Topically Active Carbonic Anhydrase Inhibitors. 3. Benzofuran- and Indole-2-sulfonamides

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Derivatives of benzofuran- and indole-2-sulfonamide were prepared for evaluation as topically active ocular hypotensive agents. These compounds were found to be excellent inhibitors of carbonic anhydrase and to lower intraocular pressure in a rabbit model of ocular hypertension. However, the development of these compounds for clinical use was precluded by the observation that they cause dermal sensitization in guinea pigs. A correlation between electrophilicity, as assessed by in vitro reactivity with reduced glutathione, and dermal sensitization potential was further documented.

The development of a topically active carbonic anhydrase inhibitor (CAI) would be a significant advance in the therapy of glaucoma and ocular hypertension. Substantial progress has been reported in this area.[1] Our initial efforts toward this goal focused on derivatives of benzothiazole-2-sulfonamide[1d,f], several of which were shown to lower intraocular pressure (IOP) in the α-chymotrypsinized (α-CT) rabbit.[2] In the course of that work we encountered two difficulties that rendered impossible the clinical development of these compounds as topical hypotensive agents. None of the molecules possessed significant water solubility, which led to problems in the preparation of a well-tolerated ophthalmic dosage form. Of a more serious nature was the finding that all derivatives of benzothiazole-2-sulfonamide were potent inducers of allergic dermatitis in the guinea pig.[1f] We deduced that this untoward effect was a reflection of the electrophilic nature of the benzothiazolesulfonamide system. On the basis of this premise, we subsequently prepared several benzo[*b*]thiophene-2-sulfonamide derivatives[1g] which were in fact nonallergenic. Members of this class of compounds also demonstrated excellent topical hypotensive activity in the α-CT rabbit. However, the solubility issue remained unresolved.

In this article we report an extension of our work to include two new structural classes of CAIs which are derivatives of benzofuran- and indole-2-sulfonamide. These compounds are excellent inhibitors of human erythrocyte carbonic anhydrase II (CA-II) and several show strong ocular hypotensive activity at concentrations where they are soluble in an aqueous dosage vehicle. Unfortunately, these benzofuran- and indole-2-sulfonamides are significantly electrophilic, as determined by their rate of reaction with reduced glutathione (GSH). We previously have noted[3] a good correlation between GSH reactivity and dermal sensitization potential. Indeed, the further development of these compounds was precluded by the observation that they are moderate (benzofuran) to strong (indole) dermal sensitizers in the guinea pig model of Magnusson and Kligman.[4]

Chemistry. The syntheses of the oxygen heterocycles are described in Scheme I. Benzofuran (**1a**) was lithiated and converted to the sulfonamide **3a** via the sulfinic acid salt **2a**.[5] The known methoxybenzofurans **1b** and **1c**, prepared by the method of Burgstahler and Worden,[6] similarly were converted to the sulfonamides **3b** and **3c**. The corresponding phenols **4b** and **4c** were obtained by demethylation of **3b** and **3c**, respectively, with pyridine hydrochloride. Esterification under standard conditions gave the acetoxy (**5b** and **5c**) and pivaloyloxy (**6b** and **6c**) derivatives. The 6-hydroxy-3-methyl derivative **8** was prepared by electrophilic sulfonation of the known[7] benzofuranol **7** and subsequent conversion to the sulfonamide.

Nitration of **3a** gave an inseparable 55:45 mixture of the 5- and 6-nitro derivatives **9a** and **9c**. The mixture was reduced to the corresponding amines **10a** and **10b**, which were separable (with difficulty) by chromatography. The structure of **10a** was assigned on the basis of the NOE observed for H-4 upon irradiation of H-3. Chemical con-

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(2) Sears, P.; Sears, M. *Am. J. Ophthalmol.* **1974**, *77*, 378.
(3) Schwam, H.; Michelson, S. R.; de Solms, S. J.; Duprat, P.; Gautheron, P.; Shepard, K. L.; Smith, R. L.; Sugrue, M. F. Abstracts of the 8th International Congress of Eye Research, San Francisco, CA, September 1988; Vol. 52, p 268.
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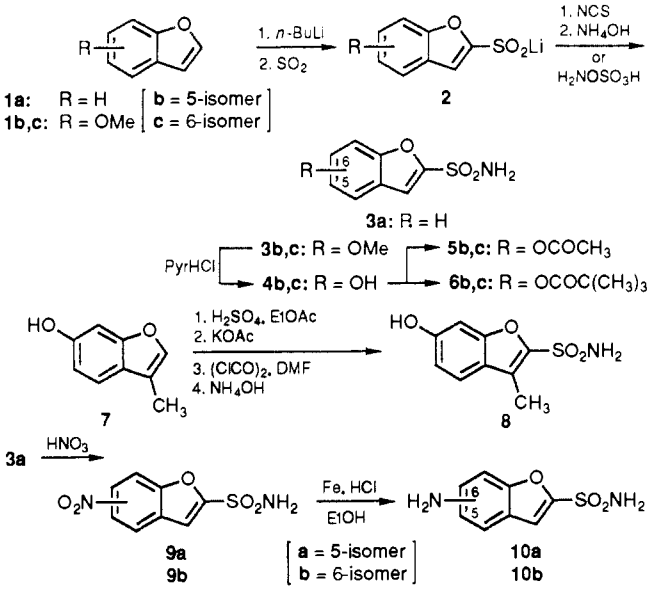
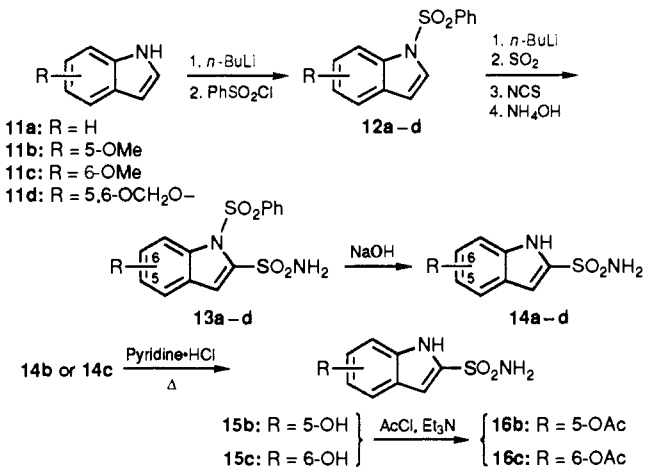
Scheme I



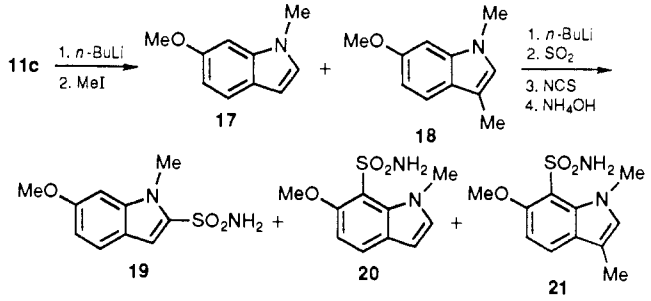
Scheme II



firmation of this assignment was obtained by heating the mixture of nitro compounds with aqueous NaOH. One of the isomeric compounds was destroyed by this treatment. This is undoubtedly the 6-nitro compound **9b** which clearly should be more susceptible to nucleophilic attack at C-2, leading to loss of the sulfonamide group. Reduction of the surviving isomer gave the 5-amino compound **10a**.

The indolesulfonamides were prepared in an analogous manner, illustrated in Scheme II. The requisite indoles were obtained commercially or were prepared readily by using the Leimgruber–Batcho synthesis.[8] Conversion of these indoles (**11a–d**) to the *N*-(phenylsulfonyl) derivatives and treatment with *n*-butyllithium gave the 2-lithio derivatives, in accord with the observations of Sundberg[9] and Gribble.[10] These carbanions were converted to the sulfonamides **13a–d** by the method described in Scheme I. Base-catalyzed hydrolysis of **13a–d** gave the indolesulfonamides **14a–d**. Phenols **15b** and **15c** were obtained by heating the corresponding methyl ethers **14b** and **14c** with pyridine hydrochloride. Attempts to liberate the catechol moiety from the methylenedioxy derivative **14d** under a variety of conditions (pyridine hydrochloride, BBr_3 or BCl_3) gave only intractable, black solids. The acetoxy

Scheme III

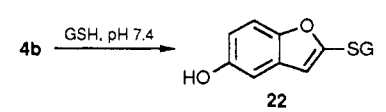


derivatives **16b** and **16c** were prepared by acetylation of the corresponding phenols.

As shown in Scheme III, methylation of 6-methoxyindole **11c** gave a 3:2 mixture of the *N*-methylindole **17** and the *N*,3-dimethylindole **18**. Lithiation of this mixture and subsequent sulfamoylation produced a mixture of three sulfonamides from which the desired compound **19** crystallized. The other sulfonamides were isolated by preparative HPLC and shown to have structures **20** and **21**. These products demonstrate that methoxy-directed lithiation at C-7 is competitive with metalation at the 2-position in *N*-methylindoles, particularly when the 3-position is also methylated. A similar observation was made by Sundberg and Parton with 5-methoxy-*N*-methylindole.[9]

In Vitro and in Vivo Evaluation. The ability of these sulfonamides to inhibit CO_2 hydration catalyzed by human erythrocyte CA-II was determined by using a pH stat assay.[1e] With the exception of those compounds bearing a substituent ortho to the sulfonamide group, excellent inhibitory activity was observed (Table I). The poor activity of the ortho-substituted compounds is well precedented by observations on the effect of ortho substitution in benzenesulfonamides[11] and may be attributed to the steric demands of the active site.[12]

A major safety issue that arose during our earlier studies of topically effective CAIs was the electrophilicity of certain structural classes of heterocyclic sulfonamides, as revealed by their propensity to cause ocular and dermal sensitization. We have reported that the electrophilicity of sulfonamides may be assessed by measuring their rate of reaction with reduced glutathione (GSH).[1f,g,3] The GSH reactivity was found to be correlated with dermal sensitization potential in guinea pigs under the Magnusson–Kligman protocol.[3] Under previously defined conditions[1f] (5 equiv of GSH, pH 7.4, 37 °C, 16–22 h), the benzofuran- and indolesulfonamides (Table I) showed much lower levels of reactivity toward GSH than benzothiazolesulfonamides, which had been demonstrated to be strong sensitizers in rabbits and guinea pigs. However, they were measurably more electrophilic than the corresponding (nonsensitizing) benzothiophene (entry 23, Table I). In the reaction between GSH and **4b**, the expected sulfonamide substitution product **22** was isolated (eq 1; GSH =



(1)

γ-Glu-Cys-Gly). It is interesting to note that the 3-methyl- and *N*-methyl-substituted derivatives of the benzofuran- and indolesulfonamides were appreciably more reactive toward GSH than the desmethyl compounds. The reason

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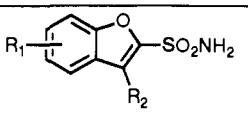
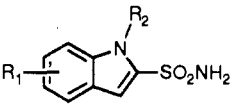
Table I. Benzofuran- and Indole-2-sulfonamides

entry	compd	R_1	R_2	mp, °C	recrystn solvent[a]	anal.[b]	sol,[c] mg/mL	I_{50},[d] nM	GSH reaction, % 5 equiv[e]	GSH reaction, % 20 equiv[f]	guinea pig sensitization[g]
					 benzofurans						
1	**3a**	H	H	153–155	A	$C_8H_7NO_3S$	0.48	8.0	0	10	
2	**3b**	5-OCH_3	H	119–120	A	$C_9H_9NO_4S$	0.54	5.1		10	
3	**3c**	6-OCH_3	H	148–149	B	$C_9H_9NO_4S$		15			
4	**4b**	5-OH	H	185–187	B	$C_8H_7NO_4S$	2.1	6.5	4.0	9	15/40
5	**4c**	6-OH	H	168–170	B	$C_8H_7NO_4S$	2.8	11	4.9	23	8/19
6	**5b**	5-$OCOCH_3$	H	178–181	A	$C_{10}H_9NO_5S$	0.19	6.5			
7	**5c**	6-$OCOCH_3$	H	138–139	C	$C_{10}H_9NO_5S$		5.5			
8	**6b**	5-$OCOC(CH_3)_3$	H	130–131	F	$C_{13}H_{15}NO_5S$		3.0	3.4		
9	**6c**	6-$OCOC(CH_3)_3$	H	122–124	A + E	$C_{13}H_{15}NO_5S$		7.0			
10	**8**	6-OH	CH_3	193–194	D + E	$C_9H_9NO_4S$		210		47	
11	**10a**	5-NH_2	H	187–188	B	$C_8H_8N_2O_3S$	0.28	9.0	11		
12	**10b**	6-NH_2	H	191 dec	B	$C_8H_8N_2O_3S$	0.08	25	11	17	
					 indoles						
13	**13c**	6-OCH_3	$PhSO_2$	171–172	J	$C_{15}H_{14}N_2O_5S_2$		$>10^4$			
14	**14a**	H	H	187–188[h]	B	$C_8H_8N_2O_2S$	0.85	45	0		
15	**14b**	5-OCH_3	H	208–209	B	$C_9H_{10}N_2O_3S$	0.15	16			
16	**14c**	6-OCH_3	H	164–165	G + B	$C_9H_{10}N_2O_3S$	0.52	60			
17	**14d**	5,6-OCH_2O–	H	235–236	H + I	$C_9H_8N_2O_4S$	0.05	25			
18	**15b**	5-OH	H	225–227	I + K	$C_8H_8N_2O_3S$	1.9	22	0.7	13	19/20
19	**15c**	6-OH	H	194–195	K + L	$C_8H_8N_2O_3S$	4.8	30	2.5	17	
20	**16b**	5-$OCOCH_3$	H	197–198	D + E	$C_{10}H_{10}N_2O_4S$	0.22	11			
21	**16c**	6-$OCOCH_3$	H	152–153	A + F	$C_{10}H_{10}N_2O_4S$		32			
22	**19**	6-OCH_3	CH_3	189–191	M	$C_{10}H_{12}N_2O_3S$	0.04	110		37	
23	6-hydroxybenzo[*b*]thiophene-2-sulfonamide (ref 1g)						0.43	9.9	0	2.9	0/20

[a] Solvents: A, dichloroethane; B, water; C, ether; D, THF; E, hexane; F, *n*-butyl chloride; G, ethanol; H, acetonitrile; I, ethyl acetate; J, ethanol; K, methanol; L, chloroform; M, dichloromethane. [b] Analysis for C, H, and N within ±0.4% of calculated values for the indicated empirical formula. [c] Solubility at pH 7.4, in 50 mM phosphate buffer. [d] Inhibition of human carbonic anhydrase II catalyzed hydration of CO_2; pH 8.3, 4 °C, enzyme concentration 0.1 nM. [e] Extent of reaction of the compound with 5 equiv of reduced glutathione (GSH) between 16 and 22 h; pH 7.4, 37 °C. [f] Extent of reaction of the compound with 20 equiv of reduced glutathione (GSH) in 20 h; pH 7.4, 37 °C. [g] Number of animals displaying sensitization/number of animals tested in the Magnusson–Kligman assay. [h] Literature mp 190–192; European Patent 0070698 (1983).

for this enhanced reactivity is not clear, but it suggests that the mechanism of the glutathione substitution reaction is not a simple addition–elimination process. A further indication of the possible mechanistic complexity of this reaction is the unusual effect upon reactivity of substituents on the six-membered ring in the benzofuran series: electron-donating substituents either did not affect or accelerated the rate of the displacement reaction. This stands in contrast to the benzothiazole series where a 6-amino or 6-hydroxy substituent markedly reduced the electrophilicity of the system.[1f,3]

Three of these compounds were evaluated in the Magnusson–Kligman dermal sensitization potential assay in guinea pigs. The benzofuransulfonamides **4b** and **4c** were moderate sensitizers, eliciting allergic responses in 15 of 40 and 8 of 19 animals, respectively. 5-Hydroxyindolesulfonamide (**15b**) was found to be a strong sensitizer, with 19 of 20 animals classified as positive responders. The occurrence of sensitization was surprising at first, given the seemingly small difference in reactivity of the compounds toward GSH in comparison to the nonsensitizing benzothiophene. However, when the sensitivity of the GSH assay was amplified by increasing the concentration of GSH 4-fold (20 equiv of GSH), it was found that each of the compounds was a factor of 3–7 times more reactive than 6-hydroxybenzo[*b*]thiophene-2-sulfonamide (Table I). While a simple linear correlation between GSH reactivity and sensitization potential does not exist, it seems clear that a compound is likely to induce an allergic response if some threshold level of electrophilicity is exceeded. It is possible that this simple in vitro assay may be useful for predicting the sensitization potential of an electrophile.

The ocular hypotensive effect of CAIs is believed to result primarily from the blockade of aqueous humor secretion into the anterior segment of the eye. Thus, the first stage in our in vivo evaluation of the ocular hypotensive potential of a CAI was to assess its effect on the rate of aqueous humor formation. An indirect method, the IOP recovery rate assay[13] in rabbits, was employed. In this model, intravenous infusion of hypertonic saline results in a prompt reduction in IOP. Upon cessation of the infusion, IOP returns to normal over a period of about 60 min. Prior treatment of the animal with an agent that suppresses the secretion of aqueous humor decreases the rate at which IOP returns to the preinfusion value. We have found that the numerical values obtained in this assay are not predictive of the magnitude of the IOP-lowering effect in the more rigorous model of ocular hypertension described below. However, the assay could be used qualitatively to select compounds for further study. Our

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Table II. Ocular Hypotensive Activity of Selected Benzofuran- and Indole-2-sulfonamides in α-CT Rabbits

entry[a]	treatment	minimum effective topical dose,[b] %	n[c]	maximum IOP reduction ± SE, mmHg (duration)[b]
1	**4b**	0.1*	12	−6.0 ± 1.5 (3)
2	**4c**	0.5*	5	−5.2 ± 2.4 (4)
3	**5b**	0.1†	6	−5.7 ± 0.6 (4)
4	**5c**	0.5†	6	−8.8 ± 1.7 (5)
5	**6c**	0.5†	6	−5.3 ± 1.5 (3)
6	**10a**	0.5†	6	−6.0 ± 0.9 (4)
7	**15b**	0.1*	6	−7.5 ± 1.2 (5)
8	**15c**	0.5*	6	−5.0 ± 2.0 (3)
9	6-hydroxybenzo[*b*]-thiophene-2-sulfonamide	0.05†	12	−5.5 ± 1.1 (3)
10	6-acetoxybenzo[*b*]-thiophene-2-sulfonamide	0.1†	17	−5.3 ± 1.0 (5)

[a] Entries 9 and 10 from ref 1g. [b] Test compounds were applied as a 50-μL drop of a suspension (†) or solution (*) of the indicated concentration (w/v) in 0.5% aqueous (hydroxyethyl)cellulose vehicle. IOP was measured (Alcon Applanation Pneumotomograph) just before (t_0) and 0.5, 1, 2, 3, 4, and 5 h after treatment. Results are expressed as the maximum fall in IOP from the t_0 value at the indicated dose level. The values in parentheses refer to the number of time points (maximum of 6) at which IOP was significantly reduced ($P \leq 0.05$, Dunnett's two-tailed test). The minimum effective dose is defined as the smallest dose that produced a statistically significant reduction in IOP at any time point. Vehicle had no effect on IOP. [c] Number of treated eyes.

best benzothiazole-[1f] and benzo[*b*]thiophenesulfonamide[1g] derivatives showed statistically significant activity in this assay when applied topically as single drops of a 1% or 2% suspension. In the current series, those compounds that were judged "active" in the IOP recovery rate assay at a 2% dose level were evaluated for hypotensive activity.

The model employed for pressure-lowering studies was the α-chymotrypsin (α-CT) treated rabbit. Intraocular injection of α-CT leads to a chronic elevation of IOP from the normal value of 20 mmHg to the range of 35–45 mmHg. With the colony of animals employed in this study, we have observed that systemically administered CAIs reduce IOP with a maximal effect of approximately 8 mmHg.[1g] In the assay for topical activity, the compounds were administered in 0.5% aqueous (hydroxyethyl)cellulose vehicle as a single 50-μL drop. IOP was measured immediately prior to treatment (t_0) and 0.5, 1, 2, 3, 4, and 5 h after dosing. The performance of the compounds in this assay was characterized (Table II) by three criteria: (1) minimum effective dose, which was the lowest dose that produced a statistically significant reduction in IOP at any time point between 0.5 and 5 h; (2) maximum pressure reduction, which was the largest decrease in IOP with respect to the pretreatment IOP at the minimum effective dose; and (3) the number of times points at which IOP was significantly lower than the value at t_0. Several of the indole and benzofuran sulfonamides showed significant hypotensive activity at topical doses as low as 25 μg (50 μL of a 0.05% suspension of **6c**). Of particular interest is the excellent activity demonstrated by several of the compounds which were instilled as solutions. For purposes of comparison, also shown in Table II is the topical activity data for two benzo[*b*]thiophenesulfonamides which previously were selected for clinical evaluation. It is evident that the activity demonstrated by the classes of compounds reported in this paper is comparable to that of their antecedents. Although the greater water solubility of the indole- and benzofuransulfonamides represented a potential advantage over the benzothiophenes, the observation of dermal sensitization induced by both structural classes precluded further development of these topically active CAIs.

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 this solvent were serum stoppered, and the solvent was transferred under nitrogen. Solvent evaporation was carried out on a rotary evaporator. Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were determined at 60 MHz (Varian T-60), 90 MHz (Varian EM-390), 300 MHz (Varian XL-300), or 360 MHz (Nicolet NT-360). Chemical shift data are reported in parts per million (ppm) downfield from tetramethylsilane as internal standard. We thank Dr. David Cochran for assistance in obtaining and interpreting ^{1}H NMR data relating to the structure determination of **22**. Elemental analyses were done by Mr. J. Moreau of the Medicinal Chemistry Department (West Point).

5-Methoxybenzofuran-2-sulfonamide (3b). A solution of 5-methoxybenzofuran (**1b**) (10.8 g, 73 mmol) in 100 mL of dry THF was cooled to −65 °C, and 50 mL of 1.6 M *n*-butyllithium in hexane was added dropwise, with stirring, at a rate such that the internal temperature did not exceed −55 °C. After 15 min, sulfur dioxide was passed into the reaction vessel via a needle positioned just above the surface of the solution, until an aliquot of the mixture gave an acidic reaction to moist pH paper. Hexane (100 mL) was added to precipitate the product, the mixture was warmed to room temperature, and the sulfinic acid salt was isolated by filtration. The salt was dried in vacuo (18 h, room temperature), yielding 15.6 g (98%) of tan powder.

The sulfinate was suspended in 150 mL of CH_2Cl_2 and cooled to 5 °C, and 10.7 g *N*-chlorosuccinimide (80 mmol) was added. The mixture was stirred at 5 °C for 15 min, the cooling bath was removed, and after an additional 15 min the mixture was filtered through a pad of Celite. The solvent was evaporated to give 17.2 g of sulfonyl chloride as a brown solid. This material was dissolved in 50 mL of acetone and added over 1 min to a cold (5 °C) solution of 15 mL of concentrated NH_4OH in 150 mL of acetone. After 30 min the solvent was evaporated and the residue was partitioned between water and EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated to give 17.2 g of a brown solid which was recrystallized twice to give 5.7 g (34% overall) of **3b** as a colorless solid: ^{1}H NMR (acetone-d_6) δ 7.5 (1 H, d, J = 9 Hz), 7.3 (1 H, s) 7.25 (1 H, d, J = 3 Hz), 7.1 (1 H, dd, J = 3, 9 Hz), 3.83 (3 H, s).

Alternatively, the direct oxidation of the sulfinate to the sulfonamide with hydroxylamine-*O*-sulfonic acid is possible. A solution of 36.2 g (0.166 mol) of the sulfinic acid and 26 g (0.31 mol) of NaOAc was prepared in 300 mL of water and cooled in an ice–water bath. Hydroxylamine-*O*-sulfonic acid (19.5 g, 0.172 mol) was added in a single portion to the solution, and the mixture was stirred for 20 min. The cooling bath was removed, and stirring was continued for 3 h. The mixture was extracted with 2 × 200 mL of EtOAc, and the combined extract was washed sequentially with 10% $NaHCO_3$ and brine. After drying (Na_2SO_4), the solvent was evaporated to afford 29.8 g (79%) of **3b** as a yellow solid.

5-Hydroxybenzofuran-2-sulfonamide (4b). A 6.2-g sample of **3b** (27 mmol) was heated with 30 g of pyridine hydrochloride at 190–200 °C for 15 min. The hot solution was poured into ice water (300 mL), and the product was extracted into EtOAc. The extract was washed with 1 N HCl, 10% $NaHCO_3$, and brine. The solution was dried (Na_2SO_4) and evaporated to give 4.4 g of a tan solid. Recrystallization with Norit A decolorization gave 2.9 g (50%) of **4b** as tan needles: ^{1}H NMR (DMSO-d_6) δ 9.48 (1 H, s), 7.91 (2 H, s), 7.50 (1 H, d, J = 9 Hz), 7.30 (1 H, s), 7.08 (1 H, d, J = 3 Hz), 6.95 (1 H, dd, J = 3, 9 Hz).

5-Acetoxybenzofuran-2-sulfonamide (5b). A mixture of **4b** (4.00 g, 18.8 mmol) and triethylamine (3.2 mL, 23 mmol) in 20 mL of THF was cooled to 3 °C, and 2.0 mL of acetic anhydride (21 mmol) was added dropwise with stirring. The cooling bath was removed and the mixture was stirred for 1.5 h. The mixture was partitioned between EtOAc and 10% $NaHCO_3$. The organic

phase was separated and washed with 10% $NaHCO_3$ and brine. After drying (Na_2SO_4), the solvent was evaporated to give 3.1 g of a brown oil which was chromatographed on silica (5% MeOH/$CHCl_3$). Fractions containing the product were pooled, evaporated, and recrystallized to give 1.62 g (34%) of **5b**.

5- and 6-Nitrobenzofuran-2-sulfonamide (9a and 9b). To 400 mL of concentrated nitric acid, cooled to 10 °C, was added 20.0 g (0.101 mol) of **3a**. The cooling bath was removed and the mixture was stirred for 2 h while warming to 20 °C. The mixture was placed in the freezer for 72 h. After warming to room temperature, the mixture was poured onto 2.2 L of ice–water slush. The solid precipitate was isolated by filtration, washed with water, and air dried to give 16.4 g (67%) of a 55:45 mixture of **9a** and **9b**. The ^1H NMR absorbances of the individual isomers were assigned by using decoupling and NOE experiments: **9a** (DMSO-d_6) δ 8.79 (1 H, d, J = 3 Hz), 8.38 (1 H, dd, J = 3, 9 Hz), 8.00 (1 H, d, J = 9 Hz), 7.67 (1 H, s); **9b** (DMSO-d_6) δ 8.70 (1 H, d, J = 2 Hz), ca. 8.28 (obsc), 8.06 (1 H, d, J = 9 Hz), 7.67 (1 H, s).

5- and 6-Aminobenzofuran-2-sulfonamide (10a and 10b). A mixture of **9a** and **9b** (4.3 g, 17.8 mmol), 6.0 g (107 mmol) of iron powder, and 110 mL of 56% aqueous EtOH was heated to reflux with vigorous (mechanical) stirring. A solution of 0.5 mL of concentrated HCl in 10 mL of aqueous EtOH was added slowly dropwise, and the mixture was refluxed for 1 h. The mixture was cooled, neutralized with concentrated NH_4OH, and filtered, and the solvent was evaporated. The product was combined with material obtained similarly from the reduction of a total of 7.3 g of the mixture of nitro compounds. The combined product was heated with 500 mL of THF, cooled to room temperature, and filtered. The filtrate was concentrated to 100 mL, and 20 g of silica gel was added. The resulting slurry was evaporated to dryness, and the product thus adsorbed was loaded on a column of 470 g of silica gel (230–400 mesh). The column was eluted with 10–60% EtOAc/hexane. The fractions containing the separated products were pooled and evaporated. The less polar compound **10b** weighed 2.0 g (20%) after recrystallization: ^1H NMR (DMSO-d_6) δ 7.2 (1 H, d, J = 9 Hz), 7.0 (1 H, s), 6.5 (2 H, m). The more polar product **10a** weighed 2.1 g (21%) after recrystallization: ^1H NMR (DMSO-d_6) δ 7.35 (1 H, d, J = 9 Hz), 7.20 (1 H, s), 6.78 (2 H, m).

Hydrolysis of the Mixture of 9a and 9b. A solution of 1.4 g of the 55:45 mixture of nitro compounds in 100 mL of 1 N NaOH was refluxed for 7.5 h. The mixture was cooled, neutralized with 1 H HCl, and extracted with EtOAc. The extract was dried (Na_2SO_4) and evaporated to give 0.40 g (52%) of **9a**.

6-Hydroxy-3-methylbenzofuran-2-sulfonamide (8).[14] A solution of 1.50 g (10.1 mmol) of 6-hydroxy-3-methylbenzofuran (**7**) and 2.87 mL (30.3 mmol) of acetic anhydride in 15 mL of EtOAc was cooled to 5 °C. A solution of 0.59 mL of concentrated H_2SO_4 in 4 mL of cold EtOAc was then added dropwise over 5 min. The mixture was stirred for 2 h while warming to 20 °C. A solution of 1.06 g of KOAc (10.8 mmol) in 5.5 mL of 95% EtOH was added dropwise with stirring. After 30 min, the precipitated potassium salt of the benzofuransulfonic acid was isolated by filtration (2.4 g, 77%).

The sulfonic acid salt was suspended in 10 mL of DMF, and 1.75 mL (20 mmol) of oxalyl chloride was added carefully, dropwise (**vigorous reaction**). After 4 h the DMF solution was added dropwise with stirring to 25 mL of concentrated NH_4OH. After 30 min the bulk of the solvent was evaporated and the residue was partitioned between EtOAc and 1 N HCl. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated to give 0.27 g of a yellow solid. The mixture was chromatographed on silica gel (5% MeOH/$CHCl_3$). The major product (0.17 g) was recrystallized to give 90 mg of **8**: ^1H NMR (DMSO-d_6) δ 7.75 (2 H, br s), 7.53 (1 H, d, J = 9 Hz), 6.93 (1 H, d, J = 2 Hz), 6.85 (1 H, dd, J = 2, 9 Hz), 2.39 (3 H, s).

6-Methoxy-1-(phenylsulfonyl)indole (12c). A solution of 1.6 M n-butyllithium in hexane (71.9 mL, 0.115 mol) was added dropwise to a solution of 6-methoxyindole (16.0 g, 0.109 mol) dissolved in 150 mL of dry THF which was cooled to −70 °C under a nitrogen atmosphere. The reaction was allowed to warm to 0 °C over 45 min. After recooling to −70 °C, benzenesulfonyl chloride (21.3 g, 0.121 mol) was added dropwise over 45 min. Again the reaction was allowed to warm to room temperature over 2 h and poured into 2% sodium carbonate solution. The product was extracted into Et_2O, washed with saturated Na_2CO_3 and brine, and dried over anhydrous K_2CO_3. This solution was filtered and the solvent evaporated under vacuum to give 25.0 g (80% yield) of crystalline **12c**, mp 137–139 °C.

6-Methoxy-1-(phenylsulfonyl)indole-2-sulfonamide (13c). A solution of 1.6 M n-butyllithium in hexane (115 mL, 0.18 mol) was added dropwise over 1 h to a solution of **12c** (50.0 g, 0.17 mol) in dry THF (200 mL), under nitrogen, and cooled to −70 °C. After an additional 0.5 h, dry sulfur dioxide gas was introduced over the solution surface for 15 min to give a copious precipitate. This suspension was allowed to warm to room temperature over 2 h diluted with hexane (500 mL), and the white precipitate was collected by filtration to give 60 g of lithium sulfinate salt. The salt was suspended in methylene chloride (200 mL) and cooled to 5 °C, and N-chlorosuccinimide (24.0 g, 0.18 mol) was added portionwise. After 2 h the mixture was filtered, and the solvent was evaporated to give a brown oil. This crude sulfonyl chloride was dissolved in THF (500 mL) and cooled to 5 °C, and dry ammonia gas was bubbled through the solution. The excess ammonia and THF were removed under vacuum, and the brown solid was triturated with water to give 51.5 g (80%) of **13c**: ^1H NMR δ 8.23 (2 H, dd, J = 1, 7 Hz), 7.72 (1 H, t, J = 6 Hz), 7.68 (1 H, d, J = 2.5 Hz), 7.63–7.43 (3 H, m), 7.44 (1 H, s), 7.0 (1 H, dd, J = 2, 9 Hz), 3.93 (3 H, s).

6-Methoxyindole-2-sulfonamide (14c). The crude **13c** (6.5 g) was dissolved in 10% sodium hydroxide (50 mL) and warmed at 80 °C for 30 min. The reaction was cooled and carefully neutralized with concentrated HCl to precipitate **14c** (3.0 g, 75% yield): ^1H NMR (acetone-d_6) δ 7.53 (1 H, d, J = 9 Hz), 7.02 (1 H, d, J = 2.5 Hz), 6.95 (1 H, s), 6.79 (1 H, dd, J = 2.5, 9 Hz), 3.78 (3 H, s).

6-Hydroxyindole-2-sulfonamide (15c). A mixture of **14c** (3.0 g, 13 mmol) and pyridine hydrochloride (15 g) was heated, under nitrogen, at 190 °C for 1 h. The hot mixture was poured on crushed ice, and the product was extracted with EtOAc (3 × 150 mL). The combined extract was washed with water and brine. The dried solution (Na_2SO_4) was evaporated under vacuum to give crude product (1.9 g, 68% yield). This material was chromatographed on silica gel eluting with 5% methanol/chloroform (v/v) to give 1.41 g of pure **15c**: ^1H NMR (acetone-d_6) δ 7.45 (1 H, d, J = 9 Hz), 6.92 (1 H, d, J = 2.5 Hz), 6.88 (1 H, s) 6.73 (1 H, dd, J = 2.5, 9 Hz).

6-Acetoxyindole-2-sulfonamide (16c). To a solution of **15c** (1.7 g, 8 mmol) in dry acetone (10 mL), cooled to 0 °C, was added triethylamine (1.23 mL, 8.8 mmol). Acetyl chloride (0.62 mL, 8.8 mmol) was then added dropwise. After 2 h, the reaction mixture was diluted with acetone and filtered and the solvent was evaporated. The solid residue was dissolved in EtOAc and washed with 5% $NaHCO_3$ and brine. After drying ($MgSO_4$), the solution was filtered and evaporated to give 1.4 g of crude **16c**. The yield after recrystallization was 1.2 g (59%): ^1H NMR (acetone-d_6) δ 7.62 (1 H, d, J = 9 Hz), 7.23 (1 H, d, J = 2.5 Hz), 6.98 (1 H, br s) 6.89 (1 H, dd, J = 2.5, 9 Hz), 2.28 (3 H, s).

1-Methyl-6-methoxyindole-2-sulfonamide (19). To a solution of 6-methoxyindole (7.35 g, 50 mmol) in dry THF (60 mL) under a nitrogen atmosphere, cooled to −70 °C, was added dropwise 1.6 M butyllithium in hexane (33 mL, 53 mmol). After 5 min, methyl iodide (3.5 mL, 56 mmol) was added and the reaction was allowed to warm gradually to room temperature. The solution was diluted with water, and the product was extracted into methylene chloride. The extract was washed with brine, dried (Na_2SO_4), and evaporated to give 9.6 g of a mixture of products and starting material. The mixture was stirred with hexane to induce fractional crystallization. Filtration afforded 3.75 g of starting material. The mother liquor was evaporated, and the residue was chromatographed on silica gel eluting with hexane. A single fraction (2.87 g) was obtained, which ^1H NMR analysis showed to be a 3:2 mixture of **17** and **18**. Conversion of this mixture to the corresponding sulfonamides was accomplished as described for **13c**, affording 1.4 g of a mixture of **19–21** after partial

(14) The electrophilic sulfonation process was developed by Dr. D. G. Melillo, Process Research, Merck Sharp & Dohme Research Laboratories, Rahway, NJ (unpublished).

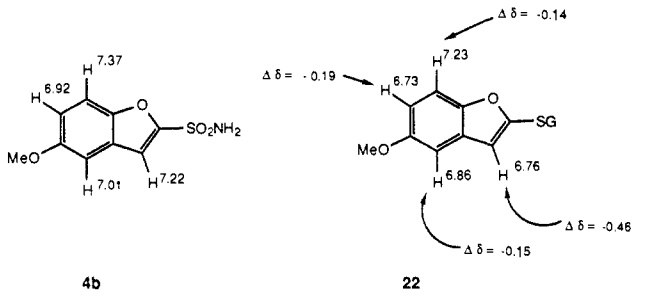

Figure 1. ^1H NMR spectral comparison (chemical shift values in ppm downfield from tetramethylsilane) of sulfonamide **4b** and glutathione adduct **22**.

purification by silica gel chromatography (40% EtOAc/hexane). Trituration with methylene chloride gave 131 mg of **19**: ^1H NMR ($CDCl_3$/DMSO-d_6) δ 7.56 (1 H, d, J = 9 Hz), 7.05 (1 H, br s), 6.90 (1 H, dd, J = 1, 9 Hz), 4.05 (3 H, s), 3.97 (3 H, s). Further trituration with Et_2O gave 650 mg of a crystalline mixture of **20** and **21**, which was separated by HPLC. There was obtained after crystallization 134 mg of **20** [^1H NMR ($CDCl_3$) δ 7.70 (1 H, d, J = 9 Hz), 7.04 (1 H, d, J = 3 Hz), 6.93 (1 H, d, J = 9 Hz) 6.50 (1 H, d, J = 3 Hz), 4.10 (3 H, s), 4.07 (3 H, s)] and 146 mg of **21** [^1H NMR ($CDCl_3$) δ 7.50 (1 H, d, J = 9 Hz), 6.72 (1 H, d, J = 9 Hz), 6.63 (1 H, s), 3.95 (3 H, s), 3.82 (3 H, s), 1.97 (3 H, s)].

Reaction of Sulfonamides with Reduced Glutathione (GSH). (A) Kinetic Determination. The protocol for the glutathione reaction as originally devised is found in ref 1f. A revised protocol[3] using 20 equiv of GSH, which better discriminates the electrophilicity of aromatic sulfonamides is described here. A solution of test sulfonamide (5×10^{-4} M) and GSH (1×10^{-2} M) in 2 mL of 0.1 M phosphate buffer (pH 7.4) was purged with N_2 and stored at 37 °C for 20 h in a well-stoppered glass vial. An aliquot (0.2 mL) was removed, and the extent of product formation was assessed by HPLC (see below).

(B) Preparative Scale Incubation of 4b with GSH. Glutathione (215 mg, 0.7 mmol) and **4b** (15 mg, 0.07 mmol) were dissolved in 70 mL of 0.05 M sodium phosphate buffer (pH 7.2). This mixture was incubated at 60 °C for 20 h under a nitrogen atmosphere. The solution was extracted with 2 volumes of EtOAc, and the aqueous phase was passed through a column of 25 g XAD-2 resin. The column was washed with water (70 mL), and the product was eluted from the column with 30 mL of methanol. Evaporation gave 1.6 mg of **22**, which was shown to be homogeneous by HPLC analysis (Waters C-18 column, eluted by 10% acetonitrile in 0.1% H_3PO_4 adjusted to pH 3.0 with trimethylamine). The chemical shifts for the aromatic protons in **4b** and adduct **22** are shown in Figure 1. The significant upfield shift of the proton bound to C-3 is indicative of replacement of the electronegative sulfamoyl group with the more neutral sulfide.

Carbonic Anhydrase Inhibition Assay. The ability of compounds to inhibit the carbonic anhydrase catalyzed hydration of CO_2 was determined by using a pH stat assay, as described in ref 1e.

IOP Recovery Rate Assay. Aqueous humor production was quantified indirectly in the conscious rabbit exactly as described by Vareilles and Lotti.[13]

IOP Studies in Ocular Hypertensive Rabbits. The α-chymotrypsinized rabbit model of Sears and Sears[2] was employed. A detailed description of this assay, as implemented in our laboratories, is found in ref 1d.

Guinea Pig Dermal Sensitization Assay. A detailed description of this assay is found in the paper by Magnusson and Kligman.[4] A summary of the procedure follows. Compounds were suspended in olive oil at a concentration of 2% and mixed with an equal volume of complete Freund adjuvant (Difco). Intradermal injection of 0.1 mL of this mixture was carried out on day 0. On day 6 a 10% suspension of sodium lauryl sulfate in petrolatum was applied to the shaved shoulder of the animal, at the injection site, followed on day 7 by application of a patch containing an 8% petrolatum suspension of the test 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 test 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 sensitization was performed 24 h after removal of the patch by both direct visual and microscopic observation. The degree of sensitizing potential was assigned according to the following scale, giving % of animals reacting, followed by classification: 0, nonsensitizer, 5–25, mild sensitizer; 30–65, moderate sensitizer; 70–100, strong sensitizer.

Registry No. **1a**, 271-89-6; **1b**, 13391-28-1; **1c**, 50551-63-8; **2a**·Li, 124043-83-0; **2b**, 124043-77-2; **2b**·Li, 124043-76-1; **2c**·Li, 124043-84-1; **3a**, 124043-72-7; **3b**, 100586-80-9; **3c**, 100586-73-0; **4b**, 100586-62-7; **4c**, 100586-63-8; **5b**, 100586-65-0; **5c**, 100586-64-9; **6b**, 100586-67-2; **6c**, 100586-66-1; 7, 3652-66-2; 8, 124043-73-8; **9a**, 124043-78-3; **9b**, 124043-79-4; **10a**, 124069-34-7; **10b**, 124043-74-9; **11a**, 120-72-9; **11b**, 1006-94-6; **11c**, 3189-13-7; **11d**, 267-48-1; **12a**, 40899-71-6; **12b**, 56995-12-1; **12c**, 56995-13-2; **12d**, 100587-74-4; **13a**, 85953-40-8; **13b**, 100587-66-4; **13c**, 100587-72-2; **13d**, 100587-77-7; **14a**, 85953-41-9; **14b**, 100587-67-5; **14c**, 100587-71-1; **14d**, 100587-73-3; **15b**, 100587-64-2; **15c**, 100587-68-6; **16b**, 100587-78-8; **16c**, 100587-79-9; **17**, 1968-17-8; **18**, 124043-80-7; **19**, 124043-75-0; **20**, 124043-81-8; **21**, 124043-82-9; **22**, 124069-36-9; carbonic anhydrase, 9001-03-0; 6-hydroxy-3-methylbenzofuransulfonic acid potassium salt, 124069-35-8; glutathione, 70-18-8.