Synthesis and Carbonic Anhydrase Inhibitory Activity of 4-Substituted 2-Thiophenesulfonamides

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A series of 4-substituted 2-thiophenesulfonamides was prepared from 3-thiophenecarboxaldehyde using metalation chemistry developed for 3-furaldehyde. Several of these compounds inhibit carbonic anhydrase II *in vitro* at concentrations of less than 10 nM. In addition, none of these compounds exhibit sensitization potential as determined from in vitro measurement of cysteine reactivity.

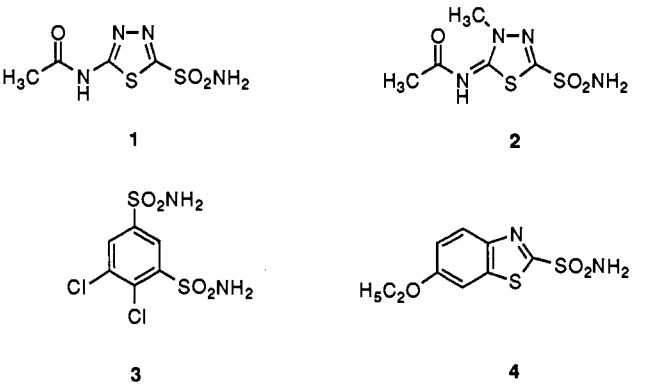
Introduction

Carbonic anhydrase inhibitors (CAIs) such as acetazolamide (1), methazolamide (2), dichlorophenamide (3), and ethoxolamide (4) have been used systemically as antiglaucoma agents for over 30 years (Scheme 1). The systemic dose of these agents required to reach the nonpigmented epithelial cells of the ciliary process and reduce aqueous humor formation is high. Consequently, a wide array of deleterious side effects such as depression, fatigue, anorexia, gastrointestinal disturbances, and weight loss[1a,b] are observed concomitantly with the reduction in intraocular pressure (IOP). A topically effective CAI administered directly to the eye would obviate these unwanted side effects since it would be localized in the target tissue. Attempts to apply systemically active CAIs topically to the eye have been unsuccessful because of poor corneal penetration.

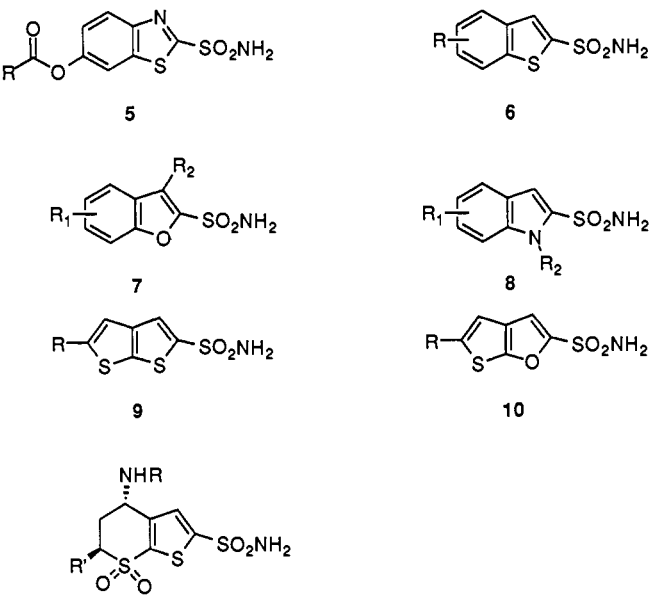
Recently, several classes of compounds have been reported to reduce IOP in animals after topical administration. Among these classes are *O*-acyl derivatives of 6-hydroxybenzothiazole-2-sulfonamides (5),[2] benzo[*b*]-thiophenesulfonamides (6),[3] benzofuran (7) and indole-2-sulfonamides (8),[4] thieno[2,3-*b*]- and -[3,2-*b*]thiophene-2-sulfonamides (9),[5] thieno[2,3-*b*]- and -[3,2-*b*]furan-2-sulfonamides (10),[6] and thienothiopyran-2-sulfonamides (11)[7] (Scheme 2). Development of five of these seven classes of compounds was precluded because of susceptibility to nucleophilic attack or lack of water solubility. Benzothiazole-2-sulfonamides, benzofuran-2-sulfonamides, indole-2-sulfonamides, and thieno[2,3-*b*]furan-2-sulfonamides demonstrated significant reactivity with glutathione (GSH). The GSH reactivity was found to correlate with dermal sensitization potential in guinea pigs under the Magnusson–Kligman protocol.[8] Thus these classes of compounds are potential sensitizers. Benzothiophene-2-sulfonamides demonstrated good in vitro activity and no reactivity with GSH but suffered from insufficient water solubility. Even though both the thieno[*b*]thiophene-2-sulfonamides and thienopyran-2-sulfonamide classes demonstrate desirable activity profiles only **11a** and **11b** in the thienothiopyran-2-sulfonamide series have been shown to lower IOP in humans after topical administration.[9]

CAIs became a logical target based on our ongoing interest in glaucoma therapy and novel heterocyclic synthesis. The reported effectiveness of topical **11** as a CAI made it a reasonable benchmark. Analysis of the structure–activity relationships of **11** and numerous other arenesulfonamides gave us some basic guidelines for design. First, a sulfonamide group must be bonded directly to an aromatic ring; second, there can be no substituents adjacent to the sulfonamide moiety; and third, compounds must demonstrate low reactivity with nucleophiles. Furthermore, if **11** is viewed as a 4-substituted 2-thiophenesulfonamide with an electron withdrawing group in the 5-position (Figure 1), then an efficient route to 4-substituted 2-thiophenesulfonamides could, quite feasibly, lead to a simple series of topically active CAIs. As with **11**,

Scheme 1



Scheme 2



11a, R = $CH_2CH(CH_3)_2$, R' = H
11b, R = CH_2CH_3, R' = CH_3

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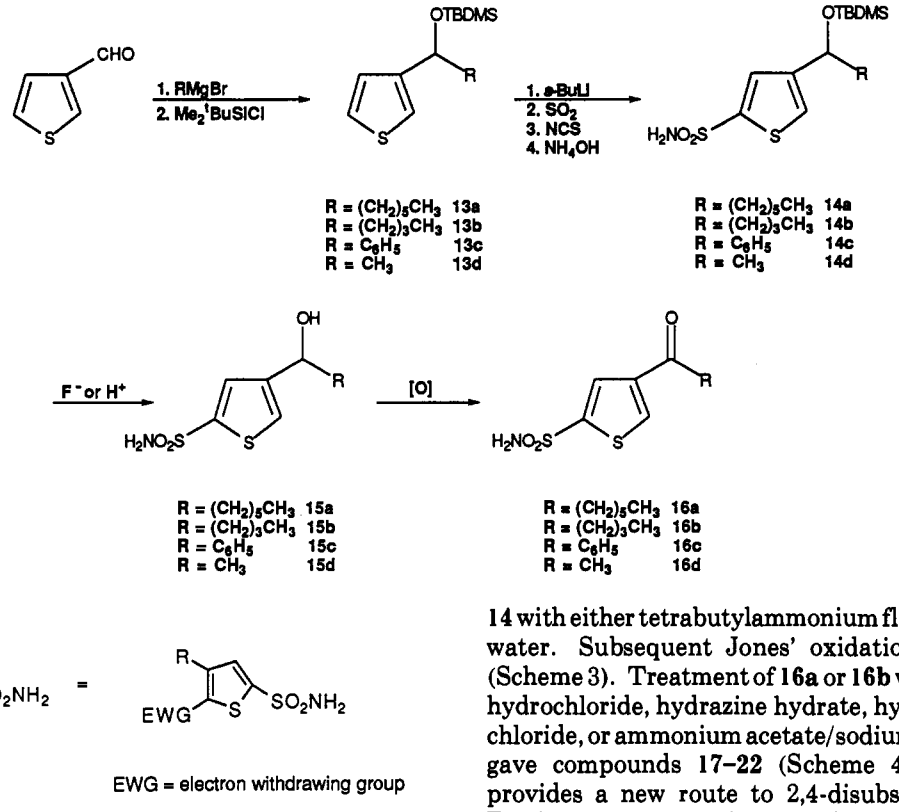
Scheme 3



11b EWG = electron withdrawing group

Figure 1.

these compounds would adhere to our basic guidelines for design. Over the course of another project, we developed methodology for direct synthesis of 2,4-disubstituted furans.[10] Extention of this methodology to thiophenes would provide an efficient route to a series of simple CAIs.[11] Here we give the details of work presented in preliminary form at the 203rd ACS national meeting[12] highlighting a novel 2,4-disubstituted thiophene synthesis. Later we became aware of related work by Hartman *et at.*[11] and discontinued our SAR efforts.

Chemistry

The chemistry used to synthesize these compounds utilizes a strategy adapted from work done by Comins[13] and revised during our work with furans. In our work with furans, we converted the aldehyde of 3-furaldehyde into an α-aminoalkoxide that would undergo metalation distal to the alkoxide. Subsequent trapping and deprotection gave 2-substituted 4-furaldehyde. A similar strategy was applied to 3-thiophenecarboxaldehyde as shown in Scheme 3. However, instead of using an α-aminoalkoxide group to mask the aldehyde and to block the 2-position of the ring, a 1-(*tert*-butyldimethylsiloxy)-alkyl group on C-4 of the thiophene was capable of directing metalation to C-2. In direct contrast, similar siloxy substituted furans gave synthetically unacceptable yields of metalated products.[10]

Thus, subsequent deprotonation at the 5-position of **13** with *s*-BuLi, followed by trapping with sulfur dioxide and treatment with NCS and ammonium hydroxide gives the desired 4-substituted 2-thiophenesulfonamide (compounds **14**) containing only 3–5% of the 3-substituted-2-thiophenesulfonamide. The pure 2,4-isomer could be obtained by careful chromatography or by recrystallization. Alcohols **15** were obtained from deprotection of compounds **14** with either tetrabutylammonium fluoride or acetic acid/water. Subsequent Jones' oxidation gave ketones **16** (Scheme 3). Treatment of **16a** or **16b** with methoxylamine hydrochloride, hydrazine hydrate, hydroxylamine hydrochloride, or ammonium acetate/sodium cyanoborohydride gave compounds **17–22** (Scheme 4). This chemistry provides a new route to 2,4-disubstituted thiophenes. Furthermore, it complements the methods used by Hartman *et al.*[11] in which a 4-electron-withdrawing substituent was used to direct electrophilic substitution at C-2.

Results and Discussion

A series of simple 4-substituted 2-thiophenesulfonamides was synthesized and evaluated for activity against human carbonic anhydrase II.[14] Although these compounds are of relatively simple structure, several exhibit IC_{50} values of less than 10 nM (Table 1). The most active compounds are those containing a ketone, hydrazone, or oxime at the 4-position of the thiophene ring (**16–19**). Compounds with an electron rich amine or siloxy ether at the 4-position of the thiophene ring exhibit very poor activity. Of the compounds with IC_{50} values below 10 nM, **15a, 16a,b, 17, 18,** and **19** were evaluated for their ability to penetrate the rabbit eye and inhibit carbonic anhydrase in a homogenate of the iris ciliary body (ex vivo).[15] Compounds were evaluated at 2% and demonstrated activities from 74 to 96%. In addition, since some arenesulfonamides have been found to cause ocular sensitization, these compounds were tested for their susceptibility to nucleophilic attack by cysteine.[16] None of these compounds were reactive (Table 2). However, compound **18** partially hydrolyzed over the course of the assay.

Conclusion

We and others[11] have now demonstrated that 4-substituted 2-thiophenesulfonamides can be appropriate CAIs for potential topical use. The thiophenesulfonamides prepared in this series established a metalation route to 2,4-disubstituted thiophenes from 4-(siloxyalkyl)thiophenes.

Experimental Section

Materials and Methods. ^1H NMR (299.943 MHz) and ^{13}C NMR spectra (75.492 MHz) were obtained in $CDCl_3$ or acetone-d_6 unless otherwise stated, and chemical shifts are reported in

Scheme 4

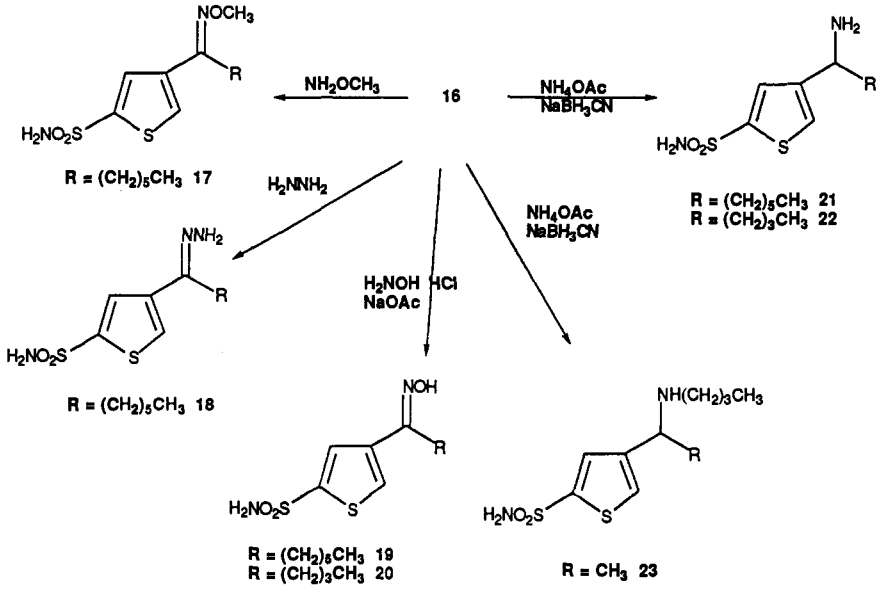
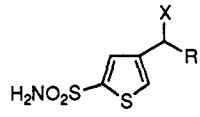
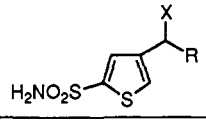


Table 1. CA Inhibition



compd	X	R	IC_{50} (nM) CA(II)
1			6
11a			4
15a	OH	$(CH_2)_5CH_3$	13
16a	O	$(CH_2)_5CH_3$	5
19	NOH	$(CH_2)_5CH_3$	5.8
17	$NOCH_3$	$(CH_2)_5CH_3$	4.7
18	NNH_2	$(CH_2)_5CH_3$	2
21	NH_2	$(CH_2)_5CH_3$	160
16b	O	$(CH_2)_3CH_3$	6.3
14b	OTBS	$(CH_2)_3CH_3$	200
22	NH_2	$(CH_2)_3CH_3$	626
20	NOH	$(CH_2)_3CH_3$	15
16d	O	CH_3	16
23	$NH(CH_2)_3CH_3$	CH_3	590
16c	O	C_6H_5	6.8

Table 2. Ex Vivo and Cysteine Reactivity Data for 4-Substituted 2-Thiophenesulfonamides



compd	X	R	ex vivo % inhibn	cysteine reactn (min^{-1})
1			67	0.000035
11a			99	<0.00005
15a	OH	$(CH_2)_5CH_3$	68	<0.00005
16a	O	$(CH_2)_5CH_3$	96	0.00005
19	NOH	$(CH_2)_5CH_3$	74	0.00005
17	$NOCH_3$	$(CH_2)_5CH_3$	82	0.00005
18	NNH_2	$(CH_2)_5CH_3$	75	0.00005
16c	O	C_6H_5	85	0.00005

δ units (parts per million) downfield from tetramethylsilane. Infrared spectra (IR) were recorded as thin films using polystyrene calibration. The frequencies are reported in cm^{-1}. Elemental analyses are within ±0.4% of calculated value and were performed at Galbraith Labratories. Analytical thin-layer chromatography (TLC) was performed on precoated 0.25-mm silica gel 60PF-254, and the spots were visualized with UV or by spraying with a solution of 5% phosphomolybdic acid in ethanol and heated at ca. 200 °C for a few minutes. All reactions involving moisture-sensitive reagents were carried out in an oven- or flame-dried apparatus under argon (Ar). THF was freshly distilled from calcium hydride or barium oxide and stored over 4-Å molecular sieves under N_2. *n*-BuLi (a 1.6 M solution in hexanes), *s*-BuLi (a 1.3 M solution in cyclohexane), and hexylmagnesium bromide (a 2.0 M solution in diethyl ether) were purchased from Aldrich and used as recieved. Unless otherwise stated, all commercial reagents were used as recieved. All chromatography was completed on silica gel unless indicated otherwise.

3-(1-Hydroxyheptyl)thiophene (12a). Hexylmagnesium bromide (24.5 mL, 48.95 mmol) was added to a solution of 3-thiophenecarboxaldehyde (3.9 mL, 44.58 mmol) in THF (150 mL) at 0 °C under Ar. After 1 h, the reaction mixture was quenched with cold 10% HCl, and the layers were separated. The aqueous phase was extracted with Et_2O. All of the organic phases were combined, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography using 25% Et_2O/hexane to give 7.49 g (85%) **12a** as a yellow oil: ^1H NMR 0.88 (t, 3 H, J = 7.0 Hz), 1.28 (m, 8 H), 1.85 (m, 2 H), 4.77 (t, 1 H, J = 6.5 Hz), 7.08 (d, 1 H, J = 5.2 Hz), 7.18 (d, 1 H, J = 2.7 Hz), 7.3 (dd, 1 H, J = 2.7, 5.2 Hz).

3-[1-(*tert*-Butyldimethylsiloxy)heptyl]thiophene (13a). A mixture of **12a** (4 g, 23.5 mmol), *tert*-butyldimethylsilyl chloride (3.9 g, 25.9 mmol), and DBU (4.2 mL, 28.2 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 15 h. The solution was poured into water, and the layers were separated. The aqueous phase was extracted with Et_2O. All of the organic phases were combined, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography using hexane to give 6.09 g (91%) of **13a** as a colorless oil: ^1H NMR −0.11 (s, 3 H), 0.02 (s, 3 H), 0.88 (m, 12 H), 1.28 (m, 8 H), 1.70 (m, 2 H), 4.75 (t, 1 H, J = 5.5 Hz), 7.02 (dd, 1 H, J = 1.2, 4.9 Hz), 7.05 (d, 1 H, J = 2.8 Hz), 7.23 (dd, 1 H, J = 2.8, 4.9 Hz).

4-[1-(*tert*-Butyldimethylsiloxy)heptyl]-2-thiophenesulfonamide (14a). *s*-BuLi (25.8 mL, 33.6 mmol) was added to a solution of **13a** (9.98 g, 32 mmol) in THF (160 mL) at −78 °C under Ar. After 45 min, SO_2 gas was bubbled over the surface of the solution for 50 min. Et_2O (75 mL) was added and the solution warmed to room temperature and stirred for 1 h. The solvent was removed in vacuo, the residue was taken up in CH_2Cl_2 (150 mL), and NCS (4.5 g, 33.6 mmol) was added. After being stirred at room temperature for 2 h, the solution was filtered and concentrated. The residue was dissolved in acetone (40 mL), and NH_4OH (64 mL) was added. After 15 min, the acetone was evaporated and the aqueous layer extracted with EtOAc (4 × 30 mL). The combined organic phases were dried ($MgSO_4$), concentrated, and purified by flash chromatography using 20% EtOAc/hexane to give 7.0 g (56%) of **14a** as an amber oil: ^1H NMR −0.08 (s, 3 H), 0.05 (s, 3 H), 0.88 (m, 12 H), 1.26 (m, 8 H),

1.6 (m, 2 H), 4.7 (t, 1 H, J = 5.9 Hz), 5.0 (br s, 2 H), 7.34 (d, 1 H, J = 2.1 Hz), 7.56 (d, 1 H, J = 2.1 Hz); ^{13}C NMR -5.3, -5.1, 13.8, 17.9, 22.3, 24.7, 25.5, 28.9, 31.7, 70.9, 126.1, 130.2, 143.0, 148.1; HRMS exact mass calcd for $C_{11}H_{33}NO_3SSi$ (M^+) 414.156, found 414.1574.

4-(1-Hydroxyheptyl)-2-thiophenesulfonamide (15a). Tetrabutylammonium fluoride (26.8 mL, 26.8 mmol. 1.0 M) was added to a solution of **14a** in dry THF (35 mL) and stirred at room temperature 16 h. The solution was quenched with water, the layers were separated, and the aqueous layer was extracted with EtOAc (5 × 40 mL). The combined extracts were dried ($MgSO_4$), concentrated, and purified by flash chromatography using 40% EtOAc/hexane to give a yellow oil. Recrystallization from EtOAC/hexane gave 3.6 g (73%) of **15a** as a white solid: mp 73-75 °C; ^{1}H NMR 0.88 (t, 3 H, J = 6.5 Hz) 1.28 (m, 8 H), 1.73 (m, 2 H), 2.35 (br s, 1 H), 4.71 (t, 1 H, J = 6.4 Hz), 5.27 (br s, 2 H), 7.41 (d, 1 H, J = 1.6 Hz), 7.62 (d, 1 H, J = 1.6 Hz); ^{13}C NMR 13.8, 22.3, 25.3, 28.8, 31.5, 37.9, 70.1, 126.9, 130.5, 143.4, 146.9; HRMS exact mass calcd for $C_{11}H_{19}NO_3S_2$ (M^+) 277.0806, found 277.0800. Anal. ($C_{11}H_{19}NO_3S_2$) C, H, N.

4-(1-Oxaheptyl)-2-thiophenesulfonamide (16a). Jones' reagent (0.15 mL, 0.4 mmol, 2.59 M) was added to a 0 °C solution of **15a** (0.10 g, 0.36 mmol) in acetone (2 mL). After 5 min, a few drops of isopropyl alcohol were added, and the solvent was removed in vacuo. The residue was taken up in water and extracted with EtOAc (4 × 10 mL). The combined extracts were dried ($MgSO_4$) and concentrated, and the crude material was purified by chromatography using 30% EtOAc/hexane to give 84 mg (85%) of **16a** as a white solid: mp 124-126 °C; ^{1}H NMR 0.90 (t, 3 H, J = 6.7 Hz), 1.33 (m, 6 H), 1.59 (m, 2 H), 2.86 (t, 2 H, J = 7.4 Hz), 5.15 (br s, 2 H), 8.05 (d, 1 H, J = 1.5 Hz), 8.22 (d, 1 H, J = 1.5 Hz); ^{13}C NMR 13.8, 22.3, 23.8, 28.7, 31.4, 39.7, 131.0, 136.1, 142.2, 144.7, 194.4; HRMS exact mass calcd for $C_{11}H_{17}NO_3S_2$ (M^+) 275.0650, found 275.0648. Anal. ($C_{11}H_{17}NO_3S_2$) C, H, N.

3-(1-Hydroxypentyl)thiophene (12b). Butyl bromide (5.3 mL, 49 mmol) was added dropwise to magnesium turnings (1.19 g, 49 mmol) in dry THF (150 mL). The mixture was refluxed for 1 h and cooled to 0 °C ,and 3-thiophenecarboxaldehyde (3.9 mL, 44.6 mmol) was added slowly. The solution was allowed to attain room temperature while being stirred for 16 h. The material was processed as described for **12a**. Purification by flash chromatography using 10% EtOAc/hexane gave 5.75 g (76%) of **12b** as a yellow oil: ^{1}H NMR 0.90 (t, 3 H, J = 7.1 Hz), 1.33 (m, 4 H), 1.78 (m, 2 H), 4.76 (t, 1 H, J = 6.6 Hz), 7.08 (dd, 1 H, J = 1.3, 5.0 Hz), 7.17 (d, 1 H, J = 2.8 Hz), 7.3 (dd 1 H, J = 2.8, 5.0 Hz); ^{13}C NMR 13.7, 22.2, 27.5, 37.8, 70.4, 120.6, 125.8, 125.9, 146.6; HRMS exact mass calcd for $C_9H_{14}OS$ (M^+) 170.0765, found 170.0765.

3-[1-(*tert*-Butyldimethylsiloxy)pentyl]thiophene (13b). This compound was prepared from **12b** (4.0 g, 23.5 mmol) as described for **13a** in 91% yield (6.1 g): ^{1}H NMR -0.11 (s, 3 H), 0.02 (s, 3 H), 0.88 (m, 12 H), 1.28 (m, 4 H), 1.7 (m, 2 H), 4.75 (t, 1 H, J = 5.5 Hz), 7.02 (dd, 1 H, J = 1.2, 5.0 Hz), 7.05 (d, 1 H, J = 2.8 Hz), 7.23 (dd, 1 H, J = 2.8, 5.0 Hz); ^{13}C NMR -5.3, -5.0, 13.8, 18.0, 22.4, 25.0, 27.4, 39.7, 71.4, 119.9, 125.3, 126.0, 147.6.

4-[1-(*tert*-Butyldimethylsiloxy)pentyl]-2-thiophenesulfonamide (14b). This compound was prepared from **13b** (6.1 g, 21.5 mmol) as described for **14a** to give 4.04 g (52%) of **14b** as a yellow oil: ^{1} HNMR -0.08 (s, 3 H), 0.05 (s, 3 H), 0,89 (m, 12 H), 1.28 (m, 4 H), 1.65 (m, 2 H), 4.71 (t, 1 H, J = 5.8 Hz), 4.97 (br, 2 H), 7.35 (d, 1 H, J = 1.6 Hz), 7.56 (d, 1 H, J = 1.6 Hz); ^{13}C NMR 13.9, 18.1, 22.5, 25.7, 27.1, 39.5, 70.9, 125.9, 130.2, 142.8, 147.9; HRMS exact mass calcd for $C_{14}H_{26}NO_3S_2Si$ ($M^+ - CH_3$) 348.1113, found 348.1112. Anal. ($C_{15}H_{29}NO_3S_2Si$) C, H, N.

4-(1-Hydroxypentyl)-2-thiophenesulfonamide (15b). To a solution of **14b** (3.8 g, 10.6 mmol) in THF (25mL) was added 25 mL of AcOH and 25 mL of water. The mixture was stirred at room temperature for 2 d. The THF was removed and the aqueous residue extracted with EtOAc (5 × 25 mL). The combined extracts were dried ($MgSO_4$), concentrated, and purified by flash chromatography using 40% EtOAc/hexane to give 1.6 g (59%) of **15b** as a pale yellow oil: ^{1}H NMR 0.91 (t, 3 H, J = 6.9 Hz), 1.34 (m, 4 H), 1.75 (m, 2 H), 2.14 (br s, 1 H), 4.37 (t, 1 H, J = 6.4 Hz), 5.15 (br s, 2 H), 7.42 (d, 1 H, J = 2 Hz), 7.63 (d, 1 H, J = 2 Hz). ^{13}C NMR 13.7, 22.2, 27.4, 37.5, 70.0, 126.9, 130.5, 143.3, 146.9; HRMS exact mass calcd for $C_9H_{15}NO_3S_2$ (M^+) 249.0493, found 249.0491.

4-(1-Oxopentyl)-2-thiophenesulfonamide (16b). This compound was prepared from **15b** (1.51 g , 6.0 mmol) as described for **16a** to give 1.09 g (79%) **16b** as a white solid: mp 139-140 °C; ^{1}H NMR 0.92 (t, 3 H, J = 7.3 Hz), 1.36 (m, 2 H), 1.65 (m, 2 H), 2.96 (t, 2 H, J = 7.2 Hz), 7.03 (br, 2 H), 7.92 (d, 1 H, J = 1.5 Hz), 8.56 (d, 1 H, J = 1.5 Hz); ^{13}C NMR 14.0, 22.8, 26.8, 39.6, 130.3, 137.1, 143.1, 147.9, 195.1; HRMS exact mass calcd for $C_9H_{13}NO_3S_2$ (M^+) 247.0336, found 247.0329. Anal. ($C_9H_{13}NO_3S_2$) C, H, N.

3-(1-Hydroxy-1-phenylmethyl)thiophene (12c). This compound was prepared from 3-thiophenecarboxaldehyde (3.9 mL, 44.6 mmol) and phenylmagnesium chloride (24.5 mL, 49.0 mmol, 2.0 M) as described for **12a** to give 5.9 g (70%) of **12c** as a pale yellow oil: ^{1}H NMR 2.2 (d, 1 H, J = 3.9 Hz), 5.9 (d, 1 H, J = 3.9 Hz), 6.9 (dd, 1 H, J = 1.3, 5.0 Hz) 7.18 (dd, 1 H, J = 1.3, 4.3 Hz), 7.35 (m, 6 H); ^{13}C NMR 72.7, 121.8, 126.3, 126.5, 126.6, 127.9, 128.6, 143.5, 145.5; HRMS exact mass calcd for $C_{11}H_{10}OS$ (M^+) 190.0452, found 190.0444.

3-[1-(*tert*-Butyldimethylsiloxy)-1-phenylmethyl]thiophene (13c). This compound was prepared from **12c** (5.9 g, 31.o mmol) as described for **12a** to give 8.4 g (95%) of **13c** as a colorless oil: ^{1}H NMR 0.01 (s, 3 H) 0.04 (s, 3 H), 0.95 (s, 9 H), 5.85 (s, 1 H), 6.95 (d, 1 H, J = 6 Hz), 7.14 (d, 1 H, J = 2.3 Hz), 7.35 (m, 6 H); ^{13}C NMR -5.22, -5.10, 18.0, 25.7, 73.4, 120.7, 125.8, 126.4, 126.5, 127.3, 128.3, 144.7, 147.0; HRMS exact mass calcd for $C_{16}H_{21}OSSi$ ($M^+ - CH_3$) 289.1082, found 289.1071.

4-[1-(*tert*-Butyldimethylsiloxy)-1-phenylmethyl]-2-thiophenesulfonamide (14c). This compound was prepared from **13c** (8.9 g, 29.4 mmol) as described for **14a** in 42% (3.7 g) yield: ^{1}H NMR -0.06 (s, 3 H) 0.04 (s, 3 H), 0.91 (s, 9 H), 4.97 (br s, 2 H), 5.73 (s, 1 H), 7.3 (m, 5 H), 7.39 (d, 1 H, J = 1.4 Hz) 7.44 (d 1 H, J = 1.4 Hz); ^{13}C NMR -5.4, -5.2, 17.9, 25.5, 73.2, 126.3, 126.4, 127.8, 128.6, 130.6, 143.4, 143.6, 147.6; LRMS ($M^+ - CH_3$) 368.

4-[1-Hydroxy-1-phenylmethyl]-2-thiophenesulfonamide (15c). This compound was prepared from **14c** (3.7 g, 10.4 mmol) as described for **15a** in 48% (1.3 g) yield: ^{1}H NMR 2.4 (br, 1 H) 5.0 (br s, 2 H), 5.83 (s, 1 H), 7.38 (m, 5 H), 7.44 (d, 1 H, J = 1.4 Hz), 7.54 (d, 1 H, J = 1.4 Hz); ^{13}C NMR 72.5, 126.9, 127.4, 128.4, 129.4, 145.4, 146.9, 148.3; HRMS exact mass calcd for $C_{11}H_{11}NO_3S_2$ (M^+) 269.0180, found 269.0139.

4-(1-Oxo-1-phenylmethyl)-2-thiophenesulfonamide (16c). This compound was prepared from **15c** (1.3 g, 4.98 mmol) as described for **16a** ito give 1.03 g (78%) of **16c** as a pale yellow solid: mp 173-174 °C; ^{1}H NMR 4.88 (s, 2 H) 7.55 (t, 2 H, J = 7.1 H z), 7.66 (t, 1 H, J = 7.6 H z), 7.83 (d, 2 H, J = 7.1 H z), 7.96 (d, 1 H, J = 1.5 Hz) 8.3 (d, 1 H, J = 1.5 Hz); ^{13}C NMR 129.8, 130.4, 131.7, 134.1, 138.9, 139.2, 141.6, 148.1, 182.4, 190.7; HRMS exact mass calcd for $C_{11}H_9NO_3S_2$ (M^+) 267.0024, found 266.9998. Anal. ($C_{11}H_9NO_3S_2$) C, H, N.

3-[1-(*tert*-Butyldimethylsiloxy)ethyl]thiophene (13d). MeLi (35.0 mL, 49.0 mmol, 1.4 M) was added to a 0 °C solution of 3-thiophenecarboxaldehyde (3.9 mL, 44.6 mmol) in dry THF (150 mL). The solution was stirred at room temperature for 1 h, TBDMSCl (8.1 g, 53.5 mmol) and HMPA (15 mL) were added, and the solution was stirred at room temperature for 16 h. The material was processed as described for **13a** to give 7.29 g (67%) of **13d** as a pale yellow oil; ^{1}H NMR 0.01 (s, 3 H) 0.06 (s, 3 H), 0.90 (s, 9 H), 1.43 (d, 3 H, J = 6.4 H z), 4.94 (q, 1 H, J = 6.4 Hz), 7.01 (d, 1 H, J = 4.9 Hz), 7.09 (d, 1 H, J = 2 Hz), 7.24 (dd, 1 H, J = 2, 4.9 Hz); ^{13}C NMR -5.1, 18.0, 25.7, 26.0, 67.4, 119.4, 125.5, 125.8, 148.6; HRMS exact mass calcd for $C_{12}H_{22}OSSi$ (M^+) 242.1160, found 242.1175.

4-[1-(*tert*-Butyldimethylsiloxy)ethyl]-2-thiophenesulfonamide (14d). This compound was prepared from **13d** (7.3 g, 30.1 mmol) as described for **14a** to give 5.8 g (60%) of **14d** as a yellow oil: ^{1} HNMR -0.00 (s, 3 H), 0.05 (s, 3 H), 0,88 (s, 9 H), 1.38 (d, 3 H, J = 6.4 Hz), 4.86 (q, 1 H, J = 6.4 Hz), 5.19 (br s, 2 H), 7.35 (s, 1 H), 7.53 (s, 1 H); ^{13}C NMR -5.2, -5.2, 17.9, 25.6, 25.8, 67.0, 125.7, 130.1, 143.2, 149.1; HRMS exact mass calcd for $C_{12}H_{23}NO_3S_2Si$ (MH^+) 321.0888, found 322.0950.

4-(1-Hydroxyethyl)-2-thiophenesulfonamide (15d). This compound was prepared from **14d** (4.14 g, 12.9 mmol) as described for **15a** to give 0.53 g of **15d** as a pale yellow solid; ^{1}H NMR 1.41

(d, 3 H, J = 6.2 Hz), 4.4 (d, 1 H, J = 4.6 Hz), 4.88 (m, 1 H), 6.83 (br s, 2 H), 7.53 (d, 1 H, J = 1.5 Hz), 7.56 (d, 2 H, J = 1.5 Hz); ^{13}C NMR 25.8, 66.2, 125.8, 130.3, 146.6, 150.1; HRMS exact mass calcd for $C_6H_9NO_3S_2$ (M^+) 207.0023, found 207.0032.

4-(1-Oxomethyl)-2-thiophenesulfonamide (16d). This compound was prepared from **15d** (0.81 g, 3.9 mmol) as described for **16a** to give 0.48 g (60%) of **16d** as a white solid: mp 132–134 °C; ^{1}H NMR 2.53 (s, 3 H), 7.03 (br s, 2 H), 7.89 (d, 1 H, J = 1.4 H z), 8.55 (d, 1 H, J = 1.4 H z); ^{13}C NMR 27.2, 130.2, 137.8, 143.0, 147.8, 192.7. Anal. ($C_6H_7NO_3S_2$) C, H, N.

4-[1-(Methoxyimino)heptyl]-2-thiophenesulfonamide (17). A solution of methoxylamine hydrochloride (0.2 g, 2.2 mmol) and NaOAc (0.2 g, 2.2 mmol) in 0.5 mL of water was added to a solution of **16a** (0.05 g, 0.18 mmol) in EtOH (0.5 mL). The solution was stirred at room temperature for 48 h. The EtOH was removed in vacuo and the aqueous layer extracted with EtOAc (6 × 5 mL). The combined extracts were dried ($MgSO_4$), concentrated, and chromatographed with 20% EtOAc/hexane to give 0.044 g (81%) of **17** as a white solid: mp 94–96 °C; ^{1}H NMR mixture of isomers 0.88 (t, 3 H, J = 6.8 Hz), 1.31 (m, 6 H), 1.52 (m, 2 H), 2.54 (t, 2 H, J = 7.9 Hz), 2.63 (t, 2 H, J = 7.7 Hz), 3.94 (s, 3 H), 5.20 (br s, 2H), 7.60 (d, 1 H, J = 1.5 Hz), 7.99 (d, 1 H, J = 1.5 Hz), 8.0 (d, 1 H, J = 1.5 Hz), 8.13 (d, 1 H, J = 1.5 Hz); ^{13}C NMR mixture of isomers 13.8, 22.3, 26.3, 26.7, 27.3, 28.7, 29.2, 31.26, 31.29, 34.3, 62.0, 128.1, 130.2, 133.0, 134.2, 138.6, 143.8, 149.5, 154.6; HRMS exact mass calcd for $C_{12}H_{21}N_2O_3S_2$ (MH^+) 305.0994, found 305.1004. Anal. ($C_{12}H_{21}N_2O_3S_2$) C, H, N.

4-(1-Hydrazonoheptyl)-2-thiophenesulfonamide (18). Hydrazine hydrate (0.08 g, 2.8 mmol) was added to a solution of **16a** (0.15 g, 0.55 mmol) in EtOH (6 mL). The solution was stirred at room temperature for 16 h. The EtOH was removed in vacuo to give a white solid which was recrystalized from MeOH/$CHCl_3$ to give 0.07 g (44%) of **18** as a white solid: mp 123–124 °C; ^{1}H NMR (CD_3OD) 0.90 (t, 3 H, J = 6.9 Hz) 1.4 (m, 8 H), 2.6 (t, 2 H, J = 8.1 Hz), 7.68 (s, 1 H), 7.86 (s, 1 H); ^{13}C NMR (CD_3OD) 14.3, 23.5, 26.3, 26.8, 30.5, 32.8, 127.1, 130.1, 143.1, 146.9, 148.4; HRMS exact mass calcd for $C_{11}H_{19}N_3O_2S_2$ (M^+) 289.0918, found 289.0904. Anal. ($C_{11}H_{19}N_3O_2S_2$) C, H, N.

4-(1-Hydroximinoheptyl)-2-thiophenesulfonamide (19). A solution of hydroxylamine hydrochloride (0.08 g, 0.11 mmol) and NaOAc (0.09 g, 0.11 mmol) in water (6 mL) was added to a solution of **16a** (0.05 g, 0.18 mmol) in EtOH (2 mL). The solution was stirred at room temperature for 16 h. The EtOH was removed in vacuo, and the residue was taken up in water and extracted with EtOAc (5 × 10 mL). The combined extracts were dried ($MgSO_4$), concentrated, and purified by chromatography using 40% EtOAc/hexane to give 0.048 g (61%) of **19** as a white solid: mp 97–100 °C; ^{1}H NMR mixture of isomers 0.86 (t, 3 H, J = 7.0 Hz), 1.31 (m, 6 H), 1.57 (m, 2 H), 2.59 (t, 1 H, J = 7.5 H z), 2.75 (t, 1 H, J = 7.9 Hz), 6.92 (br s, 2 H), 7.86 (d, 1 H, J = 1.5 Hz), 7.92 (d, 1 H, J = 1.7 Hz), 8.12 (d, J = 1 H, 1.5 Hz), 8.0 (d, 1 H, J = 1.5 Hz), 8.34 (d, 1 H, J = 1.7 Hz), 10.31 (s, 1 H), 10.33 (s, 1 H); ^{13}C NMR mixture of isomers 14.1, 14.2, 23.1, 26.3, 27.1, 28.0, 32.3, 34.9, 128.4, 129.3, 132.9, 133.9, 139.8, 147.3, 150.2, 155.1; HRMS exact mass calcd for $C_{11}H_{18}N_2O_3S_2$ (M^+) 290.0759, found 290.0755. Anal. ($C_{11}H_{18}N_2O_3S_2$) C, H, N.

4-(1-Hydroximinopentyl)-2-thiophenesulfonamide (20). This compound was prepared as described for **19** from **16a** (0.10 g, 0.41 mmol), hydroxylamine hydrochloride (0.17 g, 2.43 mmol), and NaOAc (0.20 g, 2.43 mmol) to give 0.053 g (50%) of **20** as a white solid: mp 105–107 °C; ^{1}H NMR mixture of isomers 0.92 (m, 3 H), 1.37 (m, 2 H), 1.57 (m, 2 H), 2.59 (t, 2 H, J = 7.7 H z), 2.75 (t, 2 H, J = 7.6 Hz), 6.92 (br s, 2 H), 6.95 (br, 2H), 7.85 (d, 1 H, J = 1.5 Hz), 7.91 (d, 1 H, J = 1.5 Hz), 8.11 (d, 1 H, J = 1.7 Hz), 8.33 (d, 1 H, J = 1.7 Hz), 10.31 (s, 1 H), 10.36 (s, 1 H); ^{13}C NMR mixture of isomers 14.0, 14.1, 22.8, 23.4, 26.1, 34.3, 128.0, 128.9, 132.6, 133.6, 139.3, 146.9, 154.6; HRMS exact mass calcd for $C_9H_{14}N_2O_3S_2$ (M^+) 262.0445, found 262.0445. Anal. ($C_9H_{14}N_2O_3S_2$) C, H, N.

4-(1-Aminoheptyl)-2-thiophenesulfonamide (21). A solution of ammonium acetate (0.28 g, 3.6 mmol) and sodium cyanoborohydride (0.32 g, 0.5 mmol) in absolute MeOH (1.1 mL) was added to a solution of **16a** (0.1g, 0.36 mmol) in absolute MeOH (1 mL) and stirred at room temperature for 48 h. The solution was acidified to pH 2 and the MeOH removed in vacuo. The residue was taken up in a minimun volume of water and extracted with Et_2O. The aqueous portion was made basic (pH = 10) with solid KOH, saturated with NaCl, and extracted with EtOAc (7 × 5 mL). The combined EtOAc extracts were dried ($MgSO_4$), concentrated, and purified by chromatography using 20% MeOH saturated with ammonia/$CHCl_3$ to give 0.42 g (42%) of **21** as a white solid: mp 87–90 °C; ^{1}H NMR 0.87 (t, 3 H, J = 6.5 Hz), 1.27 (m, 8 H), 1.64 (m, 2 H), 3.1 (br, 4 H), 3.96 (t, 1 H, J = 6.8 Hz), 7.35 (d, 1 H, J = 1.6 Hz), 7.63 (d, 1 H, J = 1.6 Hz); ^{13}C NMR 13.8, 22.3, 25.9, 28.9, 31.5, 36.7, 51.7, 126.2, 130.5, 143.9, 148.1; HRMS exact mass calcd for $C_{11}H_{20}N_2O_2S_2$ (M^+) 276.0966, found 277.1047. Anal. ($C_{11}H_{20}N_2O_2S_2$) C, H, N.

4-(1-Aminopentyl)-2-thiophenesulfonamide (22). This compound was prepared as described for **21** from **16a** (0.15 g, 0.61 mmol), ammonium acetate (0.47 g, 6.7 mmol), and sodium cyanoborohydride (0.03 g, 0.43 mmol) to give 0.87 g (53%) of **22** as a white solid: mp 108–109 °C; ^{1}H NMR (CD_3OD) 0.89 (t, 3 H, J = 7.3 Hz), 1.25 (m, 4 H), 1.7 (m, 2 H), 3.91 (t, 1H, J = 6.9 Hz), 4.88 (br, 2 H), 7.48 (d, 1 H, J = 1.6 Hz), 7.6 (d, 1 H, J = 1.6 Hz); ^{13}C NMR (CD_3OD) 14.3, 23.6, 29.5, 39.2, 52.8, 127.0, 130.5, 146.6, 148.5; HRMS exact mass calcd for $C_9H_{17}N_2O_2S_2$ (M^+) 249.0731, found 249.0699. Anal. ($C_9H_{17}N_2O_2S_2$) C, H, N.

4-[1-(*N*-Butylamino)ethyl]-2-thiophenesulfonamide (23). To a solution of *n*-butylamine (0.6 mL, 5.9 mmol) in MeOH (2.4 mL) was added 4 mL of 5 N methanolic HCl followed by **16d** (0.2 g, 0.98 mmol) and sodium cyanoborohydride (0.04 g, 0.59 mmol). The solution was stirred at room temperature for 16 h, but the reaction was incomplete. The pH of the solution was adjusted to 6, another 0.08 g of sodium cyanoborohydride was added, and the solution was stirred at room temperature for 16 h. The material was processed as described for **21** to give 0.081 g (37%) of **23** as a white solid: mp 122–123 °C; ^{1}H NMR 0.85 (t, 3 H, J = 7.1 Hz), 1.29 (d, 3 H, J = 6.5 Hz), 1.4 (m, 4 H), 2.45 (m, 2 H), 2.75 (br, 1 H), 3.83 (q, 1 H, J = 6.5 Hz), 6.8 (br s, 2 H), 7.5 (d, 1 H, J = 1.6 Hz), 7.57 (d, 1 H, J = 1.6 Hz); ^{13}C NMR 14.2, 21.0, 23.8, 33.1, 48.0, 54.6, 126.4, 130.8, 146.7, 149.7. Anal. ($C_{10}H_{18}N_2O_2S_2$) C, H, N.

In Vitro Inhibition of Human Carbonic Anhydrase II. Inhibition of human carbonic anhydrase II (Sigma C6156) was assessed by using a modified version of the changing pH principle of Philpot and Philpot.[14] The IC_{50} is determined from the enzyme activity. The enzyme activity is the time required to neutralize a buffered solution of enzyme, and where desired, inhibitor by a 100 mL/min^2 stream of CO_2 gas. A stock solution of the buffer consists of 30.0 mL of 1 M Na_2CO_3 and 20.6 mL of 1 M $NaHCO_3$ made up to a volume of 100 mL. All reagents are kept below 5 °C. To a reaction vessel in which CO_2 flow has been carefully stabilized is added 0.4 mL of phenol red, followed by the enzyme (dilution and volume will vary), and where desired, a 0.01–100 mg/L solution of inhibitor. The volume is immediately made up to 0.7 mL with water. Buffer (0.1 mL) is added rapidly and timing begun with a stop watch. The run ends when the indicator turns from red to yellow. The IC_{50} is obtained from a plot of time (s) versus inhibitor concentration (nM).

Ex Vivo Studies. Rabbit iris and ciliary body was obtained and prepared as described in ref 15. Instead of using the pH stat assay to determine the carbonic anhydrase activity in the iris + ciliary body homogenate, the changing pH principle of Philpot and Philpot described in ref 14 was used.

Reaction of Sulfonamides with Cysteine. The protocol used in this assay is described in ref 16.

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