cu anode (λ = 1.5418 Å) operating at 50 kV and 22 mA utilizing an R-Axis IV^{+2} image plate detector (Rigaku, USA). Each data set was processed using HKL2000.[23] All data sets were scaled to a $P2_1$ space group with statistics summarized in Table 2. Initial phases for each data set were determined using molecular replacement methods using PDB 3KS3[24] as a search model. Molecular replacement, model refinements, and generation of ligand restraint files were performed using Phenix[25] suite of programs. Models for ligand−protein complexes and PDB files for ligands were generated using Coot.[26] Coot[26b] was also used to determine bond lengths and angles used for analysis. Figures for publication were generated using PyMol.[27]

CA Inhibition Assay. An Applied Photophysics stopped-flow instrument was used for assaying the CA-catalyzed CO_2 hydration activity.[28] 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.[29] The inhibition constants (K_i) were then derived by using the Cheng−Prusoff equation 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 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.[30] The concentrations of enzymes used in the assay were: hCA I, 10.4 nM; hCA II, 8.3 nM; hCA IX, 8.0 nM; hCA XII, 12.4 nM.

■ ASSOCIATED CONTENT

Supporting Information

^1H and ^{13}C NMR spectra of compounds **2f**, **2g**, **3a−3g**, **4a−4g**, and **5b−5e**. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

Coordinates and structure factors for CA IX-mimic_**5d**, CA IX-mimic_**5e**, and CA II_**5e** have been deposited with the PDB, with accession code 4R59, 4R5A, and 4R5B, respectively.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CA, carbonic anhydrase; K_i, inhibition constant; ZBG, zinc binding group

■ REFERENCES

(1) (a) Wykoff, C. C.; Beasley, N. J.; Watson, P. H.; Turner, K. J.; Pastorek, J.; Sibtain, A.; Wilson, G. D.; Turley, H.; Talks, K. L.; Maxwell, P. H.; Pugh, C. W.; Ratcliffe, P. J.; Harris, A. L. Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer Res.* **2000**, *60*, 7075−7083. (b) 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. (c) Li, Y.; Wang, H.; Tu, C.; Shiverick, K. T.; Silverman, D. N.; Frost, S. C. Role of hypoxia and EGF on expression, activity, localization and phosphorylation of carbonic anhydrase IX in MDA-MB-231 breast cancer cells. *Biochim. Biophys. Acta* **2011**, *1813*, 159−167.

(2) (a) Mahon, B. P.; McKenna, R. Regulation and role of carbonic anhydrase IX and use as a biomarker and therapeutic target in cancer. *Res. Trends Curr. Top. Biochem. Res.* **2013**, *15*, 1. (b) Neri, D.; Supuran, C. T. Interfering with pH regulation in tumours as a therapeutic strategy. *Nature Rev. Drug Discovery* **2011**, *10*, 767−777. (c) Supuran, C. T. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nature Rev. Drug Discovery* **2008**, *7*, 168−181.

(3) (a) Höckel, M.; Vaupel, P. Tumor hypoxia: Definitions and current clinical, biologic, and molecular aspects. *J. Natl. Cancer Inst.* **2001**, *93*, 266−276. (b) Moulder, J. E.; Rockwell, S. Tumor hypoxia: its impact on cancer therapy. *Cancer Metastasis Rev.* **1987**, *5*, 313.

(4) McDonald, P. C.; Winum, J.-Y.; Supuran, C. T.; Dedhar, S. Recent developments in targeting carbonic anhydrase IX for cancer therapeutics. *Oncotarget* **2012**, *3*, 84−97.

(5) (a) 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. (b) Krall, N.; Pretto, F.; Decurtins, W.; Bernardes, G. J. L.; Supuran, C. T.; Neri, D. A small-molecule drug conjugate for the treatment of carbonic anhydrase IX expressing tumors. *Angew. Chem., Int. Ed. Engl.* **2014**, *53*, 4231−4235.

(6) (a) 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. (b) Wilkinson, B. L.; Bornaghi, L. F.; Houston, T. A.; Innocenti, A.; Vullo, D.; Supuran, C. T.; Poulsen, S.-A. Carbonic anhydrase inhibitors: inhibition of isozymes I, II, and IX with triazole-linked *O*-glycosides of benzene sulfonamides. *J. Med. Chem.* **2007**, *50*, 1651−1657. (c) Wilkinson, B. L.; Bornaghi, L. F.; Houston, T. A.; 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. (d) Wilkinson, B. L.; Innocenti, A.; Vullo, D.; Supuran, C. T.; Poulsen, S.-A. Inhibition of carbonic anhydrases with glycosyltriazole benzene sulfonamides. *J. Med. Chem.* **2008**, *51*, 1945−1953. (e) Lopez, M.; Bornaghi, L. F.; Innocenti, A.; Vullo, D.; Charman, S. A.; Supuran, C. T.; Poulsen, S.-A. Sulfonamide linked neoglycoconjugates—a new class of inhibitors for cancer-associated carbonic anhydrases. *J. Med. Chem.* **2010**, *53*, 2913−2926. (f) Singer, M.; Lopez, M.; Bornaghi, L. F.; Innocenti, A.; Vullo, D.; Supuran, C. T.; Poulsen, S.-A. Inhibition of carbonic anhydrase isozymes with benzene sulfonamides incorporating thio, sulfinyl and sulfonyl glycoside moieties. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2273−2276.

(7) (a) Winum, J.-Y.; Poulsen, S.-A.; Supuran, C. T. Therapeutic applications of glycosidic carbonic anhydrase inhibitors. *Med. Res. Rev.* **2009**, *29*, 419−435. (b) Winum, J.-Y.; Colinas, P.; Supuran, C. T. Glycosidic carbonic anhydrase IX inhibitors: a sweet approach against cancer. *Bioorg. Med. Chem.* **2013**, *21*, 1419−1426.

(8) (a) Pinard, M. A.; Boone, C. D.; Rife, B. D.; Supuran, C. T.; McKenna, R. Structural study of interaction between brinzolamide and dorzolamide inhibition of human carbonic anhydrases. *Bioorg. Med. Chem.* **2013**, *21*, 7210. (b) 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. (c) Supuran, C. T. Structure-based drug discovery of carbonic anhydrase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 759−772. (d) Supuran, C. T.; McKenna, R. *Carbonic Anhydrase: Mechanism, Regulation, Links to Disease, And Industrial Applications*; McKenna, R.; Frost, S., Eds.; Springer Verlag: Heidelberg, 2014; Vol. *75*, pp 291−323.

(9) (a) Lopez, M.; Trajkovic, J.; Bornaghi, L.; Innocenti, A.; Vullo, D.; Supuran, C.; Poulsen, S. Design, synthesis, and biological evaluation of novel carbohydrate-based sulfamates as carbonic anhydrase inhibitors. *J. Med. Chem.* **2011**, *54*, 1481−1489. (b) Lopez, M.; Vu, H.; Wang, C. K.; Wolf, M. G.; Groenhof, G.; Innocenti, A.; Supuran, C. T.; Poulsen, S.-A. Promiscuity of carbonic anhydrase II. Unexpected ester hydrolysis of carbohydrate-based sulfamate inhibitors. *J. Am. Chem. Soc.* **2011**, *133*, 18452−18462.

(10) Spillane, W.; Malaubier. Sulfamic acid and its *N*- and *O*-substituted derivatives. *Chem. Rev.* **2014**, *114*, 2507−2598.

(11) Lopez, M.; Bornaghi, L. F.; Driguez, H.; Poulsen, S.-A. Synthesis of sulfonamide-bridged glycomimetics. *J. Org. Chem.* **2011**, *76*, 2965−2975.

(12) Zemplén, G. Degradation of the reducing bioses. I. Direct determination of the constitution of cellobiose. *Ber. Dtsch. Chem. Ges.* **1926**, *59*, 1254−1266.

(13) Hasegawa, T.; Numata, M.; Asai, M.; M, T.; Shinkai, S. Colorimetric calcium-response of b-lactosylated *m*-oxo-bis-[5,15-meso-diphenylporphyrinatoiron(III)]. *Tetrahedron* **2005**, 7783−7788.

(14) Yeom, C.-E.; Lee, S. Y.; Kim, Y. J.; Kim, B. M. Mild and chemoselcective deacetylation method using a catalytic amount of acetyl chloride in methanol. *Synlett* **2005**, *10*, 1527−1530.

(15) González, A. G.; Brouard, I.; León, F.; Padrón, J. I.; Bermejo, J. A facile chemoselective deacetylation in presence of benzoyl and *p*-bromobenzoyl groups using *p*-toluenesulfonic acid. *Tetrahedron Lett.* **2001**, *42*, 3187−3188.

(16) (a) Carroux, C. J.; Moeker, J.; Motte, J.; Lopez, M.; Bornaghi, L. F.; Katneni, K.; Ryan, E.; Morizzi, J.; Shackleford, D. M.; Charman, S. A.; Poulsen, S.-A. Synthesis of acylated glycoconjugates as templates to investigate in vitro biopharmaceutical properties. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 455−459. (b) 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.

(17) 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.

(18) Aggarwal, M.; Kondeti, B.; McKenna, R. Insights towards sulfonamide drug specificity in alpha carbonic anhydrases. *Bioorg. Med. Chem.* **2013**, *21*, 1526−1533.

(19) Ibatullin, F. M.; Shabalin, K. A.; Jänis, J. V.; Shavvac, A. G. Reaction of 1,2-*trans*-glycosyl acetates with thiourea: a new entry to 1-thiosugars. *Tetrahedron Lett.* **2003**, *44*, 7961−7964.

(20) (a) Okada, M.; Iwashita, S.; Koizumi, N. Efficient general method for sulfamoylation of a hydroxyl group. *Tetrahedron Lett.* **2000**, *41*, 7047−7051. (b) Neres, J.; Labello, N. P.; Somu, R. V.; Boshoff, H. I.; Wilson, D. J.; Vannada, J.; Chen, L.; Barry, C. E.; Bennett, E. M.; Aldrich, C. C. Inhibition of siderophore biosynthesis in *Mycobacterium tuberculosis* with nucleoside bisubstrate analogues: structure−activity relationships of the nucleobase domain of 5′-*O*-[*N*-(salicyl)sulfamoyl]-adenosine. *J. Med. Chem.* **2008**, *51*, 5349−5370.

(21) Lopez, M. L.; Bornaghi, L. F.; Poulsen, S.-A. Synthesis of sulfonamide-conjugated glycosyl-amino acid building blocks. *Carbohydr. Res.* **2014**, *386*, 78−85.

(22) Genis, C.; Sippel, K. H.; Case, N.; Cao, W.; Avvaru, B. S.; Tartaglia, L. J.; Govindasamy, L.; Tu, C.; Agbandje-McKenna, M.; Silverman, D. N.; Rosser, C. J.; McKenna, R. Design of a carbonic anhydrase IX active-site mimic to screen inhibitors for possible anticancer properties. *Biochemistry* **2009**, *48*, 1322−1331.

(23) Otwinowski, Z.; Minor, W. *Processing of X-ray Diffraction Data Collected in Oscillation Mode*; Elsevier: New York, 1997; Vol. *276*.

(24) Avvaru, B. S.; Kim, C. U.; Sippel, K. H.; Gruner, S. M.; Agbandje-McKenna, M.; Silverman, D. N.; McKenna, R. A short, strong hydrogen bond in the active site of human carbonic anhydrase II. *Biochemistry* **2010**, *49*, 249−251.

(25) Golovanov, A. P.; Hautbergue, G. M.; Wilson, S. A.; Lian, L.-Y. A simple method for improving protein solubility and long-term stability. *J. Am. Chem. Soc.* **2004**, *126*, 8933.

(26) (a) Debreczeni, J. É.; Emsley, P. Handling ligands with Coot. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2012**, *68*, 425−430. (b) Emsley, P.; Cowton, K. Coot: Model-building tools for molecular graphics. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2004**, *60*, 2126−2132.

(27) *The PyMOL Molecular Graphics System*, version 1.5.0.4; Schrödinger, LLC.

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

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

(30) (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.