# New Isomeric Classes of Topically Active Ocular Hypotensive Carbonic Anhydrase Inhibitors: 5-Substituted Thieno[2,3-*b*]thiophene-2-sulfonamides and 5-Substituted Thieno[3,2-*b*]thiophene-2-sulfonamides

John D. Prugh,* George D. Hartman,* Pierre J. Mallorga, Brian M. McKeever, Stuart R. Michelson, Mark A. Murcko, Harvey Schwam, Robert L. Smith, John M. Sondey, James P. Springer, and Michael F. Sugrue

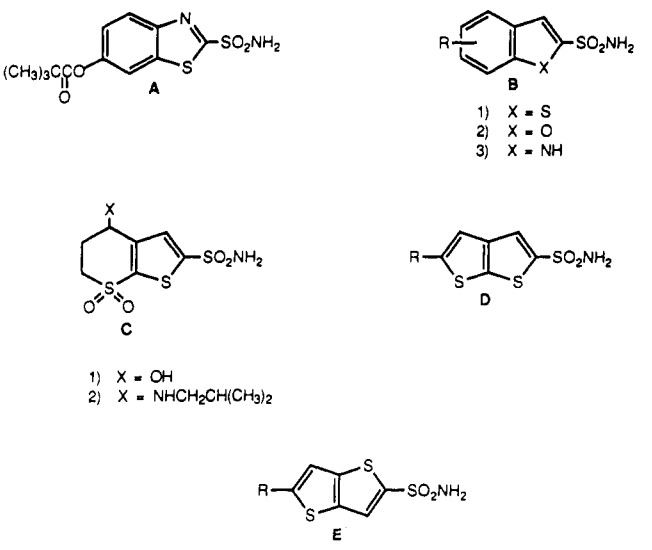
*Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486.* *Received October 26, 1990*

A series of 5-substituted thieno[2,3-*b*]- and thieno[3,2-*b*]thiophene-2-sulfonamides was prepared and evaluated for topical ocular hypotensive activity in glaucoma models. The 5-substituents were varied to maximize both inhibitory potency against carbonic anhydrase and water solubility. At the same time, these substituents were varied in order to obtain compounds with the appropriate $pK_a$ to minimize pigment binding in the iris. All of these variables were optimized in the best compound, 5-[[(methoxyethyl)[(methoxyethyl)ethyl]amino]methyl]thieno[2,3-*b*]thiophene-2-sulfonamide hydrochloride (**55**).

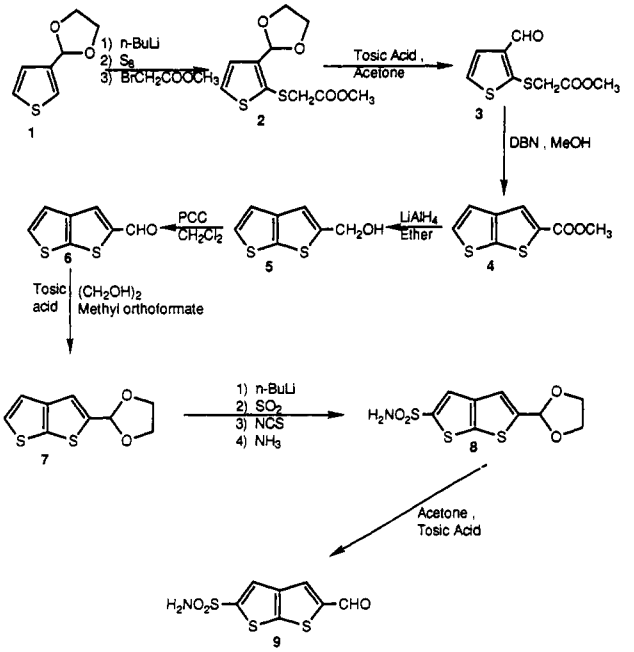
A variety of carbonic anhydrase inhibitors (CAIs) have been shown to reduce intraocular pressure (IOP) when taken orally.[1] However, their use in controlling IOP is limited by their modest activity and substantial side effects.[2] A topically active CAI would have the advantage of acting locally at the ciliary process and thereby obviate the systemic side effects of orally administered CAIs.

The development of a topically active CAI has been an objective in these laboratories for several years. Relevant compounds prepared in pursuit of this objective include the benzothiazole-2-sulfonamides[3] (A) (Chart I); the benzo[*b*]thiophene-2-sulfonamides[4] (B1), the benzofuran[5] (B2) and indole-2-sulfonamides[5] (B3), and the 4-hydroxy and 4-aminothienothiopyrans[6] (C1,C2). Fused 5- and 6-membered rings with a sulfonamide attached to the 2-position of the 5-membered ring (A,B1–3)[3–5] showed good activity but were poorly soluble in water. Prior work in the thienothiopyrans[6b] (C2) established the superior biological activity of sulfonamide inhibitors containing basic amine functionality; in addition, the amine salts allowed the preparation of 2–4% aqueous solutions. This prior experience suggested that the thienothiophene system incorporating a sulfonamide in the 2-position with a basic amine located elsewhere in the molecule would be a fertile area for development. The 5-position was selected for the attachment of the amine since in that position the amine and its substituents would not interact adversely with the confining walls of the cone-shaped[6b] active site of carbonic anhydrase and would be involved in productive noncovalent binding along the cone's lower aliphatic region. We report now the details of the development of the 5-sub-

**Chart I**




**Scheme I**




(1) Maren, T. H. *Drug Dev. Res.* **1987**, *10*, 255–276.
(2) Lichter, P. R. L. P.; Wheeler, N. C.; Beall, O. V. *Am. J. Ophthalmol.* **1978**, *85*, 495–502.
(3) (a) Woltersdorf, O. W., Jr.; Schwam, H.; Bicking, J. B.; Brown, S. L.; deSolms, S. J.; Fishman, D. R.; Graham, S. L.; Gautheron, P. D.; Hoffman, J. M.; Larson, R. D.; Lee, W. S.; Michelson, S. R.; Robb, C. M.; Share, N. N.; Shepard, K. L.; Smith, A. M.; Smith, R. L.; Sondey, J. M. *J. Med. Chem.* **1989**, *32*, 2486. (b) Sugrue, M. F.; Gautheron, P.; Schmitt, C.; Viader, M. P.; Conquet, P.; Smith, R. L.; Share, N. N.; Stone, C. A. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 534.
(4) Graham, S. L.; Shepard, K. L.; Anderson, P. S.; Baldwin, J. J.; Best, D. B.; Christy, M. E.; Freedman, M. B.; Gautheron, P.; Habecker, C. N.; Hoffman, J. M.; Lyle, P. A.; Michelson, S. R.; Ponticello, G. S.; Robb, C. M.; Schwam, H.; Smith, A. M.; Smith, R. L.; Sondey, J. M.; Strohmaier, K. M.; Sugrue, M. F.; Varga, S. L. *J. Med. Chem.* **1989**, *32*, 2548.
(5) Graham, S. L.; Hoffman, J. M.; Gautheron, P.; Michelson, S. R.; Scholz, T. H.; Schwam, H.; Shepard, K. L.; Smith, A. M.; Smith, R. L.; Sondey, J. M.; Sugrue, M. F. *J. Med. Chem.* **1990**, *33*, 749.
(6) (a) Ponticello, G. S.; Freedman, M. B.; Habecker, C. N.; Lyle, P. A.; Schwam, H.; Varga, S. L.; Christy, M. E.; Randall, W. C.; Baldwin, J. J. *J. Med. Chem.* **1987**, *30*, 591. (b) Baldwin, J. J.; Ponticello, G. S.; Anderson, P. S.; Christy, M. E.; Murcko, M. A.; Randall, W. C.; Schwam, H.; Sugrue, M. F.; Springer, J. P.; Gautheron, P.; Grove, J.; Mallorga, P.; Viader, M. P.; McKeever, B. M.; Navia, M. A. *J. Med. Chem.* **1989**, *32*, 2510.

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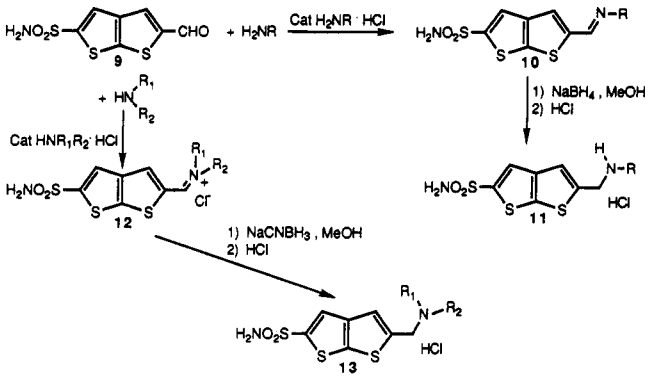
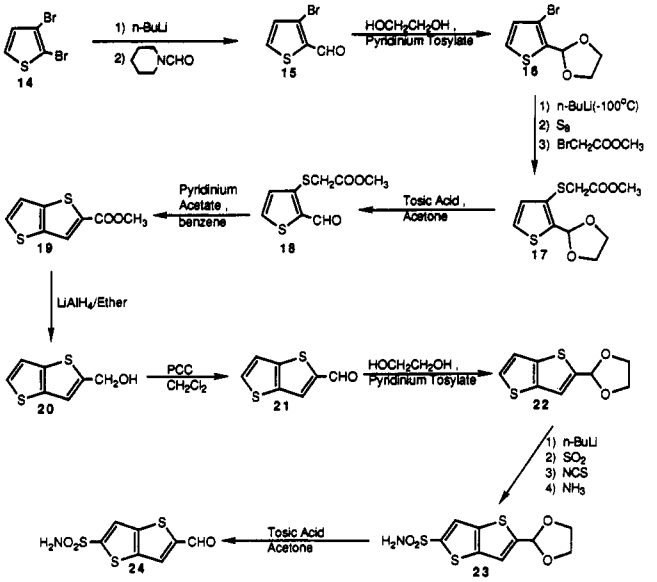
Scheme II



Scheme III



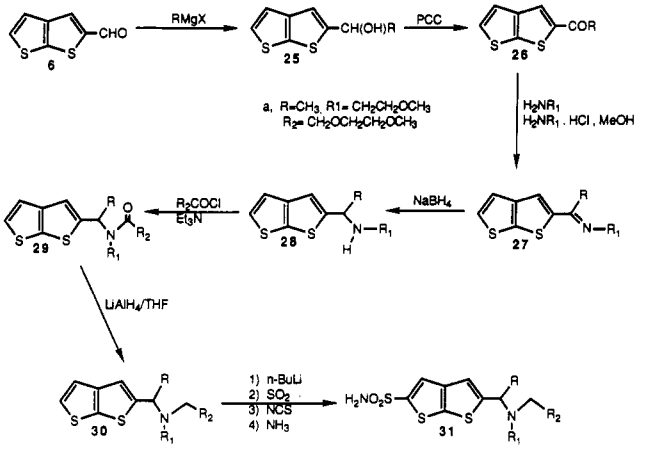
stituted thieno[2,3-*b*]thiophene-2-sulfonamides (D), and the 5-substituted thieno[3,2-*b*]thiophene-2-sulfonamides (E) as topically active, ocular hypotensive CAIs.

## Chemistry

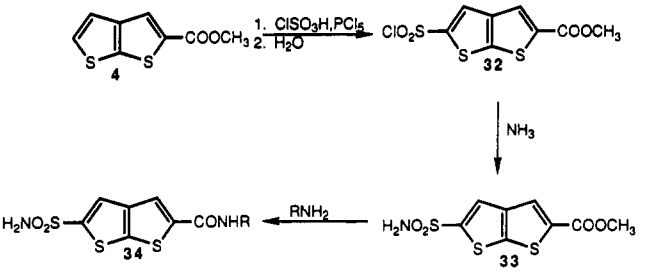
Although the parent acid of the ester **4** is a known compound,[7] the attainment of reproducible yields of **4** utilizing the published methodology was problematic. As shown in Scheme I, compound **3**, whose synthesis was optimized with commercially available reagents, was efficiently converted to **4** via DBN-catalyzed cyclization. Reduction of **4** to carbinol **5** with $LiAlH_4$ followed by pyridinium chlorochromate[8] oxidation gave **6**. Tosic acid catalyzed acetalization of **6** with ethylene glycol and methyl orthoformate[9] then provided crystalline acetal **7**. Sulfamoylation[5] of **7** was effected by metalation at C5 with *n*-butyllithium followed sequentially by treatment with $SO_2$, NCS, and ammonia to provide **8**. Deprotection to afford aldehyde **9** was effected in high yield under transacetalization conditions.

Aldehyde **9** underwent facile reaction with a wide variety of primary amines to form the imines **10** (Scheme II). Reduction with $NaBH_4$ followed by treatment with ethanolic HCl provided the target amine hydrochlorides **11** which were used in testing. Aldehyde **9** also reacted with secondary amines to form the immonium salts **12**, albeit at a slower rate than observed for primary amines. Reduction of **12** with $NaCNBH_3$ provided the corresponding amines which were converted to the desired HCl salts **13** with ethanolic HCl.

Scheme IV



Scheme V



Synthesis of the thieno[3,2-*b*]thiophene synthon **24** followed a synthetic approach similar to that designed for **9** and is outlined in Scheme III. This synthesis is well precedented[10] and is straightforward with the exception of the very delicate halogen–metal exchange to form the 3-lithio derivative from **16**. Lithium–halogen exchange and subsequent reaction of the lithio derivative of **16** at −100 °C provided the desired adduct **17** in regioselective fashion. However, lithium–halogen exchange of **16** and reaction at −78 °C was complicated with translithiation to the acidic proton in the 5-position affording a mixture of **17** and the undesired 2,5-isomer. The synthon **24** was carried on to the desired amines **59** through **62** in the same way as outlined in Scheme II.

The method employed to prepare compounds substituted at the aryl methylene carbon is outlined in Scheme IV. This route was dictated by the fact that secondary amines would not efficiently form immonium adducts with the ketone **26**. It was necessary then to form the imine **27** from **26** and a primary amine; subsequent reduction with $NaBH_4$ gave **28**. The resulting amine was acylated with the desired group and the resulting amide **29** was reduced with $LiAlH_4$ to the amine **30**. Sulfamoylation in the usual manner gave the desired sulfonamides **31**.

The amide derivatives **34** were prepared by chlorosulfonation[11] of the ester **4** (Scheme V); treatment of the

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(8) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647.
(9) Glatz, B.; Helmehen, G.; Muxfeldt, H.; Porcher, H.; Prewo, R.; Senn, J.; Stezowski, J. J.; Stojda, R. J.; White, R. R. *J. Am. Chem. Soc.* **1979**, *101*, 2171.
(10) (a) Gronowitz, S.; Bagge, A. *Acta Chem. Scand.* **1968**, *22*, 59. (b) Olah, G. A.; Arvanaghi, M. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 878.
(11) Barnish, I. T.; Cross, P. E.; Dickinson, R. P.; Parry, M. J.; Randall, M. J. *J. Med. Chem.* **1981**, *24*, 959.

resulting sulfonyl chloride with ammonia gave the sulfonamide ester **33**. This synthon reacted with a wide variety of amines at elevated temperature to provide the desired amides **34**.

## Biological Results and Discussion

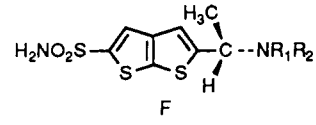
On the basis of previous experience,[3-6] we anticipated that ocular absorption would be favorable for compounds possessing an octanol–water partition coefficient of 0.2–20. The ideal $pK_a$ of the amine was not known initially but later became a key point in the design process vida infra. The in vitro potency of good compounds had to be in the nanomolar range both with respect to binding to carbonic anhydrase, expressed as $K_i$, and inhibition of the enzyme, expressed as $IC_{50}$. A goal of greater than 90% inhibition at 0.5% concentration in the first line ex vivo assay[12] was also considered ideal. Compounds that showed promise in these initial tests were selected for additional biological evaluation which included testing for the ability of the compound to concentrate in the iris plus ciliary body;[13] for sensitization liability in the guinea pig maximum sensitization test[14] (GPMST); mutagenic liability in the Ames test;[15] and pharmaceutical formulateability.[16] At the same time selected compounds were further evaluated for in vivo activity in IOP modulation of ocular normotensive albino rabbits,[5] ocular normotensive pigmented rabbits,[17] ocular hypertensive pigmented rabbits,[18] and finally laser-treated ocular hypertensive monkeys.[19]

Following the precedence of the highly active secondary aliphatic amines in the C2[6b] series, we prepared secondary amines **35–42**. These compounds were highly active in the initial biological tests, and three of them (**35**, **36**, and **42**) were selected for in-depth biological evaluation. None of the three compounds was a contact sensitizer (GPMT), or a mutagen in the Ames test. The compounds were particularly interesting because they were shown to concentrate in the iris plus ciliary body[13] (the active site tissue).

However, at this time a compound in a closely related series[19] which showed good in vitro and preliminary in vivo activity was found to be topically inactive in ocular hypertensive pigmented monkeys,[20,12] ostensibly due to its high order of binding to ocular pigment.[19] It was anticipated that this interaction with pigment might render the compound unavailable for binding with carbonic anhydrase. Subsequently, a relationship between basicity and pigment binding was found for a wide variety of topically active CAIs possessing basic amine substituents where it was shown that the greater the basicity, the greater the pigment binding.[21] A plot of $pK_a$ verses pigment binding for the thienothiophene series of the initial compounds, **35–42** ($pK_a$ = 6–8), also showed that the greater the amine basicity, the greater the pigment binding. Accordingly, efforts were directed toward compounds that were weaker bases but still basic enough to be water soluble at pH 5.2, the minimum pH selected for acceptable ophthalmic formulations. Although a wide variety of compounds (**45–58**) were made, the greatest success in this phase of the work was obtained with a series of polyethers. It was reasoned that polyether appendages would lower the $pK_a$ of the amine, provide additional water solubility, and at the same time interact in productive binding with an aliphatic trough at the active site of carbonic anhydrase (as suggested by molecular modeling). The best of this group of compounds were the secondary amines **54** and **55**, which had both appropriate $pK_a$ values (5.34 and 5.55, respectively) and ocular pigment binding values (50% and 38.5%, respectively). Both compounds had good aqueous solubility and thus could be formulated into ophthalmic solutions at pH 5.2. These compounds displayed acceptable activity in preliminary tests, and were selected for in-depth biological evaluation. As a part of this evaluation, compounds **54** and **55** were found to be nonmutagenic in the Ames test and to lack contact sensitization potential in the GPMST.

Molecular modeling of the 5-substituted thieno[3,2-*b*]-thiophene-2-sulfonamide system showed that the sulfamoyl moiety and the 5-substituent project from the ring in a manner analogous to that seen in the 2,3-*b* system. Further, the greater difficulty of preparing the 3,2-*b* system led us to prepare only those derivatives possessing substituents that had already shown promise in the 2,3-*b* system. The compounds selected and prepared along with their test results are displayed in Table II. Clearly the best compound of this series is the tertiary amine **62** with *N*,*N*-dimethoxyethyl substitution.

Molecular modeling suggested that a methyl group on the aryl methylene carbon might influence the orientation of the amine side chain in a favorable manner. Specifically, it was anticipated that $\alpha$-methyl substitution would enhance the stability of that rotamer in which the amine is projected nearly vertically out of the plane of the aryl ring. This preferred rotamer is shown as compound F. This

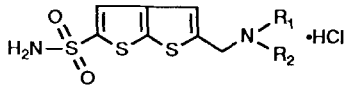


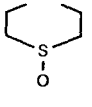

F

is the orientation of the amine side chain that binds to the enzyme vida infra and therefore it was hoped that this might enhance activity in an entropic sense by reducing the degrees of freedom of the amino side chain orientation. The $K_i$ of 1.54 nM displayed by compound **64** was found to be the same as the desmethyl compound **55**, and therefore it can be concluded that if there is a preferred orientation caused by the addition of a methyl group it is not reflected in enhanced binding of **64** to the enzyme under our assay conditions. Molecular modeling also suggested that a fluorine atom situated an appropriate distance from the aryl methylene group would bind to the imidazole NH of His-64 at the active site of carbonic anhydrase. Compound **65** is an attempt to implement this thinking; however, the decrease in activity as exhibited by

(12) Sugrue, M. F.; Gautheron, P.; Mallorga, P.; Nolan, T. E.; Graham, S. L.; Schwam, H.; Shepard, K. L.; Smith, R. L. *Br. J. Pharmacol.* **1990**, *99*, 59.
(13) The iris plus ciliary body from the ex vivo assay was homogenized and extracted with ethyl acetate. The extract was spiked with a known amount of standard and the unknown determined by HPLC.
(14) Wahlberg, J. E.; Boman, A. *Curr. Probl. Derm.* **1985**, *14*, 59.
(15) (a) Maron, D. M.; Ames, B. N. *Mutat. Res.* **1983**, *113*, 173, and references therein. Matsushima, T.; Takamoto, Y.; Shirai, A.; Sawamura, M.; Sugimura, T. *Prog. Mut. Res.* **1981**, *1*, 387.
(16) The compounds were formulated as 2% isotonic ophthalmic solution with appropriate stabilizers and at pH of 5.2 or greater.
(17) Vareilles, P.; Cougnet, P.; LeDouarec, J. C. *Exp. Eye Res.* **1977**, *24*, 369.
(18) (a) Sears, D.; Sears, M. *Am. J. Ophthalmol.* **1974**, *77*, 378. (b) Sugrue, M. F.; Gautheron, P.; Schmitt, C.; Viader, M. P.; Cougnet, P.; Smith, R. L.; Share, N. N.; Stone, C. A. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 534.
(19) Hartman, G. D.; Halczenko, W. Unpublished results.
(20) (a) Lee, P. Y.; Podos, S. M.; Howard-Williams, J. R.; Severin, C. H.; Rose, A. D.; SIegel, M. J. *Cur. Eye Res.* **1985**, *4*, 775.
(21) Randall, W. C. Unpublished results.

**Table I**

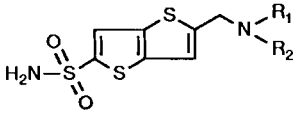


| compd | $R_1$ | $R_2$ | analysis[a] | mp, °C | solubility[b] (2% sol, pH 5.2) | percent yield | partition coeff[c] | $pK_a$[d] | CAI,[e] $I_{50} \times 10^{-9}$ M | CA binding,[e] $K_i \times 10^{-9}$ M | pigment binding,[f] % | ex vivo CA inhib,[g] 0.5% (0.1%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **35** | H | $CH_2CH(CH_3)_2$ | $C_{11}H_{17}ClN_2O_2S_2$ (C, H, N) | 251–2 | + | 87 | 8.4 | 7.90 | 2.5 | 4.4 | | 97 |
| **36** | H | $CH_3$ | $C_8H_{11}ClNO_2S_3$ (C, H, N) | 263–4 | + | 79 | 0.29 | 8.02 | 3.0 | 20.9 | 92.1 | 99 |
| **37** | H | $C(CH_3)_3$ | $C_{11}H_{17}ClN_2O_2S_3$ (C, H, N) | 292–3 | – | 83 | 0.84 | 8.28 | 10 | 36.8 | | 70 |
| **38** | H | $(CH_2)_4OH$ | $C_{11}H_{17}ClN_2O_3S$ (C, H, N) | 200–2 | + | 73 | 0.21 | 7.70 | 3 | | | 96 |
| **39** | H | $(CH_2)_2OH$ | $C_9H_{13}ClN_2O_3S_3$ (C, H, N) | 209–10 | + | 92 | 0.19 | 7.18 | 6.3 | | | 95 |
| **40** | H | $CH_2$–  | $C_{11}H_{15}ClN_2O_2S_3$ (C, H, N) | 264–5 | – | 73 | 2.5 | 7.72 | 3.3 | 6.7 | | 97 |
| **41** | H | $CHCH(OH)CH_2(OH)$ | $C_{10}H_{15}ClN_2O_4S_3$ (C, H, N) | 284–6 | | 66 | | 6.95 | 8.4 | | | 82 |
| **42** | H | $CH_2CH_2OCH_3$ | $C_{10}H_{15}ClN_2O_3S_3$ (C, H, N) | 223–4 | + | 82 | 2.11 | 7.0 | 5.0 | 3.9 | 88 | 100 |
| **43** | H | $(CH_2)_3OCH_3$ | $C_{11}H_{17}ClN_2O_3S_3$ (C, H, N) | 230–2 | + | 91 | 1.6 | 7.52 | 4.9 | | 85.4 | (72) |
| **44** | H | $(CH_2)_3O(CH_2)_2OCH_3$ | $C_{13}H_{21}ClN_2O_4S_3$ (C, H, N) | 154–5<br>244–5 | + | 82 | .73 | 7.4 | 6.5 | | 87.5 | (89) |
| **45** | H | $CH_2CF_3$ | $C_9H_{10}ClF_3N_2O_2S_3$ (C, H, N) | 230–2 | | 6 | | 3.75 | 2.2 | | 21.1 | |
| **46** | H | H | $C_7H_8N_2O_2S_2 \cdot C_2HF_3O_2$ (FAB-MS) | | | 8 | | 7.30 | 14 | | 92 | |
| **47** | H | $CH_2CH_2SCH_3$ | $C_{10}H_{15}ClN_2O_2S_4$ (C, H, N) | 224–5 | + | 48 | 15.2 | 6.85 | 4.0 | 1.92 | 92.7 | 97 |
| **48** | H | $CH_2CH_2F$ | $C_9H_{12}ClFN_3O_2S_3 \cdot {}^1/_2H_2O$ (C, H, N) | 221–2 | – | 95 | 5.04 | 6.40 | 2.7 | 1.98 | 81.9 | (94) |
| **49** | H | $CH_2CH_2S(O)CH_3$ | $C_{10}H_{15}ClN_2O_3S_4$ (C, H, N) | 110–2 | + | 85 | 0.36 | 5.58 | 8.4 | 5.5 | 67.5 | 62 |
| **50** | H | $CH_2CH_2SO_2CH_3$ | $C_{10}H_{15}ClN_2O_4S_4$ | 252–3 | – | 83 | .55 | 5.20 | 4.5 | 4.8 | 56.8 | (65) |
| **51** | |  | $C_{11}H_{15}ClN_2O_3S_3$ (C, H, N) | 260–2 | – | 26 | 14.8 | 5.25 | 3.0 | 1.1 | 42.9 | 96 |
| **52** | |  | $C_{11}H_{15}ClN_2O_2S_4$ (C, H, N) | 248–50 | – | 71 | 13.7 | 5.05 | 2.6 | 0.44 | 69.9 | (57) |
| **53** | |  | $C_{11}H_{15}N_2O_3S_4 \cdot C_2HF_3O_2$ (C, H, N) | 213–5 | – | 25 | 1.29 | 4.23 | 4.0 | 3.4 | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 54 | $CH_2CH_2OCH_3$ | $CH_2CH_2OCH_3$ | $C_{13}H_{21}ClN_2O_4S_3$ (C, H, N) | 155–7 190–2 | + | 33 | 37.8 | 5.34 | 4.2 | 1.34 | 50 | 92 |
| 55 | $CH_2CH_2OCH_2CH_2OCH_3$ | $CH_2CH_2OCH_3$ | $C_{15}H_{25}ClN_2O_5S_3$ (C, H, N) | 147–8 | + | 36 | 13.9 | 5.55 | 2.8 | 1.58 | 38.5 | (85) |
| 56 | $CH_2CH_2OH$ | $CH_2CH_2OH$ | $C_{11}H_{17}ClN_2O_4S_3$ (C, H, N) | 176–8 | + | 36 | 1.7 | 5.50 | 2.27 | 2.6 | 71.8 | 83 |
| 57 | $CHCOHCH_3$ | $CHCHOHCH_3$ | $C_{13}H_{21}ClN_2O_4S_3$ (C, H, N) | glass | + | 45 | 14.8 | 5.75 | 2.71 | 1.49 | 49.7 | 91 |
| 58 | $CH_2CH_2OCH_2CH_2OCH_3$ | $CH_2CH_2OCH_2CH_2OCH_3$ | $C_{17}H_{29}ClN_2O_6S_3 \cdot {}^1/_2H_2O$ (C, H, N) | 99–100 | + | 34 | 9.81 | 5.72 | 3.95 | 2.77 | 40.3 | 77 |

[a] Analyses for C, H, and N are within ±0.4% of calculated values for the indicated empirical formulas. [b] Solubility at pH 7.4, in 50 mM phosphate buffer. [c] Partition coefficients were determined by equilibrating each test compound between 1-octanol and 0.1 ionic strength pH 7.4 buffer (ref 6a). [d] The half-neutralization point ($pK_a$) was determined as described in ref 6a. [e] In vitro inhibition of ($IC_{50}$) and binding (CA binding) to human carbonic anhydrase II, see ref 6a. [f] The binding of each test compound to bovine iris and ciliary body was determined as described in ref 28. [g] The ability of each test compound to inhibit albino rabbit iris and ciliary body carbonic anhydrase post topical instillation was assessed ex vivo as described in ref 12.

**Table II**




| compd | $R_1$ | $R_2$ | analysis[a] | mp, °C | solubility[b] (2% sol, pH 5.2) | percent yield | partition coeff[c] | $pK_a$[d] | CAI,[e] $I_{50} \times 10^{-9}$ M | CA binding,[e] $K_i \times 10^{-9}$ M | pigment binding,[f] % | ex vivo CA inhib,[g] 0.5% (0.1%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 59 | $CH_2CH(CH_3)_2$ | H | $C_{11}H_{17}ClN_2O_2S_3$ (C, H, N) | 257–9 | – | 79 | 17.4 | 7.60 | 2.8 | 2.7 | 86.5 | (87) |
| 60 | $CH_3$ | H | $C_8H_{11}ClN_2O_2S_3$ (C, H, N) | 249–50 | + | 83 | 0.32 | 7.65 | 3.2 | 11.8 | 87.3 | (98) |
| 61 | $CH_2CH_2OCH_3$ | H | $C_{10}H_{15}ClN_2O_3S_3$ (C, H, N) | 224–5 | + | 67 | 3.23 | 6.65 | 2.8 | 3.1 | 81.9 | (92) |
| 62 | $CH_2CH_2OCH_3$ | $CH_2CH_2OCH_3$ | $C_{13}H_{31}ClN_2O_4S_3$ (C, H, N) | 138–40 | + | 40 | 36 | 5.48 | 2.5 | 0.95 | 41.4 | 71 |

[a] Analyses for C, H, and N are within ±0.4% of calculated values for the indicated empirical formulas. [b] Solubility at pH 7.4, in 50 mM phosphate buffer. [c] Partition coefficients were determined by equilibrating each test compound between 1-octanol and 0.1 ionic strength pH 7.4 buffer (ref 6a). [d] The half-neutralization point ($pK_a$) was determined as described in ref 6a. [e] In vitro inhibition of ($IC_{50}$) and binding (CA binding) to human carbonic anhydrase II; see ref 6a. [f] The binding of each test compound to bovine iris and ciliary body was determined as described in ref 28. [g] The ability of each test compound to inhibit albino rabbit iris and ciliary body carbonic anhydrase post topical instillation was assessed ex vivo as described in ref 12.

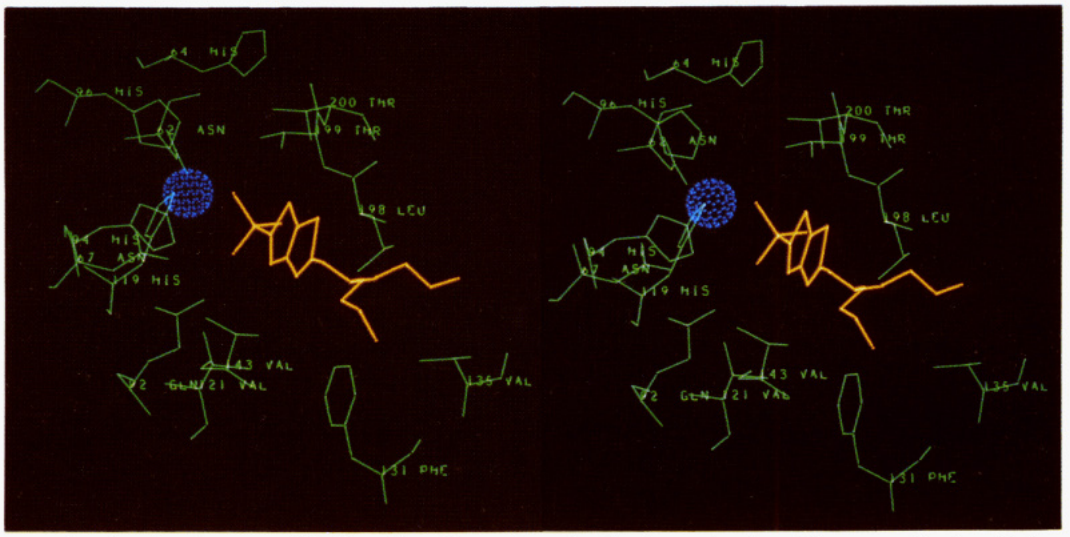



**Figure 1.** A stereo drawing showing one of the bound conformations of **54** in the active site of human carbonic anhydrase II (HCA-II). Compound **54** is in orange, the important amino acid residues lining the enzyme active site are in green, and the zinc atom is highlighted in blue. Crystals of the complex of **54** with HCA-II were formed at 4 °C in 50 mM Tris-HCl at pH 8.5 with 150 mM NaCl, 3 mM $NaN_3$, and 1 mM $CH_3HgCl$ in microdialysis buttons with 50–58% saturated ammonium sulfate used as the precipitant.[29] Before data collection, the crystal was backsoaked with 10 mM cysteine for 2 days to remove the mercurial. The crystal had space group symmetry $p2_1$ with cell constants of $a$ = 42.80, $b$ = 41.70, $c$ = 72.91 Å and $\beta$ = 104.61°. A total of 27 815 symmetry independent reflections to 1.6 Å were measured with $I > \sigma(I)$. Difference electron density maps were generated with native coordinates of CA-II that had 10 waters removed from the active-site cavity.[30] A model of **54** was generated[31] and fit into the difference maps.[32] Several rounds of constrained least-squares refinement[33] followed by difference map inspection and additional water molecule placement were used to define the structure. The present crystallographic residual value is 0.174.

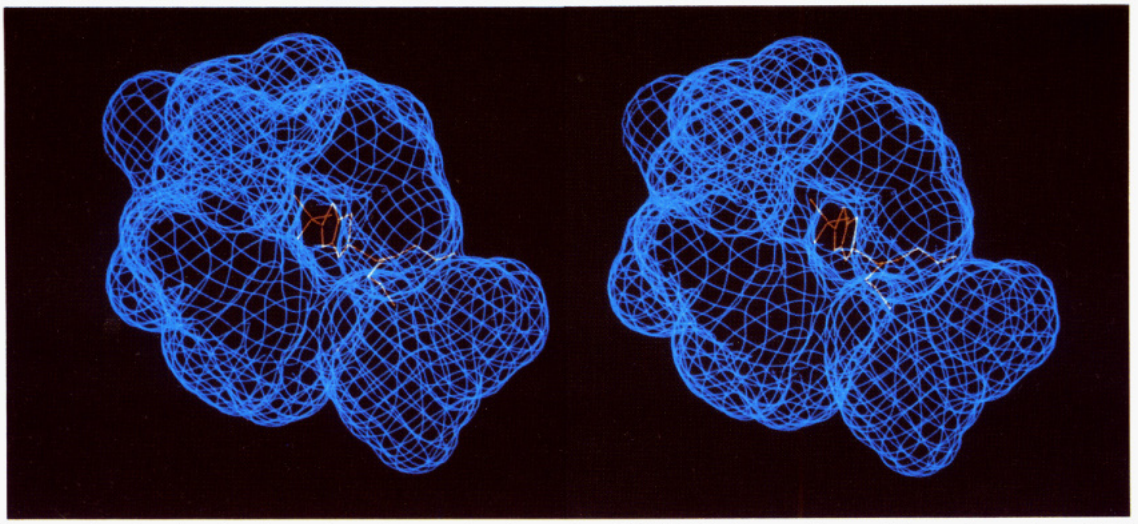


**Figure 2.** A stereo drawing showing the surface of the active site cavity of HCA-II in blue and one of the conformations of **54** in orange.

a $K_i$ of 6.54 nM for compound **65** clearly shows that the objective of enhanced activity was not achieved with this one example.

The amide compounds depicted in Table IV proved to be good inhibitors of carbonic anhydrase function. However, without a basic amine, they lacked appreciable aqueous solubility. When a basic amino function was appended and the desired solubility was obtained, the compounds displayed high pigment binding values. Further, when the basicity of the appended amine was reduced (compounds **72** and **73**), the pigment binding values remained high and the aqueous solubility decreased markedly.

Table V summarizes the comparative in vivo testing of the best compounds in the series. The best of this group is compound **55** which has about the same activity as MK-927[6b] in a head-to-head comparison in normotensive pigmented rabbits. Significantly, compound **55** retains good activity in this assay suggesting that pigment binding has been reduced to an appropriate level. This compound is quite potent and therefore was tested in hypertensive (laser-treated)[20] and normotensive cynomologus monkeys where it was found to maximally lower IOP by −8.0 mmHg[22] in the hypertensive eye and −2.7 mmHg[22] in the normotensive eye.

The verity of our modeling[23] vida supra with these compounds is shown in the 3D depiction of the single-crystal X-ray analysis of compound **54** cocrystalized with

(22) These values are quite similar to the values obtained for MK-927 in the same test.

(23) Modeling here refers to the computer driven docking of the proposed molecule with the active site of CAII generated from the crystal structure of the enzyme. Fit of the docked molecule (inhibitor) and productive binding was estimated by relative energies calculated from the interaction of the molecule with the amino acid residues defining the active site.

(24) Sugrue, M. F.; Mallorga, P.; Schwam, H.; Baldwin, J. J.; Ponticello, G. S. *Cur. Eye Res.*, in press.

Table III



| compd | $R_1$ | $R_2$ | $R_3$ | analysis[a] | mp, °C | solubility[b] (2% sol, pH 5.2) | percent yield | partition coeff[c] | $pK_a$[d] | CAI,[e] $I_{50} \times 10^{-9}$ M | CA binding,[e] $K_i \times 10^{-9}$ M | pigment binding,[c] % | ex vivo CA inhib,[g] 0.5 (0.1%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **63** | $CH_2CH_2OCH_2CH_2OCH_3$ | H | $CH_3$ | $C_{13}H_{31}ClN_2O_4S_3$ (C, H, N) | amorph | + | 47 | 5.1 | 7.13 | 0.7 | 3.05 | 71.9 | 89 |
| **64** | $CH_2CH_2OCH_2CH_2CH_2OCH_3$ | $CH_2CH_2OCH_3$ | $CH_3$ | $C_{16}H_{37}ClN_2O_5S_3$[h] (C, H, N) | amorph | | 23 | | | 0.5 | 1.54 | | |
| **65** | $CH_2CH_2OCH_2CH_2CH_2OCH_3$ | $CH_2CH_2OCH_3$ | $CH_2$–(4-F-phenyl) | $C_{24}H_{30}F_4N_2O_7S_3$[i] (C, H, N) | glass | | | | | 1.15 | 6.54 | | |

[a] Analyses for C, H, and N are within ±0.4% of calculated values for the indicated empirical formulas. [b] Solubility at pH 7.4, in 50 mM phosphate buffer. [c] Partition coefficients were determined by equilibrating each test compound between 1-octanol and 0.1 ionic strength pH 7.4 buffer (ref 6a). [d] The half-neutralization point ($pK_a$) was determined as described in ref 6a. [e] In vitro inhibition of ($IC_{50}$) and binding (CA binding) to human carbonic anhydrase II, see ref 6a. [f] The binding of each test compound to bovine iris and ciliary body was determined as described inref 28. [g] The ability of each test compound to inhibit albino rabbit iris and ciliary body carbonic anhydrase post topical instillation was assessed ex vivo as described in ref 12.

enzyme (Figures 1 and 2). The sulfonamide is coordinated to the Zn at the active site and the thienothiophene ring interacts with the walls of the cavity. The amine is unprotonated and its ether substituents are involved in productive binding along the aliphatic trough in the lower half of the cavity of the active site. The sulfonamide and aromatic ring system of **54** are bound to the active site in a similar manner to previously characterized inhibitors.[6b] However, difference electron density maps revealed that the thienothiophene ring system is disordered. This disorder is satisfactorily explained by two equally populated conformations differing by a 180° rotation in the dihedral angle between the sulfamide and the thienothiophene ring system. This arrangement causes the two populations of the aromatic ring system to be superimposed and was included in the crystallographic refinement. No gross movements of the amino side chains of the enzyme were noted upon binding of **54** except for the movement of His64 in a similar manner to that seen for previous inhibitors.[6b] Presumably, this movement is mediated by water molecule rearrangement since there are no direct interactions between **54** and His64. Preliminary molecular modeling (docking) of this compound in the active site (generated by computer display of X-ray crystal structure of native carbonic anhydrase II) prior to the actual synthesis showed substantially the same thing.

## Conclusions

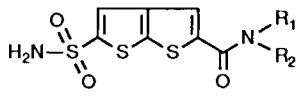
An extensive structure activity investigation of 5-substituted thieno[2,3-*b*]thiophene-2-sulfonamides and 5-substituted thieno[3,2-*b*]thiophene-2-sulfonamides has led to the identification of a single outstanding compound, **55**.

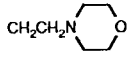
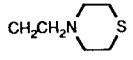
## Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. $^1H$ NMR spectra were recorded on a EM-390, XL-300, or NT 360 spectrometer. Chemical shifts are reported in parts per million relative to $Me_4Si$ as the internal standard. Elemental analysis for carbon, hydrogen, and nitrogen were determined with a Perkin-Elmer Model 240 elemental analyzer and are within ±0.4% of the theoretical values unless noted otherwise. All starting materials were commercially available and used as received unless so indicated. X-ray diffraction data were collected by using a Siemans multiwire area detector with Cu K$\alpha$ radiation ($\lambda$ = 1.5418 Å) generated from a Rigaku Ru-200 rotating anode.

**Methyl [[3-(2-Dioxolanyl)thiophene-2-yl]thio]acetate (2).** Thiophene-2-carboxaldehyde ethylene acetal (15.62 g, 0.1 mol) was dissolved in dry THF (200 mL) in an inert atmosphere and cooled to −74 °C. *n*-Butyllithium (44 mL of a 2.3 M solution in hexane; 0.1 mol) was added at a rapid drip rate over 20 min (the temperature rose to −68 °C). After the addition was complete, the mixture was stirred at −60 to −74 °C for 35 min. After about 5 min, the lithium derivative began to crystallize. Most crystallized after 35 min. Sulfur (as a fine powder; 3.21 g, 0.1 mol) in 1- and 2.21-g portions was added 5 min apart (temperature rose from −74 to −65 °C). The reaction was stirred at −74 °C for 15 min and slowly warmed with stirring to −50 °C where it was held for 15 min and then warmed to −38 °C over 15 min. Methyl bromoacetate (9.9 mL, 16.1 g, 0.105 mol) was added at a slow drip rate over a period of 8 min (temperature rose from −40 to −25 °C) and the reaction mixture was allowed to stir at ambient temperature until the temperature rose to 0 °C. After warming to 24 °C the reaction mixture was stirred at room temperature for 2 h. The reaction was worked up by evaporating the solvent in vacuo and partitioning the residue between ether (250 mL) and water (100 mL). The ether was extracted with water (3 × 25 mL), dried ($MgSO_4$), and filtered, and the solvent evaporated in vacuo to leave 23.36 g of crude product which was used in the next step without purification: $^1H$ NMR ($CDCl_3$) $\delta$, 3.56 (2 H, s), 3.71 (3 H, s), 4.04 (2 H, m), 4.15 (2 H, m), 6.08 (1 H, s), 7.16 (1 H, d, $J$ = 6 Hz), 7.37 (1 H, d, $J$ = 6 Hz). Theoretical mass for $C_{10}H_{12}O_4S_2$ 260.017 715 0; found 260.017 715 0.

**Table IV**



| compd | $R_1$ | $R_2$ | analysis[a] | mp, °C | solubility[b] (2% sol, pH 5.2) | percent yield | partition coeff[c] | $pK_a$[d] | CAI,[e] $I_{50} \times 10^{-9}$ M | CA binding,[e] $K_1 \times 10^{-9}$ M | pigment binding,[f] % | ex vivo CA inhib,[g] 0.5% (0.1%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **66** | $CH_2CH_2N(CH_3)_2$ | H | $C_{11}H_{16}ClN_3O_3S_3$ (C, H, N) | 254–5 | + | 62 | 0.40 | 7.48 | 8.8 | 11 | 97 | 82 |
| **67** | $CH_3$ | H | $C_8H_8N_2O_3S$ (C, H, N) | 172–3 | – | 27 | | | 5.2 | | | 40 |
| **68** | $CH_2CH_2S(O)CH_3$ | H | $C_{10}H_{12}N_2O_4S_4$ (C, H, N) | 242–3 | – | 71 | 0.70 | | 4.6 | 2.39 | 35.6 | (39) |
| **69** | $CH_2CH(OH)CH_2OH$ | H | $C_{10}H_{12}N_2O_5S_3$ (C, H, N) | 217–8 | – | 64 | 0.45 | | 3.2 | 3.4 | 36.7 | (18) |
| **70** | $CH_2CH_2OH$ | $CH_2CH_2OH$ | $C_{11}H_{14}N_2O_5S_3$ (C, H, N) | 155–6 | – | 11 | | | 6.6 | 9.0 | | |
| **71** | $CH_2CH_2N(CH_2CH_2OCH_3)_2$ | H | $C_{15}H_{24}ClN_3O_5S_3$ (C, H, N) | 75–80 | – | 15 | | | 2.5 | | 87.5 | |
| **72** |  | H | $C_{13}H_{18}ClN_3O_4S_3$ (C, H, N) | 267–8 | – | 70 | 3.86 | 5.80 | 2.9 | 6.38 | 91.6 | (22) |
| **73** |  | | $C_{13}H_{18}ClN_3O_3S_4$ (C, H, N) | 214–5 | – | | 31.8 | 6.03 | 2.9 | 4.02 | 96.4 | 78 |

[a] Analyses for C, H, and N are within ±0.4% of calculated values for the indicated empirical formulas. [b] Solubility at pH 7.4, in 50 mM phosphate buffer. [c] Partition coefficients were determined by equilibrating each test compound between 1-octanol and 0.1 ionic strength pH 7.4 buffer (ref 6a). [d] The half-neutralization point ($pK_a$) was determined as described in ref 6a. [e] In vitro inhibition of ($IC_{50}$) and binding (CA binding) to human carbonic anhydrase II; see ref 6a. [f] The binding of each test compound to bovine iris and ciliary body was determined as described in ref 28. [g] The ability of each test compound to inhibit albino rabbit iris and ciliary body carbonic anhydrase post topical instillation was assessed ex vivo as described in ref 12.

**Table V.** Comparative in Vivo Testing

| compd | normotensive albino rabbits: IOP, mmHg | normotensive pigmented rabbits: IOP, mmHg[a,c] | comparison with MK-927 | hypertensive pigmented rabbits: IOP, mmHg[a,c] | comparison |
|---|---|---|---|---|---|
| **5** | −3.0 (1) | | | | |
| **6** | −1.3 (1) | −1.6 (2) | | | |
| **2** | −1.4 (1) | −1.6 (3) | −3.9 (4) | −2.1 (1) | −4.8 (3)[b] |
| **54** | −2.6 (2) | −4.1 (5) | −4.6 (5) | | |
| **55** | −3.3 (5) | −4.8 (3) | −5.3 (3) | −4.4 (5) | |

[a] Number of significant points is in parentheses. [b] MK-927.[6b] [c] Intraocular pressure studies in normotensive albino and pigmented rabbits, hypertensive pigmented rabbits, and normotensive and hypertensive cynomologus monkeys were conducted as described in ref 12.

**Methyl [(3-Formylthiophene-2-yl)thio]acetate (3).** To a solution of crude methyl [[3-(2-dioxolanyl)thiophene-2-yl]thio]-acetate (23.36 g) in acetone (100 mL) was added *p*-toluenesulfonic acid (0.25 g) and the reaction mixture was stirred at room temperature for 1 h. A saturated aqueous solution of sodium bicarbonate (4 mL) was added with stirring followed by the addition of water (50 mL). After the mixture was stirred for 10 min, the acetone was evaporated in vacuo to leave a gum and water. The gum was dissolved in ether (500 mL) and extracted with water (4 × 50 mL), dried ($MgSO_4$), and filtered, and the solvent was evaporated in vacuo to leave 19.77 g of oily methyl [(3-formylthiophene-2-yl)thio]acetate which was used in the next step without purification.

**Methyl Thieno[2,3-*b*]thiophene-2-carboxylate (4).** To a solution of crude methyl [(3-formylthiophene-2-yl)thio]acetate (19.31 g, 91 mmol) in methanol (150 mL) was added 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) (1 mL, 1 g, 8.05 mmol) and the stirred reaction flask was immediately immersed in an ice–water bath. Stirring was continued for 30 min and the mixture was filtered to give a tacky solid product which was washed with a little cold (−20 °C) methanol. The solvent was evaporated in vacuo from the mother liquor and the residue was partitioned between ether and aqueous sodium bicarbonate. The water layer was washed with ether twice, and the combined ether extracts were dried ($MgSO_4$) and filtered, and the solvent evaporated in vacuo to give an oil which solidified when triturated with a little cold (−20 °C) methanol. This was combined with the original solid product to give a total of 14.55 g of methyl thieno[2,3-*b*]-thiophene-2-carboxylate, mp 101–105 °C. Recrystallization from methanol gave product with mp 106–107 °C: $^1H$ NMR ($CDCl_3$) $\delta$ 3.93 (3 H, s), 7.28 (1 H, d, $J$ = 5.37 Hz), 7.42 (1 H, d, $J$ = 5.37 Hz), 7.96 (1 H, s). Anal. ($C_8H_6O_2S_2$) C, H.

**2-(Hydroxymethyl)thieno[2,3-*b*]thiophene (5).** A solution of methyl thieno[2,3-*b*]thiophene-2-carboxylate (19.8 g, 0.1 mol) in dry ether (75 mL) was added at a rapid drip rate (1.75 h) to a suspension of lithium aluminum hydride (7.59 g, 0.2 mol in ether (500 mL) cooled in an ice–water bath. During the addition precipitated material on the inside of the flask was kept suspended in the reaction medium. After the addition was complete stirring was continued at room temperature for 3 h. The reaction mixture was cooled in an ice–water bath and there was added in succession, slowly, dropwise with vigorous stirring: water (7.6 mL), 20% aqueous NaOH (22.8 mL), water (7.6 mL), 20% NaOH (12 mL), and water (4 mL). Vigorous stirring was continued until a granular precipitate was obtained. The ether was decanted and the solids were washed by decantation three times with ether. The combined ether fractions were dried ($MgSO_4$), filtered, and evaporated in vacuo to leave 16.33 g of white, solid product, mp 85–87 °C. Recrystallization from hexane gave material with mp 86–87 °C: $^1H$ NMR ($CDCl_3$) $\delta$ 4.86 (2 H, d, $J$ = 2.69 Hz), 7.15–7.19 (2 H, m), 7.32 (1 H, d, $J$ = 5.23 Hz). Anal. ($C_7H_6OS_2$) C, H.

**Thieno[2,3-*b*]thiophene-2-carboxaldehyde (6).** 2-(Hydroxymethyl)thieno[2,3-*b*]thiophene (16.33 g, 95.9 mmol) dissolved in methylene chloride (165 mL) was added all at once to a stirred suspension of pyridinium chlorochromate (31.0 g, 143.9 mmol) in methylene chloride (172 mL) and stirring was continued at ambient temperature for 2 h. The mixture was diluted with ether (288 mL) and the supernatant was decanted. The solids were washed three times by trituration with ether. The combined ether extracts were filtered through a 60 × 150 mm silica gel (230–400 mesh) column under pressure and followed with three portions of ether. The ether phase of the combined filtrates was evaporated in vacuo to give 13.86 g of thieno[2,3-*b*]thiophene-2-carboxaldehyde, mp 43–45 °C. Sublimation at 108 °C bath temperature and 0.5 mm pressure gave 13.10 g: mp 47–48 °C; $^1H$ NMR ($CDCl_2$) $\delta$ 7.34 (1 H, d, $J$ = 5.37 Hz), 7.46 (1 H, d, $J$ = 5.37 Hz), 7.91 (1 H, s), 9.94 (1 H, s). Anal. ($C_7H_4OS_2$) C, H.

**2-(2-Dioxolanyl)thieno[2,3-*b*]thiophene (7).** *p*-Toluenesulfonic acid (150 mg) was added to a stirred two-phase mixture of thieno[2,3-*b*]thiophene-2-carboxaldehyde (8.06 g, 47.91 mmol), ethylene glycol (21.4 g, 345 mmol), methyl orthoformate (30.5 g, 287.5 mmol), and toluene (50 mL). The reaction quickly became homogeneous and stirring was continued with the reaction immersed in an oil bath at 45–50 °C. A gentle vacuum was applied through an air condenser every 30–60 min for 5 h and the reaction was stirred at 45–50 °C overnight. The reaction was cooled in an ice–water bath and pyridine (0.5 mL) was added. The solvent and volatiles were evaporated in vacuo. The remaining oil was dissolved in ether (250 mL) and extracted with a saturated solution of sodium bicarbonate (2 × 50 mL) and then water (4 × 50 mL), dried ($MgSO_4$), and filtered, and the volume of ether reduced to about 75 mL in vacuo as the product began to crystallize. The crystals were collected and washed with a little 40% ether in hexane giving 4.08 g of white, solid 2-(2-dioxolanyl)thieno[2,3-*b*]thiophene, mp 92–93 °C. The ether from the mother liquors was further reduced in volume and then allowed to crystallize, to give another 0.76 g of pure product, mp 96–97 °C. Both fractions were homogeneous by TLC and were combined (4.84 g) for use in the next step:[25] $^1H$ NMR ($CDCl_3$) $\delta$ 4.01–4.18 (4 H, m), 6.15 (1 H, s), 7.18 (1 H, d, $J$ = 5.37 Hz), 7.31 (1 H, s), 7.32 (1 H, d, $J$ = 5.37 Hz). Anal. ($C_9H_8O_2S_2$) C, H.

**5-(2-Dioxolanyl)thieno[2,3-*b*]thiophene-2-sulfonamide (8).** To a cooled mixture of 2-(2-dioxolanyl)thieno[2,3-*b*]thiophene (2.12 g, 10 mmol) in dry THF (20 mL) in a nitrogen atmosphere was added butyllithium (4.4 mL of a 2.3 M solution in hexane; 10 mmol) dropwise by syringe over a period of 30 min with magnetic stirring. Stirring was continued at −75 °C for 30 min. A rapid stream of dry gaseous $SO_2$ was directed at the surface of the stirred mixture at −75 °C. The internal reaction temperature rose to −40 °C then cooled to −75 °C again. The $SO_2$ stream was continued for 30 min and then the reaction was allowed to warm to 10 °C while stirring with use of the $SO_2$ stream. The excess $SO_2$ and solvent were then evaporated in vacuo to leave 7.09 g of lithium sulfonic acid salt. The salt was dissolved in a saturated solution of sodium bicarbonate (15 mL) and cooled in an ice–water bath. *N*-Chlorosuccinimide (2.00 g, 15 mmol) was added in small portions over a 15-min period. The reaction was stirred in an ice–water bath for 1 h and was then extracted with chloroform three times. The combined extracts were dried ($MgSO_4$) and filtered, and the solvent was removed in vacuo to leave 2.73 g of sulfonyl chloride. This intermediate was dissolved in acetone (5 mL) and added dropwise over 30 min to an ice-cold concentrated ammonium hydroxide solution (15 mL), which was then stirred at ice bath temperature for 1.5 h. The acetone and ammonia were removed in vacuo, leaving a suspension of crystalline product in water. The crystals were collected, washed with water, and dried in a vacuum oven at room temperature with a slow stream of air through the oven to give 1.76 g of crude crystalline product. Recrystallization from nitromethane (Aldrich-Gold label) gave 1.43 g of product, which partially melted at 220 °C, resolidified, and melted >320 °C: $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.98 (2 H, m), 4.06 (2 H, m), 6.09 (1 H, s), 7.50 (1 H, s), 7.73 (s H, s). Anal. ($C_9H_9NO_4S_3$) C, H.

**5-Formylthieno[2,3-*b*]thiophene-2-sulfonamide (9).** 5-(2-Dioxolanyl)thieno[2,3-*b*]thiophene-2-sulfonamide (2.59 g, 8.89 mmol) was suspended in acetone and *p*-toluenesulfonic acid (2.50 g, 13.1 mmol) was added. The mixture was stirred at room temperature for 1 h at which time a solution was obtained. Water (2.0 mL) was added and stirring continued for 3 h. A saturated solution of sodium bicarbonate (30 mL) was added dropwise, followed by the slow addition of water (40 mL). The resulting solution or mixture was seeded with a few crystals of product and the acetone was evaporated in vacuo. The crystals were collected, washed with water and dried in vacuo to give 2.10 g of product, mp 188–189 °C. This product was used in the next step without further purification: $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.86 (2 H, s), 7.91 (1 H, s), 8.29 (1 H, s), 9.95 (1 H, s). Anal. ($C_7H_5NO_3S_3$) C, H.

**Method A (for Secondary Amines). 5-[(Isobutylimino)-methyl]thieno[2,3-*b*]thiophene-2-sulfonamide (10a).** 5-Formylthieno[2,3-*b*]thiophene-2-sulfonamide (1.48 g, 6 mmol) was suspended in methanol (15 mL) and isobutylamine (4.2 mL, 3.07 g, 42 mmol) was added all at once. After solution was obtained, methanolic HCl (1.8 mL of a 6.70 M solution of HCl in methanol) was added rapidly. The resulting mixture was stoppered and heated to near reflux for 2–5 min and then allowed to stand at room temperature until the product crystallized. After cooling, the crystals were collected and washed with a little cold (−20 °C)

(25) This reaction proceeds through the intermediate thieno[2,3-*b*]thiophene-2-carboxaldehyde dimethyl acetal which was not isolated.

methanol. The crystals were dried in vacuo to give 1.31 g of product: mp 204–206 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.90 (6 H, d, $J$ = 6.54 Hz), 1.87 (1 H, m), 3.35 (2 H, d, $J$ = 6.35), 7.71 (1 H, s), 7.79 (3 H, s), 8.45 (1 H, s).

**5-[(Isobutylamino)methyl]thieno[2,3-*b*]thiophene-2-sulfonamide.** 5-[(Isobutylimino)methyl]thieno[2,3-*b*]-thiophene-2-sulfonamide (1.30 g, 4.30 mmol) was dissolved in methanol (15 mL) and THF (15 mL) and the solution was cooled in an ice–water bath. To this cooled solution was added sodium borohydride 95 mg (2.5 mmol) three times at 30-min intervals with 30 min of stirring after the last addition. Water was added and the mixture was stirred at room temperature for 1 h. The methanol and THF were evaporated in vacuo, and the resulting crystalline mass was collected and washed twice with water by trituration. The product was dried in vacuo at room temperature to give 1.20 g of product which was used in the next step without further purification.

**5-[(Isobutylamino)methyl]thieno[2,3-*b*]thiophene-2-sulfonamide Hydrochloride (35).** 5-[(Isobutylamino)-methyl]thieno[2,3-*b*]thiophene-2-sulfonamide (0.95 g, 3.12 mol) was dissolved in 58 mL of absolute ethanol and filtered. Ethanolic HCl (0.80 mL as a 5.10 M solution of HCl in ethanol; 4.08 mmol) was added and the reaction swirled and then allowed to stand. The resulting crystals were collected, washed with absolute ethanol twice, then washed with ether twice, and dried in vacuo to give 0.98 g of product: mp 251–252 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.93 (6 H, d, $J$ = 6.51 Hz) 2.01 (1 H, m), 2.73 (2 H, m), 4.41 (2 H, m), 7.61 (1 H, s), 7.78–7.79 (3 H, m), 9.31 (2 H, m). Anal. ($C_{11}H_{17}ClN_2O_2S_3$) C, H.

**Method B (for Secondary Amines). 5-[(*tert*-Butylamino)methyl]thieno[2,3-*b*]thiophene-2-sulfonamide.** *tert*-Butylamine (0.736 mL, 0.512 g, 7 mmol) was added to a suspension of 5-formylthieno[2,3-*b*]thiophene (0.247 g, 1 mmol) in methanol (2.5 mL). The mixture was warmed gently to obtain solution. Methanolic HCl (0.30 mL of a 6.70 M solution of HCl in methanol; 2 mmol) was added and the mixture was warmed with stirring to 50 °C and stirred at room temperature for 1 h. THF (2.5 mL) was added to effect solution, and the mixture was cooled in an ice–water bath. Sodium borohydride (0.183 g, 4.84 mmol) was added all at once as stirring was continued for 30 min, when TLC (10% methanol in chloroform saturated with ammonia and water; silica gel) showed the reaction to be complete. The product was worked up by adding water (50 mL) and 1 N HCl to pH 9. The crystalline product was collected, washed with water, and dried in vacuo to give 0.25 g of product: mp 208–209 °C dec; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.07 (9 H, s), 3.90 (2 H, s), 7.18 (1 H, s), 7.63 (1 H, s), 7.66 (2 H, bs).

**Method $B_1$ (for Highly Polar Secondary Amines).** Method A was followed with the following changes in workup. Upon addition of water and evaporation of organic solvents in vacuo a small amount of precipitate was obtained which was filtered off. The pH of the clear resulting solution was adjusted to 8.5 with diluted aqueous sodium hydroxide solution and nearly all of the water was evaporated in vacuo. The resulting gum was extracted by shaking and decanting with ethyl acetate 10 times or until no more UV positive material was extracted. The combined ethyl acetate extracts were dried ($MgSO_4$) and filtered and the solvent evaporated, leaving the desired amine.

**Method C (for Tertiary Amines). 5-[[(Methoxyethyl)-[(methoxyethyl)ethyl]amino]methyl]thieno[2,3-*b*]-thiophene-2-sulfonamide.** (Methoxyethyl)[(methoxyethoxy)-ethyl]amine[26a] (36.0 g, 203 mmol) was dissolved in methanol (112 mL). To this solution was added methanolic HCl (5.54 mL, MeOH) (28.2 mL, 157 mmol). The mixture was stirred for 10 min and then 5-formylthieno[2,3-*b*]thiophene-2-sulfonamide (8.80 g, 35.6 mmol) was added, and the mixture was allowed to stir for 24 h, stoppered under Argon. To the mixture was added 4 Å sieves (23.7 g), and the mixture was allowed to stir for 6 days. Sodium cyanoborohydride (4.47 g, 71.2 mmol) was added, and the mixture was stirred under argon for 48 h. Methanol (250 mL) and THF (250 mL) were added to dissolve all of the product. The mixture was filtered through a pad of Celite and then all of solvent was evaporated in vacuo. $H_2O$ (100 mL) was added and then slowly concentrated HCl (100 mL) was added. The product was decanted and filtered from the solid. (Save the acid solution of products.) More product was recovered from insoluble material by dissolving it in THF (100 mL) and concentrated HCl (25 mL) was added and the solution heated to reflux three times in a 15-min interval. Water (100 mL) was added and the THF evaporated. The clear solution was decanted from the insoluble material. To the combined decanted solutions and saved acidic water solution of product was added concentrated $NH_4OH$ until pH was 8.5 (~125 mL). The solution was extracted five times with ether (150 mL each). The combined extracts were dried ($MgSO_4$). The solvent was filtered and evaporated in vacuo to leave 10.16 g of crude product. This was chromatographed on 2.4 kg of silicon gel with 3% methanol in chloroform as eluent to give 7.7 g of highly pure product, which was used directly in the next step.

**5-[[(Methoxyethyl)[(methoxyethoxy)ethyl]amino]-methyl]thieno[2,3-*b*]thiophene-2-sulfonamide Hydrochloride (55).** 5-[[[(Methoxyethoxy)ethyl]amino]methyl]thieno[2,3-*b*]-thiophene-2-sulfonamide (6.42 g, 15.71 mmol) was dissolved in 2-propanol (550 mL) and filtered and methanolic HCl (5.54 M) (3.00 mL; 16.6 mmol) was added, swirled, and allowed to stand for 15 min. The mixture was boiled down to 200 mL and seeded. On cooling most of the product oiled out. A crust of crystals formed and was broken up by trituration. The remainder was allowed to crystallize. A total of 5.64 g of pure product was collected: mp 147–148 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.26 (3 H, s), 3.31 (3 H, s), 3.26–3.39 (4 H, m), 3.46–3.52 (2 H, m), 3.55–3.62 (2 H, m), 3.70–3.90 (4 H, m), 4.70 (2 H, m), 7.69 (1 H, s), 7.79 (2 H, m), 7.80 (1 H, s). Anal. ($C_{15}H_{25}ClN_2O_5S_3$) C, H.

**(Methoxyethoxy)ethyl *p*-Toluenesulfonate.** (Methoxyethoxy)ethanol (65.4 mL, 0.50 mol) was dissolved in dry ether (500 mL). To this solution was added powdered potassium hydroxide (28 g, 0.50 mol). *p*-Toluenesulfonyl chloride (95.3 g, 0.50 mol) was added. Quickly a copius precipitate formed and the mixture was stirred under nitrogen for 4 h. The incomplete reaction was filtered and charged with powdered potassium hydroxide (28 g, 0.50 mol) and 5 mL more of the alcohol and stirred under nitrogen for 2 h when TLC (30% EtOAc–hexanes) showed the reaction complete. The reaction was filtered and extracted with water (2 × 30 mL), dried ($MgSO_4$), and filtered, and the solvent was evaporated in vacuo to leave 127.8 g (93%) of the product which was used in the next step without purification.

**(Methoxyethoxy)ethyl Iodide.** (Methoxyethoxy)ethyl *p*-toluenesulfonate (127.8 g, 0.466 mol) was dissolved in acetone (1.5 mL) and sodium iodide (250 g, 1 mol) was added. The reaction mixture was stirred at room temperature under nitrogen in the dark for 24 h when TLC (30% EtOAc–hexane) showed the reaction complete. The reaction was filtered and most of the acetone evaporated in vacuo. The residue was dissolved in ether (500 mL) and water (100 mL). The ether was extracted with water (2 × 50 mL) containing a few crytals of sodium thiosulfate (decolorize). The ether was dried ($MgSO_4$) and filtered and the ether evaporated in vacuo to leave 107.2 g (96%) of the product which was distilled to give 86.61 g (81%): bp 107–109 °C at 10–12 mm; $^1$H NMR ($CDCl_3$) $\delta$ 3.28 (2 H, t), 3.42 (2 H, s), 3.55 (2 H, m), 3.68 (2 H, m), 3.75 (2 H, t).

**(2-Methoxyethyl)[(2-methoxyethoxy)ethyl]amine (5-Aza-2,8,11-trioxadodecane).** To a solution of (methoxyethyl)amine (101.3 g, 1.349 mol) in dry THF (80 mL) was added (methoxyethoxy)ethyl iodide (62.65 g, 0.2697 mol). There was a slow exotherm which was controlled below 45 °C with periodic cooling with an ice–water bath. The reaction mixture was stirred at room temperature for 24 h. The THF was then evaporated in vacuo, and 40% aqueous sodium hydroxide (20 mL) and water (50 mL) were added, and the product was extracted with ethyl acetate (15 × 50 mL). This solution was dried ($MgSO_4$) and filtered and the solvent evaporated in vacuo. The residue was first distilled at atmospheric pressure to remove the excess (methoxyethyl)amine (bath temperature to 260 °C). After cooling, the product was then distilled at reduced pressure to give 36 g, bp 58 °C at 0.1 mm (75%). A satisfactory elemental analysis could not be obtained because the compound was too hygroscopic: TLC

(26) (a) See following experiments after HCl salt formation for preparation. (b) Use of pyridinium acetate as a catalyst for this type of sensitive cyclization: Williams, T. M. Unpublished work.

(27) Fournari, P.; Guilard, R.; Person, M. *Bull. Soc. Chim. Fr.* **1967**, *11*, 4115.

homogeneous, $R_f$ (silica, chloroform saturated with ammonia and water) 0.36; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.66 (4 H, m) 3.23 (3 H, s), 3.24 (3 H, s), 3.36 (2 H, t), 3.4–3.52 (6 H, m). Exact mass + 1 (FAB) calculated for $C_8H_{20}NO_3$ 178.1443187, found 178.144043.

**3-Bromothiophene-2-carboxaldehyde (15).** *n*-Butyllithium dissolved in hexane (2.3 M; 44.3 mL, 0.102 mol) was added at a slow drop from a syringe to a solution of 2,3-dibromothiophene (24.2 g; 0.1 mol) in anhydrous ether (100 mL) maintained at −70 to −64 °C internal temperature. The mixture was stirred for 15 min at this temperature, followed by the addition of *N*-formylpiperidine (11.7 mL; 11.95 g, 0.105 mol) at a slow drip over approximately 20 min, maintaining a reaction temperature of −70 °C. Stirring was continued at −70 °C for 30 min. The cold bath was removed, and stirring was continued at ambient temperature until internal temperature rose to 0 °C. HCl (3 N, 50 mL) was added while keeping the internal temperature at 0 °C. The mixture was then poured into a separatory funnel, adding about 50 mL of 3 N HCl in the transfer, and shaken vigorously. The combined ether extracts were extracted with water first and then with saturated sodium bicarbonate solution and dried ($MgSO_4$). The ether solution was filtered and evaporated in vacuo to leave 18.73 g of crude 3-bromothiophene-2-carboxaldehyde which was used in the next step without purification.

**3-Bromo-2-(2-dioxolanyl)thiophene (16).** Pyridinium tosylate (1 g, 4 mmol) was added to a mixture of 3-bromothiophene-2-carboxaldehyde (18.7 g, 97.9 mmol), ethylene glycol (22 mL, 24.8 g, 400 mmol), and dry toluene (100 mL). The mixture was refluxed and the water removed by a Dean–Stark trap. After 1 h, TLC (10% EtOAc–hexane) showed the reaction was complete. The mixture was cooled to room temperature and partitioned between ether (100 mL) and water (100 mL). The organic layer was separated and extracted with water (3 × 50 mL) and then with a saturated solution of sodium bicarbonate (25 mL). The resulting extract was dried ($MgSO_4$), filtered, and evaporated in vacuo to leave 21.5 g of crude product. This product was distilled to give 17.6 g of 3-bromo-2-(2-dioxolanyl)thiophene: bp 86–87 °C (0.5 mm); $^1$H NMR ($CDCl_3$) $\delta$ 4.03–4.17 (8 H, m), 6.14 (1 H, s), 6.96 (1 H, d, $J$ = 5.50 Hz), 7.30 (2 H, d, $J$ = 5.50 Hz). Anal. ($C_7H_7BrO_2S$) C, H.

**Methyl 3-[2-(2-Dioxolanyl)thiophene-3-yl]-3-thiapropionate (17).** 3-Bromo-2-(2-dioxolanyl)thiophene (47.02 g, 0.2 mmol) was dissolved in dry THF (600 mL) under $N_2$ and cooled to 105 °C, internal temperature (cooling bath composed of methanol and ether (equal parts) frozen to a slush with liquid $N_2$). *n*-Butyllithium (83 mL or a 2.4 M solution in hexane; 0.2 mmol) was added at a rapid drip while maintaining the internal temperature at −96 to −105 °C for 5 min. Sulfur was then added (6.57 g, 205 mmol) all at once. The cooling bath was removed and the mixture allowed to warm to −78 °C until all but excess sulfur was consumed. (Not longer than 15 min.) The mixture was cooled to −90 °C and methyl bromoacetate (19 mL, 31 g, 204 mmol) was added at a rapid drip. The mixture was allowed to warm to −78 °C and was held there for 15 min. The cooling bath was removed and the mixture was stirred at ambient temperature until the internal temperature rose to 0 °C. A warming bath was then used to adjust the internal temperature to room temperature. THF was evaporated in vacuo and the residue was dissolved in ether and extracted with water four times, dried ($MgSO_4$), and filtered and the solvent evaporated in vacuo to leave 53.73 g of crude product which was used in the next step without purification.

**Methyl 3-[2-(2-Formylthiophene-3-yl)]-3-thiapropionate (18).** Methyl 3-[2-(2-dioxolanyl)thiophene-3-yl]-3-thiapropionate (117.63 g, 0.515 mol) was dissolved in acetone (500 mL), and *p*-toluenesulfonic acid hydrate (1.30 g, 6.83 mmol) was added and stirred over 1 h. A saturated solution of sodium bicarbonate (20 mL) was added followed by water (250 mL). The acetone was evaporated, leaving a tacky solid in water. This tacky solid was dissolved in ether and washed with water four times, dried ($MgSO_4$), and filtered and the solvent evaporated in vacuo to leave 96.91 g of crude product which was used in the next step without purification.

**Methyl Thieno[3,2-*b*]thiophene-2-carboxylate (19).** Piperidine (37.9 g, 445 mmol) was added to a solution of acetic acid (26.7 g, 445 mmol) in benzene (1 L) and stirred for 5 min.[26b] Methyl 3-(2-(2-formylthiophene-3-yl)-3-thiapropionate (18) (96.91 g, 448 mmol) was added in benzene to a total volume of benzene (2.2 L). The solution was refluxed with stirring and water was collected via a Dean–Stark trap. After 5 h of reflux 7.8 mL of water was collected. The mixture was cooled to room temperature and extracted with water two times, then 1 N HCl, followed by water, and then by a saturated solution of sodium bicarbonate two times. The resulting benzene solution was dried ($MgSO_4$), filtered, and evaporated to leave 88 g of crude product. This was extracted with room temperature ether and then three times with boiling ether, leaving a tarry gum. Silica gel (100 g) was added to the ether solution of the product. The solvent was evaporated and the clumps were broken up, leaving a free flowing product absorbed on silica gel. This was chromatographed on silica gel with 50% hexane in toluene as eluent. The solvent was evaporated from the fraction containing the product and after trituration with hexane, 36.93 g of product, mp 96–97 °C, was isolated: $^1$H NMR ($CDCl_3$) $\delta$ 3.91 (3 H, s), 7.27 (1 H, d, $J$ = 5.50 Hz), 7.58 (1 H, d, $J$ = 5.50 Hz), 8.00 (1 H, s). Anal. ($C_8H_6O_2S_2$) C, H.

**2-(Hydroxymethyl)thieno[3,2-*b*]thiophene (20).** A solution of methyl thieno[3,2-*b*]thiophene-2-carboxylate (37.04 g, 186.9 mmol) in ether (850 mL) was added at a rapid drip (1.75 h) to a suspension of lithium aluminum hydride (14.18 g, 373.7 mmol) in ether (500 mL) which had been cooled in an ice–water bath. After the addition was completed, the mixture was stirred at room temperature for 3 h. The reaction was worked up by cooling in an ice–water bath with successive dropwise addition of water (14 mL), 15% aqueous sodium hydroxide (14 mL), and water (42 mL) with vigorous stirring. Vigorous stirring was continued until the salts were well granulated. The ether solution of the product was decanted and the salts washed further with ether. The combined ether solutions were dried ($MgSO_4$) and filtered, and the solvent was evaporated in vacuo to leave 31.4 g of product, mp 91–93 °C, which was used in the next step without purification. A small sample was recrystallized from hexane for analysis (mp 93–94 °C): $^1$H NMR ($CDCl_3$) $\delta$ 4.87 (2 H, d, $J$ = 6.15 Hz), 7.19 (1 H, d, $J$ = 0.68 Hz), 7.22 (1 H, dd, $J$ = 0.68, 5.24 Hz), 7.35 (1 H, d, $J$ = 5.24 Hz). Anal. ($C_7H_6OS_2$) C, H.

**Thieno[3,2-*b*]thiophene-2-carboxaldehyde (21).** A solution of 2-(hydroxymethyl)thieno[3,2-*b*]thiophene (31.4 g, 184 mmol) in methylene chloride (430 mL) was added all at once to a stirred suspension of pyridinium chlorochromate (59.5 g, 276 mmol) which had been ground in a mortar and pestle and partially dissolved in methylene chloride with vigorous stirring. Stirring was continued for 2 h, at which point TLC (30% ethyl acetate in hexane on silica gel) showed the reaction about 60% complete. Pyridinium chlorochromate (15.86 g, 73.6 mmol) was added, and the mixture was stirred vigorously for 30 min. The solution was worked up by adding ether (2 L) and then filtering through an 80 × 300 mm column of silica gel. The gum in the flask was washed with ether (3 × 300 mL) and these washings were passed through the column. The combined ether and methylene chloride solvents were evaporated in vacuo to leave 24.79 g of crude dark purple product which was sublimed at bath temperature 100–107 °C at 0.1 mm pressure to give 23.30 g of white crystalline product: mp 53–54 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.34 (1 H, d, $J$ = 5.37 Hz), 7.46 (1 H, d, $J$ = 5.37 Hz), 7.91 (1 H, s). Anal. ($C_7H_4OS_2$) C, H.

**2-(2-Dioxolanyl)thieno[3,2-*b*]thiophene (22).** A mixture of thieno[3,2-*b*]thiophene-2-carboxaldehyde (**21**) (23.30 g, 138.5 mmol), ethylene glycol (31 mL, 34.39 g, 554.0 mmol), pyridinium tosylate (2.5 g, 10 mmol), and benzene (200 mL) was stirred and refluxed, and the water (3.2 mL) was removed by a Dean–Stark trap (~4 h). The reaction was cooled to room temperature, extracted with water three times and then with a saturated solution of sodium bicarbonate. The organic layer was dried ($MgSO_4$) and filtered, and the solvent was evaporated to leave a crystalline mass. Trituration with a small amount of ether removed the color to give 24.72 g of a white crystalline product, mp 88–90 °C. An additional 1.43 g of product was collected from the ether washing, mp 88–89 °C, yielding a total of 26.15 g of product. This product was used in the next step without further purification: $^1$H NMR ($CDCl_3$) $\delta$ 4.01–4.09 (4 H, m), 4.10–4.18 (4 H, m), 6.15 (1 H, s), 7.19 (1 H, d, $J$ = 5.4 Hz), 7.31 (1 H, s), 7.32 (1 H, d, $J$ = 5.4 Hz). Anal. ($C_9H_8O_2S_2$) C, H.

**5-(2-Dioxolanyl)thieno[3,2-*b*]thiophene-2-sulfonamide (23).** To 1.0 g (4.72 mmol) 2-(2-dioxolanyl)thieno[3,2-*b*]thiophene in 10 mL of THF cooled to −78 °C under $N_2$ was added 4.72 mmol

*n*-butyllithium (hexane) dropwise at <-70 °C, and the clear amber solution was stirred at -78 °C for 45 min during which time the reaction mixture become a tan suspension. Sulfur dioxide gas was then bubbled onto the surface of this suspension, and the temperature was maintained at -65 °C for 0.5 h. The temperature was then allowed to gradually rise to -10 °C over 0.5 h and the $SO_2$ was stopped. The solvent was then removed at <30 °C under vacuum to provide a brown solid that was dissolved in 10 mL of saturated $NaHCO_3$. This was cooled to 0–10 °C and 0.86 g (6.5 mmol) of *N*-chlorosuccinimide was added portionwise and stirring was continued for 1.5 h. The reaction mixture was then extracted with two 40-mL portions of ethyl acetate. The combined organics were washed with water and brine and then were dried and stripped to give the sulfonyl chloride as a tan solid. This was dissolved in 20 mL of acetone, cooled to 0–10 °C, and treated with 5 mL of concentrated $NH_4OH$ added in one portion. This was stirred for 45 min, and after most of the acetone was removed in vacuo, 10 mL of $H_2O$ was added to give a tan solid. This solid was collected, washed well with cold water, and dried to give the product, which was used in the next step, without further purification: $^1H$ NMR (DMSO-$d_6$) $\delta$ 4.01–4.10 (4 H, m), 4.10–4.16 (4 H, m), 6.13 (1 H, s), 7.69 (1 H, s), 7.79 (2 H, bs), 7.92 (1 H, s).

**5-Formylthieno[3,2-*b*]thiophene-2-sulfonamide (24).** 5-(2-Dioxolanyl)thieno[3,2-*b*]thiophene-2-sulfonamide (**23**) 8.50 g (0.029 mmol) was dissolved in 500 mL of acetone at room temperature and 0.9 g of *p*-toluenesulfonic acid monohydrate was added in one portion. After 5 h at room temperature all starting material was consumed. The reaction was quenched with 125 mL of saturated $NaHCO_3$ solution and the acetone was stripped on a rotary evaporator. The resulting viscous mass was extracted with 3 × 300 mL ethyl acetate and these combined extracts were dried and stripped to give 6.55 g (91%) of products as a tan solid: mp 203–204 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.97 (2 H, s), 8.05 (1 H, s), 8.44 (1 H, s), 10.04 (1 H, s). Anal. ($C_7H_5NO_3S_3$) C, H, N.

**2-(1-Hydroxyethyl)thieno[2,3-*b*]thiophene (25a).** To a solution of thieno[2,3-*b*]thiophene-2-carboxaldehyde (4.32 g, 26 mmol) in dry ether (80 mL) and dry THF (50 mL) cooled to a -78 °C under nitrogen was added methyllithium (21.4 mL of a 1.4 M solution in ether; 30 mmol) dropwise over 10 min to give a pale yellow solution which was stirred at -78 °C for 45 min. Water (10 mL) was added dropwise and the reaction warmed to room temperature. Ether (100 mL) and brine (75 mL) were added, the organic phase was separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated in vacuo. The residue was chromatographed on silica gel 80 × 150 mm column with 20% EtOAc–hexane as eluent to give 4.40 g of product as a gum: $^1H$ NMR ($CDCl_3$) $\delta$ 1.62 (3 H, d), 5.15 (1 H, q), 7.10 (1 H, s), 7.16 (1 H, d), 7.31 (1 H, d).

**2-(1-Oxoethyl)thieno[2,3-*b*]thiophene (26a).** To a stirred suspension of pyridinium chlorochromate (12.6 g, 58.4 mmol) in methylene chloride (150 mL) at 0–10 °C was added 2-(1-hydroxyethyl)thieno[2,3-*b*]thiophene (4.3 g, 23.4 mmol) dissolved in methylene chloride (100 mL). The reaction mixture was stirred vigorously at 8–10 °C for 2 h. An additional 2.0 g (9 mmol) of PCC was added and stirring at 0–10 °C continued for 1 h. Ether (100 mL) was added and the mixture filtered through a column of silica on a pad of Celite. The solvent was evaporated in vacuo to give 3.80 g (89%) of product which was homogeneous on TLC (70% EtOAc–hexane silica): $^1H$ NMR $\delta$ 2.60 (3 H, s), 7.28 (1 H, d), 7.40 (1 H, d), 7.83 (1 H, s).

**2-[1-[(2-Methoxyethyl)amino]ethyl]thieno[2,3-*b*]thiophene (28a).** (Methoxyethyl)amine (5.26 g, 70 mmol) was added to a suspension of 2-[(1-oxoethyl)]thieno[2,3-*b*]thiophene (1.82 g, 10 mmol) in methanol (22 mL). Methanolic HCl (3.6 mL of a 5.54 M solution, 20 mmol). The mixture was heated to reflux when all went into solution, stoppered, and let stir at a bath temperature of 45–50 °C for 2 days. To the mixture was added 3 Å sieves (10 g), the mixture was allowed to stir for one additional day, then cooled in an ice bath, $NaBH_4$ (1.89 g, 50 mmol) was added in individual portions over a 4-h period, and then the mixture was allowed to stir for an additional 2 h. The reaction mixture was worked up by diluting with methanol (50 mL) and THF (50 mL) and then filtering through Celite to remove the powdered sieves. Water (50 mL) was added, and the organic solvents were evaporated. Concentrated HCl (10 mL) was added and the mixture was swirled for 10 min. The mixture was extracted with ether (2 × 50 mL), then made basic with $NH_4OH$ (N 20 mL), extracted with ether (5 × 75 mL), dried ($MgSO_4$), and filtered, and the solvent was evaporated in vacuo to leave 2.0 g of crude product. The whole reaction was repeated with 2.0 g of starting ketone to give 2.29 g of additional product. These were combined and chromatographed on a 70 × 180 mm silica column with 2% methanol in chloroform as eluent to give 3.52 g of pure product as a gum: $^1H$ NMR ($CDCl_3$) $\delta$ 1.49 (3 H, d), 2.77 (2 H, m), 3.36 (3 H, s), 3.48 (2 H, m), 4.12 (1 H, q), 7.06 (1 H, s), 7.13 (1 H, d), 7.28 (1 H, d). Exact mass calcd for $C_{11}H_{16}NOS_2$ 242.067 330; found 242.067 459.

***N*-[1-(Thieno[2,3-*b*]thiophene-2-yl)ethyl]-*N*-(2-methoxyethyl)(methoxyethoxy)acetamide (29a).** (Methoxyethoxy)acetyl chloride (1.23 g, 8.10 mmol) was added with stirring to 2-[1-[(2-methoxyethyl)amino]ethyl]thieno[2,3-*b*]thiophene dissolved in THF (25 mL). Triethylamine (0.82 g, 8.11 mmol) was added dropwise with stirring. The reaction mixture was stoppered and stirred at room temperature for 3 h. The reaction was then worked up by evaporating the solvent in vacuo and partitioning the residue between ether (100 mL) and water (25 mL). The resulting ether solution was dried ($MgSO_4$) and filtered, and the solvent was evaporated in vacuo to leave 2.25 g of a gum which was used in the next step without purification.

**2-[1-[(Methoxyethyl)[(methoxyethoxy)ethyl]amino]ethyl]thieno[2,3-*b*]thiophene (30a).** *N*-[1-(Thieno[2,3-*b*]-thiophene-2-yl)ethyl]-*N*-(2-methoxyethyl)(methoxyethoxy)acetamide (2.25 g, 6.33 mmol) dissolved in ether (50 mL) was added dropwise to a suspension of lithium aluminum hydride (0.48 g, 12.7 mmol) in ether (50 mL) and THF (150 mL). The mixture was stirred 24 h when TLC (5% MeOH–$CHCl_3$ silica) of a mini workup showed the reaction incomplete. Lithium aluminum hydride (0.58 g, 15.3 mmol) was added and stirring continued over night. The reaction was worked up by adding dropwise successively 1 mL of $H_2O$, 4.4 mL of 15% NaOH in water. The mixture was filtered and the salts were washed with THF. The combined organic phase was evaporated in vacuo to leave a tacky solid which was triturated with ether giving 0.84 g of solid which was not product. The filtrate was evaporated in vacuo to leave 1.2 g of crude oily product. Flash chromatography eluting with 40% ethyl acetate in hexane gave 0.46 g of pure product as an oil: $^1H$ NMR ($CDCl_3$) $\delta$ 1.43 (3 H, d), 2.75 (4 H, m), 3.33 (3 H, s), 3.37 (3 H, s), 3.45–3.62 (8 H, m), 4.22 (1 H, q), 6.99 (1 H, s), 7.13 (1 H, d), 7.27 (1 H, d). Exact mass calcd for $C_{16}H_{26}NO_3S_2$ (M + 1; FAB): 344.135 412; found 344.135 193.

**5-[1-[(Methoxyethyl)[(methoxyethoxy)ethyl]amino]ethyl]thieno[2,3-*b*]thiophene-2-sulfonamide and HCl Salt (64).** Following the same sulfamoylation procedure used to prepare **8**, but substituting 2-[1-[(methoxyethyl)[(methoxyethoxy)ethyl]amino]ethyl]thieno[2,3-*b*]thiophene (0.361 g, 1.10 mmol) as the substrate gave 0.220 g of pure product isolated by flash chromatography with 5% methanol–$CHCl_3$ as eluent. The amine thus purified was converted into the hydrochloride by dissolving the pure gummy amine in 2-propanol (50 mL) to which was added 0.185 mL of a 5.62 M solution of HCl in ethanol (1.04 mmol). The 2-propanol was boiled down to about 5 mL and diluted with 45 mL of filtered ether and placed in the freezer. The amorphous powder was collected and dried to give 0.120 g of product hydrochloride: $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.77 (3 H, bd), 3.1–4.0 (18 H, m), 5.15 (1 H, m), 7.75 (1 H, s), 7.79 (3 H, m). Anal. ($C_{16}H_{27}ClN_2O_5S_3$·0.1 ether·0.1 2-propanol) C, H.

**5-(Methoxycarbonyl)thieno[2,3-*b*]thiophene-2-sulfonyl Chloride (32).** Crystals of phosphorus pentachloride (9.80 g, 47.1 mmol) were added in portions to chlorosulfonic acid (9 mL, 15.4 g, 132 mmol) in an inert atmosphere. The solution was stirred for 15 min. To this solution small portions of methyl thieno-[2,3-*b*]thiophene-2-carboxylate (8.49 g, 42.8 mmol) were slowly added, allowing for subsiding of effervescence between additions. After the addition was complete, the solution was stirred in an inert atmosphere for 25 min. The resulting solution was poured carefully onto ice–water. The resulting mixture was triturated and the off-white crystals were collected, washed with water, and dried in vacuo over phosphorus pentoxide to give 11.58 g of 5-(methoxycarbonyl)thieno[2,3-*b*]thiophene-2-sulfonyl chloride. This was used in the next step without purification.

**Preparation of 5-(Methoxycarbonyl)thieno[2,3-*b*]-thiophene-2-sulfonamide (33).** To stirred ammonium hydroxide

(150 mL) was added dropwise 5-(methoxycarbonyl)thieno[2,3-*b*]thiophene-2-sulfonyl chloride (11.58 g, 39.02 mmol) dissolved in acetone (140 mL). After the addition was complete, the solution was stirred for 30 min. The reaction was worked up by evaporating the ammonia and acetone in vacuo. The crystals were collected and dried (9.66 g) (89%). Recrystallization from nitromethane gave 7.02 g: mp 219–220 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.87 (3 H, s), 7.84 (3 H, s), 8.12 (1 H, s). Anal. ($C_8H_7NO_4S_3$) C, H.

**5-[*N*-[2-(Dimethylamino)ethyl]carbamoyl]thieno[2,3-*b*]thiophene-2-sulfonamide.** 5-(Methoxycarbonyl)thieno[2,3-*b*]thiophene-2-sulfonamide (0.55 g, 2 mmol) was suspended in methanol (5 mL). (dimethylamino)ethyl amine (0.53 g, 2 mmol) was added and the mixture refluxed for 3 days. The mixture was cooled in an ice–water bath and the product collected and washed with cold methanol. The dried product weighed 0.45 g which was used directly to make the HCl salt.

**5-[*N*-[2-(Dimethylamino)ethyl]carbamoyl]thieno[2,3-*b*]thiophene-2-sulfonamide Hydrochloride (66).** 5-[*N*-[2-(Dimethylamino)ethyl]carbamoyl]thieno[2,3-*b*]thiophene-2-sulfonamide (0.45 g, 1.35 mmol) was dissolved in hot ethanol (100 mL), filtered, and cooled. To this solution was added 0.265 mL of 5.10 M HCl in methanol. The resulting solution was stirred and scratched then cooled in a refrigerator overnight. The resulting crystalline product, 0.46 g, mp 254–255 °C dec, was collected and dried: $^1$H NMR (DMSO-$d_6$) $\delta$ 2.17 (6 H, s), 2.39 (2 H, t), 3.34 (2 H, m), 7.77 (2 H, m), 7.84 (1 H, s), 8.00 (1 H, s), 8.62 (1 H, bt). Anal. ($C_{11}H_{16}ClN_3O_3S_3$) C, H, N.

**5-[*N*-(3-Thia-*n*-butyl)carbamoyl]thieno[2,3-*b*]thiophene-2-sulfonamide.** 5-(Methoxycarbonyl)thieno[2,3-*b*]thiophene-2-sulfonamide (1.11 g, 4 mmol), 2-thia-*n*-butylamine (2.92 g, 32 mmol) and methanol were refluxed with stirring for 5 days. The solution was cooled in the freezer for 2 h, and the product was filtered off to yield 1.10 g of product which was used in the next step without further purification.

**5-[*N*-(3-Oxo-3-thia-*n*-butyl)carbamoyl]thieno[2,3-*b*]thiophene-2-sulfonamide (68).** Sodium periodate (1.40 g, 6.54 mmol) was dissolved in water (20 mL). THF (20 mL) was added followed by 5-[*N*-(3-thia-*n*-butyl)carbamoyl]thieno[2,3-*b*]thiophene-2-sulfonamide (1.10 g, 3.27 mmol), and the mixture was stirred at room temperature under argon for 24 h. The solution was filtered and then stripped of the THF and all but 2–3 mL of water. The product was then crystallized, collected, and dried. Recrystallization from nitromethane gave 1.0 g of product: mp 242–243 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.61 (3 H, s), 2.89 (1 H, m), 3.06 (1 H, m), 3.63 (2 H, m), 7.78 (2 H, s), 7.86 (1 H, s), 8.01 (1 H, s), 9.00 (1 H, bt). Anal. ($C_{10}H_{12}N_2O_4S_4$) C, H, N.

**2-Morpholinoacetamide.** Morpholine (244 g, 2.8 mmol) and 2-chloroacetamide (32.73 g, 0.35 mmol) were dissolved in dry THF (300 mL), stoppered, and stirred for 3 days. Workup by evaporating the THF in vacuo. The remainder was dissolved and suspended in EtOH (500 mL). NaOMe (1 equiv, 18.9 g, 0.34 mmol) was added, and the mixture was stirred for 15 min and filtered from salt. Excess morpholine was distilled in vacuo leaving 55.7 g of crude product which was used in the next step without purification.

**2-Morpholinoethylamine.** To a suspension of $LiAlH_4$ (26.3 g, 0.692 mmol) in ether (700 mL) and THF (500 mL) was added a solution of 2-morpholinoacetamide (41.65 g, 0.35 mmol) in hot THF (700 mL) at a rapid drip and stirred overnight at room temperature. The mixture was then cooled in an ice–water bath, $H_2O$ (26 mL) and then 15% NaOH (104 mL) were added dropwise with vigorous stirring in succession, and the mixture was stirred until well granulated. The mixture was filtered from the salts, and the salts were washed with THF. The solvent was stripped leaving 41.65 g of crude product. Distillation gave 24.05 g, bp 49–50 °C at 0.5 mm: $^1$H NMR (CDCl) 2.44 (6 H, m), 2.80 (2 H, t), 3.72 (4 H, t).

**5-[*N*-(2-Morpholinoethyl)carbamoyl]thieno[2,3-*b*]thiophene-2-sulfonamide.** A mixture of 5-(methoxycarbonyl)thieno[2,3-*b*]thiophene-2-sulfonamide (0.83 g, 3 mmol), 2-morpholinoethylamine (1.17 g, 9. mmol), and methanol (4 mL) was refluxed for 72 h. The methanol was evaporated in vacuo and the residue dissolved in hot THF. The product was absorbed onto silica gel and chromatographed with 10% methanol in chloroform as eluent to give 1.37 g of product.

**5-[*N*-(2-Morpholinoethyl)carbamoyl]thieno[2,3-*b*]thiophene-2-sulfonamide Hydrochloride (72).** 5-[*N*-(2-Morpholinoethyl]carbamoyl]thieno[2,3-*b*]thiophene-2-sulfonamide (1.07 g, 2.04 mmol) was dissolved in hot methanol (150 mL) and ethanol (150 mL). This solution was cooled to room temperature, mixed with cold 5.62 M HCl in ethanol (0.51 mL, 2.8 mmol), and allowed to stand for 15 min. The mixture was filtered and boiled down to 100 mL. Ethanol (150 mL) was added and the solution was boiled down to 100 mL again. This was repeated and allowed to crystallize to give 0.87 g of product: mp 267–268 °C dec; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.14 (2 H, bq), 3.2–3.4 (4 H, m), 3.56 (2 H, bd), 3.68 (2 H, bd), 3.81 (2 H, bt), 3.91 (2 H, bd), 7.80 (2 H, s), 7.86 (1 H, s), 8.15 (1 H, s), 9.18 (1 H, bt). Anal. ($C_{13}H_{18}ClN_3O_4S_3$) C, H, N.

**Partition Coefficients.** Partition coefficients were determined by equilibrating each test compound between 1-octanol and 0.1 ionic strength pH 7.4 buffer as described in ref 6a.

**p$K_a$.** The half-neutralization point was determined for each test compound as described in ref 6a.

**In Vitro Inhibition of and Binding to Human Carbonic Anhydrase II.** $IC_{50}$ and $K_i$ values were determined for each test compound with human carbonic anhydrase II as described in ref 6a.

**In Vitro Binding to Bovine Ocular Pigment.** The binding of each test compound to bovine iris and ciliary body pigment was determined as described in ref 28.

**Ex Vivo Inhibition of Rabbit Iris and Ciliary Body Carbonic Anhydrase.** The ability of each test compound to inhibit albino rabbit iris and ciliary body carbonic anhydrase post topical instillation was assessed ex vivo as described in ref 12.

**Intraocular Pressure Studies.** Intraocular pressure studies in normotensive albino and pigmented rabbits, hypertensive pigmented rabbits, and normotensive and hypertensive cynomologous monkeys were conducted as described in ref 12.

**Registry No.** **1**, 13250-82-3; **2**, 122267-20-3; **3**, 19685-82-6; **4**, 20969-37-3; **5**, 122267-21-4; **6**, 31486-85-8; **7**, 122289-59-2; **8**, 122267-22-5; **9**, 122267-23-6; **10a**, 122267-24-7; **15**, 930-96-1; **16**, 56857-02-4; **17**, 127025-33-6; **18**, 1723-29-1; **19**, 98800-10-3; **20**, 127025-34-7; **21**, 31486-86-9; **22**, 127025-35-8; **23**, 133445-56-4; **24**, 133445-57-5; **25a**, 133445-58-6; **26a**, 39076-86-3; **28a**, 133445-59-7; **29a**, 133445-60-0; **30a**, 133445-61-1; **32**, 129949-97-9; **33**, 129949-98-0; **35**, 122267-03-2; **35**-free base, 122266-88-0; **36**, 122267-05-4; **36**-free base, 122266-89-1; **37**, 122267-04-3; **37**-free base, 122266-91-5; **38**, 122267-06-5; **38**-free base, 122266-92-6; **39**, 122267-07-6; **39**-free base, 122266-93-7; **40**, 122267-09-8; **40**-free base, 122266-95-9; **41**, 122267-08-7; **41**-free base, 122266-94-8; **42**, 122267-10-1; **42**-free base, 122266-90-4; **43**, 122267-11-2; **43**-free base, 122289-58-1; **44**, 122267-12-3; **44**-free base, 122266-96-0; **45**, 122267-14-5; **45**-free base, 122266-98-2; **46**, 133445-63-3; **46**-free base, 133445-62-2; **47**, 122267-15-6; **47**-free base, 122266-99-3; **48**, 122267-16-7; **48**-free base, 122267-00-9; **49**, 122267-18-9; **49**-free base, 122267-01-0; **50**, 133445-64-4; **50**-free base, 133445-74-6; **51**, 122267-13-4; **51**-free base, 122266-97-1; **52**, 122267-19-0; **52**-free base, 133445-75-7; **53**, 133445-66-6; **53**-free base, 133445-65-5; **54**, 122267-17-8; **54**-free base, 122267-02-1; **55**, 128620-93-9; **55**-free base, 128620-92-8; **56**, 133445-67-7; **56**-free base, 133445-76-8; **57**, 133445-68-8; **57**-free base, 133445-77-9; **58**, 133445-69-9; **58**-free base, 133445-78-0; **59**, 127025-26-7; **59**-free base, 127025-25-6; **60**, 127025-28-9; **60**-free base, 127025-27-8; **61**, 127025-30-3; **61**-free base, 127025-29-0; **62**, 127025-32-5; **62**-free base, 127025-31-4; **63**, 133445-70-2;

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# Flavins as Potential Antimalarials. 2. 3-Methyl-10-(substituted-phenyl)flavins

William B. Cowden,* Peter K. Halladay, Ross B. Cunningham,† Nicholas H. Hunt,‡ and Ian A. Clark

*Division of Virology and Cellular Pathology, The John Curtin School of Medical Research, The Australian National University, Canberra, A.C.T., 2601 Australia.* *Received November 2, 1990*

A series of 3-methyl-10-(substituted-phenyl)flavins was prepared and tested for antimalarial activity against the lethal parasite *Plasmodium vinckei* in mice. Several of these analogues were found to be effective antimalarial agents. A quantitative structure–activity relationship study was undertaken with 44 analogues and no satisfactory relationship could be established.

We have previously found a number of 10-(halophenyl)-3-methylflavins to possess antimalarial activity both in vivo[1,2] against rodent malarias and in vitro against *Plasmodium falciparum*.[2] These agents are potent inhibitors of both human and plasmodial glutathione reductase;[3] inhibition of the latter may account for the antimalarial properties of these agents.[4] In part 1 of this series we examined compounds with only a limited number of 10-phenyl substituents which covered a relatively narrow range of electronic, steric, and lipophilic properties. In the present work we have prepared and tested a number of 3-methyl-10-(substituted-phenyl)flavin analogues with a wide range of physicochemical properties in an attempt to identify the most effective substituent pattern in the 10-phenyl moiety.

## Chemistry

The majority of 3-methyl-10-phenylflavins (**2a–ff**; Table II) were prepared by the action of nitrosobenzene on 6-anilinouracils in the presence of acetic anhydride, essentially according to the method of Yoneda et al.[5] 6-[4′-(Dimethylamino)anilino]-3-methyluracil (**1o**; Table I) failed to condense with nitrosobenzene in the expected manner. An alternate approach involving the condensation of alloxan with the 2-aminodiphenylamine reduction product of **3** to give the corresponding 10-[4′-(dimethylamino)phenyl]flavin (**4**), followed by methylation, was successful in giving 3-methyl-10-[4′-(dimethylamino)-phenyl]flavin (**2o**; Table II). The 6-anilino-3-methyluracils (**1a–cc**; Table I) were prepared by heating the appropriate aniline with 6-chloro-3-methyluracil and isolated from EtOH and recrystallized from MeOH or acetic acid. This method, described previously,[1,5] called for an excess of the aniline (3 equiv) to be reacted with 6-chloro-3-methyluracil. This procedure, in synthetic terms, is satisfactory; however, in the present work some of the aniline starting materials were either expensive or less readily available synthetically; therefore, we devised a method to help conserve them. The latter consisted of reacting molar equivalents of the chlorouracil and appropriate aniline nucleophile in the pres-

**Table I.** Physical Properties of 3-Methyl-6-(substituted-anilino)uracils **1**

| no. | X | mp, °C | method (% yield) | formula | anal. |
|---|---|---|---|---|---|
| **1a** | H | 330–331[a] | B (78) | $C_{11}H_{11}N_3O_2$ | C,H,N |
| **1b** | 2-Me | 237–239 | B (67) | $C_{12}H_{13}N_3O_2$ | C,H,N |
| **1c** | 3-Me | 273–274[b] | B (76) | $C_{12}H_{13}N_3O_2$ | C,H,N |
| **1d** | 4-Me | 311–313[c] | A (70) | $C_{12}H_{13}N_3O_2$ | C,H,N |
| **1e** | 2-Et | 223–226 | B (22) | $C_{13}H_{15}N_3O_2$ | C,H,N |
| **1f** | 3-Et | 246–248 | A (71) | $C_{13}H_{15}N_3O_2$ | C,H,N |
| **1g** | 4-Et | 304–307 | B (78) | $C_{13}H_{15}N_3O_2$ | C,H,N |
| **1h** | 4-Bu | 268–270 | A (62) | $C_{15}H_{19}N_3O_2$ | C,H,N |
| **1i** | 3-$CF_3$ | 257–259 | B (56) | $C_{12}H_{10}F_3N_3O_2$ | C,H,N |
| **1j** | 4-$CF_3$ | 300–302 | B (42) | $C_{12}H_{10}F_3N_3O_2$ | C,H,N |
| **1k** | 3-OMe | 268–269[d] | A (69) | $C_{12}H_{13}N_3O_3$ | C,H,N |
| **1l** | 4-OMe | 310–312[e] | A (75) | $C_{12}H_{13}N_3O_3$ | C,H,N |
| **1m** | 3-SMe | 257–259 | B (87) | $C_{12}H_{13}N_3O_2S$ | C,H,N |
| **1n** | 4-SMe | 327–328 | B (80) | $C_{12}H_{13}N_3O_2S$ | C,H,N |
| **1o** | 4-$NMe_2$ | dec 320[f] | A (92) | $C_{13}H_{16}N_4O_2$ | C,H,N |
| **1p** | 4-OH | 330–333[g] | B (80) | $C_{11}H_{11}N_3O_3$ | C,H,N |
| **1q** | 4-CN | 353–354[h] | B (30) | $C_{12}H_{10}N_4O_2$ | C,H,N |
| **1r** | 2,4-$Me_2$ | 290–292 | B (59) | $C_{13}H_{15}N_3O_2$ | C,H,N |
| **1s** | 3,4-$Me_2$ | 309–312[i] | B (88) | $C_{13}H_{15}N_3O_2$ | C,H,N |
| **1t** | 3,5-$Me_2$ | 289–290[j] | B (53) | $C_{13}H_{15}N_3O_2$ | C,H,N |
| **1u** | 3,5-$(CF_3)_2$ | 305–306 | A (81) | $C_{13}H_9F_6N_3O_2$ | C,H,N |
| **1v** | 3,5-$(OMe)_2$ | 298–299 | B (88) | $C_{13}H_{15}N_3O_4$ | C,H,N |
| **1w** | 3-Cl,4-Me | 299–300 | B (88) | $C_{12}H_{12}ClN_3O_2$ | C,H,N |
| **1x** | 3-Cl,5-Me | 303–304 | B (60) | $C_{12}H_{12}ClN_3O_2$ | C,H,N |
| **1y** | 4-Cl,3-Me | 334–335 | B (76) | $C_{12}H_{12}ClN_3O_2$ | C,H,N |
| **1z** | 2-Cl,5-$CF_3$ | 311–312 | B (68) | $C_{12}H_9ClF_3N_3O_2$ | C,H,N |
| **1aa** | 3-$CF_3$,4-Cl | 283–285 | A (75) | $C_{12}H_9ClF_3N_3O_2$ | C,H,N |
| **1bb** | 2-F,4-Cl | 335–336 | B (42) | $C_{11}H_9ClFN_3O_2$ | C,H,N |
| **1cc** | 3,4-$F_2$ | 333–337 | B (71) | $C_{11}H_9F_2N_3O_2$ | C,H,N |

[a] Literature[5] mp 336–338 °C. [b] Literature[5] mp 291 °C. [c] Literature mp 325 °C (Yoneda, F; Tsukuda, K.; Shinozuka, K.; Hirayama, F.; Uekama, K.; Koshiro, A. *Chem. Pharm. Bull.* **1980**, *28*, 3049). [d] Literature mp 276 °C (Grauert, R. W. *Arch. Pharm. Ber. Dtsch. Pharm. Ges.* **1982**, *315*, 949). [e] Literature mp 290–292 °C (Shinkai, S.; Kawanabe, S.; Kawase, A.; Yamaguchi, T.; Manabe, O.; Harada, S.; Nakamura, H.; Kasai, N. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2095). [f] Literature mp >330 °C (Grauert, R. W. *Ibid*). [g] Literature mp >330 °C (Grauert, R. W. *Ibid*). [h] Literature[5] mp 353 °C. [i] Literature mp 309 °C (Grauert, R. W. *Ibid*). [j] Literature mp 275–277 °C (Kurreck, H.; Bock, M.; Bretz, N.; Elsner, M.; Kraus, H.; Lubitz, W.; Müller, F.; Geissler, J.; Kroneck, P. M. H. *J. Am. Chem. Soc.* **1984**, *106*, 737).

* To whom correspondence should be sent.
† Statistics, The Australian National University.
‡ Department of Pathology, Sydney University, Sydney, Australia.

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