Topically Active Carbonic Anhydrase Inhibitors. 2. Benzo[*b*]thiophenesulfonamide Derivatives with Ocular Hypotensive Activity†

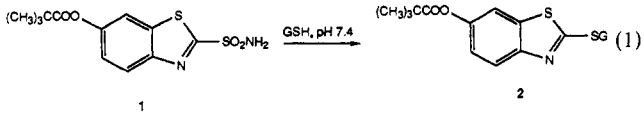
Samuel L. Graham,* Kenneth L. Shepard,* Paul S. Anderson, John J. Baldwin, Darryl B. Best, Marcia E. Christy, Mark B. Freedman, Pierre Gautheron,‡ Charles N. Habecker, Jacob M. Hoffman, Paulette A. Lyle, Stuart R. Michelson, Gerald S. Ponticello, Charles M. Robb, Harvey Schwam, Anthony M. Smith, Robert L. Smith, John M. Sondey, Kim M. Strohmaier, Michael F. Sugrue,‡ and Sandor L. Varga

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, and Centre De Recherche MSD-Chibret, Riom, France. *Received January 23, 1989*

Derivatives of benzo[*b*]thiophene-2-sulfonamide were prepared to investigate their potential utility as topically active inhibitors of ocular carbonic anhydrase. Such an agent would be useful in the treatment of glaucoma. Among the compounds described are 6-hydroxybenzo[*b*]thiophene-2-sulfonamide (**16**) and its acetate ester (**23**), which are among the most potent ocular hypotensive agents in this class, as assessed in the α-chymotrypsinized rabbit. These compounds were selected for clinical evaluation.

The central concept in the treatment of glaucoma is that reduction of intraocular pressure (IOP) will preserve visual function. While a variety of pharmacological and surgical methods have evolved for lowering IOP in glaucoma patients, none of these is entirely satisfactory.[1] It is well-established that effective reduction of IOP can be achieved by the systemic administration of carbonic anhydrase inhibitors (CAIs), which act by reducing the rate of aqueous humor secretion. Unfortunately, systemic therapy with CAIs leads to significant side effects, which have limited their utility in treating glaucoma. These side effects are a direct result of inhibition of carbonic anhydrase in extraocular tissues.

An attractive approach to achieving an adequate ocular hypotensive response, while minimizing side effects, is the development of a topically active CAI. Although this has been a goal for over 30 years, only recently has any measure of success been recorded.[2] For example, our group reported that the benzothiazolesulfonamide **1** is a topically active ocular hypotensive agent in rabbits.[2d,f] However, clinical development of **1** was precluded by two observations. First, benzothiazolesulfonamides undergo rapid metabolism involving nucleophilic displacement of the sulfonamide group by reduced glutathione (GSH).[3] For compound **1**, the half-life of this process under simulated physiological conditions (leading to **2**) is less than 1 h (eq 1). Of greater consequence was the observation, during



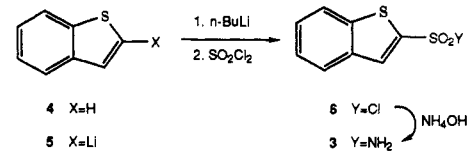
(1)

1 2

a 3-month ocular safety study in rabbits, that a significant number of animals developed an allergic reaction to **1**. Subsequent evaluation of **1** in a guinea pig model for dermal-sensitization potential[4] revealed that **1** was a potent allergen. A number of other benzothiazolesulfonamides were found to share this property.[5] Thus, the electrophilic nature of benzothiazolesulfonamides is manifested by both rapid metabolism and a presumed arylation reaction of biological macromolecules leading to an immune response.

In this paper are described the syntheses and properties of derivatives of benzo[*b*]thiophene-2-sulfonamide (**3**). The decision to pursue these studies was based primarily on the premise that the electrophilic nature of **1** (and related benzothiazoles) is due to the presence of the imino group in the ring system. Deaza analogues of **1**, such as **3**, therefore might prove less reactive toward nucleophilic substitution. It also was hoped that removal of the nitrogen atom in the ring would lead to improved topical bioavailability. It has been postulated that translocation through the cornea is the major route of access of topically applied drugs to the anterior segment of the eye.[6] Since

Scheme I



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‡ Centre De Recherche MSD-Chibret.

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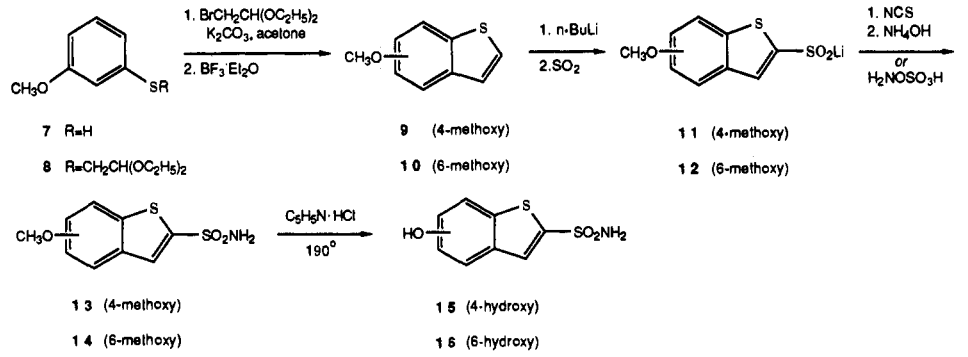
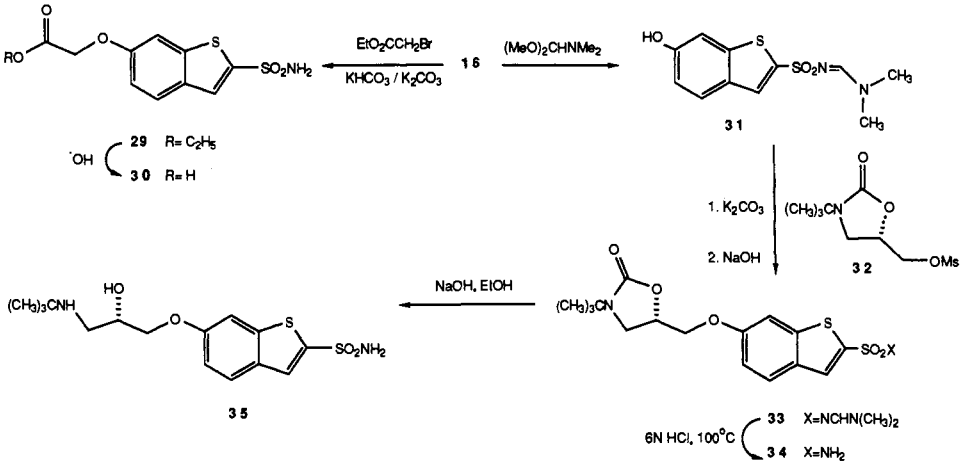
Scheme II



Scheme III



the initial corneal barrier is a highly lipophilic epithelial cell layer, we felt that the intrinsically more lipophilic benzo[*b*]thiophenes should be more readily absorbed than the corresponding benzothiazole. This might obviate the need for a lipophilic prodrug strategy (i.e., the requirement for the pivaloate ester in **1**), which was required to achieve suitable activity in the benzothiazole series.[2f] However, since the factors governing the drug-transport process are not well-defined, we included in this study compounds with quite diverse structural and physical characteristics. In general, we have found that these benzothiophenesulfonamides are excellent inhibitors of human carbonic anhydrase II. They are practically inert toward nucleophiles such as glutathione. Significantly, they are devoid of dermal sensitization potential as assessed in the guinea pig. Several of the compounds show excellent ocular hypotensive activity in rabbits. Among these, compounds **16** and **23** have been selected for clinical evaluation.

Chemistry. The key element in devising syntheses of benzo[*b*]thiophene-2-sulfonamides is the regiospecific introduction of the sulfamoyl group. Sulfonation of benzo[*b*]thiophenes under electrophilic conditions is not a general solution to this problem since many benzo[*b*]thiophenes react with electrophiles at C-3.[7] However, deprotonation of benzo[*b*]thiophenes with lithium amides or lithium alkyls invariably leads to lithiation at C-2. As shown in Scheme I, reaction of benzo[*b*]thiophene (**4**) with *n*-BuLi in THF gave **5**. Addition of **5** to sulfuryl chloride in hexane[8] yielded chlorosulfonyl compound **6**, in moderate yield. Sulfonamide **3** was obtained by treating **6** with aqueous ammonia in acetone.

Benzo[*b*]thiophenes oxygenated at the 4- and 6-positions were obtained by the route shown in Scheme II. *m*-Methoxybenzenethiol (**7**) was alkylated with bromoacetaldehyde diethyl acetal. The product (**8**) was cyclized with boron trifluoride etherate, in a modification of a published synthesis.[9] A 1:10 mixture of the 4- and 6-methoxybenzo[*b*]thiophenes (**9** and **10**, respectively) was obtained. This cyclization must be carried out at a concentration of ≤0.05 M to avoid formation of polymeric material. Since the mixture of **9** and **10** was separable only with great difficulty, introduction of the sulfonamide was carried out on the crude product of the cyclization. Lithiation and chlorosulfonation with sulfuryl chloride was possible, but only in modest yield. Substantial amounts of ring-chlorination products were observed, in addition to the desired sulfonyl chloride. However, metalation followed by reaction with sulfur dioxide provided the easily isolable sulfinic acid salts **11** and **12**. Typically, these sulfinates were chlorinated with *N*-chlorosuccinimide and the resulting sulfonyl chlorides were treated with aqueous ammonia in acetone, to give the sulfonamides **13** and **14**. Alternatively, the direct amination of the sulfinates with hydroxylamine-*O*-sulfonic acid was possible.[10] Sulfonamides **13** and **14** were readily separable by fractional crystallization from dichloroethane. The separated isomers were demethylated by heating with pyridine hydrochloride to furnish phenols **15** and **16**. 5- and 7-methoxybenzo[*b*]thiophenes (**17**[11] and **18**[12]) were synthesized by literature

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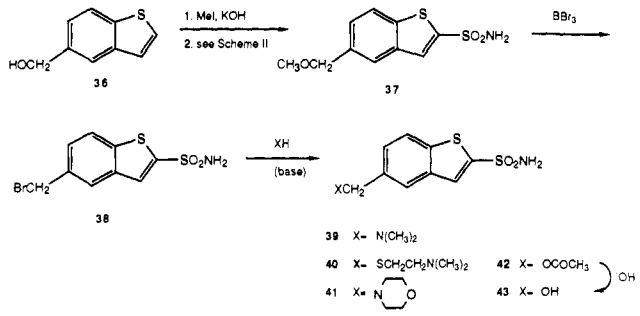
Table I. Esters of Compounds 16 and 21

no.	R	synthesis	% yield
23	6-$OCOCH_3$	$(CH_3CO)_2O$, pyridine, ethyl acetate, room temp	80
24	6-$OCOOCH_2CH(CH_3)_2$	$(CH_3)_2CHCH_2OCOCl$, Et_3N, acetone, −5 °C	70
25	6-$OCOC(CH_3)_3$	$[(CH_3)_3CCO]_2O$	66
6	5-$OCON(CH_3)_2$	$(CH_3)_2NCOCl$	53
27	6-OSO_3Na	(1) H_2NSO_3H, pyridine, 100 °C,[13] (2) NaOH	50
28	6-OPO_3Na_2	(1) $POCl_3$, pyridine, 0 °C,[14] (2) NaOH	53

Scheme IV



procedures. These compounds were converted to sulfonamides **19–22** by the method described above.

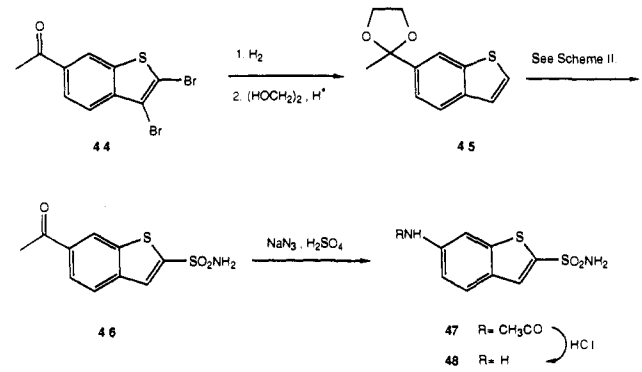
Several ester derivatives of the 5- and 6-hydroxy compounds **21** and **16** were prepared (Table I). Conditions for the esterification reaction are shown in the table.

Alkylation of phenol **16** with various electrophiles was carried out as shown in Scheme III. Phenoxyacetic acid derivatives **29** and **30** were obtained by direct alkylation of **16** with ethyl bromoacetate followed by ester hydrolysis. It was noted in those syntheses that sulfonamide *N*-alkylation was competitive with O-alkylation. To circumvent this problem in the synthesis of ethers **34** and **35**, the sulfonamide was converted to the formamidine (**31**) by treatment with dimethylformamide dimethyl acetal. Alkylation of **31** with mesylate **32**[15] and subsequent acid hydrolysis gave ether **34**. Finally, base-catalyzed hydrolysis of the cyclic carbamate gave **35**.

Benzo[*b*]thiophene-2-sulfonamides with a variety of substituents in the 5-position were prepared from the known[16] hydroxymethyl compound **36** (Scheme IV). The hydroxy group was methylated and the resulting ether was converted to sulfonamide **37** by the previously described method. The ether was cleaved with boron tribromide to give benzylic bromide **38**. Alkylation of several nucleophiles by **38** afforded compounds **39–43**.

The 6-amino derivative **48** was prepared from the known[17] dibromide **44** (Scheme V). After dehalogenation and protection of the ketone, ethylene ketal **45** was converted to the sulfonamide by the sulfinic acid route. The ketone was regenerated and acetyl compound **46** was subjected to the Schmidt reaction. Amide hydrolysis gave **48**.

Scheme V



Selected characterization data for the compounds prepared in this study are shown in Table II.

In Vitro Evaluation

The ability of these benzo[*b*]thiophene-2-sulfonamides to inhibit CO_2 hydration catalyzed by human erythrocyte carbonic anhydrase II was determined using a pH-stat assay.[2e] In general, the compounds are excellent inhibitors with I_{50} values in the 1–20 nM range (Table II). One interesting observation on the effect of structure upon CAI activity concerns inorganic esters **27** and **28**. While monoanionic sulfate **27** and neutral phenol **16** are essentially equipotent as CAIs, the dianionic phosphate **28** is more than 1 order of magnitude less active. Although the origin of this effect is not certain, it may simply reflect the desolvation of the highly charged phosphate upon entering the hydrophobic enzyme active site.[18]

The next critical issue to be addressed was the electrophilicity of benzo[*b*]thiophene-2-sulfonamides. This was gauged by the extent of reaction of the compounds with reduced glutathione under simulated physiological conditions.[2f] Thus, a solution of **16** (0.5 mM) and reduced glutathione (2.5 mM) in 0.1 M phosphate buffer (pH 7.4) was incubated for 16 h at 37 °C. The sulfonamide (**16**) was unchanged. A similar lack of reactivity was noted for all benzo[*b*]thiophene-2-sulfonamides that we evaluated. In contrast, benzothiazole **1** is converted completely to the glutathione conjugate **2** under these conditions.

In Vivo Evaluation

The sensitization potential of benzo[*b*]thiophene-2-sulfonamides **16** and **23** was evaluated in the guinea pig.[5] Neither **16** nor **23** elicited an allergic response as assessed by visual inspection and histopathology. In contrast, benzothiazole **1** was a strong sensitizer when assayed under identical conditions.[2f]

An IOP recovery rate assay[19] was used as a qualitative method to determine if the topical administration of these CAIs affected the rate of aqueous humor secretion. In this assay, compound **1** inhibited secretion upon administration of a single drop (50 μL) of a 2% suspension.[2d] Therefore, only those compounds that were active in this screening assay at a dose of 2% or less were considered as candidates for more in-depth study. The required level of efficacy was found only for phenols **16** and **21** and esters of these compounds.

The IOP-lowering effect of **16**, **23**, and **25** was studied in the α-chymotrypsin (α-CT) treated rabbit model of ocular hypertension (Table III). In this model,[20] the in-

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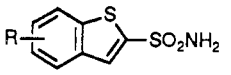
Table II. Benzo[*b*]thiophene-2-sulfonamide Derivatives and Reference CAIs



no.	R	mp, °C	recryst[a] solvent	analysis[b]	I_{50},[c] nM
	acetazolamide				10.0
	dichlorphenamide				40.0
	ethoxzolamide				3.0
	methazolamide				21.0
3	H	202–204	A	$C_8H_7NO_2S_2$ (C, H, N)	5.2
13	4-OCH_3	180–182	B	$C_9H_9NO_3S_2$ (C, H, N)	12.0
14	6-OCH_3	166–167	B	$C_9H_9NO_3S_2$ (C, H, N)	8.8
15	4-OH	212–213	C	$C_8H_7NO_3S_2$ (C, H, N)	12.0
16	6-OH	212–215	B	$C_8H_7NO_3S_2$ (C, H, N)	8.8
19	5-OCH_3	125–126	B	$C_9H_9NO_3S_2$ (C, H, N)	23.0
20	7-OCH_3	195–197	B	$C_9H_9NO_3S_2$ (C, H, N)	50.0
21	5-OH	192.5–193.5	D	$C_8H_7NO_3S_2$ (C, H, N)	6.2
22	7-OH	196–198	D	$C_8H_7NO_3S_2$ (C, H, N)	11.0
23	6-$OCOCH_3$	171–173	E	$C_{10}H_9NO_4S_2$ (C, H, N)	4.0
24	6-$OC(O)OCH_2CH(CH_3)_2$	128–130	J	$C_{13}H_{15}NO_5S_2$ (C, H, N)	5.6
25	6-$OCOC(CH_3)_3$	166–167	A	$C_{13}H_{15}NO_4S_2$ (C, H, N)	2.6
26	5-$OCON(CH_3)_2$	193–194	K	$C_{11}H_{12}N_2O_4S_2$ (C, H, N)	5.0
27	6-OSO_3Na		L	$C_8H_6NNaO_6S_3 \cdot 0.11NaCl$ (C, H, N, Cl)	6.8
28	6-OPO_3Na_2		M	$C_8H_6NNa_2O_6PS_2 \cdot H_2O$ (C, H, N, S)	100
29	6-$OCH_2C(O)OC_2H_5$	182–184	F	$C_{12}H_{13}NO_5S_2$ (C, H, N)	
30	6-OCH_2COOH	205–208	F	$C_{10}H_9NO_5S_2$ (C, H, N)	14
34	6-$OCH_2CHCH_2N[C(CH_3)_3]COO$	147–149	B	$C_{16}H_{20}N_2O_5S_2$ (C, H, N)	8.0
35	6-$OCH_2CH(OH)CH_2NHC(CH_3)_3 \cdot HCl$	266–268	G	$C_{15}H_{22}N_2O_4S_2 \cdot HCl \cdot 0.4NaCl \cdot 0.4H_2O$ (C, H, N, Cl)	18
37	5-CH_2OCH_3	125–126	B	$C_{10}H_{11}NO_3S_2$ (C, H, N)	9
39	5-$CH_2N(CH_3)_2$	172–174	D	$C_{11}H_{14}N_2O_2S_2$ (C, H, N)	12
40	5-$CH_2S(CH_2)_2NC(CH_3)_2$	132–133	C	$C_{13}H_{18}N_2O_2S_3$ (C, H, N)	15
41	5-$CH_2NCH_2CH_2OCH_2CH_2 \cdot HCl$	244–245	D	$C_{13}H_{16}N_2O_3S_2 \cdot HCl$ (C, H, N)	15
42	5-CH_2OCOCH_3	113–115	H	$C_{11}H_{11}NO_4S_2$ (C, H, N)	10
43	5-CH_2OH	174–176	D	$C_9H_9NO_3S_2$ (C, H, N)	9
46	6-$COCH_3$	154–155	H	$C_{10}H_9NO_3S_2$ (C, H, N)	6
47	6-$NHCOCH_3$	231–232	I	$C_{10}H_{10}N_2O_3S_2$ (C, H, N)	4.8
48	6-NH_2	239–240	I	$C_8H_8N_2O_2S_2$ (C, H, N)	10

[a] Recrystallization solvents: A, ethyl acetate/hexane; B, dichloroethane; C, water; D, nitromethane; E, THF/hexane; F, ethyl acetate; G, methanol/ethanol; H, chloroform; I, acetonitrile; J, *n*-chlorobutane; *K*, 2-propanol; L, aqueous NaCl; M, acetone/water. [b] Combustion analysis was within ±0.4% of values calculated for the indicated empirical formula. [c] Concentration of inhibitor required to reduce the rate of carbonic anhydrase II catalyzed CO_2 hydration by 50%, pH 8.3, 4 °C, enzyme concentration 1/nM.

traocular injection of α-CT leads to a chronic elevation of intraocular pressure from an average of 20 mmHg to the range of 35–45 mmHg. A dose–response study with **16** using an oral route of administration established a maximal IOP-lowering response of 8 mmHg in our colony of animals. The lowest dose of **16** that produced this response was 1.25 mg/kg (average 5 mg per animal). For comparison, the response to oral acetazolamide was determined at the same dose level and found to be comparable. Use of a topical route of administration revealed a clear difference between the hypotensive activity of **16** and acetazolamide. Topical administration of a single 50-μL drop of a 0.1% suspension (0.05 mg) of **16** in 0.5% (hydroxyethyl)cellulose vehicle gave a response (7 mmHg reduction) that was similar to the maximum achieved by systemic dosing. Only a slight diminution in activity was seen employing a 0.05% suspension. Lower doses did not significantly reduce IOP. Acetazolamide, on the other hand, had no effect on IOP when adminstered as a 10% suspension. Other established CAIs, those of methazolamide, ethoxzolamide, and dichlorphenamide, clearly were much less active than **16** following topical administration. It is worth noting that **16** shows hypotensive activity at lower topical doses than is seen for **1**, without resorting to derivatization as a lipophilic prodrug as had been required in the case of **1**. At the inception of this work, we had proposed that some improvement in topical bioavailability would be realized due to the increased intrinsic lipophilicity of a benzothiophene vis-à-vis the corresponding benzothiazole.

Table III. Hypotensive Effect of Topically Applied CAIs in the α-Chymotrypsinized Rabbit

entry[a]	treatment	% minimum effective[b] topical dose	n[c]	maximum IOP reduction,[d] mmHg ± SE
1	acetazolamide	10	6	*e*
2	acetazolamide	1.25 mg/kg[f]	6	−6.3 ± 1.2
2	dichlorphenamide	10	6	−4.7 ± 1.4
3	ethoxzolamide	10	6	*e*
4	methazolamide	5	11	−5.1 ± 1.5
5	**1**	0.25	6	−7.8 ± 0.8
6	**16**	1.25 mg/kg[f]	6	−7.7 ± 3.4
7	**16**	0.10	18	−6.6 ± 5.1
8	**16**	0.05	12	−5.6 ± 3.8
9	**23**	0.10	17	−5.3 ± 4.1
10	**25**	0.05	6	−4.7 ± 3.3

[a] Data for entries 1–5 are from ref 2d. [b] Test compounds applied as a 50-μL drop of suspension of the indicated concentration (w/v) in 0.5% (hydroxyethyl)cellulose vehicle. [c] Number of eyes. [d] Significantly different than pretreatment value using Dunnett's two-tailed test ($P \leq 0.05$). IOP determined by pneumatic tonometry (Alcon Applanation tonometer). [e] No effect. [f] Oral dosing. Approximately 5-mg total dose per animal.

The expected increase in lipophilicity is illustrated by the measured octanol/buffer partition coefficients for 6-hydroxybenzothiazole-2-sulfonamide and **16**, which were 13 and 19, respectively. However, it is clear that attributing the improved IOP response to a single variable such as lipophilicity is naive. For example, converting **16** to the acetate (**23**) or pivaloate (**25**) derivatives did not afford a

more effective hypotensive agent.

Summary

A series of potent carbonic anhydrase inhibitors derived from benzo[b]thiophene-2-sulfonamide has been prepared. These compounds possess significant advantages over other topically active CAIs that have been proposed as useful for treating glaucoma. In addition to excellent hypotensive activity in the α-CT rabbit, the benzo[b]-thiophenes are devoid of the toxicity (allergenicity) associated with benzothiazolesulfonamides. On the basis of these results, compounds **16** and **23** were selected for clinical evaluation in human patients with glaucoma.[21]

Experimental Section

Unless otherwise noted, starting materials were obtained from commercial suppliers and were used without further purification. All reactions involving air-sensitive materials were carried out under an atmosphere of dry nitrogen. Tetrahydrofuran (THF) was Fisher anhydrous grade. Freshly opened bottles of the solvent were serum stoppered and the solvent was transferred under nitrogen. Solvent evaporation was carried out on a rotary evaporator. Melting points (uncorrected) were determined in open capillary tubes on a Thomas-Hoover apparatus. ^{1}H NMR spectra were determined at 60 (Varian T-60), 90 (Varian EM-390), or 300 MHz (Varian XL-300). Chemical shift data are reported in ppm downfield from tetramethylsilane as internal standard. Elemental analyses were done by J. Moreau of the Medicinal Chemistry Department (West Point) and are within ±0.4% of calculated values for the indicated elements.

Benzo[*b*]thiophene-2-sulfonamide (3). Benzo[b]thiophene (**4**, 2.95 g, 21.9 mmol) was dissolved in THF (15 mL) and cooled to 0 °C. To this solution was added slowly 15.4 mL of 1.6 M *n*-BuLi in hexane, and the temperature was maintained at 0 °C. After addition was complete, the mixture was stirred for 10 min. The heterogeneous mixture was diluted with 15 mL of THF, and the suspension of **5** was transferred by canula to a well-stirred solution of sulfuryl chloride (3.6 mL, 45 mmol) in hexane (15 mL) at 0 °C. After 1 h, the yellow suspension was concentrated in vacuo to approximately 20 mL and diluted with 25 mL of acetone and the resulting solution of acid chloride **6** was added slowly to a solution of 15 mL of NH_4OH in 50 mL of acetone. The mixture was diluted with water (500 mL) and acidified with 12 M HCl. Precipitated product **3** was isolated by filtration. The sulfonamide was dissolved in 200 mL of 0.5 N KOH and extracted with ether. The aqueous solution was acidified and the product was extracted into EtOAc. The extract was washed with water and brine, dried (Na_2SO_4), and evaporated to give 2.50 g (53%) of nearly pure **3** as a white solid, mp 201–202 °C. Recrystallization from hexane/EtOAc gave 1.25 g of **3**. ^{1}H NMR (DMSO-d_6) δ 8.08 (1 H, d, J = 8 Hz), 8.04 (1 H, J = 8 Hz), 7.93 (1 H, s), 7.90 (2 H, s), 7.51 (2 H, m).

4- and 6-Methoxybenzo[*b*]thiophene-2-sulfonamides (13 and 14). Bromoacetaldehyde diethyl acetal (16.5 mL, 0.11 mol) was added dropwise to a mixture of *m*-methoxybenzenethiol (**7**, 15.0 mL, 0.12 mol) and K_2CO_3 (16.6 g, 0.12 mol) in acetone (150 mL) at room temperature. The reaction mixture was stirred for 16 h and then filtered. The solid was washed with acetone, and the combined filtrate and washes were concentrated in vacuo. The residue was diluted with H_2O and extracted with Et_2O. The Et_2O extract was washed with 0.5 M KOH, H_2O, and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to give 27.4 g of **8** as a dark yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.18 (6 H, t), 3.13 (2 H, d), 3.43–3.73 (4 H, m), 3.77 (3 H, s), 4.67 (1 H, t), 6.60–7.27 (4 H, m).

A solution of **8** (13.0 g, 0.051 mol) in CH_2Cl_2 (100 mL) was added dropwise to a solution of BF_3·Et_2O (6.7 mL, 0.054 mol) in CH_2Cl_2 (1000 mL) at room temperature under a nitrogen atmosphere. The reaction mixture was stirred for 0.5 h, treated with aqueous $NaHCO_3$ solution, and stirred until both phases were clear. The CH_2Cl_2 layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extract was dried over Na_2SO_4, filtered, and concentrated in vacuo to give 8.68 g of an approximately 1:10 mixture of 4- and 6-methoxybenzo[b]thiophene (**9** and **10**) as a dark brown oil. Major isomer (**10**): ^{1}H NMR ($CDCl_3$) δ 3.85 (3 H, s), 6.98 (1 H, dd, J = 4.5, 1.5 Hz), 7.23 (2 H, s), 7.35 (1 H, d, J = 1.5 Hz), 7.68 (1 H, d, J = 4.5).

A 1.6 M solution of *n*-BuLi (420 mL, 0.67 mol) was added at −20 °C to a stirred solution of 100.0 g (0.610 mol) of a crude mixture of **9** and **10** in 500 mL of THF, with 20 mg of α,α′-bipyridyl. Sulfur dioxide gas was introduced through a needle positioned just above the surface of the solution, at such a rate so as to maintain the mixture at less than −10 °C. After the reaction mixture changed from a red to a yellow suspension, the introduction of SO_2 gas was discontinued, and 500 mL of ether was added to fully precipitate the sulfinic acid salts **11** and **12**. The reaction product was isolated by filtration and dried in vacuo overnight. There was obtained 139.2 g (98%) of a yellowish powder. Major isomer (**11**): ^{1}H NMR (DMSO-d_6) δ 7.6 (1 H, d, J = 9 Hz), 7.4 (1 H, d, J = 3 Hz), 7.1 (1 H, s), 6.9 (1 H, dd, J = 3,9 Hz), 3.75 (3 H, s).

The crude sulfinate was suspended in 1.4 L of methylene chloride, and the mixture was cooled on ice. *N*-Chlorosuccinimide (85 g, 0.63 mol) was added in a single portion at 5 °C. The cooling bath was removed and stirring was continued for 1 h. The solution was washed with water (500 mL) and the water layer was extracted with 250 mL of methylene chloride. The combined organic extract was washed with brine, dried (Na_2SO_4), and evaporated to give 156 g of a mixture of 4- and 6-methoxy benzo[b]thiophene-2-sulfonyl chloride, as a yellow solid. Major isomer: ^{1}H NMR ($CDCl_3$) δ 8.0 (1 H, s), 7.8 (1 H, d, J = 5 Hz), 7.28 (1 H, d, J = 3 Hz), 7.1 (H, dd, J = 3, 9 Hz), 3.9 (3 H, s).

The sulfonyl chloride, dissolved in a minimum volume of THF, was added portionwise to a 5 °C solution of 100 mL of NH_4OH in 700 mL of acetone. The temperature fluctuated between 5 and 18 °C. After 30 min, the bulk of the solvent was evaporated and the residue was partitioned between water (500 mL) and ethyl acetate (1 L). The phases were separated, and the aqueous was extracted with ethyl acetate. The combined extracts were washed with brine, dried, and evaporated to give 145 g of impure sulfonamide. This solid was stirred with boiling dichloroethane (250 mL). After cooling, filtration gave 88 g (59%) of pure sulfonamide **14**: ^{1}H NMR (DMSO-d_6) 7.85 (1 H, dd, J = 9 Hz), 7.77 (1 H, s), 7.72 (2 H, br s), 7.60 (1 H, dd, J = 2 Hz), 7.05 (1 H, dd, J = 9 and 2 Hz), 3.81 (3 H, s).

Concentration of the mother liquors from the recrystallization to 150 mL by boiling and subsequent cooling afforded a second crop of crystals (4.70 g), which was largely **13**. Recrystallization of this material gave 3.68 g of pure **13**: ^{1}H NMR (DMSO-d_6) δ 7.83 (1 H, br s), 7.80 (1 H, s), 7.60 (1 H, d), 7.44 (1 H, t), 6.95 (1 H, d), 3.92 (3 H, s).

Alternatively, a suspension of **11** and **12** (1.85 g, 7.9 mmol) in 30 mL of 5% $NaHCO_3$ solution was treated with 1.1 g (10 mmol) of hydroxylamine-*O*-sulfonic acid. After stirring overnight, the product was isolated by ethyl acetate extraction. The extract was washed with brine and dried. Evaporation of the solvent gave 1.8 g of a mixture of **13** and **14**. Recrystallization of the mixture from dichloroethane gave 0.77 g (40%) of **14**.

6-Hydroxybenzo[*b*]thiophene-2-sulfonamide (16). A mixture of **14** (41.8 g) and pyridine hydrochloride (200 g) was heated under N_2 in an oil bath at 190–200 °C for 2.5 h. The mixture was allowed to cool to 140 °C and poured onto 250 g of ice and 250 mL of brine. The mixture was extracted with ethyl acetate (3 × 250 mL). The combined extract was washed with 1 N HCl and brine. After drying (Na_2SO_4), the solvent was evaporated to give 29.7 g of phenol **16**, as a tan solid. Recrystallization from water, decolorizing with Norit A, gave a white solid: ^{1}H NMR (DMSO-d_6) δ 10.02 (1 H, br s), 7.81 (1 H, d, J = 9 Hz), 7.75 (1 H, s), 7.71 (2 H, s), 7.33 (1 H, d, J = 2 Hz), 6.96 (1 H, dd, J = 9 and 2 Hz).

6-Acetoxybenzo[*b*]thiophene-2-sulfonamide (23). A suspension of **16** (20 g, 87.2 mmol) in 100 mL of ethyl acetate was treated sequentially with pyridine (7.4 mL, 96 mmol) and acetic anhydride (9.1 mL, 96 mmol). An exothermic reaction ensued and the mixture transiently became homogeneous. After 3 h, TLC analysis (silica, 20% methanol/chloroform) of the resulting

(21) Preliminary studies in normal volunteers failed to demonstrate a hypotensive effect. Werner, E. B.; Gerber, D. S.; Yoder, Y. J. *Can. J. Ophthalmol.* **1987**, *22*, 316.

suspension showed complete consumption of the starting phenol. The mixture was filtered to give 21.0 g of a white powder. Recrystallization from THF/hexane gave 17.5 g of pure **23**.

6-[(Ethoxycarbonyl)methoxy]benzo[*b*]thiophene-2-sulfonamide (29). To a solution of ethyl bromoacetate (1.2 mL, 11 mmol) and **16** (2.29 g, 10 mmol) in DMSO (10 mL) was added dropwise a solution of potassium bicarbonate (1.0 g, 10 mmol) and potassium carbonate (0.13 g, 1 mmol) in water (7 mL). The mixture was stirred for 1–2 days and then diluted with water. The precipitated product was collected, dissolved in ethyl acetate, dried (Na_2SO_4), and evaporated to dryness. The residue was recrystallized from hot ethyl acetate to give 1.2 g (38%) of **29**.

6-(Carboxymethoxy)benzo[*b*]thiophene-2-sulfonamide (30). Solid **29** (1.6 g, 5 mmol) was added to 10% NaOH (40 mL) and stirred at room temperature for 4–6 h. The solution was acidified and extracted with ethyl acetate. The extract was dried (Na_2SO_4) and evaporated. The residue was recrystallized from hot ethyl acetate to give 0.94 g (65%) of **30**.

(*S*)-6-[[3-(1,1-Dimethylethyl)-2-oxooxazolidin-5-yl]methoxy]benzo[*b*]thiophene-2-sulfonamide (34). To a partial suspension of **16** (2.29 g, 10 mmol) in acetonitrile (10 mL) under a nitrogen atmosphere was added dropwise a solution of *N,N*-dimethylformamide dimethyl acetal (1.6 mL, 12 mmol) in acetonitrile (10 mL). After $^1/_2$ h, water was added and the product was extracted into ethyl acetate. The extract was dried (Na_2SO_4) and evaporated to dryness. The residue was crystallized from 1,2-dichloroethane with charcoal treatment to give 2.1 g (74%) of **31**, mp 164–166 °C.

A solution of (*S*)-3-(1,1-dimethylethyl)-5-(hydroxymethyl)oxazolidinone mesylate[15] (**32**, 4.26 g, 17 mmol) in DMSO (15 mL) was added dropwise to a solution of **31** (4.26 g, 15 mmol) in DMSO (15 mL) containing potassium carbonate (2.8 g, 20 mmol) at 70 °C. After 20–24 h at 70 °C, the reaction mixture was poured into ice water and extracted with ethyl acetate/acetonitrile. The extract was dried (Na_2SO_4) and concentrated to a small volume. The product (5.5 g, 84%) was recrystallized twice from ethyl acetate to provide pure **33**, mp 121–25 °C. Anal. ($C_{19}H_{25}N_3O_5S_2$) C, H, N.

A suspension of **33** (5.4 g, 14.5 mmol) was heated at 100 °C in 6 N HCl (75 mL) for 3–4 h, cooled with ice, and then extracted with ethyl acetate. The extract was dried (Na_2SO_4) and evaporated to give 5.8 g of crude product. The mixture was stirred with hot 1,2-dichloroethane and filtered while hot to remove some insoluble material. The solvent was evaporated and the residue (4.5 g) was chromatographed on silica gel eluting with a gradient of 1.5–6% methanol/chloroform. The chromatographed material was recrystallized from 1,2-dichloroethane to give 3.2 g (57%) of **34**.

(*S*)-6-[3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]benzo[*b*]thiophene-2-sulfonamide Hydrochloride (35). A suspension of **34** (2.47 g, 6.4 mmol) in a solution of water/ethanol (2:1 (v/v), 20 mL) containing 40% NaOH (w/v, 5 mL) was heated at 100 °C for 3 days. The reaction mixture was cooled, acidified with hydrochloric acid, filtered to remove insoluble material, and evaporated to dryness. The residue was repeatedly extracted with hot ethanol, and the combined ethanol extracts were evaporated. Trituration with ethanol/diethyl ether gave 1.95 g of a solid. After several recrystallizations from a mixture of methanol and ethanol, 1.2 g (44%) of **35** was obtained as a partial hydrate in admixture with sodium chloride.

5-(Methoxymethyl)benzo[*b*]thiophene-2-sulfonamide (37). To a stirred mixture of powdered KOH (18.9 g, 0.336 mol) in dimethyl sulfoxide (100 mL) was added 13.8 g (0.084 mol) of 5-(hydroxymethyl)benzo[*b*]thiophene (**36**) in 25 mL of dimethyl sulfoxide. Methyl iodide (23.9 g, 0.168 mol) was added dropwise at ambient temperature over several minutes. Stirring was continued for 1.5 h. The mixture was filtered, diluted with water (150 mL), and extracted with methylene chloride (3 × 70 mL). The combined extract was washed with water and dried. Evaporation of the solvent gave 14.1 g of amber liquid. Distillation gave 10.47 g (70%) of 5-(methoxymethyl)benzo[*b*]thiophene as a colorless liquid, bp 110–111 °C (2.2 Torr). This compound was sulfamoylated, as described for the preparation of **13** and **14**, to provide **37** in 90% yield.

5-(Bromomethyl)benzo[*b*]thiophene-2-sulfonamide (38). To a suspension of **37** (7.4 g, 0.029 mol) in 300 mL of dry methylene chloride at −30 °C was added boron tribromide (30 mL of a 1 M solution in methylene chloride, 0.03 mol) over a 20-min period. The resulting solution was stirred for 1.5 h as the temperature rose to become ambient. The solution was cooled to 0 °C and 150 mL of water was added dropwise to keep the temperature below 20 °C. The methylene chloride layer was separated and the aqueous suspension was extracted with 1:1 (v/v) methanol/chloroform (3 × 200 mL). The combined extracts were washed with ice water, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give 8.8 g of **38** as a tan solid, which was recrystallized from 1,2-dichloroethane (mp 171–173 °C): ^{1}H NMR (DMSO-d_6) δ 7.6–8.15 (5 H, m), 7.5 (1 H, dd), 4.83 (2 H, s).

5-[(Dimethylamino)methyl]benzo[*b*]thiophene-2-sulfonamide (39). To an ice-cold, stirred solution of **38** (2.0 g, 6.5 mmol) in 25 mL of methanol was added an excess of anhydrous dimethylamine. The flask was sealed and the mixture was stirred at room temperature for 1 h. The methanol was removed in vacuo. The solid residue was taken up in chloroform (100 mL) and saturated $NaHCO_3$ solution (30 mL). The chloroform layer was separated and the aqueous mixture was extracted with 1:1 (v/v) chloroform/methanol (50 mL). The combined extract was dried (Na_2SO_4) and evaporated to provide 1.57 g of **39** (90%).

5-[[[2-(Dimethylamino)ethyl]thio]methyl]benzo[*b*]thiophene-2-sulfonamide (40). To a stirred solution of 2-(dimethylamino)ethanethiol hydrochloride (14.17 g, 0.10 mol) in 125 mL of dry DMF under a nitrogen atmosphere was added portionwise 8.0 g (0.2 mol) of sodium hydride (60% in mineral oil) over 0.5 h with warming on the steam bath. The mixture was stirred an additional 0.5 h and then cooled in ice. To the cold suspension was added dropwise a solution of **38** (7.66 g, 0.025 mol) in 25 mL of DMF. The mixture was stirred for an additional hour at ice-bath temperature. The mixture was filtered and the filtrate was concentrated in vacuo (0.5 Torr) at room temperature. The combined solid residue and the filtered solid were dissolved in 3 N HCl (100 mL). The acid solution was first extracted with $CHCl_3$ (2 × 50 mL) and then neutralized with $NaHCO_3$ and concentrated to dryness in vacuo. The residual solid was extracted with $CHCl_3$ (3 × 100 mL). The $CHCl_3$ extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo at room temperature. Trituration with hot water gave pure **40** (2.8 g, 34%).

5-(4-Morpholinylmethyl)benzo[*b*]thiophene-2-sulfonamide (41). To a stirred solution of morpholine (0.91 g, 10.5 mmol) and triethylamine (1.01 g, 10 mmol) in 20 mL of methanol was added **38** (3.06 g, 10 mmol) portionwise over 5 min at ambient temperature. Stirring was continued for 1.25 h. The resulting suspension was cooled and stirred for 1 h. Filtration gave 2.76 g (88%) of **41** as a yellow solid. Concentration of the filtrate in vacuo followed by trituration of the residue with water and filtering gave an additional 0.3 g (10%) of **41**.

5-(Acetoxymethyl)benzo[*b*]thiophene-2-sulfonamide (42). To a mixture of **38** (3.06 g, 0.01 mol), anhydrous sodium acetate (0.98 g, 0.01 mol), and glacial acetic acid (15 mL) was added three drops of triethylamine. The mixture was refluxed for 6 h and was left at room temperature overnight. The acetic acid was removed in vacuo and the residual gum was diluted with 25 mL of ice water.

The product was extracted into ether (3 × 50 mL). The combined extract was washed with water, dried (Na_2SO_4), filtered, and concentrated in vacuo to give 2.5 g (88%) of **42** as a yellow solid.

5-(Hydroxymethyl)benzo[*b*]thiophene-2-sulfonamide (43). A solution of **42** (5.6 g, 19.6 mmol) in 25 mL of 1 N aqueous KOH and 25 mL of methanol was stirred at room temperature for 2 h and at reflux for 2.5 h. The mixture was filtered and the methanol was removed in vacuo. The remaining aqueous suspension was acidified with excess 6 N HCl and the precipitated solid was isolated by filtration, washed with ice water, and dried. The crude product (3.1 g) was chromatographed on silica (5% methanol/chloroform). There was obtained 1.85 g (41%) of **45** as a yellow solid.

6-Acetylbenzo[*b*]thiophene-2-sulfonamide (46). A mixture of 6-acetyl-2,3-dibromobenzo[*b*]thiophene (**44**, 6.68 g, 20 mmol), MgO (1.6 g), and 20% $Pd(OH)_2$/C (800 mg) in 200 mL of 1:1 ethanol/ethyl acetate was shaken in a Parr bottle under an H_2 atmosphere (40 psi) for 3 h. The mixture was filtered and

evaporated. The residue was partitioned between $CHCl_3$ (100 mL) and water (50 mL). The aqueous layer was back-extracted with $CHCl_3$ (30 mL). The $CHCl_3$ solutions were combined and dried over Na_2SO_4, and the solvent was evaporated in vacuo to yield 3.8 g of 6-acetylbenzo[*b*]thiophene.

A mixture of 6-acetylbenzo[*b*]thiophene (10.57 g, 60 mmol), *p*-toluenesulfonic acid (1.65 g), and ethylene glycol (33 mL) was heated to reflux in toluene (250 mL) for 2 h with the continuous removal of H_2O with a Dean–Stark trap. The reaction mixture was cooled to room temperature, and washed with 20% saturated $NaHCO_3$ (150 mL) and H_2O (2 × 150 mL). After drying (Na_2SO_4), the solvent was evaporated in vacuo and the residue was crystallized from hexanes (50 mL), yielding 9.66 g (73%) of **45**. Compound **45** was converted to the sulfonamide as described in the synthesis of **13** and **14**, in 42% yield. The resulting ketalized sulfonamide (4.4 g, 14.7 mmole) and *p*-toluenesulfonic acid (400 mg) were dissolved in acetone (90 mL). The mixture was stirred overnight. The solvent was removed in vacuo and the residue was partitioned between ethyl acetate (200 mL) and 10% saturated $NaHCO_3$ solution (100 mL). The organic layer was washed with H_2O (2 × 100 mL) and dried (Na_2SO_4). The solvent was removed in vacuo and the residue was triturated with hot $CHCl_3$ (100 mL). Upon cooling, 3.4 g (91%) of **46** was obtained.

6-Acetamidobenzo[*b*]thiophene-2-sulfonamide (47). A solution of **46** (5.1 g, 0.02 mol) in glacial acetic acid (50 mL) and concentrated H_2SO_4 (20 mL) was stirred and heated to 65 °C. Sodium azide (5.0 g, 0.077 mol) was added in portions over a 1-h period. After heating of the mixture at 80 °C for another 3 h, it was poured into a stirred, saturated NaOAc solution (500 mL) cooled in an ice bath. The resulting mixture was refrigerated overnight. The precipitated product was isolated by filtration and washed by resuspension in 300 mL of H_2O and filtration. The solid was recrystallized from methanol with charcoal decolorization and subsequently from acetonitrile to provide **47** (1.5 g, 28%).

6-Aminobenzo[*b*]thiophene-2-sulfonamide (48). A suspension of **47** (0.8 g, 3 mmol) in 1 N HCl (24 mL) was heated to reflux for 1 h. The resulting clear solution was cooled to room temperature, diluted with water (2.5 mL), and neutralized with saturated $NaHCO_3$ solution. Precipitated product **48** (0.57 g, 83%) was isolated by filtration: ^1H NMR (DMSO-d_6) δ 7.45–7.7 (4 H, m) 7.0 (1 H, br s) 6.75 (1 H, dd) 5.50 (2 H, br s).

Carbonic Anhydrase Inhibition Assay. The ability of compounds to inhibit the carbonic anhydrase catalyzed hydration of CO_2 was determined using a pH-stat assay, as described in ref 2e.

IOP Recovery Rate Assay. Aqueous humor production was quantified indirectly in the conscious rabbit as described by Vareilles and Lotti.[19] Infusion of hypertonic saline into the marginal ear vein leads to a rapid fall in IOP which subsequently returns to normal over approximately 60 min. CAIs reduce the rate of return of pressure to normal, by blocking aqueous humor secretion.

IOP Studies in Ocular Hypertensive Rabbits. The α-chymotrypsinized rabbit model of Sears and Sears[20] was employed. A detailed description of this assay, as implemented in our laboratories, is found in ref 2d.

Guinea Pig Dermal Sensitization Assay.[4] The assay was conducted essentially as described by Magnusson and Kligman.[4] Intradermal dosing (20 animals) of a 2% oil suspension of the test compound (8 mg) was followed one week later by application of a patch containing an 8% petrolatum suspension of the compound for 48 h. Two weeks after this induction regimen, sensitization was assayed by application of a patch containing an 8% petrolatum suspension of the compound for 24 h. Evaluation of the degree of sensitization was performed 24 h after removal of the patch, by both visual and microscopic observation. The degree of sensitizing potential was assigned according to the percentage of animals giving a positive response according to a modified scale originally designed by Magnusson:

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

Registry No. **3**, 123126-59-0; **4**, 95-15-8; **7**, 15570-12-4; **8**, 96803-85-9; **9**, 3781-90-6; **10**, 90560-10-4; 11, 123126-60-3; **12**, 123126-61-4; **13**, 96803-88-2; **14**, 96803-87-1; **15**, 96803-90-6; **16**, 96803-89-3; **23**, 96803-92-8; **24**, 96803-29-1; **25**, 123126-62-5; **26**, 96802-95-8; **27**, 96803-44-0; **28**, 96803-45-1; **29**, 96803-75-7; **30**, 96803-76-8; **31**, 96803-67-7; **32**, 96803-68-8; **33**, 96803-69-9; **34**, 96803-70-2; **35**, 96803-72-4; **35**·HCl, 123126-63-6; **36**, 20532-34-7; **37**, 96803-57-5; **38**, 96803-58-6; **39**, 96803-59-7; **40**, 96803-11-1; **41**, 96803-60-0; **41**·HCl, 96803-61-1; **42**, 96803-62-2; **43**, 96803-63-3; **44**, 6179-22-2; **45**, 96803-39-3; **45** sulfonamide, 96803-40-6; **46**, 96803-41-7; **47**, 96803-02-0; **48**, 96803-03-1; $(CH_3)_2CHCH_2OCOCl$, 543-27-1; $[(CH_3)_2CCO]_2O$, 1538-75-6; $(CH_3)_2NCOCl$, 79-44-7; 5-methoxybenzo[*b*]thiophenesulfonamide, 20532-30-3; 7-methoxybenzo[*b*]thiophenesulfonamide, 88791-08-6; 5-methoxy-2-benzo[*b*]thiophenesulfonamide, 96804-00-1; 7-methoxy-2-benzo[*b*]thiophenesulfonamide, 96803-65-5; 5-hydroxy-2-benzo[*b*]thiophenesulfonamide, 96804-01-2; 7-hydroxy-2-benzo[*b*]thiophenesulfonamide, 96803-66-6; 2-(dimethylamino)ethanethiol hydrochloride, 13242-44-9; ethyl bromoacetate, 105-36-2; 4-methoxy-2-benzo[*b*]thiophenesulfonyl chloride, 96803-86-0; 6-methoxy-2-benzo[*b*]thiophenesulfonyl chloride, 96814-31-2; 5-(methoxymethyl)benzo[*b*]thiophene, 96803-56-4; 6-acetylbenzo[*b*]thiophene, 29813-41-0; carbon anhydride II, 9001-03-0; bromoacetaldehyde diethyl acetal, 2032-35-1.