Sulfonylmethanesulfonamide Inhibitors of Carbonic Anhydrase

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A series of sulfonylmethanesulfonamide derivatives is described, which are inhibitors of carbonic anhydrase (CA). The most potent of these is the racemic fluoro sulfone **9**, which inhibits carbon dioxide hydration catalyzed by human CA II (CA-II) with an IC_{50} of 3 nM. Binding competition studies versus dansylamide indicate that the enantiomers of **9** have different affinities for CA-II, with equilibrium dissociation constants of 3.6 and 0.6 nM. QSAR analysis suggests that the key factors involved in achieving high affinity in this series are sulfonamide acidity, hydrophobicity, and minimization of steric demands at the carbon atom adjacent to the sulfonamide group.

The carbonic anhydrases[1] are zinc-containing proteins that catalyze the equilibrium

$$H_2O + CO_2 \rightleftharpoons H^+ + HCO_3^-$$

Efficient biological catalysis of this simple chemical event is important for several reasons.[2] In addition to an obvious role in facilitating the excretion of carbon dioxide evolved in respiration, carbonic anhydrases have evolved as key components of the fluid translocating machinery of several secretory tissues. For example, aqueous humor secretion in the eye is carbonic anhydrase dependent. Since the rate of aqueous humor secretion is a factor in determining intraocular pressure, carbonic anhydrase inhibitors (CAIs) have an ocular hypotensive effect that is useful in the treatment of glaucoma.[3] While CAIs have been employed clinically for several decades, significant adverse reactions have limited their use to a fraction of glaucoma patients who might benefit from CAI therapy.[4] Many of these side effects are mechanism-based, resulting from inhibition of carbonic anhydrase in nonocular tissues. Therefore, a major theme in current research on CAIs is the development of topically active compounds.[5] It is hoped that the improved drug localization obtained by topical application will significantly lower the incidence of side effects. Significant progress has been made toward obtaining a topical CAI[6] and several agents have now been evaluated in clinical trials.[7]

Our earliest efforts to obtain a topical CAI led to the benzothiazolesulfonamide 1.[8] However, clinical evaluation

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of 1 was not attempted because its chronic administration to rabbits caused an ocular inflammatory response. The possibility that this was an allergic reaction was confirmed in the Magnusson–Kligman test for dermal sensitization in guinea pigs.[9] Sensitization was observed in other classes of CAIs, including derivatives of benzofuran-2-sulfonamide and indole-2-sulfonamide[10] as well as thiadiazolesulfonamides such as acetazolamide.[11] While dermal sensitization is itself a significant problem, we also were concerned about the suggested role of the immune response in the etiology of some of the rarer but more serious side effects (e.g., bone marrow toxicity) observed during long-term therapy with acetazolamide and related sulfonamides.[12] Thus, a key goal in our effort to develop topical CAIs was to reliably predict and minimize the propensity of a compound to elicit an immune response. One path leading to allergic responses to small molecules involves formation of a covalent adduct between the allergen and biological macromolecules prior to recognition by the immune system.[13] In the case of aromatic sulfonamides, the most likely mode of reactivity is nucleophilic aromatic substitution with displacement of the sulfonamide group by a protein thiol.[14] Therefore, a compound's electrophilicity can be a useful parameter for predicting its sensitization potential. An estimate of electrophilicity in a physiological setting can be obtained by determining approximate rates of reaction in vitro between a compound and reduced glutathione (GSH) at pH 7.4 and 37 °C. In the examples cited above,[8,10] dermal sensitizers such as 1 reacted rapidly with GSH, while nonsensitizers such as 6-hydroxybenzo[*b*]thiophene-2-sulfonamide[15] were only slightly reactive. Acetazolamide, a moderate dermal sensitizer, reacts at an intermediate rate with GSH.[16] In general, potent, nonelectrophilic CAIs that were not dermal sensitizers were readily obtained in the aromatic sulfonamide series by choosing systems such as benzo[*b*]thienyl,[15] phenyl, and thienyl[6] that offer relatively weak stabilization of the tetrahedral intermediate encountered in nucleophilic aromatic substitution reactions. This successful strategy to obtain safe compounds relies on the modulation of the rates of reactions, even though it does not entirely preclude the substitution mechanism.

An alternative way to obtain nonelectrophilic CAIs is to design compounds that lack the *mechanistic potential* to undergo nucleophilic aromatic substitution reactions. An aliphatic sulfonamide passes this test, since the sulfamoyl group is not a leaving group in the S_N2 sense. When this work was begun the literature contained little information to suggest that an aliphatic sulfonamide could be a potent CAI.[17] For example, while benzenesulfonamide inhibits carbon dioxide hydration catalyzed by crude dog red blood cell carbonic anhydrase with an IC_{50} of 2 μM, α-toluenesulfonamide is a 30-fold poorer inhibitor. Simpler aliphatic sulfonamides such as methane- or ethanesulfonamide are yet weaker CAIs, with IC_{50}'s near 1 mM.[18] Described below is a potentially general route to obtaining nonaromatic sulfonamide CAIs. These compounds are sulfonylmethanesulfonamide derivatives[19] that inhibit

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Scheme I

Scheme II

Scheme III

human carbonic anhydrase II (CA-II) with IC_{50} values in the nanomolar range.

Chemistry. The possibility that an aliphatic sulfonamide could be a potent CAI was discovered during the exploration of the chemistry and structure–activity relationships of the benzo[*b*]thiophenesulfonamide class of CAIs.[15] Oxidation of **2** with *m*-chloroperoxybenzoic acid gave the benzo[*b*]thiophene dioxide **3** (Scheme I). The 2,3-double bond in **3** is an avid Michael acceptor and was reduced with sodium borohydride in ethanol to give the aliphatic sulfonamide **4**. As shown below, **4** has an affinity for human CA-II of less than 1 μM. This result inspired the synthesis of other compounds containing the sulfonylmethanesulfonamide substructure (Scheme II). Alkylation[20] of a thiol with bromomethanesulfonamide (**5**) gave sulfides **6a–d**, which were oxidized to the corresponding sulfones **7a–d** with *m*-chloroperoxybenzoic acid or Oxone.[21]

Modifications of the methylene group adjacent to the sulfamoyl group in **7d** are described in Scheme III. The monochloro derivative **8** was obtained by conversion of **6d** to the sulfoxide **12**, Pummerer rearrangement to the chlorosulfide with thionyl chloride, and oxidation with *m*-chloroperoxybenzoic acid. For the preparation of the monofluoro analog **9**, sulfoxide **12** was converted to the formamidine **13** prior to reaction with diethylamidosulfur trifluoride (DAST). The intermediate fluoro sulfide was oxidized to the sulfone and the formamidine was hydrolyzed to give **9**. Treatment of the formamidine (**14**) of **7d** with sodium hypochlorite in aqueous solution followed by hydrolysis gave the dichlorination product **10**. Methylation of **7d** was also accomplished using **14** as an intermediate. Deprotonation of **14** with sodium hydride and addition of methyl iodide provided a mixture of compounds from which **11** was obtained after formamidine hydrolysis. Finally, the polyether-containing inhibitor **7e** was prepared as shown in Scheme IV. Diethylene glycol monomethyl ether was converted to the methylthiomethyl

Scheme IV

Table I. Carbonic Anhydrase Inhibitors Related to Methanesulfonamide

	R	*n*	mp, °C	analysis[a]	IC_{50} (nM)[b]	pK_a
4	(see text)		223–226	$C_9H_{11}NO_5S_2$	800	8.8
6a	4-hydroxyphenyl	0	140–141	$C_7H_9NO_3S_2$	2900	10.1
7a	4-hydroxyphenyl	2	206–208	$C_7H_9NO_5S_2$	400	9.1
6b	2-phenylethyl	0	69–70	$C_9H_{13}NO_2S_2$	2000	9.5
7b	2-phenylethyl	2	184–186	$C_9H_{13}NO_4S_2$	100	8.4
6c	3-phenylpropyl	0	87–88	$C_{10}H_{15}NO_2S_2$	1200	9.4
7c	3-phenylpropyl	2	140–141	$C_{10}H_{15}NO_4S_2$	190	8.4
6d	*n*-octyl	0	92–94	$C_9H_{21}NO_2S_2$	480	nd
7d	*n*-octyl	2	140–141	$C_9H_{21}NO_4S_2$	22	9.4
7e	2,5,8-trioxanonyl	2	oil	$C_7H_{17}NO_7S_2$	1300	nd
	acetazolamide				10	

	X	Y	mp, °C	analysis[a]	IC_{50} (nM)[b]	pK_a
8	H	Cl	73–74	$C_9H_{20}ClNO_4S_2$	12	7.7
9	H	F	93–94	$C_9H_{20}FNO_4S_2$	3	7.5
10	Cl	Cl	72–74	$C_9H_{19}Cl_2NO_4S_2$	200	nd
11	CH_3	H	105–106	$C_{10}H_{23}NO_4S_2$	2500	8.9

[a] Satisfactory combustion analysis (C, H, N ± 0.4%) was obtained for each compound. [b] Concentration required to reduce the rate of carbonic anhydrase-catalyzed carbon dioxide hydration by 50%, pH 8.3, 4 °C, 1 nM enzyme concentration.

ether and oxidized to the sulfone **15**. Metalation of the sulfone occurred selectively at the methyl group and the resulting carbanion was converted to the sulfonamide **7e** by treatment with sulfur dioxide and hydroxylamine-*O*-sulfonic acid.[22]

Structure–Activity Relationships. Table I contains the structures, the IC_{50} values for inhibition of carbon dioxide hydration catalyzed by CA-II, and the pK_a values of these methanesulfonamide derivatives. Viewed in the light of the literature[17] on the structural requirements for carbonic anhydrase inhibition, the prototypal sulfonylmethanesulfonamide CAI, dihydrobenzo[*b*]thiophene dioxide **4**, is a surprisingly potent compound. The cyclic structure is not required for activity as evidenced by the similar potency of **4** and **7a**. However, comparing the potency of sulfides **6a–d** with the corresponding sulfones **7a–d** reveals that the sulfur oxidation state is a critical determinant of activity: the sulfones are invariably more active than the sulfides. We believe that the better potency of the sulfones is related to the enhancement of the acidity of the sulfonamide by the electronegative sulfonyl group. A positive correlation of sulfonamide acidity with CA inhibitory activity has been noted in several published studies[23] of quantitative structure–activity relationships for CA inhibition. This aspect of the SAR probably reflects the thermodynamics of the proton transfer from the sulfonamide to the zinc-bound hydroxide that occurs during sulfonamide binding.

The second qualitative trend revealed in Table I is the increase in binding of these inhibitors to CA with increasing size and hydrophobicity of the substituent R. Of particular note is the good inhibitory activity displayed by the simple

aliphatic inhibitor **7d**. The octyl group of this compound was chosen on the basis of the X-ray crystal structure[24] of carbonic anhydrase: the octyl group is of the correct size to span the hydrophobic wall of the CA active site without protruding into the surrounding solvent. The importance of the hydrophobic contribution to enzyme–inhibitor complex formation is underscored by the reduced activity of the polyether **7e**, which is roughly isosteric to the octyl compound **7d**, but is considerably more hydrophilic. This effect of inhibitor hydrophobicity on affinity for CA is not surprising given the elegant studies of King and Burgen[25] on the kinetics and thermodynamics of binding of several types of aromatic sulfonamides to CA. Those studies quantitatively showed the important contribution of hydrophobic forces to the formation of the enzyme–inhibitor complex.

The relationship between structure and activity of the halogenated and methylated derivatives of **7d** is particularly enlightening. While monochlorination (providing **8**) led to a significant increase in sulfonamide acidity (and presumably to some extent an increase in hydrophobicity), only marginal enhancement in enzyme inhibitory activity was observed. The dichloro derivative **10** is an order of magnitude *weaker* in its affinity for the enzyme. At first, these results appear to contradict the hypotheses made above as to the critical elements responsible for forming strong complexes with CA. However, the extremely weak binding of the methylated compound **11** helps to resolve this paradox. Clearly, binding at the enzyme active site is very sensitive to the steric effects of branching at the methylene adjacent to the sulfonamide, although this effect can be somewhat reduced by cyclization as in **4**. In the case of the chlorine substituent, which has similar spatial requirements to a methyl group, the acidification effect balances the steric inhibition of binding. The steric demands of the dichloro compound clearly dominate the binding of **10** and overwhelm the inductive effect of the second chlorine atom. α-Substitution with fluorine, an electronegative but sterically undemanding group, gives **9**, the most potent inhibitor in the series. This observation strongly supports our interpretation of the factors that influence the interaction between the aliphatic sulfonamide inhibitors and CA.

The binding of the halogenated inhibitors **8** and **9** was further characterized by determination of the equilibrium dissociation constant (K_D) of its CA complex by observing the decrease in fluorescence[26] upon displacement of CA-bound dansylamide by these inhibitors. The K_D for the chlorinated compound (**8**) was 17 nM, in reasonable agreement with the IC_{50} value determined for the inhibition of carbon dioxide hydration. The fluorescence intensity curve determined for the fluorinated derivative **9** deviated systematically from the theoretical curve for an equilibrium involving a single inhibitory species. Addition of a term for the presence of a second species in equilibrium with the enzyme gave an improvement in the curve fitting that was significant at the 1% level (n = 3, partial F-test). These data can be interpreted as showing differential binding of the enantiomers of **9**.[27] The deduced K_D's for the enantiomers are 3.57 ± 0.22 and 0.64 ± 0.29 nM. Thus, one of the enantiomers of **9** represents not only an extremely potent aliphatic sulfonamide CAI but also one of the most potent CAIs reported in any class! The enantioselectivity of inhibition is remarkable considering

Table II. QSAR Analysis

compd	$ClogP_R$[a]	$\Sigma\sigma_I$[b]	Δsize[c]	log IC_{50} obs	log IC_{50} calcd[d]	residual
6a	1.475	0.19	0	3.46	3.81	−0.35
7a	1.475	0.62	0	2.60	2.56	0.04
6b	3.320	0.19	0	3.30	3.22	0.08
7b	3.320	0.62	0	2.00	1.96	0.04
6c	3.849	0.19	0	3.08	3.04	0.04
7c	3.849	0.62	0	2.28	1.79	0.49
6d	4.926	0.19	0	2.68	2.70	−0.02
7d	4.926	0.62	0	1.34	1.44	−0.10
7e	−0.059	0.62	0	3.11	3.05	0.06
8	4.926	1.09	1.3	1.08	1.74	−0.67
9	4.926	1.14	0.46	0.48	0.52	−0.04
10	4.926	1.56	2.6	2.30	2.05	0.25
11	4.926	0.57	1.27	3.40	3.22	0.18

[a] Calculated log P for substituent R in Table I. [b] Sum of σ_I values for α-substituents, respectively: S, from MeS = 0.19; SO_2, from $MeSO_2$ = 0.62; Cl = 0.47; F = 0.52; Me = −0.05. [c] Difference in size (bond length + van der Waals radius) between each substituent X and hydrogen: F = 0.46 Å; Cl = 1.30 Å; Me = 1.27 Å. [d] From eq 3.

the similar sizes of a proton and fluorine atom, and efforts are underway to define the physical basis of this observation.

Quantitative Structure–Activity Analysis. The qualitative model of the binding of sulfonylmethanesulfonamides to CA presented above suggested three key contributors to enzyme inhibitor complex formation: the hydrophobicity of the substituent R, the acidity of the sulfonamide, and the size of substituents added to the methylene adjacent to the sulfonamide (the α-carbon). Linear regression analysis was performed on the set of compounds (excluding **4**) utilizing three parameters related to these factors: the calculated[28] log P value for octanol–water partitioning of a hydrocarbon corresponding to the substituent R ($ClogP_R$); the sum of the inductive effect, characterized by the Taft σ_I parameter, of all substituents on the α-carbon ($\Sigma\sigma_I$); and a steric parameter, Δsize, characterizing the increased size of an α-substituent, X versus hydrogen, as defined by the sum of the bond length (b), and van der Waals radius (r): Δsize = $(b_X + r_X) - (b_H + r_H)$. For the purposes of the regression analysis, the hypothetical steric effect of the two α-chlorine substituents in **10** was assumed to be simply additive. The parameter values are presented in Table II. Of the equations obtained in attempting to correlate log IC_{50} with any single parameter, only the relationship characterizing the inductive effect of substituents adjacent to the sulfonamide was statistically significant at the 5% level:

$$\log IC_{50} = 3.29 - (1.43 \pm 0.53)\sum \sigma_I \quad (1)$$

$$n = 13, r^2 = 0.40, s = 0.77, F_{1,11} = 7.34$$

Of the possible two parameter equations, only that involving the inductive and steric parameters, $\Sigma\sigma_I$ ($p < 0.01$) and Δsize ($p < 0.02$) improved upon eq 1 in a statistically significant manner:

$$\log IC_{50} = 3.78 - (2.85 \pm 0.66)\sum \sigma_I + (0.95 \pm 0.34)\Delta size \quad (2)$$

$$n = 13, r^2 = 0.66, s = 0.60, F_{2,10} = 9.80$$

Finally, addition of the term describing the hydrophobicity of the substituent R gave eq 3, in which each of the

parameters contributed in a statistically significant fashion ($p < 0.001$):

$$\log IC_{50} = 4.84 - (0.32 \pm 0.06)ClogP_R - (2.91 \pm 0.35)\sum\sigma_I + (1.28 \pm 0.19)\Delta size \quad (3)$$

$$n = 13, r^2 = 0.91, s = 0.32, F_{3,9} = 31.89$$

Values of log IC_{50} obtained with eq 3 are compared to experimental values in Table II.

The success of eq 3 in predicting the affinity of these molecules for carbonic anhydrase provides substantial support to the qualitative SAR analysis given above. The dominant factor is the inductive effect of substituents adjacent to the sulfonamide, which impacts primarily on sulfonamide acidity. A Hammett relationship in terms of σ_I was found to apply to the 10 compounds in Table I for which experimental pK_a data were obtained:

$$pK_a = 10.14 \pm 0.24 - (2.22 \pm 0.36)\sigma_I \quad (4)$$

$$n = 10, r^2 = 0.82, s = 0.37, F_{1,8} = 37.5$$

It is interesting to note that the coefficients of σ_I in eq 4 (describing ΔpK_a) and of $\Sigma\sigma_I$ in eq 3 (describing log IC_{50}) are of nearly the same size. A similar result was obtained by Hansch and co-workers in their analysis of the series of benzenesulfonamides studied by Kakeya.[23] Thus, in these two classes of CAIs, log IC_{50} and pK_a are similarly related by a linear function with a slope near 1.

The next most important term in eq 3 is the steric parameter described by Δsize. Equation 3 amply confirms the intuitive notion that the sulfonamide binding site is fairly restricted in size. For each angstrom increase in size of an α-substituent compared to hydrogen, more than 1 order of magnitude is lost in potency.

Finally, a relatively modest effect of the hydrophobicity of the R group on activity is indicated by the significant contribution to eq 3 of the calculated log P value for these substituents ($ClogP_R$). This contribution (coefficient of 0.32) is somewhat smaller than might have been anticipated on the basis of King and Burgen's homologous series of *p*-alkylbenzenesulfonamides, where Hansch and co-workers[23a] found a coefficient for log P between 0.64 and 0.72. This may reflect occupancy of somewhat different binding domains in the enzyme active site for the two classes of compounds. However, the hydrophobic binding element clearly can be exploited in optimizing the activity of the methanesulfonamide class of CAIs.

During the course of this work, a patent[29] was issued that noted an ocular hypotensive response to topical administration of salts of trifluoromethanesulfonamide (16) in water-loaded rabbits. Inhibition of carbonic

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anhydrase was suggested to be mechanistically involved in this event, although the CA inhibitory activity of 16 was not reported. Given a $ClogP_R$ value of 0 (i.e., no substituent R), eq 3 predicts an IC_{50} value of 30 nM for 16. Under the conditions of our assay, the IC_{50} of 16 is 13 nM. Using the fluorescence quenching method, a K_D value of 50 nM was obtained. This further demonstrates the dominant nature of the inductive effect, which emerged from the QSAR analysis. At the other end of the spectrum, eq 3 predicts an IC_{50} of 0.07 mM for methanesulfonamide. This can be roughly compared to the literature value of 0.30 mM, which was measured using a crude dog red blood cell CA preparation.[18]

Table III. Inhibitioin of Ciliary Process Carbonic Anhydrase by 9 in an ex Vivo Assay[a]

dose (mg)	CA activity (mL NaOH min^{-1}) vehicle	treated	% inhibition
0.25	0.481 ± 0.021	0.043 ± 0.006	91
0.05	0.509 ± 0.036	0.147 ± 0.026	71

[a] A suspension of 9 or vehicle (0.5% (hydroxyethyl)cellulose) was instilled (one 50-μL drop) into both eyes of an albino rabbit 1 h prior to the removal of the iris–ciliary body. Carbonic anhydrase activity was assayed in the homogenized tissue by the pH stat assay described in the Experimental Section. The enzyme activity is expressed as the volume per minute of 0.025 N NaOH solution required to maintain the pH of the assay mixture. Values are group means ± SD for $n = 6$.

Biological Evaluation

(Octylsulfonyl)methanesulfonamide (**7d**) was evaluated for dermal sensitization potential in the Magnusson–Kligman protocol[9] and found not to be a sensitizer. This is consistent with the hypothesis that allergenicity in sulfonamide inhibitors of CA has its origins in the electrophilic nature of certain of the aromatic sulfonamides. The sensitization potential of the halogenated derivatives has not been evaluated. Compound **7d** was also found to be nonmutagenic in the Ames assay.[30]

The fluorinated inhibitor **9** was evaluated in rabbits in an ex vivo experiment for its ability after topical administration to inhibit CA activity in a homogenate of the iris–ciliary body.[31] A suspension of **9** was prepared in 0.5% (hydroxyethyl)cellulose vehicle and applied as a single 50-μL drop to the eyes of albino rabbits. After a 1-h period, the animals were sacrificed and the iris–ciliary body was excised and homogenized. Residual CA activity was determined and expressed as a percent inhibition with respect to a vehicle-treated control animal (Table III). Dosing with 0.05 and 0.25 mg of **9** gave 71% and 91% inhibition, respectively. Thus, **9** is somewhat less active than MK-927, a topically active CAI that lowers intraocular pressure in humans, which is 90% inhibitory in this ex vivo assay at a dose of 0.05 mg.[32] However, the activity is comparable to that shown by the thiophenesulfonamide 17, which gives 63% and 96% inhibition at the same dose

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levels. The latter compound is active in a number of pharmacological models of ocular hypertension.[31] The observation of even this moderate level of activity for **9** in this assay is encouraging, since its design did not consider any structural features that might contribute to ocular bioavailability.

Conclusions

A new class of CAIs, the sulfonylmethanesulfonamides, was discovered. These inhibitors are unusual in that the sulfonamide moiety is attached to an sp^3 carbon atom, in violation of one of the historically accepted rules[17] for the

structural requirements of CAIs. The apparent key to successfully violating that rule is the introduction of the sulfonyl substituent that acidifies the sulfonamide relative to the typical aliphatic sulfonamide (e.g., methanesulfonamide, pK_a = 10.8[33]). Acidification compensates for a decrement in activity engendered by inserting a tetrahedral carbon atom into the sterically congested neighborhood of the active-site zinc atom. For optimal activity, additional electronegative substituents are beneficial, provided that they are small, again a consequence of the limited volume of the enzyme active site near the zinc atom.

A three-parameter QSAR equation derived from the study of the methanesulfonamide inhibitors in Table I accounts for 91% of the variance in the measured log IC_{50}. This analysis indicated that the sum of the inductive effects of substituents adjacent to the sulfonamide ($\sum\sigma_I$) is the principle determinant of binding energy. The dominance of inductive effects is clearly manifested in the good CA inhibitory activity of trifluoromethanesulfonamide (16, IC_{50} = 13 nM, pK_a = 6.3[33]). Furthermore, the activity of 16 was successfully predicted by the QSAR equation (eq 3). A positive correlation between sulfonamide acidity and affinity for CA is not surprising and has been previously noted in the aromatic sulfonamides. In fact, the slope of the correlation of pK_a and log IC_{50} is similar in the aromatic and aliphatic series. The second parameter in the QSAR equation revealed a significant steric inhibition to binding by substituents on the carbon α to the sulfonamide. Finally, the third parameter in the QSAR equation indicated a beneficial effect on enzyme inhibitory activity of hydrophobic elements, which mirrors the SAR established for aromatic inhibitors. The combination of these three features of the structure–activity relationship clearly accounts for the exceptional activity of the fluorinated sulfonamide 9 (IC_{50} = 3 nM).

A key goal in pursuing nonaromatic sulfonamide CAIs was the elimination of electrophilic reactivity by mechanistically precluding the nucleophilic substitution reactions that were implicated in the development of dermal and ocular sensitization by some heterocyclic sulfonamides. Sulfonylmethanesulfonamides, in general, should not be electrophilic, since neither sulfinate nor the sulfonamide group should be leaving groups in the S_N2 sense. While the chloro and fluoro derivatives 8 and 9 fail to satisfy the goal of excluding reactivity on mechanistic grounds, the fluorinated compound 9 should not be particularly electrophilic, given the poor leaving-group potential of fluoride.

Finally, while the principles outlined above suggest several ways to improve the intrinsic activity of these CAIs, the structural features required to obtain compounds suitable for topical administration remain undefined.

Experimental Section

Unless otherwise noted, solvents and reagents were obtained from commercial sources and were used as received. Reactions involving air-sensitive reagents were carried out under an inert atmosphere of nitrogen or argon. THF (Fisher anhydrous grade) was further purified by distillation from sodium–benzophenone ketyl. Standard workup referred to in the experimental procedures involved dilution with an organic solvent and washing with dilute hydrochloric acid, 10% sodium bicarbonate, and brine. The organic solution was dried over sodium sulfate, filtered, and evaporated on a rotary evaporator. Melting points were determined in open capillaries on a Thomas-Hoover apparatus. ^{1}H-NMR spectra were obtained at 90 or 300 MHz. Elemental analyses were performed by Mr. J. Moreau of the Medicinal Chemistry Department. Note that many of the thiols employed in the syntheses have objectionable odors. Synthetic manipulations should be carried out in an efficient fume hood, and glassware should be cleaned with bleach before it is removed from the hood.

2,3-Dihydro-6-methoxy-2-sulfamoylbenzo[*b*]thiophene 1,1-Dioxide (4). A mixture of 2.36 g (9.7 mmol) of 6-methoxy-2-sulfamoylbenzo[*b*]thiophene (2) and 4.6 g (21.3 mmol) of *m*-chloroperoxybenzoic acid in 25 mL of methylene chloride was refluxed for 15 h. The mixture was cooled and diluted with sufficient ethyl acetate to dissolve all the solids present. The solution was washed with ice-cold 5% $NaHCO_3$ solution, 10% HCl, and brine. The solution was dried and evaporated to give a solid, which was triturated with ether. Filtration gave 2.0 g of 6-methoxy-2-sulfamoylbenzo[*b*]thiophene 1,1-dioxide (3): ^{1}H NMR (DMSO-d_6) δ 8.11 (2H, br s), 8.05 (1H, s), 7.76 (1H, d, J = 8 Hz), 7.60 (1H, d, J = 2 Hz), 7.30 (1H, dd, J = 2, 8 Hz), 3.90 (3H, s).

Compound 3 (200 mg, 0.73 mmol) was dissolved in 1.5 mL of DMSO and added rapidly dropwise to a stirred solution of 200 mg of $NaBH_4$ in 5 mL of 95% ethanol. After 30 min, the mixture was poured into 10% HCl, extracted with ethyl acetate, and worked up in the standard manner to provide 0.20 g 4 as a white solid: EI mass spectrum m/z 277; ^{1}H NMR (DMSO-d_6) δ 7.50 (3H, m), 7.25 (2H, m), 5.30 (1H, t, J = 8 Hz), 3.80 (3H, s), 3.60 (2H, t, J = 8 Hz).

Representative Procedure for the Alkylation of Thiols with Bromomethanesulfonamide: [(4-Hydroxyphenyl)thio]methane sulfonamide (6a). A solution of 3.6 g (28.7 mmol) of 4-hydroxybenzenethiol in 40 mL of DMF was added dropwise under a nitrogen atmosphere to a suspension of 1.2 g (31 mmol) of a 60% mineral oil dispersion of sodium hydride in 50 mL of DMF. **Caution: gas evolution and exothermic reaction.** After the reaction had subsided, 5.0 g of bromomethanesulfonamide[20] was added and the mixture was heated at 70 °C for 2 h. The mixture was cooled and extracted with hexane to remove the mineral oil (from the sodium hydride dispersion) and the DMF was evaporated under vacuum. The residue was partitioned between ethyl acetate and water and the organic phase was worked up in the standard way. The solid residue was purified by trituration with ether to give 4.1 g of **6a**: ^{1}H NMR (DMSO-d_6) δ 8.60 (1H, br s), 7.35 (2H, d, J = 9 Hz), 6.90 (2H, br s), 6.70 (2H, d, J = 9 Hz), 4.21 (2H, s).

[(2-Phenylethyl)thio]methanesulfonamide (6b): ^{1}H NMR (DMSO-d_6) δ 7.25 (5H, m), 6.97 (2H, s), 4.03 (2H, s), 3.02 (2H, t), 2.86 (2H, t).

[(3-Phenylpropyl)thio]methanesulfonamide (6c): ^{1}H NMR (DMSO-d_6) δ 7.02 (5H, m), 4.93 (2H, br s), 3.93 (2H, s), 2.73 (4H, m), 1.97 (2H, m).

(Octylthio)methanesulfonamide (6d): ^{1}H NMR (DMSO-d_6) δ 4.84 (2H, br s), 3.97 (2H, s), 2.86 (2H, t), 1.60 (2H, m), 1.30 (10H, m), 0.88 (3H, t).

Representative Procedure for the Oxidation of Thiomethanesulfonamides with *m*-Chloroperoxybenzoic Acid: [(4-Hydroxyphenyl)sulfonyl]methanesulfonamide (7a). To a solution of **6a** (3.5 g, 18.7 mmol) in 25 mL of ethyl acetate was added 7.9 g of 85% *m*-chloroperoxybenzoic acid (37.4 mmol) as a solution in 50 mL of ethyl acetate. After 10 min the solution was washed with 5% sodium bisulfite and then worked up in the standard manner. The crude solid was triturated with boiling toluene and subsequently recrystallized from water affording 1.7 g of **7a**: ^{1}H NMR (DMSO-d_6) δ 7.80 (1H, br s), 7.70 (2H, d, J = 9 Hz), 7.20 (2H, br s), 6.90 (2H, d, J = 9 Hz), 4.97 (2H, s).

[(2-Phenylethyl)sulfonyl]methanesulfonamide (7b): ^{1}H NMR (DMSO-d_6) δ 7.38 (2H, br s), 7.26 (5H, br s), 4.97 (2H, s), 3.58 (2H, m), 3.03 (2H, m).

[(3-Phenylpropyl)sulfonyl]methanesulfonamide (7c): ^{1}H NMR (DMSO-d_6) δ 7.32 (2H, br s), 7.22 (5H, m), 4.93 (2H, s), 3.33 (2H, t), 2.70 (2H, t), 2.06 (2H, m).

(Octylsulfonyl)methanesulfonamide (7d): ^{1}H NMR (DMSO-d_6) δ 7.34 (2H, s), 4.92 (2H, s), 3.32 (2H, t), 1.70 (2H, m), 1.36 (2H, m), 1.24 (8H, m), 0.86 (3H, t).

(2,5,8-Trioxanonylsulfonyl)methanesulfonamide (7e). Diethylene glycol monomethyl ether (20 g, 170 mmol) was dissolved in 100 mL of DMSO with 12.5 mL of acetic acid. The mixture

was cooled to 10 °C and 70 mL of acetic anhydride was added over a 5-min period. Stirring was continued for 1 h in the ice bath and 48 h at room temperature. The mixture was poured into 1.2 L 10% $NaHCO_3$, stirred for 9 h, and extracted with ether (4 × 200 mL). The extract was washed with brine, dried, and evaporated. The residual oil, which contained a substantial amount of acetoxymethyl methyl sulfide, was redissolved in 100 mL methanol. Water (270 mL) was added followed by 30 mL of 10 N NaOH over a 60-s period. After 45 min the solution was extracted with ether (2 × 200 mL). The extract was washed with brine, dried, and evaporated to give 10.2 g of a colorless oil: ^1H NMR ($CDCl_3$) δ 4.7 (2H, s), 4.75–4.55 (8H, m), 3.40 (3H, s), 2.17 (3H, s). The oil (3.5 g, 19 mmol) was added dropwise over 10 min to a solution of 26 g of Oxone (42 mmol) in 200 mL of water. After 18 h, the mixture was given a standard workup to afford 1.9 g of methyl 2,5,8-trioxanonyl sulfone (15). ^1H NMR (acetone-d_6) δ 4.58 (2H, s), 3.98 (2H, m), 3.67–3.45 (6H, m), 3.29 (3H, s), 2.90 (3H, s).

Sulfone 15 (1.5 g, 7.1 mmol) was dissolved in THF with 5 mg of bipyridyl as an indicator, the solution was cooled to −65 °C and 5.2 mL of 1.5 M *n*-butyllithium was added over a 10-min period, resulting in a pink suspension. After 10 min, sulfur dioxide gas was introduced into the flask until the red color of the solution was discharged, while the temperature was maintained at ≤−60 °C. The solution was diluted with ether and the solid sulfinic acid salt was isolated by filtration. This salt was dissolved in 25 mL of 5% $NaHCO_3$ solution and 0.90 g of hydroxylamine-*O*-sulfonic acid (HOSA) (8.0 mmol) was added. Standard workup gave 0.9 g of a viscous oil. The aqueous phase was treated with another 0.5 g portion of HOSA for 18 h to afford an additional 0.26 g of the desired product. The combined materials were partially purified by chromatography on silica gel (1–5% methanol/chloroform). This material (0.50 g) was further purified by adsorption on an IRA-400 column (hydroxide form), washing the column with water, and elution of the title compound with 1 N HCl. The water-soluble product was extracted into ethyl acetate, the extract was washed with brine, dried, and evaporated to yield 96 mg of pure sulfonamide 7e. ^1H NMR (acetone-d_6) δ 6.60 (2H, br s), 4.88 (2H, s), 4.86 (2H, s), 4.03 (2H, s), 3.67–3.47 (6H, s), 3.29 (3H, s).

(Octylsulfonyl)chloromethanesulfonamide (8). A solution of 5.9 g of 6d in THF was cooled to −70 °C and a solution of *m*-CPBA in THF was added dropwise. After addition was complete, the cooling bath was removed and the mixture was allowed to warm to room temperature. Filtration gave 5.4 g of (octylsulfinyl)methanesulfonamide (12) as a white solid: ^1H NMR (DMSO-d_6) δ 7.26 (2H, s), 4.64 (1H, d, J = 14 Hz), 4.28 (1H, d, J = 14 Hz), 2.94 (2H, m), 1.66 (2H, m), 1.28 (10H, m), 0.86 (3H, t).

To a suspension of 5.4 g of 12 in 250 mL of methylene chloride was added 2.4 mL of thionyl chloride, dropwise with stirring. The mixture was refluxed for 3 h, cooled, and extracted with 10% sodium bicarbonate. The solution was dried and evaporated to provide a somewhat unstable chloro sulfide intermediate. This intermediate was dissolved in THF and cooled in an ice bath and a solution of 10.1 g of *m*-CPBA in THF was added. The cooling bath was removed and the mixture was stirred at ambient temperature for 16 h. The solvent was evaporated and the residual oil was dissolved in ether. The ether solution was washed with 5% sodium carbonate and brine. Workup was continued as in the standard method, affording 2.5 g of crude product. Purification on silica gel gave 0.42 g of 8: ^1H NMR ($CDCl_3$) δ 5.56 (1H, s), 5.33 (2H, br s), 3.83 (2H, m), 1.93 (2H, m), 1.30 (10H, m), 0.88 (3H, t).

(Octylsulfonyl)fluoromethanesulfonamide (9). A mixture of 1.10 g (4.3 mmol) of 12 and 2.8 mL (21 mmol) of dimethylformamide dimethyl acetal in 30 mL of acetonitrile was stirred overnight at room temperature. The solvent was removed and the residue was dried under vacuum overnight to provide 1.55 g of *N*-[[(octylsulfinyl)methyl]sulfonyl]formamidine (13).

To a stirring solution of 1.35 g (4.3 mmol) of 13 in 40 mL of chloroform was added 1.5 mL of diethylamidosulfur trifluoride (DAST) and 4 mg of ZnI_2. The mixture was stirred for 16 h at room temperature in the dark and poured into a mixture of 20 mL of saturated sodium bicarbonate and 25 g of ice. The organic phase was separated, dried, and evaporated. The residue was dissolved in 50 mL of chloroform and 2.4 g of *m*-CPBA was added. The mixture was stirred overnight at room temperature, then refluxed for 2 h, and evaporated and the residue was partitioned between ether and dilute sodium bisulfite. The organic phase was washed with sodium carbonate solution and worked up in the standard manner. Crystallization from ethyl acetate/hexane gave 0.81 g of *N*-[[(octylsulfonyl)methyl]sulfonyl]formamidine (14). The mother liquors were evaporated and chromatographed to provide another 0.88 g of 14, and 0.30 g of the formamidine of 9. This material was dissolved in 20 mL of absolute ethanol at room temperature and 3 mL of 0.5 N NaOH was added. After 3 h the ethanol was evaporated and the residue was partitioned between ether and 1 N HCl. Standard workup and silica gel chromatography gave 120 mg of 9: ^1H NMR ($CDCl_3$) δ 5.78 (1H, d, J = 45 Hz), 5.30 (2H, br s), 3.35 (2H, m), 1.92 (2H, m), 1.50 (2H, m), 1.30 (8H, m), 0.88 (3H, t).

(Octylsulfonyl)dichloromethanesulfonamide (10). A suspension of 1.00 g (3.68 mmol) of 7d in 10 mL of ethyl acetate was treated with 0.54 mL (4.0 mmol) of dimethylformamide dimethyl acetal. An exothermic reaction ensued and the reaction mixture transiently became homogeneous. The mixture was diluted with 75 mL of ethyl acetate and the resulting solution was worked up in the standard way to afford 1.20 g of N-[[(octylsulfonyl)methyl]sulfonyl]formamidine (14): ^1H NMR ($CDCl_3$) δ 7.99 (1H, s), 4.45 (2H, s), 3.35 (2H, t, J = 9 Hz), 3.20 (3H, s), 3.09 (3H, s), 1.84 (2H, m), 1.45 (2H, m), 1.29 (8 H, m), 0.87 (3H, t, J = 6 Hz).

To a stirring solution of 500 mg (1.5 mmol) of 14 in 25 mL of dioxane was added 4.5 mL (3.4 mmol) of 5.25% sodium hypochlorite solution. After 30 min, sufficient 5.25% sodium hypochlorite solution was added to obtain a homogeneous reaction mixture and stirring was continued for 3 h. The mixture was diluted with ethyl acetate. Standard workup and chromatography gave the formamidine of 10, which was converted to 10 as described in the synthesis of 9. Crystallization from ether/hexane gave 0.38 g of 10: ^1H NMR ($CDCl_3$) δ 5.70 (2H, br s), 3.68 (2H, m), 2.00 (2H, m), 1.48 (2H, m), 1.32 (8H, m), 0.88 (3H, t).

1-(Octylsulfonyl)ethanesulfonamide (11). Formamidine 14 (1.062 g, 3.25 mmol) was dissolved in 3 mL of dry DMF, and 130 mg of a 60% oil dispersion of sodium hydride (3.25 mmol) was added portionwise. When the foaming and gas evolution had ceased, methyl iodide (0.20 mL, 3.2 mmol) was added to the suspension. An exothermic reaction ensued. The mixture was stirred at ambient temperature for 45 min, and 3 mL of 10 N sodium hydroxide solution and 25 mL of water were added. After stirring overnight, the mixture was diluted with 25 mL of water and acidified with 6 N hydrochloric acid. The precipitated product was isolated by filtration. ^1H NMR of the crude product shows a mixture of 11, the product of dimethylation, and unreacted starting material. The products were separated by chromatography (silica gel, 3% $MeOH/CHCl_3$). Fractions containing the desired product were pooled and recrystallized from hexane/ethyl acetate to afford 0.18 g of 11 as white plates: ^1H NMR ($CDCl_3$) δ 5.21 (2H, br s), 4.34 (1H, q, J = 8 Hz), 3.40 (2H, m), 1.87 (3H, d, J = 8 Hz), 1.45 (2H, m), 1.30 (8H, m), 0.87 (3H, t, J = 6 Hz).

Purification of Carbonic Anhydrase. The enzyme was isolated as described in ref 26b.

In Vitro Inhibition of Carbonic Anhydrase. Inhibition of the purified human erythrocyte carbonic anhydrase II was assessed by using a pH stat assay.[34] This assay measures the rate of hydration of CO_2 by determining the rate at which a standard solution of NaOH has to be added to a lightly buffered solution to maintain a constant pH as CO_2 is bubbled into the buffer. Enzymatic activity is proportional to the volume of a standard NaOH solution that is required to maintain the pH at a given value, e.g., 8.3. To 4 mL of 0.02 M Tris-chloride buffer, pH 8.6, in a 5-mL Radiometer V531 jacketed assay vessel equilibrated at 2 °C was added buffer-diluted enzyme (25 μL). CO_2/air (5:95) was bubbled into the assay vessel at a rate of 150 mL/min. The pH stat end point was set at pH 8.3, and the volume of 0.025 N NaOH added over a 3-min period in order to maintain pH 8.3 was measured. Enzyme inhibition was measured by the addition of an inhibitor in 0.1–3.9 mL of buffer followed by the addition of enzyme and titration with NaOH. Results were expressed as IC_{50} values, which were obtained from semilog plots of percent inhibition against log concentration.

In Vitro Binding for Human Carbonic Anhydrase II. The binding of test compounds to purified human erythrocyte carbonic anhydrase II was determined by a fluorescence competition assay employing the fluorescent CA inhibitor dansylamide. This compound has been shown to produce a large increase in fluorescence upon binding to the active site of carbonic anhydrase.[26] A fluorescence cuvette containing 1×10^{-7} M human CA II (HCA II) and 2×10^{-5} M dansylamide in pH 7.4, 0.1 ionic strength phosphate buffer was placed in the thermostated cell holder of a Perkin-Elmer MPF-44B fluorescence spectrophotometer. The temperature was maintained at 37 °C by using a constant-temperature water circulator. The excitation and emission wavelengths were set at 280 and 460 nm, respectively. Fluorescence intensities were recorded following addition, with stirring, of small, measured aliquots of a solution of the test compound in pH 7.4 buffer. The resulting data were converted to fluorescence intensity vs compound concentration, corrected for dilution by the titrant, and fitted by nonlinear least squares to a model in which the compound and dansylamide compete for a single binding site on HCA II. The dissociation constant of the dansylamide–HCA II complex, which is needed for these calculations, was found to be 1.98×10^{-6} M under these conditions. All binding determinations were done a minimum of three times.

Guinea Pig Sensitization Test. The Magnusson–Kligman test as implemented in our laboratories is described in ref 10.

Ex Vivo Inhibition of Carbonic Anhydrous in a Homogenate of Rabbit Iris–Ciliary Body. The experimental conditions are described in detail in ref 31.

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References

(1) Pocker, Y.; Sarkanen, S. Carbonic anhydrase: structure, catalytic versatility, and inhibition. *Adv. Enzymol. Relat. Areas Mol. Biol.* 1978, *47*, 149–274.

(2) Maren, T. H. Carbonic anhydrase: chemistry, physiology and inhibition. *Physiol. Rev.* 1967, *47*, 595–781.

(3) (a) Becker, B. Decrease in intraocular pressure in man by a carbonic anhydrase inhibitor, Diamox. *Am. J. Ophthalmol.* 1954, *37*, 13–14. (b) Maren, T. H. The rates of movement of Na^+, Cl^- and HCO_3^- from plasma to posterior chamber. Effect of acetazolamide and relation to the treatment of glaucoma. *Invest. Ophthalmol. Visual Sci.* 1976, *15*, 356–364.

(4) (a) Epstein, D. L.; Grant, W. M. Carbonic anhydrase inhibitor side-effects; serum chemical analysis. *Arch. Ophthalmol.* 1977, *95*, 1378–1382. (b) Lichter, P. R.; Newman, L. P.; Wheeler, N. C.; Beal, O. V. Patient tolerance to carbonic anhydrase inhibitors. *Am. J. Ophthalmol.* 1978, *85*, 495–502.

(5) Maren, T. H. The development of ideas concerning the role of carbonic anhydrase in the secretion of aqueous humor: relation to the treatment of glaucoma. In *Glaucoma: Applied Pharmacology in the Medical Treatment*. Drance, S. M., Ed., Grune and Stratton, New York, 1984; pp 325–355.

(6) For leading references, see: Shepard, K. L.; Graham, S. L.; Hudcosky, R. J.; Michelson, S. R.; Scholz, T. H.; Schwam, H.; Smith, A. M.; Sondey, J. M.; Strohmaier, K. M.; Smith, R. L.; Sugrue, M. F. Topically active carbonic anhydrase inhibitors. 4. [(Hydroxyalkyl)sulfonyl]benzene and [(hydroxyalkyl)sulfonyl]thiophenesulfonamides. *J. Med. Chem.* 1991, *34*, 3098–3105.

(7) (a) Baldwin, J. J.; Ponticello, G. S.; Anderson, P. S.; Christy, M. E.; Murcko, M. A.; Randall, W. C.; Schwam, H.; Sugrue, M. F.; Springer, J. P.; Gautheron, P.; Grove, J.; Mallorga, P.; Viader, M. P.; McKeever, B.; Navia, M. A. Thienothiopyran-2-sulfonamides: novel topically active carbonic anhydrase inhibitors for the treatment of glaucoma. *J. Med. Chem.* 1989, *32*, 2510–2513. (b) Baldwin, J. J.; Ponticello, G. S.; Sugrue, M. F. MK-507. *Drugs Future* 1990, *15*, 350–351.

(8) Woltersdorf, O. W., Jr.; Schwam, H.; Bicking, J. B.; Brown, S. L.; deSolms, S. J.; Fishman, D. R.; Graham, S. L.; Gautheron, P. D.; Hoffman, J. M.; Larson, R. D.; Lee, W. S.; Michelson, S. R.; Robb, C. M.; Share, N. N.; Shepard, K. L.; Smith, A. M.; Smith, R. L.; Sondey, J. M.; Strohmaier, K. M.; Sugrue, M. F.; Varga, S. L. Topically active carbonic anhydrase inhibitors. 1. O-Acyl derivatives of 6-hydroxybenzothiazole-2-sulfonamide. *J. Med. Chem.* 1989, *32*, 2486–2492.

(9) Magnusson, B.; Kligman, A. M. The identification of contact allergens by animal assay. The guinea pig maximization test. *J. Invest. Dermatol.* 1969, *53*, 268–276.

(10) Graham, S. L.; Hoffman, J. M.; Gautheron, P.; Michelson, S. R.; Scholz, T. H.; Schwam, H.; Shepard, K. L.; Smith, A. M.; Smith, R. L.; Sondey, J. M.; Sugrue, M. F. Topically active carbonic anhydrase inhibitors. 3. Benzofuran- and indole-2-sulfonamides. *J. Med. Chem.* 1990, *33*, 749–754.

(11) Acetazolamide is a moderate sensitizer (dermal reaction in five of 11 animals) in the Magnusson–Kligman test using a protocol described in ref 10. Personal communication, P. Gautheron, Centre de Recherche MSD-Chibret, Riom, France.

(12) Reviewed: Zimran, A.; Beutle, E. Can the risk of acetazolamide induced aplastic anemia be decreased by periodic monitoring of blood cell counts. *Am. J. Ophthalmol.* 1987, *104*, 654–658.

(13) Eisen, H. N. *Immunology*; Harper and Row: Hagerstown, MD, 1980.

(14) (a) Conroy, C. W.; Schwam, H.; Maren, T. H. The nonenzymatic displacement of the sulfamoyl group from different classes of aromatic compounds by glutathione and cysteine. *Drug Metab. Dispos.* 1984, *12*, 614–618. (b) Colluci, D. F.; Buyske, P. A. The biotransformation of a sulfonamide to a mercaptan and to mercapturic acid and glucuronide conjugates. *Biochem. Pharmacol.* 1965, *14*, 457–466.

(15) Graham, S. L.; Shepard, K. L.; Anderson, P. S.; Baldwin, J. J.; Best, D. R.; Christy, M. E.; Freedman, M. B. Gautheron, P.; Habecker, C. N.; Hoffman, J. M.; Lyle, P. A.; Michelson, S. R.; Ponticello, G. S.; Robb, C. M.; Schwam, H.; Smith, A. M.; Smith, R. L.; Sondey, J. M.; Strohmaier, K. M.; Sugrue, M. F.; Varga, S. L. Topically active carbonic anhydrase inhibitors. 2. Benzo[b]thiophenesulfonamide derivatives with ocular hypotensive activity. *J. Med. Chem.* 1989, *32*, 2548–2554.

(16) Using the protocol described in ref 10, acetazolamide reacts with GSH to the extent of 16 ± 1% (n = 3) in 20 h. Michelson, S. R.; Schwam, H. unpublished.

(17) "The criteria for activity [of CAIs] are the simplest in pharmacology; all unsubstituted aromatic sulfonamides...inhibit carbonic anhydrase, and no other class of organic compounds approaches these in activity" from: Maren, T. H. Relations between structure and biological activity of sulfonamides. *Annu. Rev. Pharmacol. Toxicol.* 1976, *16*, 309–327.

(18) Maren, T. H.; Wiley, C. E. The in vitro activity of sulfonamides against red blood cell carbonic anhydrases. Effect of ionic and substrate variation on the hydration reaction. *J. Med. Chem.* 1968, *11*, 228–232.

(19) Graham, S. L.; Scholz, T. H. Alkanesulfonamides as antiglaucoma agents. U.S. Patent 4,812,463, 1989.

(20) (a) Goralski, C. T.; Klingler, T. C. 1-(Arylthio)methanesulfonamides *J. Chem. Eng. Data* 1976, *21*, 237–238. (b) Schlor, H. H.; Grewe, F. Fungicidal composition containing phenylmercaptomethane sulfonamide and method of using the same. U.S. Patent 3,501,578, 1970.

(21) Trost, B. M.; Curran, D. P. Chemoselective oxidation of sulfides to sulfones with potassium hydrogen persulfate. *Tetrahedron Lett.* 1981, *22*, 1287–1290.

(22) Graham, S. L.; Scholz, T. H. The reaction of sulfinic acid salts with hydroxylamine-O-sulfonic acid. A convenient synthesis of primary sulfonamides. *Synthesis* 1986, 1031–1032.

(23) (a) Hansch, C.; McClarin, J.; Klein, T.; Langridge, R. A. Quantitative structure-activity relationship and molecular graphics study of carbonic anhydrase inhibitors. *Mol. Pharmacol.* 1985, *27*, 493–498. (b) Kakeya, N.; Aoki, M.; Kamada, A.; Yata, N. Biological activities of drugs. VI. Structure–activity relationship of sulfonamide carbonic anhydrase inhibitors. *Chem. Pharm. Bull. (Tokyo)* 1969, *17*, 1010–1018. (c) Kakeya, N.; Yata, N.; Kamada, A.; Aoki, M. Biological activities of drugs. VIII. Structure-activity relationship of sulfonamide carbonic anhydrase inhibitors. *Chem. Pharm. Bull. (Tokyo)* 1969, *17*, 2558–2564.

(24) Kannan, K. K.; Vaara, I.; Notstrand, B.; Lövgren, S.; Borell, A.; Fridborg, K.; Petef, M. Structure and function of carbonic anhydrase: comparative studies of sulphonamide binding to human erythrocyte carbonic anhydrases B and C. In *Drug Action at the Molecular Level*; Roberts, G. C. K., Ed.; MacMillan Press: London, 1977, pp 73–91. The crystallographic coordinates of the enzyme are available through the Brookhaven Protein Data Bank File: ICAC (May 26, 1976).

(25) King, R. W.; Burgen, A. S. V. Kinetic aspects of structure-activity relations: the binding of sulphonamides by carbonic anhydrase. *Proc. R. Soc. London B.* 1976, *193*, 107–125.

(26) (a) Chen, R. F.; Kernohan, J. C. Combination of bovine carbonic anhydrase with a fluorescent sulfonamide. *J. Biol. Chem.* 1967, *242*, 5813. (b) Ponticello, G. S.; Freedman, M. B.; Habecker, C. N.; Lyle, P. A.; Schwam, H.; Varga, S. L.; Christy, M. E.; Randall, W. C.; Baldwin, J. J. Thienothiopyran-2-sulfonamides: novel topically active carbonic anhydrase inhibitors for the treatment of glaucoma. *J. Med. Chem.* 1987, *30*, 591–597.

(27) This type of behavior was seen previously when measuring the affinity of CA-II for the racemic compound **i**.[7a] In that case, resolution of **i** allowed determination of the K_d values for each antipode in separate experiments. The true K_d values of the enantiomers were correctly predicted by the curve-fitting procedure

employed to analyze the fluorescence curve observed for the racemate.

(28) MEDCHEM Software, Release 3.33, Pomona Medicinal Chemistry Project, Pomona College, Pomona, CA.
(29) Kvam, D. C. Antiglaucoma use of trifluoromethanesulfonamide. U.S. Patent 4,824,866, 1989. We thank a referee for bringing this work to our attention.
(30) Personal communication, Sina, J. F.; Cook, M. M.; Bradley, M. O. Department of Safety Assessment, Merck Research Laboratories.
(31) Sugrue, M. F.; Gautheron, P.; Mallorga, P.; Nolan, T. E.; Graham, S. L.; Schwam, H.; Shepard, K. L.; Smith, R. L. L-662,583 is a topically effective ocular hypotensive carbonic anhydrase inhibitor in experimental animals. *Br. J. Pharmacol.* **1990**, *99*, 59–64.
(32) Sugrue, M. F.; Gautheron, P.; Grove, J.; Mallorga, P.; Schwam, H.; Viader, M. P.; Baldwin, J. J.; Ponticello, G. S. MK-927: A topically effective ocular hypotensive carbonic anhydrase (CA) inhibitor in rabbits. *Invest. Ophthalmol. Visual Sci.* **1988**, *29* (*Supp.*), 81.
(33) Trepka, R. D.; Harrington, J. K.; Belisle, J. W. Acidities and partition coefficients of fluoromethanesulfonamides. *J. Org. Chem.* **1974**, *39*, 1094–1097.
(34) Leibman, K. C.; Alford, D.; Boudet, R. A. Nature of the inhibition of carbonic anhydrase by acetazolamide and benzthiazide. *J. Pharmacol. Exp. Ther.* **1961**, *131*, 271.