Comparison of Sulfamate and Sulfamide Groups for the Inhibition of Carbonic Anhydrase-II by Using Topiramate as a Structural Platform†

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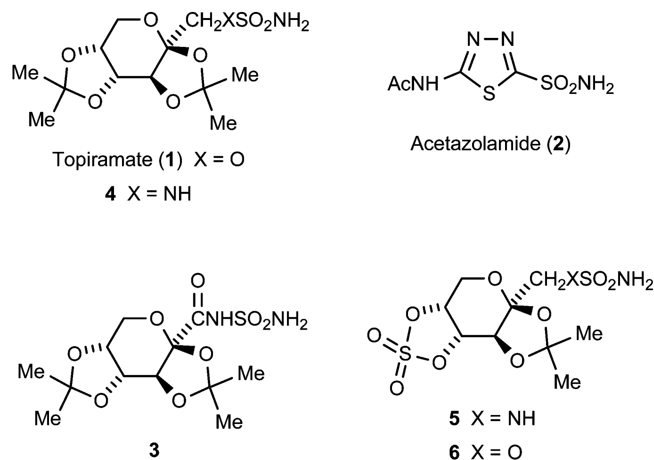
This paper examines the relative effectiveness of sulfamate and sulfamide groups for the inhibition of carbonic anhydrase-II (CA-II). Topiramate (**1**) and its sulfamide analogue **4**, and 4,5-cyclic sulfate **6** and its sulfamide analogue **5**, were compared for inhibition of human CA-II. A colorimetric assay, based on the pH shift that accompanies hydration of carbon dioxide, and an esterase assay were used. For these bioisosteric pairs, **1/4** and **6/5**, the sulfamate compound was markedly more potent than its sulfamide counterpart. A similar, large difference in potency was also observed for the sulfamate/sulfamide pairs **14/15** and **16/17**. These results indicate that the sulfamide moiety is not particularly suitable for obtaining potent carbonic anhydrase inhibition. A discussion of this structure−activity relationship with respect to the interactions of **1** and **6** with CA-II from published X-ray data is presented. A metabolic acidosis study was performed in rats with **1**, **4**, **6**, and **2**, and the results are discussed with respect to the degree of inhibition of CA-II in vivo.

Carbonic anhydrase (CA, EC 4.2.1.1) is a zinc-containing enzyme that catalyzes a reversible reaction involving the hydration of carbon dioxide and the dehydration of bicarbonate: $CO_2 + 2H_2O \rightleftarrows HCO_3^- + H_3O^+$.[1−4] This enzymatic reaction is essential to many physiological anion-exchange processes,[5] and CA inhibitors are effective drugs for the treatment of glaucoma by reducing aqueous humor formation to lower intraocular pressure.[3,6,7] Of the three CA families, designated α, β, and γ, the mammalian α-CA family encompasses 14 genetically distinct isozymes, CA-I through CA-XIV, with different tissue and intracellular distribution.[4] CA-II, the most widely studied form, which is found in many different organs and cell types, has very high catalytic activity for CO_2 hydration. Numerous potent inhibitors of CA-II, generally having a key primary sulfonamide functionality, $R{-}SO_2NH_2$, have been reported.[3,8,9] The sulfonamide in these compounds coordinates with the tetrahedral Zn^{II} atom in the enzyme active site, which is also coordinated to the imidazole groups of three histidine residues (His-94, His-96, and His-119 in human CA-II), thereby displacing the water/hydroxide ligand that is intimately involved in CO_2 hydration.[9,10]

Our interest in carbonic anhydrase enzymes derives from working with topiramate (**1**),[11] a novel sugar sulfamate anticonvulsant that is marketed worldwide for the treatment of epilepsy and migraine.[12] Following our discovery of topiramate,[11b,c] we were prompted to investigate its potential to inhibit CA because of the presence of a sulfamate functionality ($R{-}OSO_2NH_2$). This effort entailed the use of CA isozymes derived from different tissues and the determination of CO_2 hydration.[11a,b,13,14] In a CO_2 hydration bioassay involving enzymes from rat blood (CA-I + CA-II) or rat myelin



(CA-II), we found that topiramate is a moderate inhibitor with IC_{50} values of 8.9 or 1.4 μM, respectively.[11a,16] Also, topiramate is reported to be a moderate inhibitor of purified human erythrocyte CA-II, with a K_i value of 5 μM, which is 125 times less potent than the value obtained with the reference sulfonamide acetazolamide (**2**).[13,17] With respect to other CA isozymes, topiramate is generally a moderate inhibitor, with IC_{50} or K_i values usually well above 100 nM.[18] However, recent publications have reported low-nanomolar inhibition of human recombinant CA-II (K_i = 5 nM) by topiramate via an esterase assay (rather than a CO_2-hydration assay), twice as potent as acetazolamide (K_i = 12 nM).[19] The study by Casini et al.[19a] also brought to light some sulfamide derivatives that are structurally related to topiramate, and they found that close analogue **3** is essentially devoid of CA-II inhibition in the esterase assay (K_i > 100 μM). By contrast, other sulfamides were found to be potent CA-II inhibitors,[19a,20] and the study by Abbate et al.[20] also suggests that the sulfamide group should be favorable for binding within the CA active site cleft. Because **3**[19a] differs from topiramate by having a

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† This paper is dedicated to the memory of Dr. Paul A. J. Janssen, one of the most prolific pharmaceutical researchers of all time.

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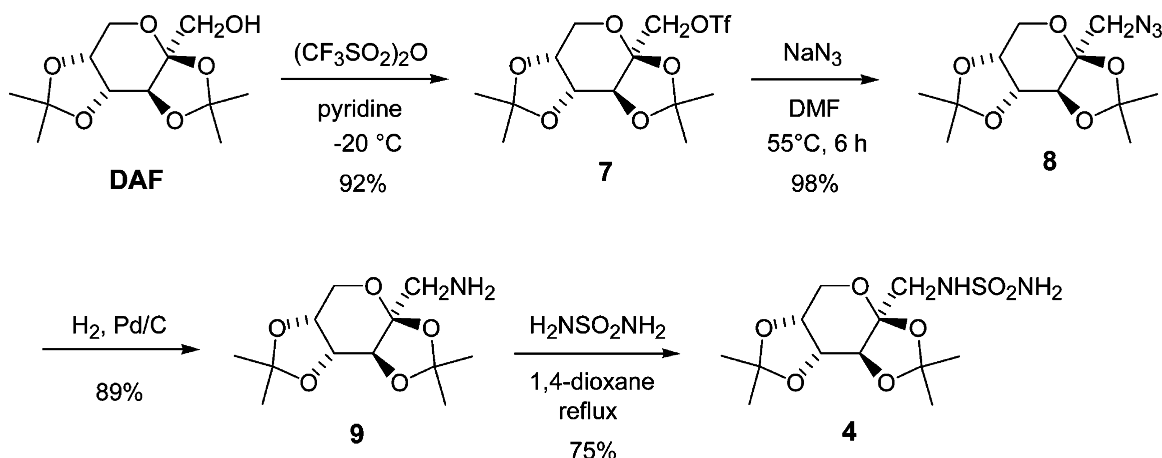
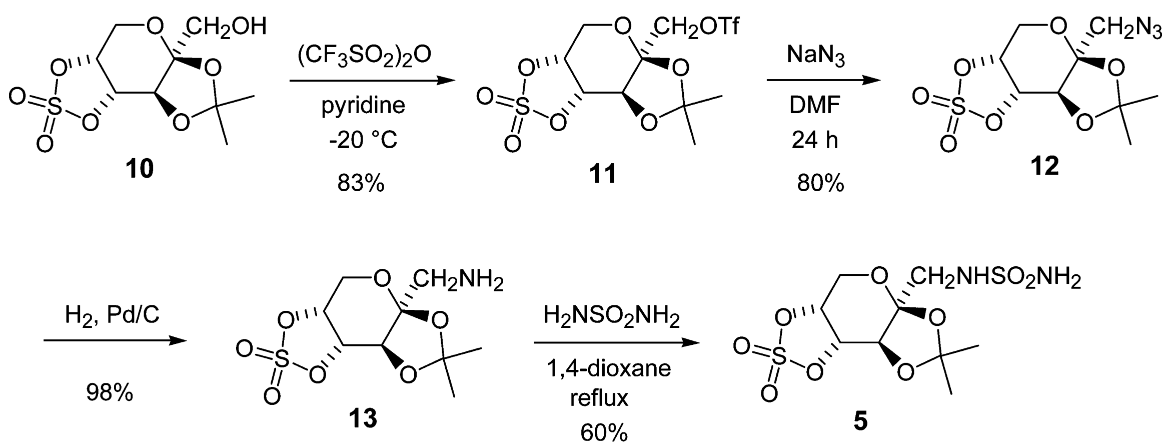
Scheme 1



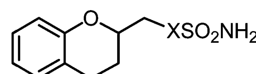
Scheme 2

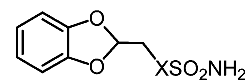


carbonyl group installed, not just by changing a sulfamate to a sulfamide, we thought that it would be important to determine the CA-II inhibition for the direct sulfamide analogue of topiramate, i.e., **4**.[11a] Herein, we report the evaluation of sulfamide **4**, as well as its 4,5-cyclic sulfate congener, **5**, which is the direct sulfamide analogue of **6**, a very potent sulfamate-based CA inhibitor.[11a] Our results for sulfamate and sulfamide bioisosteric pairs indicate that the sulfamide moiety is not particularly suitable for obtaining potent carbonic anhydrase inhibition.

Results and Discussion

Synthesis. Sulfamide **4**, reported earlier,[11a] was synthesized by an improved method, as outlined in Scheme 1. Commercially available diacetone fructose (DAF) was treated with triflic anhydride to give triflate **7**, which was immediately converted to azide **8** in near quantitative yield. Catalytic hydrogenation cleanly afforded the desired amine **9** in a superior yield (ca. 80% from DAF) compared with the previously reported method (ca. 50%).[11a] Reaction of amine **9** with sulfamide in refluxing 1,4-dioxane gave target **4** in 75% yield, which was far superior to the 20% yield obtained in the original synthesis that employed sulfamoyl chloride and sodium hydride. Sulfamide **5** was synthesized in good overall yield from alcohol **10**[11a] in a similar manner, via **11**−**13** (Scheme 2). It is remarkable that the sulfamide reaction could be effected with high efficiency despite the presence of the cyclic sulfate functionality, which can be sensitive to nucleophilic ring opening, especially at higher temperatures such as 100 °C. Because of the cyclic sulfate group, the azide displacement step was carried out at a lower temperature (23 °C) than that used in the synthesis of **4**. Compounds **14**−**17** were prepared in a straightforward manner (Supporting Information).





14 X = O
15 X = NH

16 X = O
17 X = NH

Enzyme Inhibition. Compounds **1**, **2**, **4**, **5**, and **6** were tested for inhibition of purified human CA-II obtained from erythrocytes (Sigma Chemical Co.) by using an assay involving the native action of the enzyme, namely, the hydration of CO_2, via a pH-shift measurement (Table 1). Sulfamates **1** and **6** inhibited CA-II with K_i values of 0.50 and 0.012 μM, respectively, which compare with a K_i value of 0.0033 μM for acetazolamide (**2**), which is an expected level of inhibition for this reference compound and serves to validate the enzyme assay (positive control). By contrast, sulfamides **4** and **5** exhibited comparatively weak CA-II inhibition, with K_i values of 650 and 20 μM, respectively. From these data, the sulfamide-based bioisosteres, **4** and **5**, are approximately *1000 times less potent* than their sulfamate counterparts, **1** and **6**.

Table 1. Carbonic Anhydrase Inhibition Data[a]

cmpd	pH-shift CA-II, K_i (μM)[b]	esterase CA-II, K_i (μM)[b]
1	0.50 (0.39−0.64)	0.43 (0.39−0.49)
2	0.0033 (0.0026−0.0043)	0.018 (0.016−0.020)
4	650 (390−1070)	340 (240−490)
5	20 (12−35)	25 (22−29)
6	0.012 (0.011−0.015)	0.038 (0.030−0.047)
L-**1**[c]	9.6 (5.3−17.2)	7.7 (6.0−9.7)
L-**6**[d]	0.91 (0.59−1.4)	0.75 (0.64−0.88)

[a] Inhibition of human CA-II, as described in the Experimental Section. [b] The 95% confidence intervals are given in parentheses. See the Experimental Section for details on the method. IC_{50} values for **1**, **2**, **4**, **5**, and **6** in the pH-shift assay: 1.5, 0.011, 1950, 60, and 0.036 μM. [c] L enantiomer of **1**. [d] L enantiomer of **6**.

With the above confirmation of our earlier results with **1** as an inhibitor of CA-II, we became curious about the much more potent CA-II inhibition reported for the esterase method.[19] In our hands, the esterase assay for topiramate (**1**) and acetazolamide (**2**) afforded K_i values of 0.43 and 0.018 μM, respectively (Table 1), instead of 0.005 and 0.012 μM, as reported by Supuran and coworkers.[19] The K_i values in the esterase assay for sulfamate **6** and sulfamides **4** and **5**, were 0.038, 340, and 25 μM, respectively. It is not clear to us what factors are behind the observed discrepancy between the K_i values for **1** and **2** measured by each group, although the source of CA-II is different (purified native vs. recombinant[21]). Fairly consistent results were obtained between the two assay modes (Table 1), except for acetazolamide (**2**), which had a pH-shift K_i value of 0.0033 and an esterase K_i value of 0.018 μM. Our results for the L enantiomer of **1** and the L enantiomer of **6** in the paired assays were also consistent (Table 1).

Sulfamates and Sulfamides as Inhibitors of CA-II. Although aryl and heteroaryl sulfonamides are archetypal structures for inhibitors of carbonic anhydrases, sulfamate and sulfamide derivatives can also give rise to potent inhibitors.[11a,e,19−21] In an X-ray crystallographic study of human CA-II complexed with sulfamic acid and sulfamide, the parent core structures for the sulfamate and sulfamide derivatives, respectively, the expected Zn^{II}−nitrogen binding is reflected, as are some new interactions resulting from the heteroatoms (O or N).[20] These new interactions were suggested to offer opportunities for the design of new inhibitors. However, because sulfamic acid and sulfamide themselves are relatively weak inhibitors of CA-II (esterase assay K_i values of 97 and 82 μM, respectively),[19a,21] the relevance of those new interactions to CA inhibitor design might be questioned. Consequently, it would be worthwhile to compare sulfamate and sulfamide molecules that are *strict bioisosteres* and that represent meaningfully potent inhibitors of CA. Given that the cyclic sulfate topiramate analogue **6** is a very potent inhibitor of human CA-II with a K_i value of 12 nM (Table 1), it provides a suitable benchmark compound for the sulfamate species. However, its sulfamide isostere, **5**, has remarkably weaker human CA-II inhibitory activity (K_i = 20 μM; 1000-fold less potent). A similar dichotomy is observed between the human CA-II inhibition of topiramate (**1**) (K_i = 0.50 μM) and that of its sulfamide analogue, **4** (K_i = 650 μM; ca. 1000-fold less potent). This pattern for the sulfamate/sulfamide pairs also applies to the CA-II inhibition results from the esterase-based assay (Table 1).

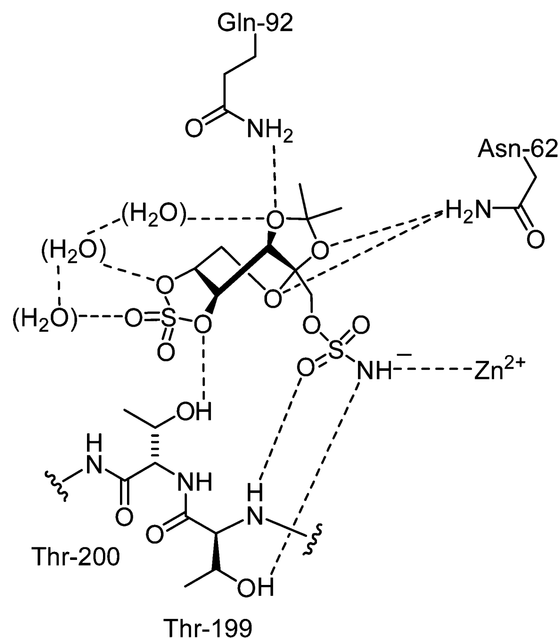


Figure 1. Schematic representation of interactions of **6** within the active site of human CA-II.

Since these substantial potency differences emanate from sulfamate/sulfamide bioisosteric pairs, it would appear that the sulfamide moiety is not especially competent for obtaining potent inhibition of CA-II. Considering the importance of this observation, we sought to probe this issue further with some additional sulfamate/sulfamide pairs. Thus, we synthesized and examined the pairs **14/15** and **16/17**, which have some structural distinction from the other pairs studied. In this manner, we could gain support for the generality of this effect. CA-II inhibition IC_{50} values for the pairs **14/15** and **16/17** were determined in the pH-shift assay. Sulfamate **14** and sulfamide **15** had IC_{50} values of 0.13 and 71 μM, respectively, and sulfamate **16** and sulfamide **17** had IC_{50} values of 0.28 and 129 μM, respectively. Again, in the case where sulfamate compounds are reasonably potent inhibitors of CA-II, the corresponding sulfamide compounds are considerably less potent, by a large factor of approximately 500.[22]

Binding Interactions of Sulfamates 1 and 6 with CA-II. We have described the X-ray structure of sulfamate **6** complexed with human CA-II,[11e] the collected interactions for which are depicted schematically in Figure 1. Casini et al. have reported on the X-ray structure of topiramate (**1**) complexed with human CA-II,[19a] the interactions for which are depicted in Figure 2. The pyranose rings of **1** and **6** adopt the $^3S_0$ skew conformation (Figures 1 and 2), which we have correlated with potent anticonvulsant activity.[11a,e] The key interactions between each inhibitor molecule and the CA active site are very similar for these two complexes and dependent on this $^3S_0$ skew conformation for a good steric fit (Figure 3). However, the two ligands manage to dock into the active site of CA-II in a different structural context (Figure 3). The sulfamate nitrogen of both **1** and **6** is coordinated to Zn^{II} and hydrogen bonded to the hydroxyl of Thr199 in basically the same manner. Likewise, the binding modes of both **1** and **6** have the sulfamate oxygen hydrogen bonding to the α-amino group of Thr199 and the C2 oxygen of the 2,3-

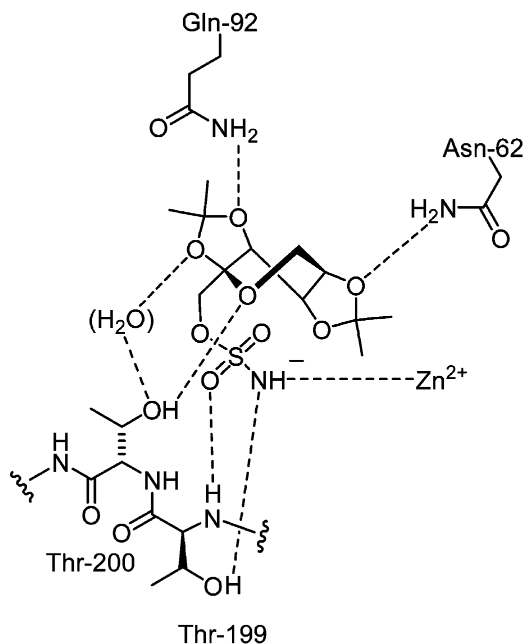


Figure 2. Schematic representation of interactions of topiramate (**1**) within the active site of human CA-II.

acetonide ring hydrogen bonding to the side chain amino group of Gln92. In addition, various hydrogen-bonding interactions occur between both inhibitors and key residues of CA-II: Asn62, Gln92, and Thr200. However, the binding modes of **1** and **6** differ in that the molecules are rotated by ca. 180°, relative to each other, about the sugar anomeric C−CH_2 bond, i.e., the 2,3-ring of one ligand has exchanged position with the 4,5-ring of the other ligand. Hence, the 4,5-cyclic sulfate of **6** is directed toward the solvent and forms hydrogen bonds with two water molecules and the hydroxyl of Thr200. In addition, the C2 oxygen of the 2,3-acetonide and the pyranose ring oxygen of **6** form hydrogen bonds with the amide side chain of Asn62. By contrast, for **1** the 2,3-acetonide is directed toward the solvent and hydrogen bonded to a water molecule and Gln92. In addition, the C5 oxygen of the 4,5-acetonide forms a hydrogen bond with the amide side chain of Asn62. The difference in binding modes for **1** and **6** can be rationalized by the structural differences between each ligand. Perhaps, it is energetically more favorable for the more polar 4,5-cyclic sulfate of **6** to be solvated (e.g., by water). This viewpoint is consistent with the fact that the 4,5-cyclic sulfate has three hydrogen bonding interactions (Figure 1) and three associated water molecules, whereas the colocalized 2,3-acetonide of **1** has only two hydrogen-bonding interactions and one associated water molecule (Figure 2). The remarkable distinction in binding modes for **1** and **6** underscores the flexibility that is available in the design of active-site-directed inhibitors of CA-II. However, it is important to note that the active site of CA-II does not accommodate the L isomers[11a] of **1** and **6** nearly as well as the D isomers, which is clearly reflective of specific steric requirements (Table 1). In the pH-shift assay, the L enantiomer of **1**[11a] was ca. 20 times less potent than D isomer **1**, and the L enantiomer of **6**[11a] was ca. 80 times less potent than D isomer **6**. The weaker affinities of the L isomers of **1** and **6** relative to the D isomers is consistent with our structural analysis.

The bridging sulfamate oxygen (CH_2−O−S) does not play a meaningful role in the binding of **1** and **6** with CA-II.[11e,19a] By contrast, in the complex of sulfamic acid with CA-II there are important interactions of the bridging sulfamate oxygen.[20] It would appear that the anionic properties of the oxygen in the sulfamic acid case govern interactions within that enzyme complex and thereby distinguish it from the sulfamate ester cases. In the absence of interactions of the bridging sulfamate oxygen of **1** and **6**, how might one explain the poor CA-II inhibitory activity of sulfamide bioisosteres **4** and **5**, respectively? Perhaps, the bioisosteric CH_2NHSO_2 group has an altered conformation in the ligand such that the terminal NH_2 (or NH^-) group cannot complex effectively with the Zn atom and still retain appropriate hydrophobic and hydrogen-bonding interactions for the rest of the molecule. Another contributing factor could be the difference in pK_a between the sulfamate and sulfamide groups. For sulfamates **1** and **6**, we determined pK_a values of 8.66 and 8.51, respectively, whereas for sulfamide **4** we determined a pK_a value of 10.7. Because **4** is much less acidic, it will have a much lower population of the anionic form that is required for binding to Zn^{II} in the active site of CA-II.[9,10,23]

CA Inhibition and Topiramate's Antiepileptic Action. Given the topic under discussion in this paper, it would be instructive to consider the relevance of CA inhibition to the antiepileptic activity of topiramate. While attempting to elucidate the mechanisms of action for topiramate, we suggested that in some forms of epilepsy the inhibition of CA-II or CA-IV may contribute to the pharmacological activity.[11d] However, a cross-tolerance study with acetazolamide and topiramate indicated that inhibition of CA-II and CA-IV is not a major factor for topiramate's anticonvulsant activity in mice.[11c] Also, when we conducted pharmacological studies in rats with sulfamate **6**, which is much more potent than topiramate as a CA-II inhibitor,[11a] we observed frank diuresis and alkalinization of the urine, which is characteristic of CA inhibition.[24] Such diuresis has not been observed with topiramate in preclinical experiments or in clinical practice. However, there have been clinical reports of metabolic acidosis, which presumably emanates from inhibition of CA-II and possibly other CA isozymes.[25] To follow up on this point, we investigated the effects of topiramate (**1**), **6**, acetazolamide (**2**, positive control), and **4** (negative control) on the pH values of arterial blood in rats. Since inhibition of CA-II is likely to result in metabolic acidosis, with a dose-dependent relationship, the blood pH readout should reflect the degree of CA-II inhibition in vivo.[26] With a normal pH for rat blood of 7.40−7.43, the threshold for metabolic acidosis would be a pH of less than 7.40.

We orally dosed adult male rats with 100 mg/kg of topiramate, **6**, acetazolamide, or **4**, sampled their arterial blood 2 h later, and measured the pH. Acetazolamide (CA-II K_i = 11 nM) gave a pH of 7.26 ± 0.06, reflecting frank acidosis, whereas **4** (K_i = 650,000 nM) gave a pH of 7.40 ± 0.03, reflecting a lack of activity (essentially the same as the value for vehicle, pH = 7.42 ± 0.07). Compound **6** (K_i = 12 nM) was very potent in inducing acidosis (pH = 7.25 ± 0.07), whereas topiramate (K_i = 500 nM) exhibited weak acidosis activity (pH

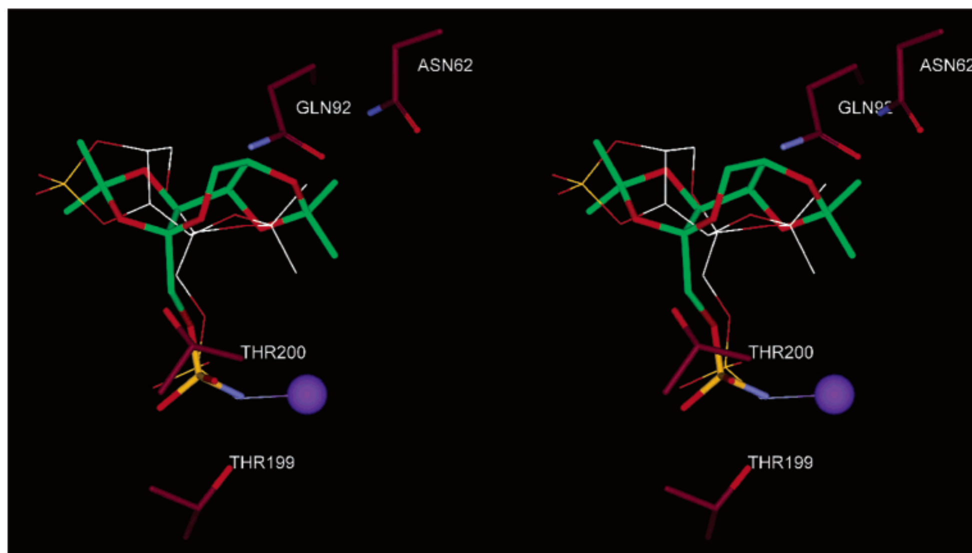


Figure 3. Stereoview of **1** (green) and **6** (white) complexed with human CA-II, from the relevant X-ray crystal structures.

= 7.37 ± 0.04). Our data indicate that the degree of blood acidification, in terms of pH, is reasonably consistent with the level of CA-II inhibition for the compounds of interest. It is clear that topiramate causes limited metabolic acidosis compared to the more potent CA-II inhibitors. Because topiramate does not manifest strong inhibition of human CA-II in vitro and induces just mild acidosis in rats in vivo, we suggest that other, CNS-based mechanisms are probably the dominant factors in topiramate's antiepileptic action.[11d]

Conclusion

We examined some sulfamate/sulfamide bioisosteric pairs to determine the relative effectiveness of the sulfamate and sulfamide groups for inhibiting human CA-II. Topiramate (**1**) and its sulfamide analogue **4**, and 4,5-cyclic sulfate **6** and its sulfamide analogue **5**, were compared, and a dramatic difference in potency was observed. This finding also applied to the sulfamate/sulfamide pairs **14**/**15** and **16**/**17**. Given our results for these sulfamate/sulfamide pairs, it would appear that the sulfamide moiety is not particularly suitable for obtaining potent carbonic anhydrase inhibition. Our study of metabolic acidosis in rats with topiramate (**1**), **6**, acetazolamide (**2**), and **4**, which reflects on the inhibition of CA-II in vivo, indicates that topiramate causes limited metabolic acidosis compared to the more potent CA-II inhibitors, **6** and **2**. Thus, we suggest that other, CNS-based mechanisms are probably the principal source of topiramate's antiepileptic action.

Experimental Section[27]

1-[(Aminosulfonyl)amino]-1-deoxy-2,3:4,5-bis-*O*-(isopropylidene)-*β*-D-fructopyranose (4).[27] Diacetone fructose (DAF) and pyridine (6 equiv) in dichloromethane were treated with triflic anhydride (3 equiv) at −20 °C, and the mixture was stirred for 2 h. The crude triflate was purified by column chromatography to give oily **7**, which was dissolved in dimethylformamide (DMF) and treated with sodium azide (1.1 equiv) at 55 °C for 6 h. Crude azide **8** in ethanol was hydrogenated in the presence of Pd/C, and acid/base workup gave amine **9**. Reaction of **9** with sulfamide (2 equiv) in refluxing 1,4-dioxane for 2 h produced crude **4**, which was purified by flash column chromatography to give pure sulfamide **4**. (MS M − 1: 337. ^{1}H NMR: C, H, N.)

1-[(Aminosulfonyl)amino]-1-deoxy-2,3-*O*-(isopropylidene)-4,5-*O*-sulfonyl-*β*-d-fructopyranose (5).[27] A solution of 2,3-*O*-(isopropylidene)-4,5-*O*-sulfonyl-*β*-D-fructopyranose (**10**)[11a] and pyridine (6 equiv) in dichloromethane was treated at −20 °C with triflic anhydride (3 equiv), and the reaction was stirred for 2 h. The crude triflate was purified by flash column chromatography to give oily **11**, which was dissolved in DMF and treated with sodium azide (1.1 equiv) at 23 °C for 24 h. Crude azide **12** in ethanol was hydrogenated in the presence of Pd/C to give amine **13**, which was reacted with sulfamide (4 equiv) in refluxing 1,4-dioxane for 30 min to produce crude **5**. Purification by flash column chromatography furnished pure sulfamide **5**. (MS M − 1: 337. ^{1}H NMR: C, H, N.)

Carbonic Anhydrase Inhibition. pH-Shift Assay. Purified human CA-II, obtained from erythrocytes, was purchased from Sigma Chemical Co. Inhibition of CA-II was determined in a "pH-shift" assay performed according to the following procedure.[28] The experiments involved ca. 0.5 mg of CA-II dissolved in 20 mL of water and then diluted 10-fold to 20-fold with water (amount of CA-II per sample = 0.1−0.2 μg). The decrease in pH resulting from CO_2 and H_2O being converted to H^+ and HCO_3^- was measured by the change in the color of the dye bromthymol blue. The reaction was performed at 0−2 °C under conditions that allowed the catalyzed reaction to reach an endpoint within 15−25 s and the uncatalyzed reaction to reach an endpoint in 90−120 s. A 10 mM aqueous HEPES solution was buffered to pH 7.7 at 23 °C with Tris containing bromthymol blue (50 mg/L) and dithiothreitol (160 mg/L). For the catalyzed reaction, the contents of each sample included 0.5 mL of the buffered dye solution, 0.1 mL of CA-II solution, 0.1 mL of test compound solution (0.1 mL of water for catalyzed control samples), and 0.3 mL of fresh seltzer water. The temperature of the solutions (final volume = 1.0 mL) was maintained at 0−2 °C. For uncatalyzed control samples, 0.1 mL of water was substituted for the 0.1 mL of enzyme solution. The seltzer water was added last to initiate the timed reaction. Each sample was gently mixed, placed in ice water, and gently mixed every 15−30 s until the solution turned yellow, at which point the time was recorded. Each compound was tested in triplicate at each specified concentration. Test compounds were dissolved in dimethyl sulfoxide at 100 mM and then diluted in water to a concentration 10 times the final concentration in the test tube. In this assay, the concentration of the substrate (CO_2) decreases from ca. 18 to 14 mM and, on average, is about twice the concentration of the K_m for CO_2. By use of the Cheng−Prusoff equation,[29] the K_i value determined is approximately $^1/_3$ of the IC_{50} value. Concentration−inhibition curves were generated for each compound (5−10 concentrations) in 4−12

independent experiments. The IC_{50} values were obtained using the PRISM (GraphPad) curve-fitting program in the competitive inhibition mode.

Carbonic Anhydrase Inhibition. Esterase Assay. This method was performed with purified enzyme (see above) and is based on procedures described previously.[30,31] Stock solutions of the substrate, 4-nitrophenyl acetate (4-NPA), were made up in 95% ethanol at 4 mM. In most experiments, the reaction medium was buffered using HEPES (10 mM) adjusted to a pH of 7.7 with Tris. Preliminary experiments showed that similar catalytic activity was obtained by using Tris Cl^- or Na^+ phosphate as buffering agents. The reaction medium was comprised as follows: 70 μL of buffer, 10 μL of test compound (see above), 10 μL of 4-NPA, and 10 μL of enzyme. Water was substituted for test compound or enzyme as appropriate to establish the control catalyzed and uncatalyzed reaction rates. In each experiment, compounds were tested in quadruplicate by using 96-well plates at 23 °C. The reaction was initiated by adding enzyme, and the reaction course was monitored at 400 nm. Data for each sample was recorded at each minute from 0 to 20 min. Because the concentration of substrate (0.4 mM) was at least 25-fold lower than the K_m (>10 mM), the IC_{50} values obtained were essentially the same as the K_i values.[29,30] The data were analyzed as described above for the pH-shift assay.

Rat Metabolic Acidosis Assay. Male Sprague–Dawley rats (250–300 g) were fasted overnight and dosed orally with vehicle (0.5% methylcellulose) or 100 mg/kg of topiramate (**1**), acetazolamide (**2**), **6**, or **4**. Arterial blood samples were collected 2 h postdosing. Blood chemistries, including pH, were measured by using an iSTAT portable clinical analyzer. The data in the text represent the mean ± SD for each treatment group, with eight rats per group ($N = 8$).

Acknowledgment. We thank Dr. Brett Tounge for technical assistance with computer modeling and Dr. Michael Parker for preparing **16** and **17**.

Supporting Information Available: General procedures, synthetic details and analytical data for **4** and **5**, detailed synthetic procedures, and analytical data for **14**–**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) The corresponding IC_{50} values for acetazolamide (**2**) were 0.037 or 0.024 μM, respectively.

(17) The corresponding K_i value for acetazolamide was 0.04 μM.

(18) For the six CA isozymes examined (CA-I, CA-II, CA-III, CA-IV, CA-V, CA-VI), topiramate inhibited CA-II and CA-IV more effectively than the others. The K_i values for CA-II and CA-IV ranged from 0.1 to 20 μM, depending on the species and the temperature.[13]

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