4-Substituted Thiophene- and Furan-2-sulfonamides as Topical Carbonic Anhydrase Inhibitors†

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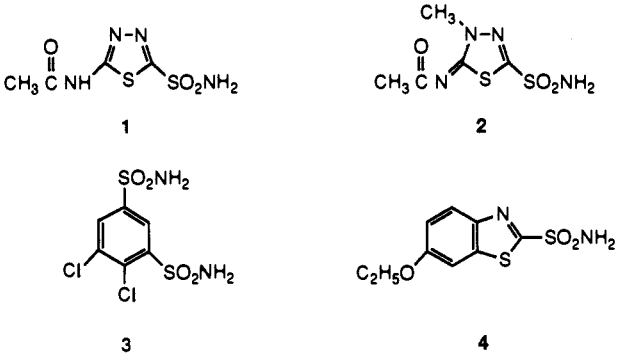
A series of 4-substituted thiophene- and furan-2-sulfonamides was prepared and was found to possess nanomolar-level potency for inhibition of human carbonic anhydrase II in vitro. Selected examples from this group were further evaluated for their potential to act as topically effective ocular hypotensive agents in the ocular normotensive albino rabbit and the ocular α-chymotrypsinized rabbit. Solubility studies in water and pH 7.4 buffer were carried out to estimate the ability of compounds to be formulated in solution. The sensitization potential of key representative structures was determined by in vitro glutathione reactivity studies and guinea pig maximization testing.

Introduction

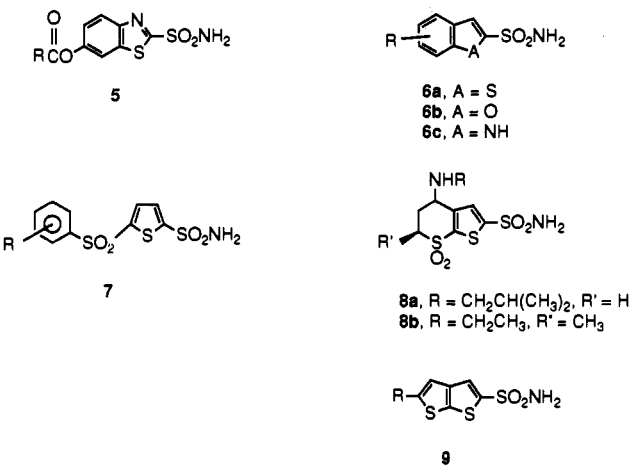
Topically effective carbonic anhydrase inhibitors (CAIs) potentially represent a significant therapeutic advance for the treatment of open-angle glaucoma[1,2] since the side effect profile[3,4] associated with systemic therapy may be dramatically improved. Clinical utility has been demonstrated for the orally active agents acetazolamide (**1**), methazolamide (**2**), dichlorophenamide (**3**), and ethoxzolamide (**4**) in the treatment of glaucoma in man[5] (Scheme I). These compounds inhibit the secretion of aqueous humor by the nonpigmented epithelial cells of the ciliary process via inhibition of carbonic anhydrase II and thereby reduce intraocular pressure (IOP).[6]

Recently, a variety of structures have been revealed that are reported to diminish IOP in animals following topical administration. Included in this group are benzo[*b*]thiazole-2-sulfonamides (**5**),[7] benzo[*b*]thiophene-2-sulfonamides (**6a**),[8] benzo[*b*]furan-2-sulfonamides (**6b**),[9] indole-2-sulfonamides (**6c**),[9] 4-(arylsulfonyl)thiophene-2-sulfonamides (**7**),[10] 4-(alkylamino)thieno[2,3-*b*]thiopyranes (**8**),[11] and thieno[2,3-*b*]thiophene-2-sulfonamides (**9**)[14] (Scheme II). In particular, **8a**[12a] and **8b**[12b] have been found to lower the IOP of glaucoma patients after topical dosing.[13]

Scheme I



Scheme II



The active site of carbonic anhydrase II (CA II) is a conical cavity approximately 12 Å deep which contains at

† This paper is dedicated to Professor Ralph Hirschmann on the occasion of his 70th birthday.

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the bottom a zinc atom in tetrahedral coordination with three histidines and a water molecule. This cylindical passage embodies both hydrophobic and hydrophilic segments.[15] In recognition of these geometic and electronic requirements, we wish to report the synthesis and ocular hypotensive profiles of a series of 4-substituted thiophene- and furan-2-sulfonamides. Members of this series exhibit nanomolar potency for inhibition of CA II and demonstrate significant ocular hypotensive activity upon topical administration in rabbits. Key compounds exhibit appreciable water solubility and are readily formulated in solution for topical administration.

Chemistry

The compounds prepared in this work can be separated into two general structural classes wherein the thiophene- or furan-2-sulfonamide contains either a 4-aroyl (Class I) or 4-arylsulfonyl (Class II) functionality. Compounds of Class I were prepared as shown in Scheme III.[16]

Metalation of the heterocyclic bromides **10**, treatment with the appropriate aryl nitrile, and acidic hydrolysis gave the ketones **11**.[17] Electrophilic aromatic sulfonation provided the expected[18] 2,4-disubstituted sulfonic acids **12**. Generation of the sulfonyl chlorides **13** was effected with oxalyl chloride/DMF, while subsequent treatment with ammonia provided **14**. Functionalized sulfonamides **16** were prepared by free radical bromination of **13** to provide bromomethyl derivative **15**, which was treated sequentially with ammonia to give the sulfonamide and then with a primary or secondary amine to afford **16**. Disubstituted aminoalkyl analogs **18** in the thiophene series were prepared by a sequence that involved generation of phenol **17** by demethylation of **14** with either 48% hydrobromic acid or boron tribromide, followed by Mannich reaction.[20]

Compounds of Class II were prepared as shown in Scheme IV. Metalation of the 3-bromo heterocycle **10** followed by treatment with the requisite disulfide[21] provided the sulfide **19**. Oxidation to the sulfone **20** was effected with *m*-chloroperbenzoic acid and chlorosulfonation[19] at 50–55 °C for 15–25 min provided the desired sulfonyl chlorides **21** in good yields. Adducts **21** were either converted to sulfonamides **22** by treatment with ammonium hydroxide or were, in the thiophene series, halogenated with *N*-bromosuccinimide to provide bromomethyl analogs **23**. These were treated, as before, with ammonia and then with a primary or secondary amine to give the aminomethyl sulfonamides **24**.

Disubstituted phenyl derivatives of methoxy sulfonamides **22b–d** were prepared by demethylation as previously described to give **25** followed by Mannich reaction under basic conditions. This sequence provided analogs **26** in modest yield.

Results and Discussion

A sequence of in vitro, ex vivo, and in vivo studies was used to evaluate the ocular hypotensive profile of this series of thiophene and furan sulfonamides. As the initial screen, all compounds were evaluated for their ability to inhibit human erythrocyte CA II-catalyzed carbon dioxide hydration (I_{50}). Selected examples were then tested for their ability to compete with dansylamide for binding to CA II (K_i). As shown in Tables I and II, several functionalized 4-substituted thiophene- and furan-2-sulfonamides showed sub-10 nM potencies. Specifically, 4-acyl analogs, such as **16b** and **16f**, bearing monosubstitution in the 3- or 4-positions of the phenyl ring, displayed equivalent or somewhat enhanced activity as compared to their corresponding sulfonyl counterparts, **24b** and **24c**. Similarly, the thiophene-based Mannich adducts **18a** and **18c** were of comparable potency to furan adducts **26f** and **26g**. Overall, potency was neither dramatically improved nor diminished by the mono- or disubstitution patterns utilized in these examples.

Compounds were evaluated in an ex vivo assay for their ability to penetrate the albino rabbit eye and to inhibit the target enzyme in an homogenate of the iris–ciliary body at 1 h postdosing.[12b] All compounds were screened at 0.5% (1 drop, 50 μL), while the more potent compounds were tested at lower concentrations. The most potent compounds proved to be **16b**, **16d**, **24c**, and **24e**, all of which showed appreciable ex vivo activity at a concentration of 0.1%. Interestingly, none of these compounds were among the most potent in the in vitro assays. A common feature of this group of actives, however, was their intermediate level of lipophilicity as demonstrated by the range of partition coefficients from 2 to 12.

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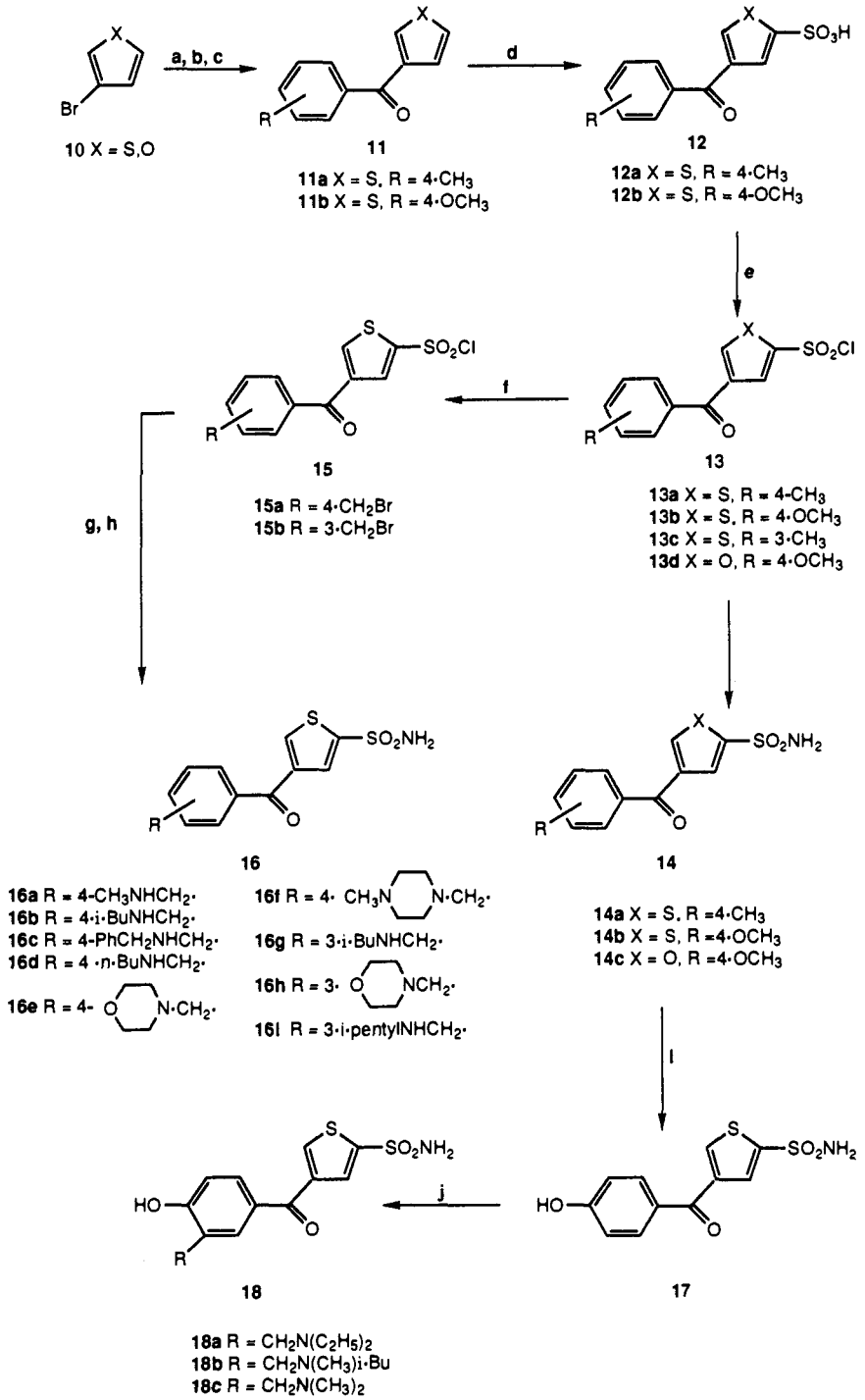
Scheme III. Synthesis of 4-Aroyl Sulfonamides[a]

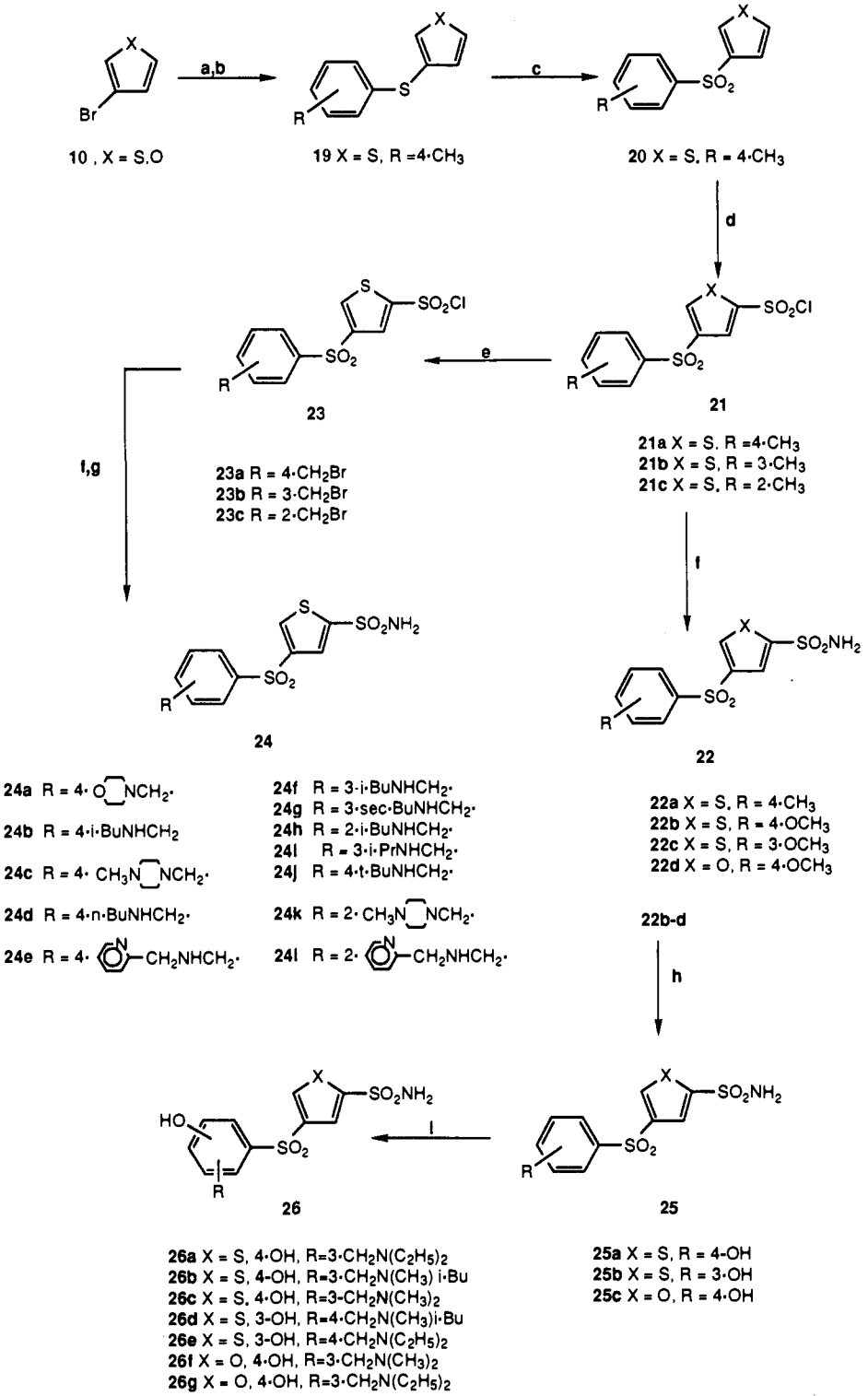


[a] Reagents: (a) *n*-BuLi, ether, -78 °C. (b) Aryl nitrile. (c) H_3O^+. (d) H_2SO_4/HOAc. (e) Oxalyl chloride/DMF, rt. (f) NBS, benzoyl peroxide, $CHCl_3$, hν. (g) NH_3·$CHCl_3$. (h) Primary or secondary amines, $CHCl_3$, rt. (i) 48% aqueous hydrobromic acid. (j) CH_2O (aq) with amine under Mannich conditions.

Compounds were further studied for their ability to lower IOP in the α-chymotrypsinized (α-CT) rabbit model of ocular hypertension.[7a] In this model, rabbits display chronic IOP elevations due to obstruction in the aqueous humor outflow channels following α-CT injection and are treated topically with a single 50-μL drop of the test compound in 0.5% aqueous (hydroxyethyl) cellulose. The IOP is then monitored at set times out to 5 h postdosing. Table III shows the maximum drop in IOP in this experiment and the time post dosing that this decline was measured. The three most potent compounds in this model were **18a**, **24h**, and **24l**, however at 0.1% dosing, **24l** proved to be much less active than the other two. Neither **18a** nor **24h** was among the most potent in terms of in vitro enzyme inhibition and both displayed moderate levels (~50%) of inhibition at 0.1% dosing in the ex vivo assay. The partition coefficients of **18a** and **24h**, 2.3 and 14.8, respectively, were again in an intermediate range. Interestingly, the magnitude of the IOP drop in the α-CT rabbit illicited by **18a** and **24h** was comparable to that seen for benzothiophene-, benzofuran-, and indole-2-sulfonamides,[9] as well as certain 5,6-dihydro-4*H*-thieno-[2,3-*b*]-thiopyran-2-sulfonamides.[11b]

Since ocular and dermal sensitization due to the electrophilicity of some heterocyclic sulfonamides has been an on-going concern,[7b,8,9] select compounds were studied

Scheme IV. Synthesis of 4-Sulfonyl Sulfonamides[a]



[a] Reagents: (a) *n*-BuLi, ether, −78 °C. (b) (Ar-S)$_2$, −78 °C to rt. (c) MCPBA, $CHCl_3$, 0–10 °C. (d) $ClSO_3H/PCl_5$, 50–55 °C. (e) NBS, $CHCl_3$, benzoyl peroxide, hν. (f) NH_3, $CHCl_3$, 0–10 °C. (g) Primary or secondary amines. (h) 48% hydrobromic acid. (i) CH_2O (aq) under Mannich conditions.

for this effect. Both **17** and **24h** were found to be unreactive to reduced glutathione (5 equiv of GSH, pH 7.4, 37 °C, 16–22 h),[7b] and **17** was found to not cause sensitization in the Magnusson–Kligman guinea pig maximum sensitization test.[22]

Finally, as a guide to the formulateability of compounds at or near physiological pH, the solubility was determined in both water and pH 7.4 buffer at 25 °C. As exemplified by examples **16a** (7.5–1.4 mg/mL), **16b** (3.5–0.09 mg/mL), **24a** (35–0.85 mg/mL), and **24e** (90–0.9 mg/mL) a significant and sometimes dramatic drop in solubility was observed in going from a determination involving an uncontrolled pH in water to pH 7.4 buffer. This latter experiment more closely approximates the solubility of the free base. On the other hand, piperazine **24c** (43–56 mg/mL) and amine **24b** (29.5–9.25 mg/mL) displayed appreciable solubility at neutrality, as did Mannich adducts **26a** (13.9 mg/mL) and **26c** (>25 mg/mL).

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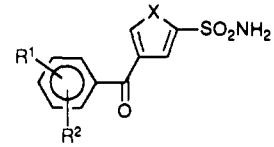
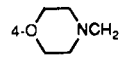
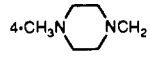
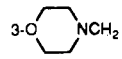
Table I. Analytical, Solubility, and Biological Data for 4-Acylthiophene- and -furan-2-sulfonamides



cmpd	X	R^1	R^2	formula	mp, °C	anal.	solubility,[a,b] mg/mL	partition coeff[c]	pK_a[d]	CA:[e] $10^{-9}I_{50}$, M	CA:[e] $10^{-9}K_i$, M	ex vivo, %
14b	S	4-OCH_3	H	$C_{12}H_{11}NO_4S_2$	173–175	C,H,N	0.01[b]	57	8.20	3.2		31 (0.5)
17	S	4-OH	H	$C_{11}H_9NO_4S_2$	192–193	C,H,N	0.39[b]	26	9.68 (OH) 7.90 (NH_2)	2.5		83 (0.5)
14a	S	4-CH_3	H	$C_{12}H_{11}NO_3S_2$	175–177	C,H,N	0.01[b]	146	9.30	2.0		26 (0.5)
16a	S	4-CH_3NHCH_2	H	$C_{13}H_{14}N_2O_3S_2$·HCl	238–240 dec	C,H,N	1.38[a]	0.14	9.57 8.39	4.5		67 (0.5) 13 (0.1)
16b	S	4-*i*-BuNHCH$_2$	H	$C_{16}H_{20}N_2O_3S_2$·HCl	237–239 dec	C,H,N	0.09[a] 3.5[b]	5.0	9.55 8.30	4.8		87 (0.5) 77 (0.1)
16c	S	4-$PhCH_2NHCH_2$	H	$C_{19}H_{18}N_2O_3S_2$·HCl	244–246 dec	C,H,N	0.01[a] 0.18[b]	52	9.35 7.64	2.4		81 (0.5)
16d	S	4-*n*-BuNHCH$_2$	H	$C_{16}H_{20}N_2O_3S_2$·HCl	243–246 dec	C,H,N	0.46[a] 2.4[b]	3.7	9.32 8.12	12.0		95 (0.5) 68 (0.1)
16e	S		H	$C_{16}H_{18}N_2O_4S_2$·HCl	232–235 dec	C,H,N	0.07[a] 12.1[b]	15.1	9.28 5.92	3.7	3.32	73 (0.5)
16f	S		H	$C_{17}H_{21}N_3O_3S_2$·2HCl	250–252 dec	C,H,N	2.0[a] 5.0[b]	2.34	9.32 7.54 3.89	4.0		90 (0.5)
18a	S	4-OH	3-Et_2NCH_2	$C_{18}H_{20}N_2O_4S_2$·HCl		C,H,N	0.08[a] 34.2[b]	2.3	10.55 9.22 6.10	7.0	18.6	55 (0.5) 54 (0.1)
18b	S	4-OH	3-CH_3(*i*-Bu)-NHCH$_2$	$C_{17}H_{22}N_2O_4S_2$·HCl	190–195	C,H,N	16.0[b]	17	10.40 9.13 5.80	9.0		58 (0.5)
18c	S	4-OH	3-$(CH_3)_2$-NCH$_2$	$C_{14}H_{16}N_2O_4S_2$·HCl	228–230 dec	C,H,N	11.0[b]	0.36	10.38 9.20 6.25	6.4	34.4	59 (0.5)
16g	S	3-*i*-BuNHCH$_2$	H	$C_{16}H_{20}N_2O_3S_2$·HCl	246–248 dec	C,H,N	1.9[a] 10.0[b]	2.63	9.50 8.35	13.0	14.5	68 (0.5)
16h	S		H	$C_{16}H_{18}N_2O_4S_2$·HCl	253–255 dec	C,H,N	4.8[b]	13	9.03 5.70	5.0	10.8	90 (0.5) 26 (0.1)
16i	S	3-isopentylNHCH$_2$	H	$C_{17}H_{22}N_2O_3S_2$·HCl	238–240	C,H,N	12.8[b]	10.6	9.45 8.28	9.0		82 (0.5)
13c	O	4-OCH_3	H	$C_{12}H_{11}NO_5S$	180–182	C,H,N	0.02[a]	33.2	9.50	2.0		67 (0.5) 13 (0.1)

[a] Solubility measured in pH 7.4 buffer at 25 °C. [b] Solubility measured in water at 25 °C. [c] Partition coefficients were determined by equilibrating each test compound between 1-octanol and 0.1 ionic strength pH 7.4 buffer. [d] pK_a was determined in 30% ethanol/H_2O. [e] In vitro inhibition of human carbonic anhydrase II (see Experimental Section for details). [f] In this assay the known carbonic anhydrase inhibitors acetazolamide and methazolamide displayed inhibition levels of 26% and 12%, respectively, when dosed as 0.1% suspensions in 0.5% aqueous (hydroxyethyl) cellulose.

Conclusion

Separate series of 4-substituted thiophene- and furan-2-sulfonamides were identified as exhibiting nanomolar-level in vitro inhibitory activity vs human CA II. Specific compounds from these series possess IOP lowering potential post topical dosing in ocular normotensive and hypertensive albino rabbits. Compounds **18a** and **24h**, which were optimum in the α-CT model and showed moderate activity in the ex vivo assay, along with **24c**, which showed good ex vivo activity and excellent solubility, are of continuing interest.

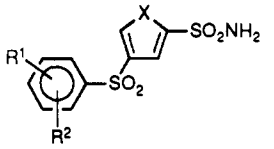
Experimental Section

Melting points were determined on a Thomas-Hoover apparatus in open capillary tubes and are uncorrected. ^1H NMR spectra were recorded in either $CDCl_3$ or Me_2SO-d_6 on an EM 390, XL-300, or NT-360 spectrometer with Me_4Si as internal standard. The elemental analyses were carried out by J. P. Moreau and all are within 0.4% of the theoretical value. Physical measurements were carried out by W. C. Randall, J. M. Sondey, S. R. Michelson, and S. J. Smith. Silica gel (230–400 mesh, E. Merck) was used for column chromatography. The mass spectral analyses were carried out by H. Ramjit and his staff using an LKB-9000 S instrument at 70 eV. *N*-Bromosuccinimide, 3-bromothiophene, 3-bromofuran, 4-methoxybenzonitrile, 3-methoxybenzonitrile, 4-methylbenzonitrile, and 2-methylbenzonitrile were purchased from Aldrich and used without purification. *n*-Butyllithium in hexane was purchased from Alfa.

3-(4-Methylbenzoyl)thiophene (11a). To a solution of 5.0 g (0.031 mol) of 3-bromothiophene in 20 mL of ether containing 5 mL of tetrahydrofuran cooled to −78 °C under nitrogen was added 0.031 mol of *n*-butyllithium (in hexane) dropwise at <−70 °C. This clear, pale yellow solution was stirred for 10 min and then 3.63 g (0.31 mol) of 4-methylbenzonitrile in 5 mL of tetrahydrofuran was added dropwise at <−70 °C. The reaction mixture was stirred at −70 °C for 0.5 h, −50 °C for 1.0 h, and then allowed to rise to −10 °C over 1.5 h as the color changed to deep cherry red.

The reation mixture was quenched with 5 mL of water followed by 30 mL of 2 N HCl, and the mixture extracted with 2 × 50 mL of ether. The acidic aqueous phase was then heated at reflux for 2 h, cooled, and extracted with 3 × 50 mL of Et_2O. The combined organic phases were washed with brine and dried and the solvent removed in vacuo to give a dark oil. This was purified by flash chromatography on silica gel eluting with 5% 2-propanol/hexane to give 0.5 g (83%) of pure **11a** as a clear oil: ^1H NMR (300 MHz,

Table II. Analytical, Solubility, and Biological Data for 4-Sulfonylthiophene- and -furan-2-sulfonamides



cmpd	X	R¹	R²	formula	mp, °C	anal.	solubility[a,b] mg/mL	partition coeff[c]	pK_a[d]	CAI:[e] $10^{-9}I_{50}$, M	CA:[e] $10^{-9}K_i$, M	ex vivo
22b	S	4-OCH_3	H	$C_{11}H_{11}NO_5S_3$	169–171	C,H,N	0.02[b]	23	8.78	45		68 (0.5)
25a	S	4-OH	H	$C_{10}H_9NO_5S_3$	192–194	C,H,N	0.5[a]	13	9.20	5.0	8.0	82 (0.5)
							0.3[b]		7.46			
22b	S	4-CH_3	H	$C_{11}H_{11}NO_4S_3$	184–186	C,H,N	0.03[a]	43	8.95	3.2		86 (0.5)
24a	S	4-O, NCH_2	H	$C_{16}H_{18}N_2O_5S_3$·HCl	238–241 dec	C,H,N	0.85[a]	9.5	8.84	7.6	1.43	83 (0.5)
							35.0[b]		5.28			79 (0.1)
24b	S	4-*i*-BuNHCH$_2$	H	$C_{16}H_{20}N_2O_4S_3$·HCl	208–209	C,H,N	9.8[a]	6.4	9.22	13.0	6.50	87 (0.5)
							29.5[b]		7.87			63 (0.1)
24c	S	4-CH_3N, NCH_2	H	$C_{16}H_{21}N_3O_4S_3$·2HCl	238–242	C,H,N	>56[a]	2.4	9.25	11.0		92 (0.5)
							>43[b]		7.40			90 (0.1)
									3.81			29 (0.05)
24d	S	4-*n*-BuNHCH$_2$	H	$C_{16}H_{20}N_2O_4S_3$·HCl	174–176	C,H,N	5.0[a]	2.1	9.35	29.0		87 (0.5)
							8.8[b]		8.12			48 (0.1)
24e	S	4-N, CH_2NHCH_2	H	$C_{17}H_{17}N_3O_4S_3$·2HCl	90–100	C,H,N	0.9[a]	11.5	8.92	8.0		92 (0.5)
							90[b]		6.25			75 (0.1)
									3.55			
25b	S	3-OH	H	$C_{10}H_9NO_5S_3$	144–146	C,H,N	0.61[b]	25	9.35	2.1		86 (0.5)
									7.93			
24f	S	3-*i*-BuNHCH$_2$	H	$C_{16}H_{20}N_2O_4S_3$·HCl		C,H,N	10.9[b]	4.1	9.10	12		84 (0.5)
									7.83			
24g	S	3-*s*-BuNHCH$_2$	H	$C_{16}H_{20}N_2O_4S_3$·HCl	171–182	C,H,N	20.0[b]	3.0	9.05	26		72 (0.5)
									7.45			
24h	S	2-*i*-BuNHCH$_2$	H	$C_{16}H_{20}N_2O_4S_3$·HCl		C,H,N	4.6[b]	14.8	8.98	18	21.1	62 (0.5)
									7.45			47 (0.1)
24i	S	3-*i*-PrNHCH$_2$	H	$C_{14}H_{18}N_2O_4S_3$·HCl	182–186	C,H,N	11.4[b]	0.91	9.20	22		71 (0.5)
									6.95			
24j	S	4-*t*-BuNHCH$_2$	H	$C_{16}H_{20}N_2O_4S_3$·HCl	272–274 dec	C,H,N	4.5[b]	0.72	9.30	36		61 (0.5)
									8.08			
24k	S	2-CH_3N, NCH_2	H	$C_{16}H_{21}N_3O_4S_3$·HCl		C,H,N	>64[b]	13.4	8.80	23		77 (0.5)
									6.40			65 (0.1)
24l	S	2-N, CH_2NHCH_2	H	$C_{17}H_{17}N_3O_4S_3$·HCl		C,H,N	5.2[a]	0.3	8.70	15		68 (0.5)
									6.10			68 (0.1)
26a	S	4-OH	3-Et_2NHCH_2	$C_{16}H_{20}N_2O_5S_3$·HCl	169–178	C,H,N	13.9[a]	0.81	10.47	9.0		71 (0.5)
							>10.2[b]		8.75			
									5.63			
26b	S	4-OH	3-*i*-Bu(CH_3)-$NHCH_2$	$C_{16}H_{22}N_2O_5S_3$·HCl		C,H,N	1.1[a]	4.8	10.45	5.0		90 (0.5)
							>50[b]		8.85			26 (0.1)
									5.43			
26c	S	4-OH	3-Me_2NCH_2	$C_{16}H_{18}N_2O_5S_3$·HCl		C,H,N	>25[a]	0.21	10.32	9.0		57 (0.5)
							>21[b]		8.95			40 (0.1)
26d	S	3-OH	4-*i*-Bu(CH_3)N	$C_{16}H_{22}N_2O_5S_3$·HCl		C,H,N	>66[b]	30	5.89	14.0		
									10.32			
									8.83			
26e	S	3-OH	4-Et_2NCH_2	$C_{16}H_{20}N_2O_5S_3$·HCl	190–198	C,H,N	1.4[b]	2.7	6.02	16.0		84 (0.5)
									8.98			
22c	O	4-OCH_3	H	$C_{11}H_{11}NO_6S_2$	117–118	C,H,N	0.3[b]	14.7	6.32	2.5		68 (0.5)
												97 (0.5)
												95 (0.1)
25c	O	4-OH	H	$C_{10}H_9NO_6S_2$	174–176	C,H,N	2.3[a]	0.79	8.50	4.2		54 (0.02)
							1.2[b]		9.35			100 (0.5)
22d	O	4-CH_3	H	$C_{11}H_{11}NO_5S_2$	184–186	C,H,N	0.05[a]	31	8.02	4.2		36 (0.1)
									9.32			96 (0.5)
26f	O	4-OH	3-Me_2NCH_2	$C_{13}H_{16}N_2O_6S_2$·HCl		C,H,N		0.11	8.12	7.0	2.34	78 (0.1)
											4.9	81 (0.5)
26g	O	4-OH	3-Et_2NCH_2	$C_{16}H_{20}N_2O_6S_2$·HCl		C,H,N	>13[a]	4.2	10.62	10.0	4.0	82 (0.5)
							>6[b]		8.72			58 (0.1)
									5.78			

[a–e] See Table I for notes.

$CDCl_3$) δ 2.50 (3 H, s), 7.32 (1 H, d, J = 10 Hz), 7.40 (1 H, dd, J = 9, 1 Hz), 7.62 (1 H, d, J = 9, 1 Hz), 7.80 (1 H, d, J = 10 Hz), 7.95 (1 H, d, J = 1 Hz).

4-(4-Methylbenzoyl)thiophene-2-sulfonic Acid (12a). To 3.0 g (0.015 mol) of **11a** in 25 mL of methylene chloride cooled to 0–10 °C was added 4.8 g (0.047 mol) of acetic anhydride followed by 1.9 g (0.016 mol) of sulfuric acid added dropwise. The resulting clear, dark solution was stirred at room temperature for 3 days and was then diluted with 50 mL of hexane. The resulting suspension was stirred in an ice bath for 0.5 h and was then

Table III. Ocular Hypotensive Activity of Selected Sulfonamides in α-CT Rabbits

compd	dose,[a] %	maximum IOP reduction, mmHg (time of peak effect, h)
16b	0.5	inactive
16d	0.1	-3.7 (3)
16e	0.5	-2.2 (1)
18a	0.1	-6.0 (2)
16g	0.1	-4.0 (3)
16i	0.1	-4.0 (3)
24a	0.1	-4.5 (0.5)
24b	0.1	-2.8 (2)
24g	0.5	-2.3 (2)
24h	0.1	-5.5 (4)
24k	0.5	-2.7 (2)
24l	0.5	-6.5 (3)
26g	0.1	-3.0 (3)

[a] Test compounds were applied as a 50-μL drop of a suspension or solution of the indicated concentration in 0.5% aqueous (hydroxyethyl) cellulose. IOP was measured just before (τ_0) and 0.5, 1, 2, 3, 4, and 5 h after treatment. Results are expressed as maximum drop in IOP from τ_0 value. Parallel control groups for each compound were run in which a 50 μ-L drop of 0.5% aqueous (hydroxyethyl) cellulose was instilled. The maximum IOP reductions shown above are all significantly different from controls.

filtered to provide **12a** as a pale, brown solid, 2.4 g (57%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.42 (3 H, s), 7.40 (1 H, d, J = 2 Hz), 7.43 (2 H, d, J = 7 Hz), 7.74 (2 H, d, J = 7 Hz), 8.13 (1 H, d, J = 2 Hz).

4-(4-Methylbenzoyl)thiophene-2-sulfonamide (14a). To 2.2 g (0.0078 mol) of **12a** suspended in 50 mL of ethyl acetate was added 1.97 g (0.0156 mol) of oxalyl chloride, and then at 0–10 °C 1.46 g (0.020 mol) of *N,N*-dimethylformamide was added. The reaction mixture was then stirred at room temperature for 16 h.

With cooling in an ice bath, the reaction mixture was quenched with 20 mL of water, and the organic phase was then extracted with 3 × 20 mL of water. The organic phase was washed with brine and dried, and the solvent was removed in vacuo to give the sulfonyl halide **13a** as a tan solid, R_f = 0.4 (5% 2-propanol/hexane): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (3 H, s), 7.35 (2 H, d, J = 8 Hz), 7.75 (2 H, d, J = 8 Hz), 8.29 (1 H, d, J = 1 Hz), 8.35 (1 H, d, J = 1 Hz). This solid was dissolved in 25 mL of chloroform, and after cooling to 0–10 °C a stream of ammonia was passed into the solution. The addition was stopped after 10 min, and after another 2 h stirring at room temperature, all starting material was consumed. The solvent was removed in vacuo and the resulting solid (R_f 0.4) purified by flash chromatography on silica gel eluting with 5% methanol/chloroform to give 1.4 g (64%) of pure **14a**: mp 175–177 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.45 (3 H, s), 7.38 (2 H, d, J = 8 Hz), 7.77 (2 H, d, J = 8 Hz), 7.86 (1 H, d, J = 2 Hz), 8.45 (1 H, d, J = 2 Hz). Anal. ($C_{12}H_{11}NO_3S_2$) C, H, N.

3-(4-Methoxybenzoyl)thiophene (11b). To 0.026 mol of *n*-butyllithium in 50 mL of ether at -78 °C under nitrogen was added a solution of 4.0 g (0.025 mol) of 3-bromothiophene in 100 mL of ether dropwise at <-70 °C. This was stirred at -78 °C for 45 min, and then a solution of 3.46 g (0.026 mol) of 4-methoxybenzonitrile in 50 mL of ether was added dropwise at <-70°. The reaction mixture was stirred at -70 °C for 0.5 h, -50 °C for 0.5 h, and then allowed to warm to -10 °C over 2.0 h. The mixture was quenched with 15 mL of H_2O and 40 mL of 2 N HCl. The acidic aqueous phase was separated, washed with 3 × 30 mL of Et_2O and then heated at reflux for 2.0 h. The cooled solution was extracted with 3 × 60 mL of ether, and the combined organic extracts were washed with brine. The solvent was removed in vacuo to give an oil that was triturated at 0–10 °C with petroleum ether to give **11b** as a tan solid, 2.5 g (52%): mp 61–65 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.82 (3 H, s), 7.00 (2 H, d, J = 8 Hz), 7.40 (1 H, dd, J = 1 Hz), 7.60 (1 H, dd, J = 7, 1 Hz), 7.85 (2 H, d, J = 8 Hz), 7.87 (1 H, d, J = 1 Hz).

4-(4-Methoxybenzoyl)thiophene-2-sulfonic Acid (12b). To a solution of 9.79 g (0.05 mol) of **11b** in 75 mL of methylene chloride cooled to 0–10 °C was added 15.3 g (0.15 mol) of acetic anhydride followed by 5.9 g (0.06 mol) of sulfuric acid added dropwise. The resulting dark solution was stirred at room temperature for 16 h at which time a solid was present. This pale yellow solid was collected by filtration and was dried to give 9.16 g (61%) crude **12b**: mp 169–179 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.90 (3 H, s), 7.15 (2 H, d, J = 8 Hz), 7.41 (1 H, d, J = 2 Hz), 7.85 (2 H, d, J = 8 Hz), 8.13 (1 H, d, J = 2 Hz).

4-(4-Methoxybenzoyl)thiophene-2-sulfonamide (14b). A suspension of 2.98 g (0.01 mol) of **12b** in 50 mL of thionyl chloride was refluxed for 1.5 h to give a homogeneous solution. Excess thionyl chloride was removed in vacuo and the residue was decomposed with 20 mL of ice–water and extracted with 2 × 40 mL of chloroform. The combined organic extracts were washed with brine and dried, and the solvent was removed in vacuo to give crude sulfonyl chloride **13b** as a yellow oil. This was taken up in 25 mL of acetone and treated at 0–10 °C with 10 mL of ammonium hydroxide. The solvent was removed in vacuo and the residue was extracted with chloroform. The organic extract was washed with brine and dried, and the solvent was removed in vacuo to give a yellow solid. This was triturated with ether to give 1.6 g (54%) of pure **14b** as a white solid: mp 173–175 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.90 (3 H, s), 7.18 (2 H, d, J = 9 Hz), 7.92 (6 H, m), 8.49 (1 H, s). Anal. ($C_{12}H_{11}NO_4S_2$) C, H, N.

4-(4-Hydroxybenzoyl)thiophene-2-sulfonamide (17). A solution of 3.0 g (0.01 mol) of **14b** in 75 mL of 48% aqueous hydrobromic acid was heated at reflux for 8 h, at which time all starting material was consumed. The cooled reaction mixture was then poured onto ice and stirred for 10 min. The violet-colored solid that formed was collected by filtration and purified by flash chromatography on silica gel eluting with hexane (55)–ethyl acetate (45). This gave a solid that was triturated with methylene chloride to give 7.0 g (74%) of pure **17**: mp 189–191 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.85 (2 H, d, J = 8 Hz), 7.66 (2 H, d, J = 8 Hz), 7.79 (1 H, d, J = 2 Hz), 8.41 (1 H, d, J = 2 Hz).

4-[3-[(Dimethylamino)methyl]-4-hydroxybenzoyl]thiophene-2-sulfonamide (18c). A solution of 1.70 g (0.006 mol) of **17**, 2.71 g (0.024 mol) of dimethylamine, and 0.73 g (0.009 mol) of formaldehyde (37% aqueous solution) in 15 mL of ethanol was heated at reflux for 16 h. The cooled reaction mixture was filtered to remove a small amount of the crude bis-Mannich product. The filtrate solvent was removed in vacuo, and the residue was flash chromatographed on silica gel eluting with chloroform (9)–methanol (1) to give 0.7 g (35%) (R_f 0.3) of pure **18c** as a pale yellow solid: mp 197–201 °C. This was suspended in 15 mL of ethanol and treated with ethanolic HCl to give a clear solution. This solution was gradually diluted with 15 mL of ether to provide after stirring at 0–10 °C for several hours, a hydrochloride of **18c** as a white powder: mp 230–232 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.33 (6 H, s), 3.62 (2 H, s), 6.95 (1 H, d, J = 8 Hz), 7.74 (2 H, bs), 7.90 (2 H, bs), 8.42 (1 H, s). Anal. ($C_{14}H_{17}ClN_2O_4S_2$) C, H, N.

Compounds **18a** and **18b** were prepared from **17** in similar fashion and had the physical properties shown below.

4-[3-[(Diethylamino)methyl]-4-hydroxybenzoyl]thiophene-2-sulfonamide (18a) gave a hydrochloride as a white powder with a broad melting range: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (6 H, t), 2.76 (4 H, q), 3.86 (2 H, s), 6.72 (1 H, d), 7.63 (2 H, m), 7.96 (2 H, d). Anal. ($C_{16}H_{20}N_2O_4S_2$·HCl) C, H, N, Cl.

4-[3-[(*N*-Isobutyl-*N*-methylamino)methyl]-4-hydroxybenzoyl]thiophene-2-sulfonamide (18b) gave a hydrochloride salt as a white powder: mp 190–195 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92 (6 H, d), 2.32 (2 H, s), 2.39 (1 H, d), 3.84 (2 H, s), 7.73 (2 H, m), 7.93 (2 H, d), 8.50 (1 H, s). Anal. ($C_{17}H_{22}N_2O_4S_2$·HCl) C, H, N.

4-(3-Methylbenzoyl)thiophene-2-sulfonyl Chloride (13c). This intermediate was prepared from 3-methylbenzonitrile and 3-bromothiophene as described above for **13a** and was used without purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.57 (2 H, s), 7.29 (1 H, s), 7.51–7.83 (4 H, m), 8.32 (1 H, dd), 8.38 (1 H, dd).

4-[4-(Bromomethyl)benzoyl]thiophene-2-sulfonyl Chloride (15a). A solution of 5.0 g (0.017 mol) of **13a**, 5.9 g (0.033 mol) of *N*-bromosuccinimide, and 10 mg of benzoyl peroxide in 100 mL of chloroform was heated at reflux and irradiated with a sunlamp (200 W) for 0.5 h. At this time almost all starting material was consumed. The cooled reaction mixture was extracted with 3 × 75 mL of water to remove the succinimide, 75 mL of 5% sodium thiosulfate, and brine and was then dried. The solvent was removed in vacuo to give crude **13a** as an oil.

A portion of this was purified by flash chromatography on silica gel eluting with 5% 2-propanol/hexane to give pure **13a** as a white solid: mp 140–145 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.57 (2 H, s), 7.60 (2 H, d, J = 7 Hz), 7.85 (2 H, d, J = 8 Hz), 8.30 (1 H, d, J = 2 Hz), 8.38 (1 H, d, J = 2 Hz). However, when large amounts of crude **13a** were chromatographed, recovered yields of pure product were very low due to the longer time on the column. In the preparative scale runs, crude **13a** was used directly in the next step. The analogous dibromomethyl compound, identified by the characteristic C*H* Br_2 absorption in the NMR at 6.70 ppm, was formed in 10% yield.

4-[4-[(Isobutylamino)methyl]benzoyl]thiophene-2-sulfonamide (16b). To a solution of 5.0 g (0.013 mol) of crude **15a** in 75 mL of chloroform at 0–10 °C was added gaseous ammonia for 10 min, and then this suspension was stirred at 0–10 °C. After 3 h no more starting material remained so the solvent was stripped, and the residue was purified by flash chromatography on silica gel eluting with 3% methanol/chloroform to give 3.0 g (~60%) pure 4-[4-(bromomethyl)benzoyl]thiophene-2-sulfonamide: mp 172–178°; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.81 (2 H, s), 7.68 (2 H, d, J = 7 Hz), 7.90 (5 H, m), 8.50 (1 H, d, J = 2 Hz).

A solution of 1.0 g (0.0028 mol) of this bromomethyl sulfonamide and 2.2 g (0.030 moles) of isobutylamine in 15 mL of tetrahydrofuran was stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue diluted with 40 mL of water and acidified with 6 N HCl. This solution was extracted with 3 × 30 mL of ethyl acetate to remove nonbasic materials. The aqueous phase was made basic with ammonium hydroxide and the bright yellow solid that appeared was collected. The yield was 0.41 g (40%) of the free base of **16b**. This was dissolved in ethanol and treated with ethanolic HCl with stirring for 0.5 h. The reaction mixture was then filtered to give the hydrochloride salt of **16b** as a white solid: mp 235–237 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.06 (6 H, d, J = 7 Hz), 2.15 (1 H, m), 4.42 (2 H, bs), 7.83 (2 H, m), 7.97 (3 H, m), 8.55 (1 H, s). Anal. ($C_{16}H_{20}N_2O_3S_2$ HCl) C, H, N.

In a similar manner the following compounds were prepared from **15a**.

4-[4-[(Methylamino)methyl]benzoyl]thiophene-2-sulfonamide Hydrochloride (16a): mp 238–240 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.70 (3 H, s), 3.45 (2 H, s), 4.34 (1 H, bs), 7.78 (2 H, d), 8.00 (3 H, m), 8.56 (1 H, d). Anal. ($C_{13}H_{14}N_2O_3S_2$·HCl) C, H, N.

4-[4-[(Benzylamino)methyl]benzoyl]thiophene-2-sulfonamide Hydrochloride (16c): mp 244–246 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6), δ 4.23 (2 H, bs), 4.35 (2 H, bs), 7.53 (3 H, m), 7.67 (2 H, m), 7.88 (2 H, d, J = 8 Hz), 7.98 (5 H, m), 8.55 (1 H, s), 9.95 (1 H, bs).

4-[4-[(*n*-Butylamino)methyl]benzoyl]thiophene-2-sulfonamide Hydrochloride (16d): mp 243–246 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90 (3 H, t), 1.34 (2 H, m), 1.66 (2 H, m), 2.92 (2 H, m), 4.26 (2 H, s), 7.79 (2 H, m), 7.90 (3 H, m), 8.45 (1 H, d). Anal. ($C_{16}H_{20}N_2O_3S_2$·HCl) C, H, N.

4-[4-(Morpholinomethyl)benzoyl]thiophene-2-sulfonamide Hydrochloride (16e): mp 232–235 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.47 (4 H, bs), 3.42 (2 H, m), 3.69 (6 H, m), 7.65 (2 H, d), 7.82 (2 H, d, 7.93 (2 H, s). Anal. ($C_{16}H_{18}N_2O_4S_2$) C, H, N.

4-[4-[(*N*-Methylpiperazino)methyl]benzoyl]thiophene-2-sulfonamide Hydrochloride (16f): mp 250–252 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.26 (3 H, s), 3.42 (4 H, m), 3.70 (2 H, s), 7.62 (2 H, d), 7.90 (2 H, d), 7.98 (2 H, s). Anal. ($C_{17}H_{21}N_3O_3S_2$·HCl) C, H, N.

The following compounds were prepared from 4-[3-(bromomethyl)benzoyl]thiophene-2-sulfonamide (**15b**) as described for **15a**.

4-[3-[(Isobutylamino)methyl]benzoyl]thiophene-2-sulfonamide Hydrochloride (16g): mp 246–248 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (6 H, d), 2.80 (2 H, m), 3.43 (2 H, s), 4.33 92 H, bs), 7.75 (1 H, t), 8.00 (5 H, m), 8.22 (1 H, m), 8.73 (1 H, s). Anal. ($C_{16}H_{20}N_2O_3S_2$·HCl) C, H, N.

4-[(3-Morpholinomethyl)benzoyl]thiophene-2-sulfonamide Hydrochloride (16h): mp 253–255 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.12 (1 H, m), 3.27 (2 H, m), 3.38 (2 H, m), 3.76 (2 H, m), 3.94 (2 H, m), 4.50 (2 H, m), 7.75 (1 H, m), 7.92 (5 H, m), 8.07 (1 H, m), 8.62 (1 H, m). Anal. ($C_{16}H_{18}N_2O_4S_2$·HCl) C, H, N.

4-[3-[(Isopentylamino)methyl]benzoyl]thiophene-2-sulfonamide Hydrochloride (16i): mp 238–240 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.94 (6 H, d), 1.68 (4 H, m), 2.96 (2 H, m), 4.26 (1 H, s), 7.66 (1 H, t), 7.88 (2 H, dd), 7.93 (2 H, t), 8.05 (1 H, bs), 8.61 (1 H, dd). Anal. ($C_{17}H_{22}N_2O_3S_2$·HCl) C, H, N.

4-(4-Methoxybenzoyl)furan-2-sulfonamide (13d). This intermediate was prepared from 3-bromofuran and benzonitrile as described for **13a**.

4-(4-Methoxybenzoyl)furan-2-sulfonamides (14c) was prepared from **13d** as described above for **14a**: mp 180–182 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.98 (3 H, s), 7.25 (2 H, d, J = 9 Hz), 7.38 (1 H, d, J = 1 Hz), 8.00 (2 H, d, J = 9 Hz), 8.09 (2 H, bs), 8.25 (1 H, d, J = 1 Hz). Anal. ($C_{12}H_{11}NO_5S$) C, H, N.

3-[(4-Methylphenyl)thio]thiophene (19). To 67.6 g (0.415 mol) of 3-bromothiophene in 225 mL of ether cooled to −78 °C under N_2 was added 0.415 mol *n*-butyllithium (in hexane) dropwise at <−70 °C. After addition was complete the reaction mixture was stirred for 45 min at −78 °C to give a white suspension. Then 51.0 g (0.207 mol) of bis(4-methylphenyl) disulfide in 75 mL ether was added dropwise at <−70 °C. The reaction mixture was then allowed to warm gradually to room temperature with stirring overnight.

The cooled reaction mixture was quenched with 250 mL of ice–water, and the organic phase was separated, washed with water and brine, and dried. The solvent was removed in vacuo to provide 42.0 g (98%) of crude **19a** as a yellow oil: R_f 0.5, silica gel eluted with 5% 2-propanol/hexane; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33 (3 H, s), 7.0–7.4 (7 H, aromatic); MS *m/e* 206.

3-[(4-Methylphenyl)sulfonyl]thiophene (20). To a solution of 20.6 g (0.1 mol) of **19a** in 150 mL of chloroform cooled to 0–10 °C was added dropwise a solution of 43.0 g (0.25 mol) of *m*-chloroperbenzoic acid portionwise over 15 min with mechanical stirring. The resulting suspension was stirred at 0–10 °C for 1.5 h at which time all starting sulfide was consumed. This suspension was then extracted with 2 × 75-mL portions of 1 N NaOH solution and brine and then dried. The solvent was removed in vacuo to give a dark oil that was triturated with 3:1 hexane/ethyl acetate to afford 18.6 g (78%) of **20a** as a white solid: mp 128–132 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.45 (3 H, s), 7.30–7.43 (4 H, m, aromatic), 7.90 (2 H, d, J = 9 Hz), 8.12 (1 H, d, J = 2 Hz); MS *m/e* 238.

4-[(4-Methylphenyl)sulfonyl]thiophene-2-sulfonyl Chloride (21a). To 1.22 g (0.010 mol) of chlorosulfonic acid under nitrogen was added 0.88 g (0.0042 mol) phosphorus pentachloride portionwise (caution, foaming), and the resulting solution was stirred at room temperature for 10 min. Then, 1.0 g (0.0042 mol) of **20a** was added in one portion, and the resulting dark suspension was heated at 55 °C for 25 min during which time foaming occurred and subsided.

The reaction mixture was then poured onto ice and the resulting suspension was extracted with chloroform. The organic phase was filtered through a Celite pad, washed with brine, and dried. The solvent was removed in vacuo to provide 1.3 g (93%) of nearly pure **21a** as a tan solid. This had R_f 0.7 on silica gel eluting with 10% 2-propanol/hexane, nearly identical to **20a**; however the iodine stain of **21a** was much darker. **21a** had mp 118–120°: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (3 H, s), 7.42 (2 H, d, J = 9 Hz), 7.90 (2 H, d, J = 9 Hz), 8.00 (1 H, d, J = 2 Hz), 8.41 (1 H, d, J = 2 Hz); ms *m/e* 336.

4-[(3-Methylphenyl)sulfonyl]thiophene-2-sulfonyl Chloride (21b). This compound was prepared from 3-bromothiophene and bis(3-methylphenyl) disulfide as described for **21a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.48 (3 H, s), 7.51 (2 H, m), 7.82 (2 H, m), 8.07 (1 H, d), 8.50 (1 H, d).

4-[(2-Methylphenyl)sulfonyl]thiophene-2-sulfonyl Chloride (21c). This compound was prepared from 3-bromothiophene and bis(2-methylphenyl)disulfide as described for **21b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.59 (3 H, s), 7.37 (1 H, d), 7.50 (1 H, t), 7.62 (1 H, t), 7.98 (1 H, d), 8.22 (1 H, dd), 8.49 (1 H, d).

4-[(4-Methylphenyl)sulfonyl]thiophene-2-sulfonamide (22a). A stream of ammonia gas was bubbled into a chloroform solution of 1.0 g (0.003 mol) of **21a**, cooled to 0–10 °C. The resulting suspension was then stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue purified by flash chromatography on silica gel eluting with 5% methanol/chloroform to give 0.75 g (80%) of pure **22a** as a white solid: mp 164–166 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.42 (3 H, s), 7.40

(2 H, d, J = 9 Hz), 7.71 (1 H, d, J = 2 Hz), 7.84 (2 H, d, J = 9 Hz), 8.53 (1 H, d, J = 2 Hz); ms m/e 317. Anal. ($C_{11}H_{11}NO_4S_3$) C, H, N.

The following sulfonamides were prepared in an analogous manner.

4-[(4-Methoxyphenyl)sulfonyl]thiophene-2-sulfonamide (22b): mp 169–171 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.92 (3 H, s), 7.25 (2 H, d, J = 9 Hz), 7.81 (1 H, d, J = 2 Hz), 8.00 (2 H, bs, SO_2NH_2), 8.04 (2 H, d, J = 9 Hz), 8.70 (1 H, d, J = 2 Hz); ms m/e 333. Anal. ($C_{11}H_{11}NO_5S_3$) C, H, N.

4-[(3-Methoxyphenyl)sulfonyl]thiophene-2-sulfonamide (22c): mp 112–113 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 3.84 (3 H, s), 7.10 (2 H, bs, SO_2NH_2), 7.25 (1 H, m), 7.54 (3 H, m), 7.81 (1 H, d, J = 2 Hz), 7.57 (1 H, d, J = 2 Hz), ms m/e 333. Anal. ($C_{11}H_{11}NO_5S_3$) C, H, N.

4-[(4-Methoxyphenyl)sulfonyl]furan-2-sulfonamide (22d): mp 117–118 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.90 (3 H, s), 7.21 (2 H, d, J = 8 Hz), 7.37 (1 H, s), 8.02 (4 H, m), 8.78 (1 H, s); ms m/e 317. Anal. ($C_{11}H_{11}NO_6S_2$) C, H, N.

4-[(4-Hydroxyphenyl)sulfonyl]thiophene-2-sulfonamide (25a). To a suspension of 3.9 g (0.0117 mol) of **22b** in 100 mL of 1,2-dichloroethane at room temperature was added 0.06 mol of boron tribromide (1 M in CH_2Cl_2) dropwise over 15 min. The reaction mixture became homogeneous for a short period, and then a precipitate appeared. This was heated under nitrogen at reflux for 16 h.

The cooled reaction mixture was carefully quenched by the dropwise addition of 50 mL of water, and the resulting two-phase mixture contained a tan solid. This was collected by filtration, washed with methylene chloride, and purified by flash chromatography on silica gel eluting with 8% methanol/chloroform to provide 3.05 g (82%) of pure **25a** as a white solid: mp 192–194 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.07 (2 H, d, J = 9 Hz), 7.80 (1 H, d, J = 2 Hz), 7.92 (2 H, d, J = 9 Hz), 7.98 (2 H, bs, SO_2NH_2), 8.67 (1 H, d, J = 2 Hz); ms m/e 319. Anal. ($C_{10}H_9NO_5S_3$) C, H, N.

The following compounds were prepared in an identical manner.

4-[(3-Hydroxyphenyl)sulfonyl]thiophene-2-sulfonamide (25b): mp 144–146 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.18 (2 H, m), 8.50 (4 H, m), 7.79 (1 H, d, J = 2 Hz), 8.58 (1 H, d, J = 2 Hz); ms m/e 319. Anal. ($C_{10}H_9NO_5S_3$) C, H, N.

4-[(4-Hydroxyphenyl)sulfonyl]furan-2-sulfonamide (25c): mp 174–176 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.00 (2 H, d, J = 9 Hz), 7.33 (1 H, d, J = 2 Hz), 7.87 (2 H, d, J = 9 Hz), 8.73 (1 H, d, J = 2 Hz); ms m/e 303. Anal. ($C_{10}H_9NO_6S_2$) C, H, N.

4-[[3-[(Dimethylamino)methyl]-4-hydroxyphenyl]sulfonyl]thiophene-2-sulfonamide (26c). A solution of 0.96 g (0.003 mol) of **25a**, 1.35 g (0.012 mol) of dimethylamine (40% aqueous solution), and 0.49 g (0.060 mol) of formaldehyde (37% aqueous solution) in 15 mL of ethanol was heated at reflux for 16 h. The solvent was then removed in vacuo, and the residue was acidified with 6 N HCl. This aqueous phase was washed with 2 × 50-mL portions of ethyl acetate and then basified with ammonium hydroxide (pH = 9). This was extracted with ethyl acetate, the organic phase was washed with brine and dried, and the solvent was stripped. The residue was purified by flash chromatography on silica gel eluting with 12% methanol/chloroform to give 0.67 g (59%) (free base) of **26c** as an oil. The hydrochloride salt was prepared by dissolving **26c** in 10 mL of ethanol and treating with ethanolic HCl. This was stripped to dryness and triturated with 10% ethanol/ether to provide pure HCl salt of **26c**: mp 75–80 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ, 6.95 (2 H, d, J = 9 Hz), 7.62 (1 H, d, J = 1 Hz), 7.79 (1 H, dd, J = 6, 1 Hz), 7.83 (1 H, d, J = 2 Hz), 8.25 (1 H, d, J = 1 Hz); ms m/e 376. Anal. ($C_{13}H_{18}N_2O_5S_3$·HCl) C, H, N.

The following compounds were prepared in the same manner.

4-[[3-[(Diethylamino)methyl]-4-hydroxyphenyl]sulfonyl]thiophene-2-sulfonamide (26a). The hydrochloride salt of **26a** was a white solid: mp 190–198 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) (free base **26a**) δ 1.13 (6 H, t), 2.56 (4 H, q), 3.87 (2 H, s), 7.65 (1 H, d, J = 8 Hz), 7.41 (3 H, bs), 7.48 (1 H, d, J = 8 Hz), 7.87 (1 H, d, J = 2 Hz), 8.25 (1 H, d, J = 2 Hz); ms m/e 404. Anal. ($C_{15}H_{20}N_2O_5S_3$·HCl) C, H, N.

4-[[3-[(*N*-Isobutyl-*N*-methylamino)methyl]-4-hydroxyphenyl]sulfonyl]thiophene-2-sulfonamide (26b): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (6 H, d), 2.28 (3 H, s), 2.32 (2 H, d), 3.75 (2 H, broad), 6.90 (1 H, d), 7.63 (1 H, m), 7.77 (1 H, dd), 7.81 (1 H, s), 8.24 (1 H, d). Anal. ($C_{16}H_{22}N_2O_5S_3$·HCl) C, H, N.

4-[[4-[(*N*-Isobutyl-*N*-methylamino)methyl]-3-hydroxyphenyl]sulfonyl]thiophene-2-sulfonamide (26d): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (6 H, d), 2.27 (3 H, s), 2.32 (2 H, d), 3.76 (2 H, broad), 7.20 (1 h, d), 7.41 (4 H, m), 7.82 (1 H, d), 8.28 (1 H, d). Anal. ($C_{16}H_{22}N_2O_5S_3$·HCl) C, H, N.

4-[[4-[(Diethylamino)methyl]-3-hydroxyphenyl]sulfonyl]thiophene-2-sulfonamide (26e): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (6 H, t), 3.20 (4 H, q), 3.82 (2 H, s), 7.15 (1 H, d), 7.33 (3 H, m), 7.89 (1 H, d), 8.25 (1 H, d). Anal. ($C_{15}H_{20}N_2O_5S_3$·HCl) C, H, N.

4-[[3-[(Dimethylamino)methyl]-4-hydroxyphenyl]sulfonyl]furan-2-sulfonamide (26f): ^{1}H NMR of free base (300 MHz, acetone-d_6) δ 1.33 (6 H, t), 4.10 (2 H, s), 7.09 (2 H, d, J = 9 Hz), 7.40 (1 H, d, J = 1 Hz), 7.95 (1 H, d, J = 1 Hz), 8.05 (1 H, dd, J = 6, 1 Hz), 8.14 (1 H, d, J = 1 Hz); ms m/e 360. Anal. ($C_{13}H_{16}N_2O_6S_2$·HCl) C, H, N.

4-[[3-[(Diethylamino)methyl]-4-hydroxyphenyl]sulfonyl]furan-2-sulfonamide (26g): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (6 H, t), 2.71 (4 H, q), 3.83 (2 H, s), 6.89 (1 H, d), 7.61 (1 H, d), 7.74 (2 H, dd), 7.79 (1 H, s), 8.23 (1 H, s).

4-[[4-(Bromomethyl)phenyl]sulfonyl]thiophene-2-sulfonyl Chloride (23a). A solution of 18.0 g (0.054 mol) of **21a**, 46.0 g (0.258 mol) of *N*-bromosuccinimide, and 20 mg of benzoyl peroxide in 300 mL of chloroform was heated at reflux and irradiated with a 200-W sunlamp. The reaction mixture was closely monitored by NMR, and the reaction was stopped (1 h) when significant amounts of the dibromo product (C*H*Br_2, δ 6.65) began to appear. The cooled reaction mixture was washed with 2 × 300 mL of water, 150 mL of 5% sodium thiosulfate, and brine and dried. The solvent was removed to afford crude **23a** as an oil, which by NMR was 80% **23a**, 10% **21a**, and 10% dibromo. **23a** had ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.51 (2 H, s), 7.61 (2 H, d, J = 8 Hz), 7.95 (2 H, d, J = 8 Hz), 8.01 (1 H, d, J = 2 Hz), 8.46 (1 H, d, J = 2 Hz); ms m/e 415.

In the same manner the following compounds were prepared.

4-[[3-(Bromomethyl)phenyl]sulfonyl]thiophene-2-sulfonyl Chloride (23b): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.55 (2 H, s), 7.50–8.20 (4 H, m), 8.53 (2 H, m).

4-[[2-(Bromomethyl)phenyl]sulfonyl]thiophene-2-sulfonyl Chloride (23c): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.88 (2 H, s), 7.63 (2 H, m), 7.76 (1 H, d), 8.04 (1 H, d), 8.27 (1 H, d), 8.60 (1 H, d).

4-[[4-[(Isobutylamino)methyl]phenyl]sulfonyl]thiophene-2-sulfonamide (24b). A stream of ammonia was bubbled into a cooled solution of 2.2 g (0.0053 mol) of **23a** in 35 mL of chloroform for 15 min and the resulting mixture was then stirred at room temperature for 3 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel eluting with 4% methanol/chloroform to give 1.2 g (57%) of 4-[[4-(bromomethyl)phenyl]sulfonyl]thiophene-2-sulfonamide as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.50 (2 H, s), 5.45 (2 H, bs, SO_2NH_2), 7.57 (2 H, d, J = 9 Hz), 7.85 (1 H, d, J = 1 Hz), 7.95 (2 H, d, J = 9 Hz), 8.28 (1 H, d, J = 1 Hz); ms m/e 396.

A solution of 18.5 g (0.047 mol) of the sulfonamide from above and 22.08 g (0.30 mol) of isobutylamine in 100 mL of tetrahydrofuran was stirred at room temperature for 48 h. The solvent and excess amine were removed at reduced pressure, and the residue was taken up in 500 mL of ethyl acetate. This solution was washed with 3 × 50-mL portions of water and brine and dried. The solvent was removed in vacuo to give an amber oil that was purified by flash chromatography on silica gel eluting with 5% methanol/chloroform to give crude **24b** (free base) as a gum. This was triturated with 20% hexane/ether to afford 5.4 g (30%) of **24b** as a tan solid. This solid was dissolved in a mixture of 50 mL of ethanol/25 mL of methanol and then treated with ethanolic HCl. Gradual dilution of the resulting solution with ether gave the hydrochloride salt of **24b** as a white solid: mp 207–209 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.06 (6 H, d, J = 7 Hz), 2.11 (1 H, m), 2.90 (2 H, bs), 7.94 (3 H, m), 8.08 (2 H, s), 8.25 (2 H, d), 8.86 (1 H, s); ms m/e 388. Anal. ($C_{15}H_{20}N_2O_4S_3$·HCl) C, H, N.

In a similar manner the following compounds were prepared from either **23a**, **23b**, or **23c**.

4-[[4-(Morpholinomethyl)phenyl]sulfonyl]thiophene-2-sulfonamide Hydrochloride (24a): mp 238–241 °C dec. Anal. ($C_{15}H_{18}N_2O_5S_3$·HCl) C, H, N.

4-[[4-[(*N*-Methylpiperazino)methyl]phenyl]sulfonyl]-thiophene-2-sulfonamide Hydrochloride (24c): mp 238–242 °C. Anal. ($C_{16}H_{21}N_3O_4S_3$·2HCl) H, N; C: calcd, 39.34; found, 39.84.

4-[[4-[(*n*-Butylamino)methyl]phenyl]sulfonyl]thiophene-2-sulfonamide Hydrochloride (24d): mp 174–176 °C. Anal. ($C_{15}H_{20}N_2O_4S_3$·HCl) C, H, N.

4-[[4-[[(2-Pyridylmethyl)amino]methyl]phenyl]sulfonyl]-thiophene-2-sulfonamide Hydrochloride (24e): mp 90–100 °C. Anal. ($C_{17}H_{19}Cl_2N_3O_4S_2$·2HCl) C, H, N, Cl.

4-[[3-[(Isobutylamino)methyl]phenyl]sulfonyl]thiophene-2-sulfonamide Hydrochloride (24f). Anal. ($C_{15}H_{20}N_2O_4S_3$·HCl·0.5H_2O) C, H, N.

4-[[3-[(*sec*-Butylamino)methyl]phenyl]sulfonyl]thiophene-2-sulfonamide Hydrochloride (24g): mp 171–182 °C. Anal. ($C_{15}H_{20}N_2O_4S_3$·HCl) C, H, N.

4-[[2-[(Isobutylamino)methyl]phenyl]sulfonyl]thiophene-2-sulfonamide Hydrochloride (24h). Anal. ($C_{15}H_{20}N_2O_4S_3$·HCl) C, H, N.

4-[[3-[(Isopropylamino)methyl]phenyl]sulfonyl]thiophene-2-sulfonamide Hydrochloride (24i): mp 182–186 °C. Anal. ($C_{14}H_{18}N_2O_4S_3$·HCl) C, H, N.

4-[[4-[(*tert*-Butylamino)methyl]phenyl]sulfonyl]thiophene-2-sulfonamide Hydrochloride (24j): mp 272–274 °C dec. Anal. ($C_{15}H_{20}N_2O_4S_3$·HCl) C, H, N.

4-[[2-[(*N*-Methylpiperazino)methyl]phenyl]sulfonyl]-thiophene-2-sulfonamide Hydrochloride (24k). Anal. ($C_{16}H_{21}N_3O_4S_3$·1.15HCl) C, H, N, Cl.

4-[[2-[[(2-Pyridylmethyl)amino]methyl]phenyl]sulfonyl]-thiophene-2-sulfonamide Hydrochloride (24l). Anal. ($C_{17}H_{17}N_3O_4S_3$·1.2HCl·H_2O) C, H, N, Cl.

H_2O Solubility. A standard solution was prepared by dissolving 1 mg of sample in 10 mL of CH_3OH. The standard solution was scanned by UV (Acta M VI Beckman spectrophotometer) to determine the wavelength of maximum absorbance, diluting as necessary. A saturated solution was prepared by stirring magnetically a small volume of pH 7.4 phosphate buffer 0.039 M (~500 μL) in the presence of excess compound. The saturated solution was checked every 30 min and additional compound added if necessary to maintain saturation. After 4 h, the solution was filtered to remove excess compound, using HA 0.45-μm Millipore filters. The saturated solution was diluted to at least 3 mL and then scanned by UV at the wavelength of maximum absorbance. Total solubility was then determined by the relationship $C' = A'C/A$ where C = concentration of saturated solution in milligrams/milliliter, A = absorbance of the standard solution (correcting for any dilutions), A' = absorbance of the saturated solution (correcting for any dilutions), and C^1 = concentration of saturated solution in milligrams/milliliter.

pK_a. The half-neutralization point was measured by titrating the organic acids and bases with 0.5 N NaOH and 0.5 N HCl in H_2O and mixed solvents, using a glass-columned electrode system. All of the compounds were run in 30% EtOH–H_2O.

Partition Coefficients. Partition coefficients were obtained by equilibrating the test compound between octanol and 0.1 ionic strength pH 7.4 phosphate buffer. The concentration in each phase was determined by UV spectrophotometry.

In Vitro Inhibition of Human Carbonic Anhydrase II. Human erythrocyte CA II was isolated from lysed red blood cells by the following affinity chromatography procedure. Citrated human blood (500 mL) was centrifuged at 5000*g* for 10 min at 4 °C and the resultant plasma decanted. Red blood cells were washed with cold 0.9% NaCl solution and then centrifuged. The supernatant was discarded and the process of washing and centrifugation repeated. Cell lysis was achieved at 4 °C by adding an equal volume of cold water and cellular debris was removed by centrifugation. Lysed human red blood cells (80 mL) were diluted 5-fold with 0.05 M Tris sulfate buffer, pH 8.8, and poured onto a 0.9- × 8-cm (4-(aminomethyl)benzenesulfonamide–CM agarose) affinity chromatography gel column. Chromatography was carried out at 4 °C, and fractions were monitored by determining optical density at 280 nm with an LKB Uvicord III.

The column was eluted with 0.2 M sodium sulfate in 0.1 M Tris sulfate buffered at pH 8.8 to remove all hemoglobin and other proteins not specifically bound. Low-activity carbonic anhydrase I was eluted as a single peak with 0.6 M potassium chloride in 0.1 M potassium phosphate buffer (pH 7.2). Elution was continued until the optical density at 280 nm was less than 0.1. Highly purified carbonic anhydrase II was eluted with 0.6 M potassium chloride in 0.1 M potassium phosphate buffer (pH 5.2). Carbonic anhydrase II purity was assessed by disk gel and starch gel electrophoresis. The gels were stained for protein and Coomassie Blue, and carbonic anhydrase II bands were visualized by fluorescein diacetate staining. The enzyme solution was desalted and concentrated to 1 mg of protein (mL of 0.1 M phosphate)$^{-1}$ pH 7.2, on an Amicon UM-10 Ultrafiltration membrane and stored at 2–5 °C.

Inhibition of the purified human erythrocyte carbonic anhydrase II was assessed by using a pH stat assay. This assay measures the rate of hydration of CO_2[17] by determining the rate at which a standard solution of NaOH has to be added to a lightly buffered solution to maintain a constant pH as CO_2 is bubbled into the buffer. Enzymatic activity is proportional to the volume of a standard NaOH solution that is required to maintain the pH at a given value, e.g., 8.3. To 4 mL of 0.02 M Tris chloride buffer, pH 8.6, in a 5-mL Radiometer V531 jacketed assay vessel equilibrated at 2 °C was added buffer-diluted enzyme (25 μL). CO_2–air (5:95) was bubbled into the assay vessel at a rate of 150 mL/min. The pH stat end point was set at pH 8.3, and the volume of 0.025 N NaOH added over a 3-min period in order to maintain pH 8.3 was measured. Enzyme inhibition was measured by the addition of an inhibitor in 0.1 mL to 3.9 mL of buffer followed by the addition of enzyme and titration with NaOH. Results were expressed as the I_{50} values, which were obtained from semilog plots of percent inhibition against log concentration.

In Vitro Binding for Human Carbonic Anhydrase II. The binding of test compounds to purified human erythrocyte carbonic anhydrase II was determined by a fluorescence competition assay employing the fluorescent CA inhibitor dansylamide. This compound has been shown to produce a large increase in fluorescence upon binding to the active site of carbonic anhydrase. A fluorescence cuvette containing 1×10^{-7} M human CA II (HCA II) and 2×10^{-6} M dansylamide in pH 7.4, and 0.1 ionic strength phosphate buffer was placed in the thermostated cell holder of a Perkin-Elmer MPF-44B fluorescence spectrophotometer. The temperature was maintained at 37 °C by using a constant-temperature water circulator. The excitation and emission wavelengths were set at 280 and 460 nm, respectively. Fluorescence intensity were recorded following addition, with stirring, of small, measured aliquot of a solution of the test compound in pH 7.4 buffer. The resulting data were converted to fluorescence intensity vs compound concentration, corrected for dilution by the titrant, and fitted by nonlinear least squares to a model in which the compound and dansylamide compete for a single binding site on HCA II. The dissociation constant of the dansylamide–HCA II complex, which is needed for these calculations, was found to be 1.98×10^{-6} M under these conditions. It was found in all cases that the data fitted well to a single-site model. There was no evidence for additional, lower-affinity binding sites. All binding determinations were done a minimum of three times.

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