Topically Active Carbonic Anhydrase Inhibitors. 1. *O*-Acyl Derivatives of 6-Hydroxybenzothiazole-2-sulfonamide

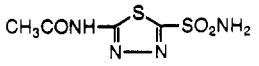
O. W. Woltersdorf, Jr.,*,† H. Schwam,*,† J. B. Bicking,† S. L. Brown,† S. J. deSolms,† D. R. Fishman,† S. L. Graham,† P. D. Gautheron,§ J. M. Hoffman,† R. D. Larson,‡ W. S. Lee,† S. R. Michelson,† C. M. Robb,† N. N. Share,† K. L. Shepard,† A. M. Smith,† R. L. Smith,† J. M. Sondey,† K. M. Strohmaier,† M. F. Sugrue,§ and M. P. Viader§

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, and Rahway, New Jersey 07065, and Centre de Recherche, Merck Sharp & Dohme-Chibret, Riom 63203, France. *Received January 23, 1989*

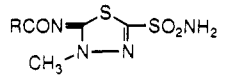
A series of *O*-acyl derivatives of 6-hydroxybenzothiazole-2-sulfonamide (**4**, L-643,799) was prepared and the potential utility of each series member as a topically active inhibitor of ocular carbonic anhydrase was determined. In vitro studies showed these esters to be substrates for ocular esterases which liberate **4** during corneal translocation. The most interesting series member, 2-sulfamoyl-6-benzothiazolyl 2,2-dimethylpropionate (**22**, L-645,151), acting as a prodrug form of **4**, was found to enhance delivery through the isolated albino rabbit cornea by 40-fold when compared to the parent phenol **4**. Studies in rabbits revealed that **22** is a potent topically active ocular hypotensive carbonic anhydrase inhibitor.

Glaucoma, the leading cause of irreversible blindness in the Western world, is characterized by optic-nerve damage associated with visual-field loss and/or abnormally high intraocular pressure (IOP). Two million Americans are estimated to have glaucoma.[1] As many as 10 million additional Americans are suspected to have ocular hypertension, i.e., elevated IOP without optic-nerve or visual-field damage.[2] No means presently exists for reliably predicting which ocular hypertensive individuals will develop glaucoma. Intraocular pressure is controlled primarily by aqueous humor (AH) dynamics, which in turn, are determined by the difference in the rates at which AH enters and leaves the eye. All known antiglaucoma drugs lower IOP by decreasing AH formation and/or increasing AH elimination and, accordingly, are ocular hypotensive agents.[3]

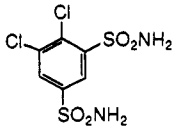
Systemically administered carbonic anhydrase inhibitors (CAIs), such as acetazolamide (**52**), methazolamide (**53a**), dichlorophenamide (**54**), and ethoxzolamide (**3**), have been used to treat glaucoma for over 3 decades. By inhibiting



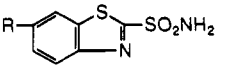
52



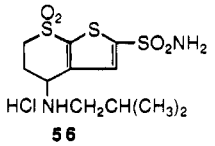
53
a, R = CH_3
b, R = CF_3



54



55
a, R = H
b, R = H_2N



56

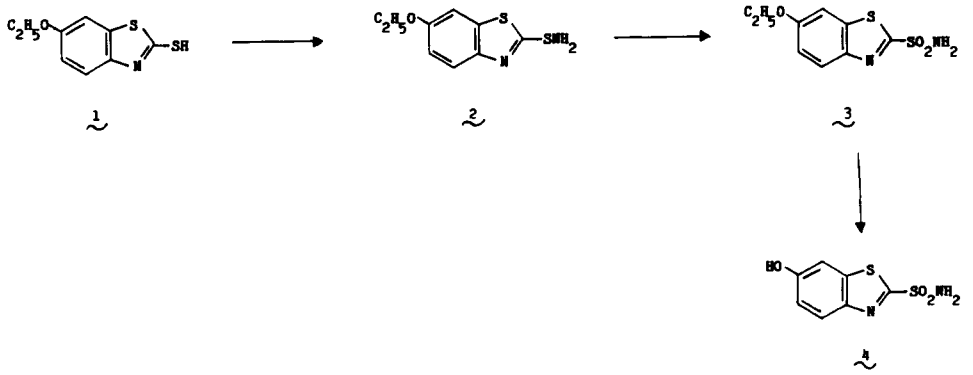
CA in the nonpigmented epithelial cells of the ciliary process, CAIs reduce AH formation and, hence, lower IOP.[4a,b] However, the large oral doses of CAIs required to obtain reductions in IOP concomitantly evoke a wide array of undesirable side effects which include altered taste, paresthesia, malaise, fatigue, depression, anorexia, nausea, weight loss, and diminished libido[5a-c] and result in poor patient compliance. These undesirable side effects are undoubtedly a consequence of the inhibition of CA in extraocular tissues and should diminish and ultimately disappear as localization of the drug to the ciliary process is improved. Toward this end, attempts have been made to lower IOP by administering CAIs directly to the eye. Trifluormethazolamide (**53b**), when topically applied to the eye, reduced IOP in rabbits[6] and cats,[7] but its chemical instability rendered it unacceptable for clinical use. A gel formulation of aminozolamide (6-aminobenzothiazole-2-sulfonamide, **55b**) has been reported to lower IOP in rabbits[8] and man.[9] However, suppression of AH flow in man by topically instilled **55b** could not be demonstrated.[10] Recently, the thienothiopyran-2-sulfonamides, a promising class of CAIs with water solubilities in the 1-2% range, have been reported.[11] A recently disclosed series member,[12a,b] 5,6-dihydro-4*H*-4-(isobutylamino)thieno[2,3-b]-thiopyran-2-sulfonamide 7,7-dioxide hydrochloride (**56**,

† Merck Sharp & Dohme Research Laboratories, West Point, PA.
‡ Merck Sharp & Dohme Research Laboratories, Rahway, NJ.
§ Merck Sharp & Dohme-Chibret, Riom, France.

(1) Remis, L. L.; Epstein, D. L. *Annu. Rev. Med.* **1984**, *35*, 195.
(2) Vision Research—A National Plan: *1983-1987*; U.S. Department of Health and Human Services. NIH: Washington, DC, 1983; Vol. 2, Part 4, Publication No. 82-2474.
(3) Sugrue, M. F.; Smith, R. L. *Annu. Rep. Med. Chem.* **1985**, *20*, 83.
(4) (a) Maren, T. H. *Invest. Ophthalmol. Visual Sci.* **1974**, *13*, 479. (b) Maren, T. H. *Invest. Ophthalmol. Visual Sci.* **1976**, *15*, 356.
(5) (a) Epstein, D. L.; Grant, W. M. *Arch. Ophthalmol.* **1977**, *95*, 1378. (b) Grant, W. M. *Symposium on Ocular Therapy*; Leopold, I. H., Ed.; C. V. Mosby Co.: St. Louis, 1973; p 19. (c) Lichter, P. R.; Newman, L. P.; Wheeler, N. C.; Beal, O. V. *Am. J. Ophthalmol.* **1978**, *85*, 495.
(6) Maren, T. H.; Jankowska, L.; Sanyal, G.; Edelhauser, H. F. *Exp. Eye Res.* **1983**, *36*, 457.
(7) Stein, A.; Pinke, R.; Krupin, T.; Glabb, E.; Podos, S. M.; Serle, J.; Maren, T. H. *Am. J. Ophthalmol.* **1983**, *95*, 222.
(8) Lewis, R. A.; Schoenwald, R. D.; Eller, M. G.; Barfknecht, C. F.; Phelps, C. D. *Arch. Ophthalmol.* **1984**, *102*, 1821.
(9) Lewis, R. A.; Schoenwald, R. D.; Barfknecht, C. F.; Phelps, C. D. *Arch. Ophthalmol.* **1986**, *104*, 842.
(10) Kalina, P. H.; Shetlar, D. J.; Lewis, R. A.; Kullerstrand, L. J.; Brubaker, R. F. *Ophthalmology* **1988**, *95*, 772.
(11) 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. *J. Med. Chem.* **1987**, *30*, 591.
(12) (a) Ponticello, G. S.; Baldwin, J. J.; Freedman, M. B.; Habecker, C. N.; Christy, M. E.; Sugrue, M. F.; Mallorga, P. J.; Schwam, H. Third Chemical Congress of the North American Continent, Toronto, Canada, Abstr. No. 134, June, 1988. (b) Baldwin, J. J.; Ponticello, G. S.; Sugrue, M. F.; Mallorga, P. J.; Randall, W. C.; Schwam, H.; Springer, J. P.; Smith, G. M.; Murcko, M. Third Chemical Congress of the North American Continent, Toronto, Canada, Abstr. No. 95, June 1988.

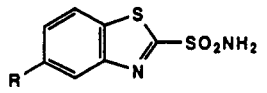
0022-2623/89/1832-2486$01.50/0

Scheme I



MK-927), when formulated as a 2.0% aqueous solution and applied topically lowered IOP in rabbits,[13] monkeys,[14] and man.[15]

In this report, we present the syntheses and pertinent in vitro and in vivo evaluation of *O*-acyl derivatives of 5- and 6-hydroxybenzothiazole-2-sulfonamide (**6** and **4**, respectively) which led to the selection of the pivaloate ester (**22**) for in-depth evaluation as a topically active ocular hypotensive CAI.[16a-c]

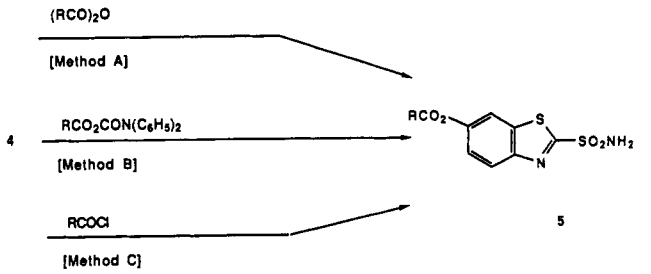


6, R = CH_3O
7, R = HO
8, R = n-$C_3H_7CO_2$

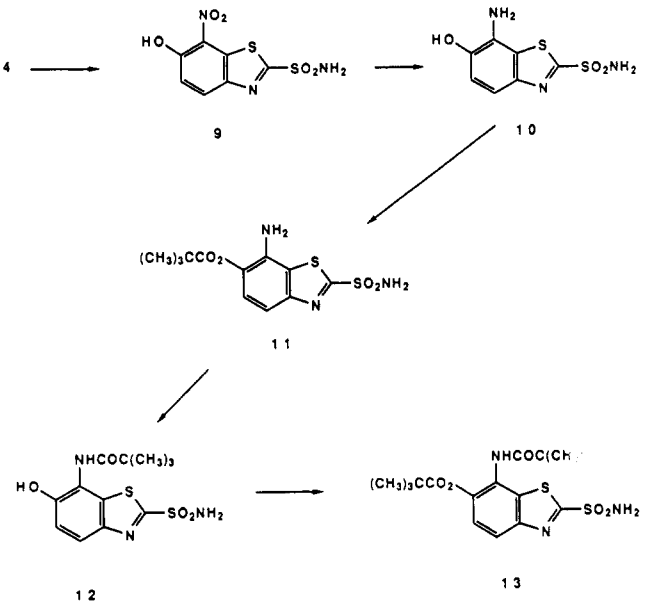
Chemistry. The esters reported here were prepared by selective O-acylation of the corresponding hydroxybenzothiazole-2-sulfonamides. The requisite phenols **4** and **6** were prepared by acid-catalyzed cleavage of an alkoxy derivative. A modification of a previously reported procedure was used to prepare 6-ethoxybenzothiazole-2-sulfonamide (**3**) (Scheme I).[17] Commercially available 6-ethoxybenzothiazole-2-thiol (**1**)[18] was converted to sulfenamide **2** with sodium hypochlorite and concentrated aqueous ammonia, followed by oxidation to **3** with aqueous potassium permangantate under controlled-pH conditions. Cleavage of the ethyl ether to generate **4** was best accomplished with aluminum chloride in dichloroethane. Preparation of 5-hydroxy isomer **7** was similarly accomplished by aluminum chloride catalyzed cleavage of the known 5-methoxy precursor (**6**).[19] Nitration of **4**, followed by catalytic reduction (Scheme III), led to 7-amino derivative **10**.

Selective O-acylation of these phenols was generally achieved with an anhydride (Scheme II, method A), a

Scheme II



Scheme III

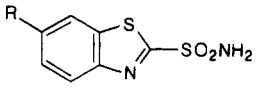


mixed anhydride (Scheme II, method B), or an acyl halide (Scheme II, method C). The acylations were conducted in pyridine, DMF, or acetone using triethylamine as the acid acceptor. In several early preparations, the utility of 4-(dimethylamino)pyridine as a catalyst was explored; however, it provided no enhancement to the reaction. A competing acylation of the sulfonamide moiety could be minimized by running the reactions in acetone at 0 °C. Any such product formed, however, could be removed by treatment with bicarbonate solution or chromatographic separation in those cases where the ester displayed significant instability to aqueous base.

It should be noted also that other synthetic complications were observed in several instances. For example, acylation of **4** to give **5** utilizing isobutyric anhydride proceeded readily in DMF solution (Scheme II, method A). When isobutyryl chloride was substituted under the same conditions, **14** was generated as the major product (Scheme IV). Use of the acyl halide, a more active acy-

(13) Sugrue, M. F.; Gautheron, P.; Grove, J.; Mallorga, P. J.; Schwam, H.; Viader, M. P.; Baldwin, J. J.; Ponticello, G. S. *Invest. Ophthalmol. Visual Sci.* **1988**, *29* (Suppl.), 81.
(14) Wang, R. F.; Serle, J. B.; Podos, S. M.; Sugrue, M. F. *Invest. Ophthalmol. Visual Sci.* **1988**, *29* (Suppl.), 16.
(15) Hennekes, R.; Pfeiffer, N.; Lippa, E.; Garus, H.; Grehn, F.; Jaeger, A. *Invest. Ophthalmol. Visual Sci.* **1988**, *29* (Suppl.), 82.
(16) (a) Schwam, H.; Michelson, S. R.; Sondey, J. M.; Smith, R. L. *Invest. Ophthalmol. Visual Sci.* **1984**, *25* (Suppl.), 181. (b) Sugrue, M. F.; Gautheron, P.; Schmitt, C.; Viader, M. P.; Conquet, P.; Smith, R. L.; Share, N. N.; Stone, C. A. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 534. (c) Bar-Ilan, A.; Pessah, N. I.; Maren, T. H. *J. Ocular Pharmacol.* **1986**, *2*, 109.
(17) Korman, J. *J. Org. Chem.* **1958**, *23*, 1768.
(18) Aldrich Chemical Co., Cat. No. 14, 217-4.
(19) U. S. Patent 4,454,148; June 12, 1984; *Chem. Abstr.* **1984**, *101* 116752e.

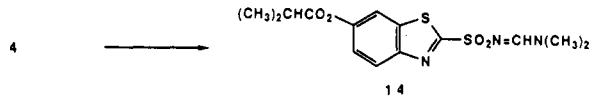
Table I. Esters of 6-Hydroxybenzothiazole-2-sulfonamide



no.	R	method	recrystn solvent	mp, °C	% yield	formula	anal.[a]
18	CH_3CO_2	A		194–196	6	$C_9H_8N_2O_4S_2$	C, H, N
19	$CH_3(CH_2)_2CO_2$	A	C_6H_6	122–123	34	$C_{11}H_{12}N_2O_4S_2$	C, H, N
20	$(CH_3)_2CHCO_2$	C	C_6H_6	140–142	71	$C_{11}H_{12}N_2O_4S_2$	C, H, N
21	$CH_3CH{=}CHCO_2$	A	$C_6H_5CH_3$	181–182	32	$C_{11}H_{10}N_2O_4S_2$	C, H, N
22	$(CH_3)_3CCO_2$	A	$C_6H_5CH_3$	171–173	52	$C_{12}H_{14}N_2O_4S_2$	C, H, N
23	$CH_3(CH_2)_4CO_2$	C	BuCl	106–109	35	$C_{13}H_{16}N_2O_4S_2$	C, H, N
24	$CH_3(CH_2)_6CO_2$	C	BuCl	114	20	$C_{15}H_{20}N_2O_4S_2$	C,[b] H, N
25	$CH_3(CH_2)_{10}CO_2$	C	BuCl	112–114	18	$C_{19}H_{28}N_2O_4S_2$	C, H, N
26	c-$C_5H_9CH_2CO_2$	C	[c]	118–120	53	$C_{14}H_{16}N_2O_4S_2$	C, H, N
27	c-$C_6H_{11}CO_2$	C	$C_6H_5CH_3$	142–144	18	$C_{14}H_{16}N_2O_4S_2$	C, H, N
28	*trans*-4-[$(C_2H_5)_2NCH_2$]-c-$C_6H_{10}CO_2$[d]	C	CH_3CN	156–158	19	$C_{19}H_{27}N_3O_4S_2$	C, H, N
29	$C_6H_5CO_2$	C	$C_6H_5CH_3$	208–210	50	$C_{14}H_{10}N_2O_4S_2$	C, H, N
30	$C_6H_5CH_2CO_2$	A	C_6H_6	177–179	21	$C_{15}H_{12}N_2O_4S_2$	C, H, N
31	4-$ClC_6H_4CH_2CO_2$	C	C_6H_6	204–206	20	$C_{15}H_{11}ClN_2O_4S_2$	C,[e] H, N, Cl
32	4-$(CH_3)_2NC_6H_4CO_2$[f]	B	CH_3CN	241–242	53	$C_{16}H_{15}N_3O_4S_2$	C, H, N
33	4-[$(CH_3)_2NCH_2$]$C_6H_4CO_2$[g]	C	CH_3CN	169	27	$C_{17}H_{17}N_3O_4S_2$	C, H, N
34	4-[$(C_2H_5)_2N(CH_2)_2O$]$C_6H_4CO_2$[h]	C	CH_3CN	173.5–174.5	64	$C_{20}H_{23}N_3O_5S_2$	C, H, N
35	$C_6H_5CH{=}CHCO_2$	C	[c]	235–238	97	$C_{16}H_{12}N_2O_4S_2$	C, H, N
36	2,6-$(CH_3O)_2C_6H_3CO_2$	C	$CHCl_3$	210–212	33	$C_{16}H_{14}N_2O_6S_2$	C, H, N
37	2,4,6-$(CH_3)_3C_6H_2CO_2$	A	CH_2Cl_2	202–204	82	$C_{17}H_{16}N_2O_4S_2$	C, H, N
38	$CH_3OCH_2CO_2$	C	CH_3CN	192–193	3	$C_{10}H_{10}N_2O_5S_2$	C, H, N
39	$CH_3OC(CH_3)_2CO_2$[i]	B	EtOAc–hexane	162.5–164	27	$C_{12}H_{14}N_2O_5S_2$	C, H, N
40	$C_2H_5OC(CH_3)_2CO_2$[j]	B	EtOAc–hexane	153–154	38	$C_{13}H_{16}N_2O_5S_2$	C, H, N
41	$CH_3O(CH_2)_2OC(CH_3)_2CO_2$[k]	B	EtOAc–hexane	118–120	30	$C_{14}H_{18}N_2O_6S_2$	C, H, N
42	$C_2H_5(OCH_2CH_2)_2OC(CH_3)_2CO_2$[i]	B	Me_2CO–hexane	134.5–136	32	$C_{17}H_{24}N_2O_7S_2$	C, H, N
43	*N*-CH_3-pyrrole-2-CO_2	B	MeOH	212.5–213.5	75	$C_{13}H_{11}N_3O_4S_2$	C, H, N
44	$C_2H_5OCO_2$	C	[c]	144–146	29	$C_{10}H_{10}N_2O_5S_2$	C, H, N
45	$(CH_3)_2CHCH_2OCO_2$	C	C_6H_6	145–146	79	$C_{12}H_{14}N_2O_5S_2$	C, H, N
46	$(CH_3)_3COCO_2$	A	Et_2O–PE	136–137	39	$C_{12}H_{14}N_2O_5S_2$	C, H, N
47	$CH_3(CH_2)_3SO_3$	C	$C_6H_5CH_3$	125–128	10	$C_{11}H_{14}N_2O_5S_3$	C, H, N
48	4-$CH_3C_6H_4SO_3$	C	$C_6H_5CH_3$	142	37	$C_{14}H_{12}N_2O_5S_3$	C, H, N
49	$(CH_3)_2NSO_3$	C	[l]	162–164		$C_9H_{11}N_3O_5S_3$	C, H, N
50	$(CH_3)_2NCO_2$	C	*i*-PrOH	208–209	63	$C_{10}H_{11}N_3O_4S_2$	C, H, N
51	$(C_2H_5O)_2PO_2$	C	1,2-$(ClCH_2)_2$	141–143	22	$C_{11}H_{15}N_2O_6PS_2$	C, H, N

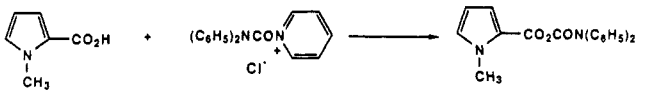
[a] All compounds analyzed as noted are within ±0.4% of the calculated values. ^1H NMR is consistent with assigned structures. [b] C: calcd, 50.59; found, 50.98. [c] Chromatographed (silica gel, EtOAc–hexane, 1:1, v/v). [d] Carboxylic acid preparation: Okano, A.; Inaska, M.; Funabashi, S.; Iwamoto, M.; Isoda, S.; Moroi, R.; Abiko, Y.; Hirata, M. *J. Med. Chem.* **1972**, *15*, 247. [e] C: calcd, 47.06; found, 46.07. [f] 4-(Dimethylamino)benzoic *N,N*-diphenylcarbamic anhydride prepared by method in ref 22, mp 176–180 °C (1-chlorobutane). Anal. ($C_{22}H_{20}N_2O_3$) C, H, N. [g] Carboxylic acid preparation: Smith, J. H.; Menger, F. M. *J. Org. Chem.* **1969**, *34*, 77. [h] Carboxylic acid preparation: Shadbolt, R. S.; Sharpe, C. J.; Brown, G. R.; Ashford, A.; Ross, J. W. *J. Med. Chem.* **1971**, *14*, 836. [i] Acid preparation: House, H. O.; Prabho, A. V.; Wilkins, J. M.; Lee, L. F. *J. Org. Chem.* **1976**, *41*, 3067. [j] Acid preparation: Weizman, C.; Sulzbacher, M.; Bergmann, E. *J. Am. Chem. Soc.* **1948**, *70*, 1153. [k] Acid preparation by method of reference of footnote j, 60% yield, bp 134–136 °C. [l] Chromatographed (silica gel, $CHCl_3$–MeOH, 9:1, v/v).

Scheme IV



14

Scheme V



15 16 17

lating agent, led to interaction with the solvent DMF to produce an iminium halide which condensed with the sulfonamide function.[20] Thus, the further use of acyl halides was confined to pyridine or acetone solution.

Use of the mixed anhydride derived from *N*-methylpyrrole-2-carboxylic acid and isobutyl chloroformate in the preparation of **43** gave the desired product in extremely low yield. The major acylation product from this procedure gave **45**, identical with the product from isobutyl chloroformate alone. Carboxylic carbamic mixed anhydride[22] **17** was formed from **15** and **16** (Scheme V) and this intermediate selectively gave **43** as the reaction product. Utilization of this latter type intermediate also was beneficial in the preparation of **32** and **39–42**.

Acylation of 7-amino derivative **10** could be regulated to give each of the three products, **11–13**. Careful examination of the reaction mixture (TLC) indicated that the initial acylation occurred at the hydroxyl function to give **11**, which could be isolated. Under extended reaction times, **11** rearranged to *N*-acyl product **12**. The use of excess anhydride produced bis-acylated derivative **13**.

Results and Discussion

The strategy of enhancing ocular penetration by converting an active parent compound to a more lipophilic prodrug has ample precedent. For example, the dipivaloyl ester of epinephrine (dipivefrin) penetrates the rabbit eye much more readily than the parent drug and upon contact with corneal esterases the parent drug is released.[22] Furthermore, IOP in man is decreased by doses of dipivefrin which are significantly lower than equivalent doses

(20) Pettit, G. R.; Kadunce, R. E. *J. Org. Chem.* **1986**, *127*, 4566.
(21) Shepard, K. L.; Halczenko, W. *J. Heterocycl. Chem.* **1979**, *16*, 321.
(22) Wei, C.; Anderson, J. A.; Leopold, I. *Invest. Ophthalmol. Visual Sci.* **1978**, *17*, 315.

Table II. Inhibition of Human CAII

no.	I_{50}[a,b] × 10^{-9} M	no.	I_{50}[a,b] × 10^{-9} M	no.	I_{50}[a,b] × 10^{-9} M
3	3	**26**	6	**42**	3.2
4	4	**27**	6	**43**	2.9
7	5	**28**	2.8	**44**	6.8
8	4	**29**	8	**45**	2.2
11	25	**30**	30	**46**	10
13	32	**31**	10	**47**	1.8
18	2	**32**	60	**48**	4.1
19	3.2	**33**	4	**49**	1.2
20	6.8	**35**	15	**50**	1.6
21	2.1	**36**	4	**51**	1.8
22	4	**38**	3.5	**52**	10
23	4	**39**	1.2	**53a**	21
24	1.6	**40**	1.2	**54**	40
25	3.1	**41**	1.4		

[a] Compound **52** was utilized as a control for each determination shown. It's I_{50} ranged between 9 and 11 × 10^{-9} M. [b] Inhibitor and CAII were preincubated for 2 min.

of epinephrine.[23] In addition, the enzymatically liberated pivalic acid is not sequestered in ocular structures and is rapidly eliminated from the eye.[24] As described in this paper, we have employed this strategy in the quest for a CAI which is capable of lowering IOP after topical ocular instillation.

Toward this objective, a series of esters derived from 6-hydroxybenzothiazole-2-sulfonamide (**4**) was prepared (Table I) and evaluated in vitro for inhibitory activity against human CA II (Table II), the isozyme present in the human ciliary process. Selected compounds then were examined for cleavage by corneal esterases, susceptibility to nucleophilic attack by reduced glutathione (GSH), and corneal permeability (Table III).

As noted in Table II, each of these derivatives exhibited excellent intrinsic inhibitory activity against human CA II. Their I_{50} values were in the range of those of ethoxzolamide (**3**), acetazolamide (**52**), and methazolamide (**53**). With few exceptions, the esters prepared in this study were cleaved when incubated at 37 °C with a freshly prepared rabbit corneal homogenate; the half-lives of some of the more active derivatives are recorded in Table III.

During the synthesis of **3** (Scheme I), it was noted that the yield of the oxidation step (**2** to **3**) was markedly dependent upon pH and temperature. A similar observation by Korman[17] indicated that the sulfamoyl moiety was replaced by a hydroxyl under certain conditions. These observations, the metabolism of benzothiazole-2-sulfonamide (**55a**) through interaction with GSH,[25] and the observation that the concentration of benzothiazole-2-sulfonamides in corneal perfusion chambers decreased rapidly when GSH was present in the buffer, led to the examination of the reaction of a variety of these derivatives with GSH. Susceptibility to nucleophilic replacement of the sulfamoyl moiety by GSH was assessed by incubating compounds in 0.1 M phosphate (pH 7.4) containing a 5-fold excess of GSH at 37 °C and monitoring GSH adduct formation by HPLC as a function of time (Table III).[26]

Excised albino rabbit corneas were utilized to determine the corneal penetration rate constants (k). Each of the carboxylate and carbonate esters shown in Table III acted as a prodrug form of the parent phenol (**4**) and were found to deliver **4** exclusively through the intact cornea when determined at 37 °C with Krebs–Ringer bicarbonate buffer (pH 7.4). Additionally, under these conditions, the k values measured with the esters were significantly greater than that determined for phenol **4** and much greater than those determined for acetazolamide (**52**) and methazolamide (**53**). The sulfonates (**47**, **48**), sulfamate (**49**), carbamate (**50**), and phosphate (**51**) derivatives were not susceptible to hydrolysis by corneal esterases. Pivaloate ester **22** had a k value that was 40-fold greater than that of the parent phenol **4** and superior to all of the other esters tested. Interestingly, as shown in Table III, the enhanced transcorneal delivery of a number of the esters was not observed in the denuded (epithelium free) cornea.

Compounds were micronized and evaluated in vivo for topical activity in the IOP recovery rate (IOPR) assay and the α-chymotrypsin (α-CT) assay using albino rabbits[16b] (Table IV). The compounds were administered topically as suspensions in 0.5% aqueous (hydroxyethyl)cellulose (HEC) vehicle with no other additives. The IOPR assay served as an initial screen to select compounds for further evaluation. The IOPR assay provided an indication of the ability of each compound to decrease AH and revealed any local irritation caused by the compound. Although essentially all of the esters decreased the IOPR when topically instilled bilaterally as 10% or 5% suspensions, only those compounds eliciting a statistically significant response at 2% or less were considered sufficiently active for further evaluation.

The α-CT assay gave an indication as to the ability of each compound to lower IOP in hypertensive eyes. As noted earlier, the minimum concentration of **4** required to significantly lower the elevated IOP of the α-CT rabbit when applied as a single 50-μL drop was 2%. This compares favorably with the 10% and 5% concentrations required of the standards dichlorphenamide (**54**) and methazolamide (**53a**), respectively. The other standard CAIs, acetazolamide (**52**) and ethoxzolamide (**3**), failed to lower IOP at concentrations up to 10%.[16b] More impressive was the activity of the esters of **4**, reported here, which displayed IOP reduction at concentrations of 0.5% or less (Table IV).

Only carboxylic esters displayed activity in the lower doses (Table IV). This activity was related to the susceptibility of the ester to cleavage by corneal esterases, delivering **4** to the appropriate ocular compartments.[16b] Those esters not cleaved during corneal penetration, **47**–**51**, failed to display any in vivo activity at less than 10% concentration. The esters that displayed the greatest activity were those that were derivatives of aliphatic acids in the C4–C7 range. 5-Phenolic compound **7** required concentrations above 5% for activity in the α-CT assay, compared to 2% for 6-phenol **4**, and butyrate ester **8** showed no IOPR activity at concentrations lower than 10%. Thus, 6-substituted derivatives were considerably more active in the in vivo models, although **4** and **7** exhibited essentially identical in vitro potency. Incorporation of a substituent into the 7-position (**11**–**13**) essentially abolished activity. No significant reduction could be seen at concentrations of 10% or less.

The excellent activity of **22** in all assays and its superior chemical stability to formulation at pH 6.5 in an acetate buffer[27] led to its selection for in-depth pharmacological[16a-c] and preliminary toxicological evaluation.

(23) Koback, M. B.; Podos, S. M.; Harbin, T. S.; Mandell, A.; Becker, B. *M. J. Ophthalmol.* **1976**, *81*, 768.

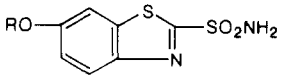
(24) Tamaru, R. D.; Davis, W. L.; Anderson, J. A. *Arch. Ophthalmol.* **1983**, *101*, 1127.

(25) Colucci, D. F.; Buyske, D. A. *Biochem. Pharmacol.* **1965**, *14*, 457.

(26) The synthesis and characterization of several products resulting from the interaction of GSH with benzothiazole-2-sulfonamides will be reported elsewhere.

(27) B. Plazonnet, internal report.

Table III. In Vitro Properties of Selected Benzothiazole-2-sulfonamides



compd	R	GSH reactivity[a]	cleavage by corneal esterase[b]	corneal penetration rate constant (k)[c] intact	corneal penetration rate constant (k)[c] denuded
3	CH_3CH_2	36 (18)		25.7	30.2
4	H	27 (18)		2.0	18.4
18	CH_3CO		22	16.5	21.7
19	$CH_3(CH_2)_2CO$		6	54.0	20.2
20	$(CH_3)_2CHCO$	50 (0.5)	9	45.0	16.2
22	$(CH_3)_3CCO$	50 (0.5)	38	80.0	11.8
23	$CH_3(CH_2)_4CO$		2	15.2	18.2
27	c-$C_6H_{11}CO$	50 (0.1)	2	71.2	17.5
29	C_6H_5CO	50 (0.4)	30	35.0	15.2
39	$CH_3OC(CH_3)_2CO$		36	22.2	19.0
40	$CH_3CH_2OC(CH_3)_2CO$		64	37.3	16.2
41	$CH_3O(CH_2)_2OC(CH_3)_2CO$		72	9.7	15.0
44	CH_3CH_2OCO		19	40.2	14.5
45	$(CH_3)_2CHCH_2OCO$	50 (0.4)	7	81.2	21.2
52	-	3 (16)		1.1	17.2
53	-	27 (16)		4.1	

[a] Percent reaction (h) at 37 °C, pH 7.4. [b] $t_{1/2}$ (min) at 37 °C, pH 7.4: compound **22** served as a control for each determination shown and had a $t_{1/2}$ value of 38 ± 1.9 min. [c] k ($\times 10^{-3}$ h^{-1}). Only **4** was detected on the endothelial side; compound **4** was utilized as a control for each determination shown and had a k value that ranged between 1.8 and 2.2 for intact cornea and between 17.8 and 19.0 for denuded cornea.

Table IV. In Vivo Activity of Topically Instilled Benzothiazole-2-sulfonamides in Albino Rabbits[e]

compd	IOP recovery rate assay[a] 10%[b]	5%[b]	2%	1%	α-CT assay[d] 5%	1%	0.5%
8	-54[c]						
18	-47*	-42*	-35*	-10		-8.8*	
19	-50*	-46*	-30*	-25*		-6.8*	-5.5*
20	-30*	-33*	-48*	-38*	-6.2*	-6.8*	-9.3*
21	-20						
22	-47*	-41*	-30*	-16	-9.2*	-8.2*	-6.5*
24	-33*	-29	-16			-5.0*	
25	-25						
27	-34*					-9.8*	-4.6*
29	-25	+11					
30	-48*	+4	+13			-6.8*	-2.5*
32	+7						
42	-22*		-21			-9.0*	
43	-12						
44	-29*				-5.5*		
45	-46*	-54*	-22*			6.7*	-5.7*
47	-14						
48	-32*	-9					
50	-32*	+2					
51	-22						

[a] Results expressed in percent change from control. [b] Compounds bilaterally instilled in three divided doses of 20, 15, and 15 μL at 5-min intervals. For all other experiments, compounds were instilled in a single dose of 50 μL. [c] Severe ocular irritation. [d] Maximum IOP decrease in mmHg from the resting levels, measured just before administration of compounds, recorded during 5-h observation period after topical instillation of test compound. Significant IOP depression recorded at more than one time point. [e] An asterisk represents values where $P \leq 0.05$, minimum of six eyes.

During the later stages of 3-month toxicological studies in rabbits and dogs, a significant number of animals treated topically with a 2% suspension formulation of **22** developed ocular toxicity. The etiology of this adverse side effect is unknown but could be rationalized to result from a drug-mediated allergic reaction. Since **22** reacts facilely with GSH ($t_{1/2}$ = 5 min) under simulated physiological conditions with displacement of the sulfamoyl moiety, a similar reaction with a macromolecular nucleophile could produce a potent allergen. When **22** was tested in a modified Magnusson and Kligman guinea pig sensitization assay,[28] 17 of 20 animals reacted positively and **22** was deemed a potent contact sensitizer. The adverse ocular side effects evoked by **22** in animals as well as its characterization as a strong contact sensitizer precluded its development as a clinical candidate. Subsequently, benzothiazole-2-sulfonamide (**55a**) and several derivatives including **3**, **4**, **19**, **45**, and aminozolamide (**55b**) were evaluated in the Magnusson and Kligman guinea pig sensitization assay and were judged to be strong contact sensitizers by visual inspection and histopathology.[29] It is of interest to note that the repeated instillation of an aminozolamide gel elicited ocular inflammatory reactions in human volunteers.[10]

In summary, the importance of **22** resides in the fact that it is the first reported topically active CAI which displays ocular hypotensive activity in generally accepted preclinical assays after the conventional, i.e. one drop, topical administration of low doses.[16a-c]

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed in the Analytical Chemistry section of the Medicinal Chemistry Department under the direction of W. C. Randall and were within ±0.4% of the theoretical values unless otherwise noted. Detailed experimental procedures are given only for selected compounds, which will serve to illustrate the general synthetic methods employed.

6-Ethoxy-2-benzothiazolesulfenamide (2). A 12-L, four-necked round-bottomed flask equipped with a mechanical stirrer, thermometer, and two dropping funnels was charged with concentrated NH_4OH (28%, 5.1 L) and cooled to 0 °C in an ice–salt bath. One dropping funnel was charged with a filtered solution of 6-ethoxy-2-benzothiazolethiol (**1**, 404 g, 1.91 mol) in H_2O (1.4 L) containing NaOH (80.3 g, 2.01 mol) and the other was charged with NaOCl (2.4 L, 5.25%; commercial laundry bleach). The two solutions were added simultaneously over 1.5 h at 0–5 °C. Stirring

(28) Magnusson, B.; Kligman, A. M. *J. Invest. Dermatol.* **1969**, *52*, 268.
(29) Schwam, H.; Michelson, S. R.; de Solms, S. J.; Duprat, P.; Gautheron, P.; Shepard, K. L.; Smith, R. L.; Sugrue, M. F. *Abstr. 8th Int. Congr. Eye Res.* (San Franciso, CA) **1988**, *52*, 268.

was continued for 15 min and then the white product was filtered and rinsed with copious amounts of ice–H_2O to remove all NH_4OH. The damp filter cake was used to prepare **3**. A small, dried sample recrystallized from $(ClCH_2)_2$ melted at 141–143 °C. Anal. ($C_9H_{10}N_2OS_2$) C, H, N.

6-Ethoxy-2-benzothiazolesulfonamide (3). A 20-L plastic bucket equipped with a thermometer, mechanical stirrer, two dropping funnels, and an electrode attached to a pH meter was charged with a suspension of **2** (prepared from 404 g of **1**), Me_2CO (4 L), and H_2O (4 L). A solution of $KMnO_4$ (374 g, 2.37 mol) in H_2O (7.48 L) was added with stirring over a 2.5-h period while a temperature of 28–30 °C was maintained by the occasional addition of crushed ice, and a pH of 8.0–8.1 was maintained by the concomitant addition of H_2SO_4 (1 N, 825 mL). The reaction mixture was stirred for 1 h, treated with decolorizing charcoal (20 g), and NaOH (10 N, 500 mL), and then stirred for 0.5 h. The MnO_2 was filtered and rinsed with H_2O. The filtrate was acidified with concentrated HCl and the product was filtered, rinsed with H_2O, and dried, affording 396 g (80%) of **3**. A sample recrystallized from $(ClCH_2)_2$ melted at 188–189 °C (lit.[17] mp 188–190.5 °C).

6-Hydroxy-2-benzothiazolesulfonamide (4). To a 5-L, four-necked, round-bottomed flask equipped with a mechanical stirrer was added **3** (129.2 g, 0.5 mol) and $(ClCH_2)_2$ (2 L). The stirred suspension was cooled to 20 °C and treated with $AlCl_3$ (300 g) over an 0.5-h period. The reaction mixture was stirred at 25 °C for 20 h and then the $(ClCH_2)_2$ was decanted. The brown, residual gum was cooled in an ice–H_2O bath and then treated with a solution of concentrated HCl (250 mL) in ice H_2O (4 L) as rapidly as possible with vigorous stirring. The solid product was filtered, washed well with H_2O, reprecipitated from NaOH (20%, 350 mL) with HCl, filtered, rinsed with H_2O, and dried, affording 76 g (66%) of **4**. A sample recrystallized from *n*-BuOH melted at 229–231 °C. Anal. ($C_7H_6N_2O_3S_2$) C, H, N.

5-Hydroxy-2-benzothiazolesulfonamide (7). A stirred suspension of 5-methoxy-2-benzothiazolesulfonamide (**6**,[19] 11.8 g, 0.048 mol) and $AlCl_3$ (28 g, 0.21 mol) in heptane (0.5 L) was heated at reflux for 2.5 h and then cooled. The heptane was decanted and the $AlCl_3$ complex was decomposed by the rapid addition of ice–H_2O (400 mL) to give 6.5 g (59%) of **7**, which melted at 224 °C after reprecipitation from dilute NaOH with dilute HCl. Anal. ($C_7H_6N_2O_3S_2$) C, H, N.

Method A. 2-Sulfamoyl-6-benzothiazolyl 2,2-Dimethylpropionate (22). Trimethylacetic anhydride (186 mL, 0.92 mol) was added dropwise at 40–50 °C over 1 h to a solution of **4** (191.2 g, 0.83 mol) and Et_3N (128 mL, 0.92 mol) in DMF (1.9 L) in a 5-L four-necked round-bottomed flask equipped with a mechanical stirrer, thermometer, addition funnel, and reflux condenser. The reaction mixture was stirred at 40–50 °C for 2 h, and then an additional 10% of Et_3N (12.8 mL) and trimethylacetic anhydride (18.6 mL) were added and stirring was continued for an additional hour. The reaction mixture was added to crushed ice–H_2O (~10 L) and stirred mechanically until a solid formed (~2 h). The solid was collected by suction filtration, washed well with H_2O, pulled dry overnight, and then dried in a vacuum oven at 80 °C overnight. The solid was dissolved in EtOAc–10% DMF and chromatographed on a silica gel (2 kg E. Merck 70–230 mesh) gravity column (14-cm diameter) eluting with EtOAc–hexane (1:2, then 1:1). Like fractions were combined, treated with Norit A for 0.5 h with stirring, and then filtered through fiberglass paper and concentrated to dryness on a rotary evaporator. Trituration with hexane gave 172.2 g of 2-sulfamoyl-6-benzothiazolyl 2,2-dimethylpropionate. One crystallization from toluene (2 L) gave 140 g of analytically pure product, mp 171–173 °C. Anal. ($C_{11}H_{12}N_2O_4S_2$) C, H, N.

2-Sulfamoyl-5-benzothiazolyl Butyrate (8). Butyric anhydride (4.7 mL, 0.03 mol) was added dropwise over 5 min to a stirred solution of **6** (6.5 g, 0.028 mol), 4-(dimethylamino)pyridine (0.2 g), and Et_3N (4 mL, 0.028 mol) in DMF (30 mL). Stirring was continued for 1.5 h and then the reaction mixture was poured into ice–H_2O (150 mL) containing excess HCl, extracted into ether, washed with H_2O, dried over $MgSO_4$, and evaporated at reduced pressure. The residue was chromatographed on silica gel eluting with EtOAc–hexane (1:1) providing 1.9 g (23%) of **8**, which melted at 132 °C after recrystallization from C_6H_6. Anal. ($C_{11}H_{12}N_2O_4S_2$) C, H, N.

Method B. *N*-Methylpyrrole-2-carboxylic *N,N*-Diphenylcarbamic Anhydride (17). A solution of *N*-methylpyrrole-2-carboxylic acid (**15**, 1.25 g, 0.01 mol) and Et_3N (1.01 g, 0.01 mol) in H_2O (10 mL) was added dropwise to a solution of *N,N*-diphenylcarbamoylpyridinium chloride[30] (**16**, 3.11 g, 0.01 mol) in H_2O. The yellow solid that formed was filtered, washed with H_2O, and dried (3.11 g), mp 108–113 °C. Recrystallization from cyclohexane gave white needles, mp 116–117 °C. Anal. ($C_{19}H_{16}N_2O_3$) C, H, N.

2-Sulfamoyl-6-benzothiazolyl *N*-Methylpyrrole-2-carboxylate (43). Et_3N (2.20 g, 0.0217 mol) and 4-(dimethylamino)pyridine (100 mg) were added to a solution of **4** (5.00 g, 0.0217 mol) in Me_2CO (60 mL), followed by the dropwise addition of **17** (6.95 g, 0.0217 mol) in Me_2CO (40 mL). When addition was complete, the mixture was heated on the steam bath for 2 h. The cooled reaction mixture was added to 1 L of H_2O and extracted with EtOAc. The organic phase was washed with H_2O (3×) and saturated brine and dried (Na_2SO_4). The filtered solvent was evaporated to dryness and the residue was stirred with hexane to remove $(C_6H_5)_2NH$: 5.48 g (75% yield), tan solid, mp 205–207 °C. Two recrystallizations from CH_3CN gave **43**, mp 212.5–213.5 °C. Anal. ($C_{13}H_{11}N_3O_4S_2$) C, H, N.

2-Sulfamoyl-6-benzothiazolyl 2-(2-Methoxyethoxy)-2,2-dimethylpropionate (41). A solution of 2-(2-methoxyethoxy)-2,2-dimethylpropionic acid (1.56 g, 0.0096 mol), *N,N*-diphenylcarbamoylpyridinium chloride[30] (3.00 g, 0.0096 mol) and Et_3N (2.69 mL, 0.019 mol) in Me_2CO (20 mL) and DMF (15 mL) was stirred at 25 °C for 15 min, treated with **4** (2.02 g, 0.0088 mol), and heated at reflux for 1 h. The Me_2CO was evaporated at reduced pressure and the residue was partitioned between EtOAc and H_2O. The EtOAc solution was dried over $MgSO_4$ and evaporated at reduced pressure. The residue was chromatographed over silica (EtOAc–hexane 1:1) to remove $(C_6H_5)_2NH$ and then rechromatographed ($CHCl_3$–*i*-PrOH 20:1) to obtain **41**. Anal. ($C_{14}H_{18}N_2O_6S_2$) C, H, N.

Method C. 2-Sulfamoyl-6-benzothiazolyl Benzoate (29). A stirred solution of **4** (2.3 g, 0.01 mol) and 4-(dimethylamino)-pyridine (0.2 g) in pyridine was cooled to 15 °C and treated with BzCl (1.2 mL, 0.01 mol) over a 5-min period. After stirring at 25 °C for 1.5 h, the solution was poured into ice–H_2O (150 mL) containing excess HCl. The crude product was filtered, rinsed with H_2O, and dried.

2-Sulfamoyl-6-benzothiazolyl 2-Methylpropionate (20). Isobutyryl chloride (10.21 g, 0.101 mol) was added dropwise to a solution of **4** (21 g, 0.091 mol) and Et_3N (10.18 g, 0.101 mol) in Me_2CO (420 mL) at −5 °C. After 0.25 h, the Et_3NHCl was filtered and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc, washed with H_2O, and dried (Na_2SO_4). Removal of the solvent gave 27 g of an off-white solid, mp 136–139 °C. Two recrystallizations from $(ClCH_2)_2$ (1.07 g of **4**, insoluble in $(ClCH_2)_2$, filtered) gave white needles, 19.30 g. Anal. ($C_{11}H_{12}N_2O_4S_2$) C, H, N.

2-[(*N,N*-Dimethylaminomethylene)sulfamoyl]-6-benzothiazolyl 2-Methylpropionate (14). Isobutyryl chloride (11.2 g, 0.105 mol) was added dropwise to a cold (0–5 °C) solution of **4** (22.0 g, 0.096 mol) and Et_3N (10.63 g, 0.105 mol) in DMF (220 mL). When addition was complete, the reaction mixture was maintained at 5 °C for 24 h. EtOAc (220 mL) was added, the Et_3NHCl was filtered, and the filtrate was treated with H_2O (1 L). The organic layer was separated and washed with saturated Na_2CO_3 solution (100 mL), saturated Na_2CO_3–H_2O (100 mL, 1:1, v/v), and H_2O (50 mL). Acidification of the basic extracts gave 4.9 g of recovered **4** after filtration and drying. The organic phase was evaporated to a glassy residue, 16.3 g. Trituration of this residue with EtOAc gave 8.23 g of white solid, mp 120–123 °C. Repeated recrystallization from EtOAc gave 4.44 g, mp 131–132 °C. Anal. ($C_{14}H_{17}N_3O_4S_2$) C, H, N.

6-Hydroxy-7-nitrobenzothiazole-2-sulfonamide (9). Fuming HNO_3 (sp gr = 1.48, 0.37 mL, 0.00868 mol) was added dropwise to concentrated H_2SO_4 (sp gr = 1.84, 2.7 mL) at 10 °C followed by the addition of **4** (1.00 g, 0.00434 mol) over a 30-min period at 5–10 °C. The cooling bath was removed and stirring was

(30) Herzog, J. *Ber. Dtsch. Chem. Ges.* **1907**, *40*, 1831.

continued for 1 h. The reaction mixture was poured onto crushed ice (15 g), affording 0.49 g (41%) of **9**, which melted at 258–259 °C after recrystallization from CH_3CN. Anal. ($C_7H_5N_3O_5S_2$) C, H, N.

7-Amino-6-hydroxybenzothiazole-2-sulfonamide (10). A 6.4 g (0.0232 mol) sample of **9** in EtOH (250 mL) containing 5% Pt on C (1 g) was hydrogenated in a Parr apparatus at 25 °C until the required amount of H_2 was consumed (3 h). The catalyst was filtered and thoroughly washed with boiling EtOH (250 mL). Evaporation of the combined solvents left 5.2 g (91%) of **10** as a yellow solid, mp >340 °C. Anal. ($C_7H_7N_3O_3S$) C, H, N.

7-Amino-2-sulfamoyl-6-benzothiazolyl 2,2-Dimethylpropionate (11). Trimethylacetic anhydride (0.38 g, 2 mmol) was added dropwise to a solution of Et_3N (0.206 g, 2 mmol) and **10** (0.500 g, 2 mmol) in DMF (5 mL) at 15–18 °C. After 5 min of additional stirring, the reaction was quenched by mixing with ice-cold H_2O (50 mL). The resulting yellow solid was filtered, washed with H_2O, and dried: 0.50 g, mp 164–166 °C; TLC, fluorescent silica, EtOAc–hexane (1:1, v/v), R_f = 0.68. ($C_{12}H_{15}N_3O_4S_2$) ($C_{12}H_{15}N_3O_4S_2$) C, H, N.

7-[(2,2-Dimethylpropionyl)amino]-6-hydroxybenzothiazole-2-sulfonamide (12). A sample of **11** stirred with silica gel suspended in EtOAc and hexane (1:1, v/v) was completely converted to **12** after 40 h: mp 184–185 °C. TLC, fluorescent silica, EtOAc–hexane (1:1, v/v), R_f = 0.27. Anal. ($C_{12}H_{15}N_3O_4S_2$) C, H, N.

7-[(2,2-Dimethylpropionyl)amino]-2-sulfamoyl-6-benzothiazolyl 2,2-Dimethylpropionate (13). Trimethylacetic anhydride (1.52 g, 0.0082 mol) was added at 45–46 °C to a stirred solution of **12** (1.0 g, 0.0041 mmol) and Et_3N (0.825 g, 0.0082 mol) in DMF (20 mL) over a 1-h period. The reaction was stirred at 45 °C for 12 h and then poured into ice–H_2O (100 mL) to give 1.1 g of **13**, which was purified by chromatography on silica (20 g) eluted with EtOAc–hexane (1:1), mp 223–224 °C. Anal. ($C_{17}H_{23}N_3O_5S_2$) C, H, N.

In Vitro Procedures. Inhibition of Human Carbonic Anhydrase II. I_{50} values against human CAII were determined by the pH stat method as described previously.[11]

Reactivity with Reduced Glutathione (GSH). A stirred solution containing 0.1 M phosphate (pH 7.4), 5×10^{-4} M test compound, and 2.5×10^{3} M GSH (5 equiv) was incubated at 37 °C. Aliquots (0.2 mL) were removed with time, immediately quenched by addition of 1% H_3PO_4 in CH_3CN, and analyzed by HPLC under isocratic conditions.

Corneal Esterase Mediated Ester Cleavage. Freshly acquired corneas from the eyes of 5 albino rabbits were homogenized in a Polytron tissue homogenizer for 90 s in 0.1 M potassium phosphate (pH 7.4) buffer (7 mL). The homogenate was centrifuged for 10 min at 4 °C in a Sorvall RC-5 refrigerated centrifuge and the supernatant was decanted away from the cellular debris. A solution of the test compound (50 μg) in CH_3CN (50 μL) was added to the corneal supernatant (750 μL), and the resulting solution was incubated at 37 °C. Aliquots (100 μL) were removed at 0, 5, 10, 20, 30, 45, 60, and 90 min and added to an equal volume of 0.1 N HCl in CH_3CN, which served to quench the esterase-mediated reaction by lowering the pH and precipitating the protein. The resulting suspension was clarified by centrifugation and analyzed by HPLC under isocratic conditions.

Determination of Corneal Penetration Rate Constants. This assay was carried out as described by O'Brien and Edelhauser[31] with the exception that GSH was not present in the buffer.

In Vivo Procedures. Intraocular Pressure Recovery Rate Assay. This assay was carried out in adult New Zealand rabbits (2.5–5 kg) as described previously.[16b]

Intraocular Pressure Assay in α-Chymotrypsinized Rabbits. This assay was carried out in adult New Zealand rabbits (2.5–5 kg) as described previously.[16b]

Guinea Pig Sensitization Assay. This assay, with minor modifications, was carried out as described by Magnusson and Kligman.[28] Animals (20) were intradermally injected with 8 mg of compound as a 2% suspension in oil. One week later a patch containing an 8% petrolatum suspension of compound was placed over the injection site for 48 h. Two weeks later an occlusive patch containing an 8% petrolatum suspension of the compound was applied to the shaved flank of the animal for 24 h. After removal of the patch, the flank was evaluated both visually and microscopically for the degree of sensitization. The sensitizing potential of a compound was assigned according to the percentage of animals giving a positive response by using the following scale:

% animals responding	classification
0	nonsensitizer
5–25	mild sensitizer
30–65	moderate sensitizer
70–100	strong sensitizer

Registry No. **1**, 120-53-6; **2**, 5304-15-4; **3**, 452-35-7; **4**, 29927-14-8; **6**, 86695-27-4; **7**, 86695-28-5; **8**, 88515-17-7; **9**, 121810-92-2; **10**, 121810-93-3; **11**, 121810-94-4; **12**, 121810-95-5; **13**, 121810-96-6; **14**, 121810-97-7; **15**, 6973-60-0; **16**, 1228-96-2; **17**, 121810-98-8; **18**, 86394-99-2; **19**, 86394-92-5; **20**, 86395-00-8; **21**, 86395-02-0; **22**, 86394-94-7; **23**, 121810-99-9; **24**, 86395-01-9; **25**, 86395-03-1; **26**, 86394-96-9; **27**, 86394-98-1; *trans*-**28**, 121811-00-5; **29**, 86395-15-5; **30**, 86394-93-6; **31**, 86394-95-8; **32**, 121811-01-6; **33**, 121811-02-7; **34**, 121811-03-8; **35**, 86394-97-0; **36**, 121811-04-9; **37**, 121811-05-0; **38**, 121811-06-1; **39**, 121811-07-2; **40**, 121811-08-3; **41**, 121811-09-4; **42**, 121811-10-7; **43**, 121811-11-8; **44**, 94855-84-2; **45**, 94855-82-0; **46**, 94855-83-1; **47**, 93105-21-6; **48**, 93105-20-5; **49**, 93509-86-5; **50**, 93460-24-3; **51**, 92064-53-4; $(CH_3CO)_2O$, 108-24-7; $(CH_3(CH_2)_2CO)_2O$, 106-31-0; $(CH_3CH{=}CHCO)_2O$, 623-68-7; $(PhCH_2CO)_2O$, 1555-80-2; $(2,4,6\text{-}(CH_3)_3C_6H_2CO)_2O$, 5745-51-7; $((CH_3)_3COCO)_2O$, 24424-99-5; $MeO(CH_2)_2OC(CH_3)_2CO_2CON(Ph)_2$, 121811-12-9; $p\text{-}Me_2NC_6H_4CO_2CON(Ph)_2$, 121811-13-0; $MeOC(CH_3)_2CO_2CON(Ph)_2$, 121811-14-1; $EtOC(CH_3)_2CO_2CON(Ph)_2$, 121811-15-2; $Et(OCH_2CH_2)_2OC(CH_3)_2CO_2CON(Ph)_2$, 121811-16-3; $p\text{-}ClC_6H_4CH_2COCl$, 25026-34-0; $p\text{-}Me_2NCH_2C_6H_4COCl$, 121811-18-5; $p\text{-}Et_2N(CH_2)_2OC_6H_4COCl$, 121811-19-6; $PhCH{=}CHCOCl$, 102-92-1; $2,6\text{-}(MeO)_2C_6H_3COCl$, 1989-53-3; $MeOCH_2COCl$, 38870-89-2; $EtOCOCl$, 541-41-3; $(CH_3)_2CHCH_2OCOCl$, 543-27-1; $CH_3(CH_2)_3SO_2Cl$, 2386-60-9; $p\text{-}MeC_6H_4SO_2Cl$, 98-59-9; Me_2NSO_2Cl, 13360-57-1; Me_2NCOCl, 79-44-7; $(EtO)_2POCl$, 814-49-3; trimethylacetic anhydride, 1538-75-6; butyric anhydride, 106-31-0; benzoyl chloride, 98-88-4; isobutyryl chloride, 79-30-1; hexanoyl chloride, 142-61-0; octanoyl chloride, 111-64-8; dodecanoyl chloride, 112-16-3; cyclopentylacetyl chloride, 1122-99-2; cyclohexylacetyl chloride, 23860-35-7; *trans*-4-(diethylaminomethyl)cyclohexanecarbonyl chloride, 121811-17-4; carbonic anhydrase, 9001-03-0; 2-(2-methoxyethoxy)2-methylpropionic acid, 121811-20-9.

(31) Edelhauser, H. F.; Maren, T. H. *Arch. Ophthalmol.* **1988**, *106*, 1110.