N-(2-Hydroxyethyl)-8-bromonorapocodeine Trifluoroacetate (5b). To a solution of N-(2-hydroxyethyl)norapocodeine 3; 1.7 g, 5.5 mmol) in 450 mL of TFA was added dropwise, at room temperature over 2 h, 556 μL of bromine in 150 mL of TFA with rapid stirring in the dark. After 2 h, the solvent was evaporated under vacuum to yield a white solid. Recrystallization from MeOH/ether gave 8a: yield 2.73 g (98%); mp 230 °C dec. HPLC, one peak. Anal. ($C_{19}H_{20}BrNO_3 \cdot CF_3COOH$) C, H, N; Br: calcd, 15.9; found, 16.25.

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Topical Carbonic Anhydrase Inhibitors

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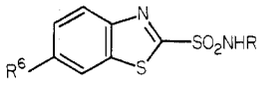
Ethoxzolamide and several derivatives (1–6) were synthesized and evaluated for carbonic anhydrase inhibition (CAI), solubility, pK_a, distribution, and corneal permeability. The 6-hydroxy (5) and, particularly, the 6-chloro (6) analogues have the best combination of properties for penetrating the site of action and reducing intraocular pressure. Both 5 and 6 exhibited topical effectiveness in the normal rabbit, with 6 showing greater potency.

Several different classes of drugs are used topically to treat glaucoma through the reduction of intraocular pressure (IOP).[1] Glaucoma is an optic condition in which the increased pressure can constrict capillaries delivering blood to the retina and optic nerve. If the IOP is not controlled, loss of peripheral vision and, eventually, blindness occurs.

Elevated IOP can be controlled by oral administration of carbonic anhydrase inhibitors (CAI), but this therapy produces serious side effects, leading to noncompliance by patients.[2] It may be possible to develop a CAI that would produce a reduced IOP after topical administration. Toward this goal we have selected ethoxzolamide (1, 6-ethoxy-2-benzothiazolesulfonamide) as the prototype CAI to be structurally altered in order to improve varous physical properties while retaining CAI activity. Compound 1 has high CAI potency and excellent corneal permeability. However, its aqueous solubility is poor, such that its solubility in tears and the resulting corneal penetration remain low upon topical dosing to the rabbit eye. Nevertheless, with a structure able to be modified, 1 is a promising candidate for optimizing penetration.

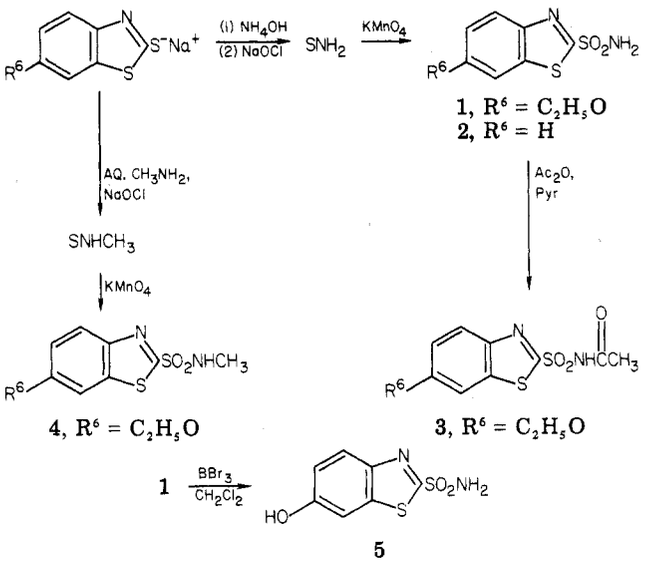


1, R = H; $R^6 = C_2H_5O$
2, R = H; R^6 = H
3, R = $COCH_3$; $R^6 = C_2H_5O$
4, R = CH_3; $R^6 = C_2H_5O$
5, R = H; R^6 = HO

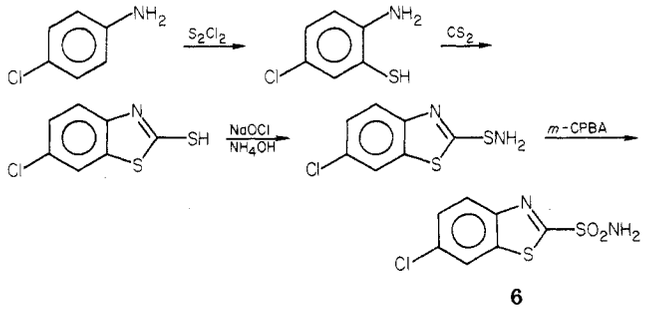
Chemistry. Most of the compounds were synthesized in a straightforward manner. The sulfonamides were prepared from the corresponding 2-mercapto derivative via the sulfenamide and subsequent oxidation. Compound 4 was made from the 2-mercapto compound via the N-methylsulfenamide in order to avoid dimethylation. Compound 5 was synthesized efficiently from 1 by ether cleavage with boron tribromide. Compound 6 was prepared by total synthesis via the Herz reaction from 4-chloroaniline.[3]

Physical Properties. The distribution coefficients, solubilities, pK_a's, and corneal permeability coefficients were determined by standard methods. The data are listed in Table I.

Scheme I



Scheme II



Biological Activity. The in vitro inhibition of carbonic anhydrase activity was determined by Maren's[4] method. The data relative to ethoxzolamide are listed in Table I. Evaluation of the ability of the compounds to reduce IOP on topical dosing to normal rabbits was accomplished.[5]

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Table I. Comparison of in Vitro Parameters for Ethoxzolamide Analogues

no.	pK_a	solubility,[a] mg/mL	log DC[b]	CAI act.[c]	corneal permeability coeff ($\times 10^6$, cm/s)
1	8.1	0.06	2.056	1	43.9 (2.10)[d]
2	7.9	1.021	1.329	1.25	36.2 (1.66)
3	2.95	11.5	−1.016	0.0041	nd[e]
4	9.1	0.064	2.59	0.0064	54.2 (8.5)
5	$pK_1 = 7.81$ $pK_2 = 9.26$	1.56	1.125	0.876	5.64 (2.1)
6	7.72	0.607	2.1	1.23	42.8 (5.12)

[a] pH 7.2 phosphate buffer, 37 °C ($N = 6$). [b] Octanol/pH 7.2 phosphate buffer distribution coefficient ($N = 2$–3). [c] Relative in vitro carbonic anhydrase activity (from human red blood cell). [d] Mean plus or minus standard deviation; all means represent three to five determinations. [e] nd = not determined due to high aqueous solubility and lack of CAI effect.

Table II. Intraocular Pressure Measurement of 5 ($N = 9$)[a] and 6 ($N = 18$)[b] in Rabbits following Topical Instillation[c]

time after dosing, min	change from base line ($t = 0$)			
	5	P, mmHg	6	P, mmHg
30	0.0	0.5	−1.2	0.019
60	−1.33	0.061	−1.1	0.033
90	−1.89	0.038	−1.0	0.032
120	−1.90	0.036	−1.1	0.056

[a] When the experiment was repeated in an additional nine rabbits, only the 120-min measurement showed a similar drop in IOP (change = −1.11); all other intervals (30-90 min) showed no significance. [b] Compound 6 has been evaluated in two additional experiments ($N = 8$ and 12) showing identical results (i.e., reduction in IOP of >1 mmHg and $p < 0.05$). [c] Compounds 1-4 exhibited no topical activity on repeated experiments.

The data are presented in Table II.

Discussion

Our approach to the development of a topically effective carbonic anhydrase inhibitor involves selecting a suitable prototype molecule, making single molecular modifications of that structure, determining various physical and biological properties of each analogue, and finally utilizing the data to design a molecule with the best combination of properties. When 1 is applied topically to the eye it is inactive. In our initial studies, we have found that removal of the 6-ethoxy group greatly enhances the aqueous solubility (2) without substantially affecting the permeability coefficient. While 2 appears to possess a very good set of physical parameters, several in vivo experiments have confirmed its topical inactivity. The inactivity may be due to metabolism, but studies to verify this explanation are not complete. Acetylation (3) yields an aqueous soluble compound, but penetration is diminished substantially, and CAI would require deacetylation. Literature reports state that an unsubstituted sulfonamide is required for CAI.[4] While we had hoped that only reduction in potency would occur with N-methylation (4), our results show a complete loss of CAI activity, which is consistent with previous reports. The data suggest that attempts to improve physical properties through modification of the sulfonamide will be nonproductive.

Compound 5 is a promising compound with increased solubility and high CAI potency. While the permeability coefficient of 5 is reduced from that of 1, the value is sufficient and when combined with the enhanced solubility permits an increased rate of penetration upon topical administration to the eye. Compound 6 is an analogue that provides polar character to the ring system, increasing water solubility tenfold, yet retaining lipophilic character. Its properties predict better penetration than 1–5, which is exemplified in Table II, showing a small but statistically significant reduction in IOP.

The applicability of our approach to the systematic modification of 1 in order to improve the physical parameters necessary for corneal penetration while maintaining the CAI pharmacophore is demonstrated by the small but significant in vivo activity of 5 upon topical dosing to the eye of normal rabbits. The borderline activity of 5 and the inactivity of 1–4 are consistent with the need to provide both aqueous and lipid solubilities. The effect of 6 strongly suggests that the approach being used to design analogues is valid. As data from more analogues are obtained, we should be able to predict better ring substituents.

The success of the in vivo rabbit assay is dependent upon maintaining the rabbits on adequate levels of sodium chloride prior to the assay.[6] A statistically significant but small reduction in IOP for 6 is not surprising, since the maximum reduction is very small in normal rabbits (maximum reduction $\cong$ 3–5 mmHg), and the physical parameters of 5 and 6 do not represent optimized values.

Current efforts are directed toward the total synthesis of other ring-substituted benzothiazole-2-sulfonamides with possibly more favorable physical parameters.

Experimental Section

Chemistry. Melting points were determined in capillary tubes in a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are uncorrected. Elemental analysis were performed by Galbraith Laboratories, Knoxville, TN. The ^1H NMR spectra were completed in Me_2SO-d_6 with Me_4Si as the internal standard on a Varian EM 360A NMR spectrometer. Infrared spectra were recorded with a Beckman IR 4240 spectrophotometer. Mass spectra were obtained with a Finnigan 3200 GC/MS at the Midwest Center for Mass Spectrometry, Department of Chemistry, University of Nebraska, Lincoln, NE.

6-Ethoxy-2-benzothiazolesulfonamide (1). A solution of ammonium hydroxide (650 mL) was cooled to 0 °C in an ice/methanol bath. A solution containing 6-ethoxy-2-mercaptobenzothiazole (16.0 g, 0.076 mol) in 170 mL of 5% NaOH and a solution of 5.25% NaOCl (150 mL) were added simultaneously to the ammonium hydroxide solution while maintaining a temperature of 0 °C. The reaction was stirred for 15 min upon completing the addition, and the sulfenamide was collected by vacuum filtration. The sulfenamide was dissolved in 1 L of acetone and oxidized by the addition of 450 mL of 5% $KMnO_4$ over 4 h. The MnO_2 was removed by filtering through Celite, and the acetone was removed under vacuum. The product was precipitated from solution by acidification with concentrated HCl to yield 11.7 g (59.7%) of 6-ethoxy-2-benzothiazolesulfonamide, which was purified by dissolving it in 5% NaOH, filtered, and precipitated with concentrated HCl: mp 190–191 °C (lit.[7] mp 188.5–190.5 °C); MS, m/e 258 (M, calcd 258).

2-Benzothiazolesulfonamide (2). The synthesis used for preparation of 1a was employed: mp 170–172 °C (lit.[8] mp 177

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°C); mass spectrum, m/e 214 (M^+). Anal. ($C_7H_6N_2O_2S_2$) C, H, N.

***N*-Acetyl-6-ethoxy-2-benzothiazolesulfonamide (3).** 6-Ethoxy-2-benxothiazolesulfonamide (1.0 g, 0.0039 mol) was dissolved in 30 mL of pyridine, and 2 mL of acetic anhydride was added. The reaction was stirred for 15 h at room temperature. It was poured into 250 mL of ice/25 mL f concentrated HCl. The precipitated product was collected by filtration to yield 1.0 g (86.0%) of *N*-acetyl-6-ethoxy-2-benzothiazolesulfonamide. It was purified by disolving it in 10% $NaCO_3$ and then filtered, and the sodium salt was dissolved in H_2O and precipitated with concentrated HCl: mp 178–180 °C; exact mass calcd for $C_{11}H_{12}N_2O_4S_2$, 300.3573; found, 300.0253.

***N*-Methyl-6-ethoxy-2-benzothiazolesulfonamide (4).** A solution of 40% aqueous methylamine (100 mL) was cooled to −10 °C in an ice/methanol bath. A solution of 6-ethoxy-2-benzothiazolesulfonamide (2.0 g, 0.0095 mol) in 20 mL of 5% NaOH and a solution of 5.25% NaOCl (40 mL) were added simultaneously to the cooled aqueous methylamine solution, maintaining a temperature of 0 °C. The reaction was stirred for 15 min upon completing the addition, and sulfenamide was collected by vacuum filtration. The sulfenamide was dissolved in 100 mL of acetone and oxidized by the addition of 50 mL of 5% $KMnO_4$ over 2 h. The MnO_2 was removed by filtration through Celite, and the acetone was removed under vacuum. The product was precipitated by acidification with 5% HCl to yield 1.3 g of product (50.3%). The product was purified by dissolving it in 5% NaOH and precipitated with 5% HCl, mp 129–131 °C. Anal. ($C_{10}H_{12}N_2O_3S_2$) C, H. N.

6-Hydroxy-2-benzothiazolesulfonamide (5). A 1 M solution of BBr_3 in CH_2Cl_2 (23 mL, 0.022 mol) was cooled to −80 °C in a dry ice/acetone bath under a N_2 atmosphere. A suspension of 6-ethoxy-2-benzothiazolesulfonamide (0.5 g, 0.002 mol) in 75 mL of CH_2Cl_2 was added slowly to the cooled BBr_3 solution. The reaction was removed from the cooling bath and stirred at room temperature for 15 h. It was poured into ice–water, stirred for 30 min, and filtered to yield 0.35 g of product (73.8%). The product was purified by recrystallization from $MeOH/H_2O$: mp 209–212 °C; M/S, m/e 230 (M, calcd 230). Anal. ($C_7H_6N_2O_3S_2$) C, H, N.

5-Chloro-2-aminobenzenethiol. This was prepared by a modified Huestis et al.[9] and Warburton[10] procedure in 60% yield, mp 70–72 °C (lit.[10] mp 100–111 °C).

6-Chloro-2-mercaptobenzothiazole. This was synthesized by Leaper's[11] general method in 89% yield, mp 243–246 °C (lit.[12] mp 245 °C).

6-Chlorobenzothiazole-2-sulfonamide (6). The 2-mercapto derivative was converted to the 2-sulfenamide by Machon's[7] procedure and oxidized directly. The sulfenamide was dissolved in dimethoxyethane (75 mL) and cooled to −5 °C. *m*-Chloroperoxybenzoic acid (85%, 4.7 g, 0.023 mol) in 1,2-dimethoxyethane (25 mL) was added to the sulfenamide solution during a 1-h period with the temperature maintained at 0 °C. After the addition was complete, the reaction mixture was stirred at room temperature for 18 h. The volatiles were removed under reduced pressure, and the *m*-chlorobenzoic acid was separated from the sulfonamide by stirring with 10% aqueous sodium bicarbonate (45 mL) for 1 h. Compound **6** was collected by vacuum filtration and purified through sodium salt formation, followed by reprecipitation of the free sulfonamide, to give 2.6 g (70% yield). Anal. ($C_7H_5ClN_2O_2S_2$) C, H, N.

Distribution Coefficients. The method of Hansch[13] for octanol and pH 7.2 phosphate buffer was used.

Solubility. High-pressure liquid chromatography was used to determine the solubility of the compounds in pH 7.2 phosphate buffer.[14]

pK_a Determination. The potentiometric pH titration procedure described by Albert and Serjeant[15] was employed for **3** and **5**. Compounds **1**, **2**, **4**, and **6** were analyzed by the pH-solubility method.

Carbonic Anhydrase Inhibition. The method of Maren[4] was used.

Measurement of Intraocular Pressure (IOP). Rabbits were maintained on 0.3% sodium chloride solution for 3 weeks prior to the tests. IOP measurement were made during this period to familiarize the rabbits with the procedure. Base-line IOP measurements were determined by tonometry, followed by the topical administration of the compounds in one eye and vehicle in the other.[5] Each drug was administered topically as a 1% aqueous suspension (pH 7.65 buffer) in a volume of 50 μL every 2 min for three doses.

Corneal Permeability Coefficients. The penetration through intact rabbit cornea was determined by the method of Schoenwald and Ward.[16]

Acknowledgment. This research was supported by NIH Research Grant EY03297.

Registry No. **1**, 452-35-7; **2**, 433-17-0; **3**, 88946-18-3; **4**, 88946-19-4; **5**, 29927-14-8; **6**, 88946-20-7; 6-ethoxy-2-mercaptobenzothiazole, 120-53-6; 6-ethoxy-2-benzothiazolesulfenamide, 5304-15-4; *N*-methyl-6-ethoxy-2-benzothiazolesulfenamide, 88946-21-8; 5-chloro-2-aminobenzenethiol, 23474-98-8; 6-chloro-2-mercaptobenzothiazole, 51618-29-2; 6-chloro-2-benzothiazolesulfenamide, 88946-22-9; carbonic anhydrase, 9001-03-0.

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