in water, and the solution was made strongly basic with concentrated NH_4OH. The aqueous mixture was extracted with 3:1 $CHCl_3$/*i*-PrOH. The organic extracts were combined, washed with saturated, aqueous NaCl, and dried over Na_2SO_4. A colorless solid remained after evaporation of the solvent. This solid was dissolved in absolute ethanol, and 1.1 equiv of D-(−)-(*tert*-butyoxycarbonyl)phenylglycine was added. The solution was heated to boiling for 10 min and then allowed to stand at room temperature. The crystals were collected by vacuum filtration and dried in the vacuum desiccator: mp 192–194 °C; $[\alpha]^{25}_D$ (H_2O) +3.79°. Anal. ($C_{13}H_{21}N_3 \cdot C_{13}H_{17}NO_4$) C, H, N, O.

The compound crystallized as colorless plates, in the space group $P2_1$, with two molecules in a unit cell having the dimensions $a = 9.557 \pm 0.002$, $b = 6.680 \pm 0.001$, $c = 20.795 \pm 0.003$ Å, and $\beta = 91.44 \pm 0.02°$. The density calculated for $C_{26}H_{38}N_4O_4$ (M_r 470.6) is 1.18 g cm^{-3}. The intensities of 1959 unique reflections were measured on a four-angle diffractometer with Cu $K\alpha$ radiation. The X-ray structure was solved by direct methods and refined by the least-squares method to a value of $R = 0.054$.

Biological Methods. Rat Locomotor Activity. Male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN) were dosed intraperitoneally with saline or with increasing concentrations of pergolide or (±)-**5** (R = Pro). Immediately after injection, the rats were placed in electronic activity monitors (Stoelting Co., Chicago, IL) for a 1-h period. The rats were not allowed to acclimatize to the monitors so that they would have a relatively high level of spontaneous locomotor activity. External stimuli were minimized by isolating the monitors in a sound-attenuated laboratory. An activity count registered each time the radio-frequency field was interrupted. The monitors were not able to differentiate vertical and horizontal movements. Each animal was used only once, and six to nine rats were employed per group. Each time activity was monitored there was one vehicle control group with the other groups receiving the various drug doses to be tested.

Lordotic Behavior in Rats. Chronic ovariectomized Long-Evans hooded rats received subcutaneous injections of 100 μg of estrone in sesame oil 48 h prior to testing. Each of the female rats was exposed to a sexually active male rat for 15 mounts prior to the injection of the drug. The compounds were injected subcutaneously, and the animals were reexposed to the male rat 90 min following injection. The results are given in Table VIII.

Acknowledgment. The authors thank Dr. G. M. Maciak and associates for the microanalyses.

Registry No. (±)-**5** (R = Pr), 74196-92-2; (−)-**5** (R = Pr), 85760-74-3; (+)-**5** (R = Pr), 85760-75-4; (−)-**5** (R = Pr) D-(−)-tartrate, 85798-07-8; (−)-**5** (R = Pr) HCl, 85798-08-9; (+)-**5** (R = Pr) L-(+)-tartrate, 85798-09-0; (+)-**5** (R = pr) D-(−)-(*tert*-butyloxycarbonyl)phenylglycine, 85798-10-3; prolactin, 9002-62-4; dopamine, 51-61-6.

Supplementary Material Available: Table IX, atomic coordinates and *Uij* values; Table X, bond lengths; Table XI, bond angles; Table XII, anisotropic temperature factors; Table XIII, hydrogen coordinates; Figure 2, X-ray numbering diagram pertaining to the X-ray structure determination (6 pages). Ordering information is given in any current masthead page.

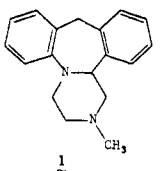
Synthesis and Biological Properties of Thiophene Ring Analogues of Mianserin

Jeffrey W. H. Watthey,*[†] Terrence Gavin,[†1] Mahesh Desai,[†] Barbara M. Finn,[†] Ronald K. Rodebaugh,[‡] and Steven L. Patt[§]

Research and Development Department, Pharmaceuticals Division, and Analytical Research Department, CIBA-GEIGY Corporation, Ardsley, New York 10502, and Varian Instrument Division, Florham Park, New Jersey 07932.
Received December 8, 1982

The synthesis of two thiophene-containing analogues of mianserin, i.e., 1,2,3,4,10,13b-hexahydro-2-methylpiperazino[1,2-*a*]thieno[2,3-*c*][1]benzazepine (**2**), and the corresponding [3,2-*c*] isomer (**12**) is described. The key step in the synthesis is the nucleophilic aromatic substitution reaction of the *N*-lithio derivative of 1-methyl-3-(2-thienyl)piperazine (**4**) with the oxazoline derivative of *o*-anisic acid (**7**) to give the *N*-phenylpiperazine **8**. This substance was converted via ethyl ester **10** to 1-[2-(hydroxymethyl)phenyl]-4-methyl-2-(2-thienyl)piperazine (**3**), which was cyclized with polyphosphate ester to a 5:1 mixture of **2** and **12**. The antidepressant potential of **2** maleate (CGS 11049A) and **12** fumarate (CGS 15413A) were compared with that of mianserin hydrochloride in a variety of biochemical and pharmacological test systems. The three substances exhibited generally similar profiles. However, the results suggest that **2** and **12** bind more strongly to central presynaptic α-receptors than does mianserin.

Mianserin (**1**), an antidepressant agent currently marketed in several countries throughout the world, has a level of efficacy comparable with that of amitryptyline and imipramine.[2] Significantly, this efficacy is associated with a lower incidence of anticholinergic side effects than that of equitherapeutic doses of the tricyclic drugs,[2a,c,3] while the liability of mianserin to cause cardiovascular side effects also appears to be less than that of the classical antidepressants.[2a,4] The neurobiochemical profile of mianserin differs significantly from that of the classical agents. Thus, mianserin causes pronounced blockade of central



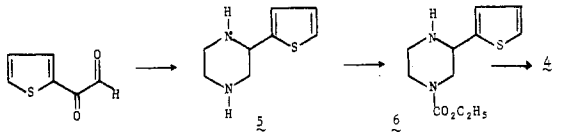
1

[†] CIBA-GEIGY Corp., Research and Development Department.
[‡] CIBA-GEIGY Corp., Analytical Research Department.
[§] Varian Instrument Division.

(1) Present Address: Department of Chemistry, Iona College, New Rochelle, NY 10801.
(2) (a) Brogden, R. N.; Heel, R. C.; Speight, T. M.; Avery, G. S. *Drugs* **1978**, *16*, 273. (b) Zis, A. P.; Goodwin, F. K. *Arch. Gen. Psychiatry* **1979**, *36*, 1097. (c) Cassano, G. B.; Conti, L.; Dell'Osso, L.; Massimetti, G. *Adv. Biochem. Psychopharmacol.* **1982**, *32*, 125. (d) Branconnier, R. J.; Cole, J. O.; Ghazvinian, S.; Rosenthal, S. *Ibid.* **1982**, *32*, 195.
(3) Kopera, H. *Br. J. Clin. Psychiatry* **1978**, *5*, 29S.
(4) Montgomery, S. A.; Burgess, C. D.; Montgomery, D. B. In "Progress in the Pharmacotherapy of Depression: Mianserin HCl"; Drykoningen, G.; Rees, L. W.; Ruiz Ogara, C., Eds., Excerpta Medica: Amsterdam, 1979; pp 60–64. Agnoli, A.; Martucci, N.; Ciabotti, P.; Caratozzolo, P.; Rulli, V.; Sollecito, A. *Adv. Biochem. Psychopharmacol.* **1982**, *32*, 375.

0022-2623/83/1826-1116$01.50/0

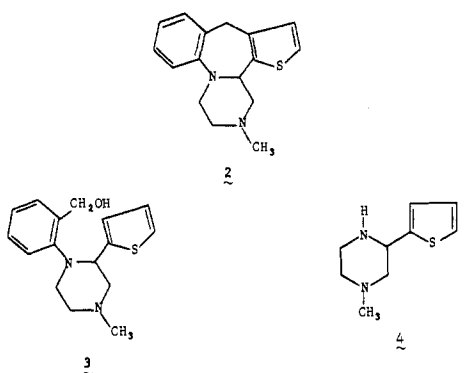
Scheme I



presynaptic α-adrenoreceptors.[5,6] This may be responsible for the increase in norepinephrine turnover observed, which is also an effect contrary to that produced by the tricyclic antidepressants.[2a,5,6] There are conflicting reports concerning the norepinephrine uptake blocking properties of mianserin, but the balance of the evidence suggests that this is not an important property of the drug in vivo.[2,6] In common with the tricyclic antidepressants, mianserin has been reported to inhibit histamine-activated adenylate cyclase[7] and to act as a central 5-HT antagonist.[2a,8]

Numerous publications have appeared that deal with the animal pharmacology or biochemistry of mianserin,[2] and, more recently, the structural requirements for presynaptic α-adrenoreceptor blockade have been described.[9] In order to gain further insight into structure–activity relationships in this area, we decided to prepare thiophene ring analogues of mianserin.

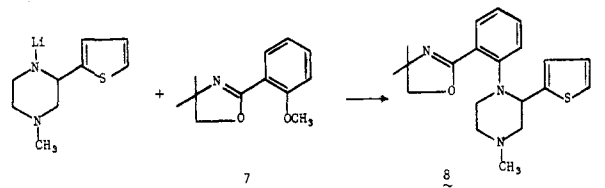
Chemistry. We envisioned that **2** could be prepared



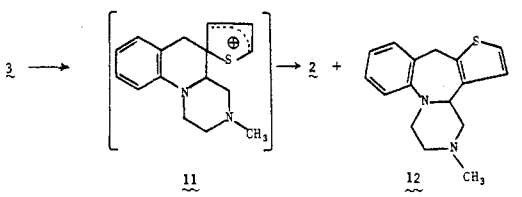
from the benzyl alcohol **3**. This, in turn, would be available from the thienylpiperazine **4** by nucleophilic aromatic substitution by using a reactant containing a substituent that could be transformed into the benzyl alcohol function of **3**.

2-(2-Thienyl)piperazine (**5**) was prepared by condensation of 2-thienylglyoxal[10] with ethylenediamine in ethanol, followed by the addition of sodium borohydride. These conditions have been used previously for the preparation of 2-arylpiperazines.[11] Methylation of **5** to give **4** was accomplished by a two-step procedure involving lithium aluminum hydride reduction of the monocarbamate **6**, which was prepared from **5** by treatment with ethyl chloroformate in acetic acid[12] (Scheme I).

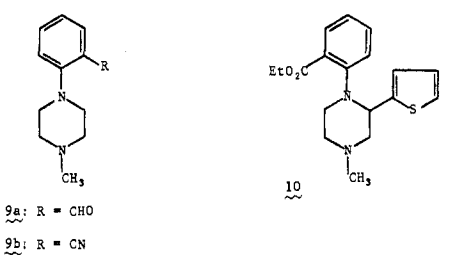
Scheme II



Scheme III



Reaction of the *N*-lithio derivative of **4** with the oxazoline **7**[13] gave the *N*-arylpiperazine **8** (Scheme II). No reaction was observed when **4** was treated with 2-fluorobenzaldehyde or 2-fluorobenzonitrile in Me_2SO at 95 °C in the presence of potassium carbonate. In contrast, both fluoro compounds reacted smoothly with *N*-methylpiperazine under these conditions[14] to give the *N*-arylpiperazines **9**.



Treatment of **8** with ethanolic sulfuric acid at the reflux gave **10**, which was reduced to **3** with lithium aluminum hydride. Cyclization of **3** was accomplished with polyphosphate ester[15] in refluxing dichloromethane. Two isomeric products were formed, in a ratio of 5:1. Both had NMR and mass spectra generally appropriate for the anticipated product. In keeping with other cyclizations onto thiophene rings,[16] it seems reasonable that the two products were formed via the spiro intermediate **11**. This process would involve formation of a six-membered ring with substitution onto the more reactive α-position of the thiophene ring, followed by collapse of the intermediate to either **2** or **12** (Scheme III). This appears to be the first case in which a rearranged product has been observed on formation of a seven-membered ring by electrophilic ring closure onto a thiophene ring.

The two isomers were separated by column chromatography on silica gel. Structural assignment was based on ^{13}C NMR data. Consideration of possible long-range $^{13}C^1H$ couplings in the two isomers led to the realization that in **2** a J_3 vicinal interaction between H_A and C_1 would be anticipated, while in **12** this would not be possible. In the spectrum of the major product, the fine structure on

(5) Baumann, P. A.; Maitre, L. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1977**, *300*, 31. Robson, R. D.; Antonaccio, M. J.; Saelens, J. K.; Liebman, J. *Eur. J. Pharmacol.* **1978**, *47*, 431. Fludder, J. M.; Leonard, B. E. *Psychopharmacology* **1979**, *64*, 329.

(6) Leonard, B. E. *Adv. Biochem. Psychopharmacol.* **1981**, *31*, 301.

(7) Kanof, P. D.; Greengard, P. *Nature (London)* **1978**, *272*, 329.

(8) Maj, J.; Sowinska, H.; Baran, L.; Gancarczyk, L.; Rawlow, A. *Psychopharmacology* **1978**, *59*, 79.

(9) Nickolson, V. J.; Wieringa, J. H. *J. Pharm. Pharmacol.* **1981**, *33*, 760. Nickolson, V. J.; Wieringa, J. H.; van Delft, A. M. L. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1982**, *319*, 48.

(10) Kipnis, F.; Ornfelt, J. *J. Am. Chem. Soc.* **1946**, *68*, 2734.

(11) U.S. Patent 4 166 180, 1979; *Chem. Abstr.* **1978**, *88*, 89714y.

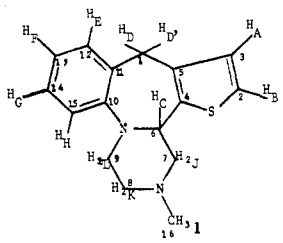
(12) Desai, M.; Watthey, J. W. H.; Zuckerman, M. *Org. Prep. Proced. Int.* **1976**, *8*, 85.

(13) Meyers, A. I.; Mihelich, E. D. *J. Am. Chem. Soc.* **1975**, *97*, 7383. See also Meyers, A. I.; Gabel, R. *J. Org. Chem.* **1977**, *42*, 2653. Ellefson, C. R.; Prodan, K. A.; Brougham, L. R.; Miller, A. *J. Med. Chem.* **1980**, *23*, 977.

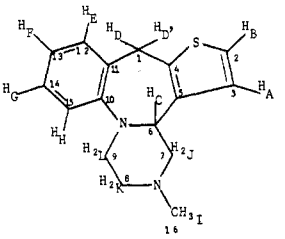
(14) Bader, H.; Hansen, A. R.; McCarty, F. J. *J. Org. Chem.* **1966**, *31*, 2319.

(15) Fieser, L. F.; Fieser, M. "Reagents for Organic Synthesis"; Wiley: New York, 1967; 892–894.

(16) Palmer, M. H.; Leitch, D. S.; Greenhalgh, C. W. *Tetrahedron* **1978**, *34*, 1015. Mackay, C.; Waigh, R. D. *J. Chem. Soc., Chem. Commun.* **1982**, 793.



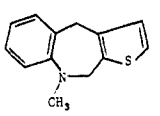
2



12

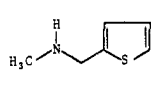
the triplet arms of the C_1 signal appeared as a doublet of doublets (J_1 = 5 Hz, J_2 = 1–2 Hz; not completely resolved). In the spectrum of the minor product, the corresponding fine structure appeared as a doublet (J = 5 Hz). The 5-Hz couplings were assigned to the H_E–C_1 interactions in **2** and **12**, while the 1–2-Hz coupling of the major product was assigned to the H_A–C_1 interaction anticipated for **2**.[17]

In order to shed further light on the chemistry and spectral data discussed above, we decided to examine the preparation of the tricyclic analogue **13**. The amine **14**,[18]

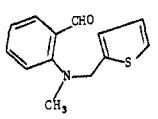


13

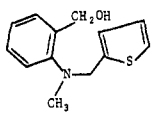
in contrast to **4**, reacted readily with 2-fluorobenzaldehyde in Me_2SO at 95 °C in the presence of potassium carbonate, to give the aldehyde **15**. The failure of the thienylpiperazine **4** to undergo nucleophilic aromatic substitution with 2-fluorobenzaldehyde or 2-fluorobenzonitrile is therefore not due to the inductive effect of the thiophene substituent but to its steric effect, which results from conformational restraints imposed by its attachment to the piperazine ring.



14



15



16

Reduction of **15** with sodium borohydride gave the alcohol **16**, which was cyclized with polyphosphate ester[15] in refluxing dichloromethane to give two isomers in a ratio of 10:1. In this case, the presence of isomers was detected by NMR, but they could not be separated by column chromatography, and no separation was apparent on TLC. Separation was finally achieved by HPLC (see Experimental Section).

The structures of the two isomers were assigned on the basis of ^1H NMR experiments. First, chemical-shift assignments at 200 MHz were made on the basis of precedent and the gross coupling information. Then, strong resolution enhancement revealed highly detailed coupling information, which, in conjunction with decoupling experiments and iterative spin simulation, allowed unequivocal assignment of every resonance in both isomers. Finally, difference NOE experiments[19] were run, irradiating CH_{2_C} and CH_{2_D} in both isomers. Irradiation of CH_{2_C} in the major isomer produced no effect on any of the thiophene or aromatic protons, while irradiations of CH_{2_D} produced an NOE of 27% to H_A and 22% to H_E. This could only be consistent with **13** as the structure of the major isomer. Irradiation of CH_{2_C} in the minor isomer produced an enhancement of 27% to H_A, and irradiation of CH_{2_D} an enhancement of 14% to H_E. This fixes **17** as the structure of the minor isomer. The ^{13}C spectra of **13** and **17** also exhibited changes in the respective chemical shifts of C_1 and C_6. These changes very closely paralleled those noted for **2** and **12** (see Table I), thus providing further support for the structures previously assigned to the tetracyclic isomers.

Table I. ^{13}C Chemical Shifts[a] of C_1 and C_6 in Compounds 2, 12, 13, and 17

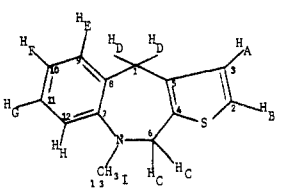
	2	12	Δ (12 − 2)	13	17	Δ (17 − 13)
C_1	32.79	31.64	−1.15	33.90	32.59	−1.31
C_6	62.00	63.23	+1.23	56.47	58.08	+1.61

[a] In parts per million.

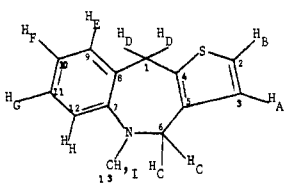
Table II. In Vitro Determinations of Receptor Binding Affinities

	IC_{50},[a] nM			
	[^{3}H]-clonidine			
compd	site A[b]	site B[b]	[^{3}H]-prazosin	[^{3}H]-serotonin
2 maleate	3	3	1400	500
12 fumarate	2	3	300	100
mianserin hydrochloride	20	6	1200	300

[a] Drugs were tested in triplicate over a wide concentration range in a single experiment. Membranes were prepared from calf cortex for [^{3}H]clonidine binding, from rat forebrain for the [^{3}H]prazosin assay, and from calf caudate nucleus for [^{3}H]serotonin experiments. See Experimental Section for assay procedures. [b] Sites A and B indicate two experimentally observed high-affinity binding sites for [^{3}H]clonidine on calf cortical membranes. Dissociation constants (K_D) are as follows: site A, 1.1 nm; site B, 5.4 nm. Although the two sites are experimentally distinguishable,[20] their functional significance is not yet known.



13



17

Biological Results

The maleate salt of **2** (CGS 11049A) and the fumarate salt of **12** (CGS 15413A) were evaluated in a variety of biological test systems, and comparative data on mianserin hydrochloride were also obtained. An in vitro assessment of the α_2-adrenoreceptor blocking properties of the substances was obtained by examination of their interaction with two high-affinity [^{3}H]clonidine binding sites of calf cortex membranes.[20,21] The IC_{50} values are given in Table

(17) Confirmation that 1–2 Hz represented a reasonable value for $J_{C_1H_A}$ in **2** was obtained by examination of the spectra of 3-methylthiophene and 2-(hydroxymethyl)thiophene. The 3-methyl compound clearly showed two 1–1.2-Hz couplings, while the hydroxymethyl compound exhibited line broadening in the fine structure consistent with a 1–2-Hz coupling.

(18) Shapiro, S. L.; Parrino, V. A.; Freedman, L. *J. Am. Chem. Soc.* **1959**, *81*, 3728.

(19) McConnell, H. M.; Robertson, R. E. *J. Chem. Phys.* **1958**, *29*, 1361.

(20) Braunwalder, A.; Stone, G.; Lovell, R. A. *J. Neurochem.* **1981**, *37*, 70.

(21) U'Prichard, D. C.; Bechtel, W. D.; Rouot, B. M.; Snyder, S. H. *Mol. Pharmacol.* **1979**, *16*, 47.

Table III. In Vivo Determinations of Central Presynaptic α-Blockade

compd	[^{3}H]rauwolscine binding:[a] ID_{50}, mg/kg ip (95% CL)	antagonism of clonidine-induced analgesia[b]	noradrenergic single unit act.[d] dose, mg/kg iv	n	clonidine ID_{50}, μg/kg
2 maleate	3.3 (1.4-7.9)	4/10 at 30 mg/kg[c]	3.0	4	82
			6.0	2	170
			10.0	5	165
12 fumarate	2.7 (1.2-5.9)	4/10 at 10 mg/kg[c]			
mianserin hydrochloride	3.7 (1.6-8.7)	ED_{50} = 14.9 mg/kg (12.9-17.2)	4.0	4	21
			8.0	4	47

[a] The experimental design always included one vehicle-treated group (n = 8) and four drug-treated groups (n = 5 per group). The four doses were chosen to cover the appropriate range for ID_{50} determination. Mice were given the test drug or vehicle intraperitoneally at zero time. [^{3}H]Rauwolscine was injected intravenously 20 min later, and the mice were sacrificed at 35 min. ID_{50} values and 95% confidence limits were determined as described by Granat et al.[24] [b] Number of mice in which the analgesic effects of 0.1 mg/kg po of clonidine, as determined in a phenylquinone-induced writhing assay, are reversed by the test substance (administered ip); n = 10 per experiment. See Experimental Section for further details. [c] Maximum effects observed. Both higher and lower doses were less effective. [d] The effectiveness of clonidine in inhibiting the firing rate of a single locus coeruleus neuron by 50% (ID_{50}) following various doses of the test substances was determined.

Table IV. In Vivo Determinations of Changes in MHPG Levels

compd	dose, mg/kg ip	MHPG-SO_4, μg/g, ± SE	% of control
2 maleate	0	0.11 ± 0.01	
	10	0.15 ± 0.02	136
	20	0.21 ± 0.03[a]	191
	30	0.27 ± 0.01[b]	245
mianserin hydrochloride	0	0.11 ± 0.01	
	30	0.19 ± 0.01[b]	180

[a] $p < 0.01$. [b] $p < 0.001$. [c] Five rats were used for each experiment. Animals were sacrificed 2 h after administration of test agent in corn starch suspension. MHPG-SO_4 levels (whole brain) were determined by the procedure of Meek and Neff.[27]

II, together with those for [^{3}H]prazosin binding sites,[22] which give an indication of α_1-adrenoreceptor blocking properties. Table II also shows the effects of the compounds on [^{3}H]serotonin receptor binding.[23]

Three in vivo assays were used to confirm the interaction of the compounds with central α_2-receptors. In the first ([^{3}H]rauwolscine binding), the ability of the test agent (administered ip) to displace rauwolscine from mouse forebrain was determined.[24] In the second (antagonism of clonidine-induced analgesia), the ability of the test substances, administered ip to mice, to antagonize analgesic effects of clonidine, as determined in a phenylquinone-induced writhing assay,[25] was determined. In the third (noradrenergic single unit activity), the effect of **2** maleate and **1**·HCl (administered intravenously) on the ability of clonidine to suppress the firing rate of a single rat locus coeruleus neuron was determined.[26] Data from these three assays are presented in Table III.

In order to supplement the data on presynaptic α-adrenoreceptor blockade, the effect of **2** maleate and **1**·HCl on norepinephrine turnover as evidenced by changes in 3-methoxy-4-hydroxyphenylglycol (MHPG) levels was determined[27] following ip administration of the test agents to rats. The data are given in Table IV.

The antihistamine activity of the two thiophene compounds and of mianserin hydrochloride were determined in vitro by using the adenylate cyclase procedures developed by Psychoyos.[28] The data obtained in these studies are presented in Table V.

Finally, an attempt was made to compare the antidepressant potential of **2** maleate with that of mianserin hydrochloride by use of the behavioral despair or acquired immobility model of Porsolt et al.[29] The data obtained are presented in Table VI.

Table V. In Vitro Determination of Antihistamine Activity

compd	IC_{50},[a] μM $H_1 + H_2$[b]	H_2[b]
2 maleate	0.28	19
12 fumarate	0.09	7.2
mianserin hydrochloride	0.19	3.8

[a] The percent inhibitions of histamine or 4-methylhistamine activation of an adenylate cyclase preparation from guinea pig cerebral cortex were determined (average of triplicates) over a wide concentration range, and IC_{50} values were calculated from the resulting data. [b] Activation of guinea pig cerebral cortex adenylate cyclase by histamine is mediated by both H_1 and H_2 receptors, whereas activation by 4-methylhistamine is mediated by H_2 receptors only.[28]

Table VI. Determination of Antidepressant Potential by Using the Behavioral Despair Model[a]

compd	duration, s
control	82.0
2 maleate	184.6[b]
control	90.8
mianserin hydrochloride	185.6[b]

[a] Sixty minutes after ip injection of the test agent at 10 mg/kg ip, ten CF1 male mice (18-22 g) were placed into cylinders containing 6 cm of tap water at 21-23 °C for a total of 6 min. Only the duration of immobility occurring during the last 4 min was counted. Significance was determined by use of the rank sum test. [b] $p \leq 0.05$.

(22) Greengrass, P.; Bremner, R. *Eur. J. Pharmacol.* **1979**, *55*, 323. Hornung, R.; Presek, P.; Glossmann, H. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1979**, *308*, 223. Langer, S. Z.; Massingham, R.; Shepperson, N. B. *Br. J. Pharmacol.* **1980**, *70*, 60P.
(23) Bennett, J. P.; Snyder, S. H. *Mol. Pharmacol.* **1976**, *12*, 373.
(24) Granat, F. R.; Stone, G. A.; Lovell, R. A. to be submitted to *Eur. J. Pharmacol.*
(25) Fielding, S.; Wilker, J.; Hynes, M.; Szewczak, M.; Novick, W. J.; Lal, H. *J. Pharmacol. Exp. Ther.* **1978**, *207*, 899.
(26) Engberg, G.; Svensson, T. H. *Commun. Psychopharmacol.* **1980**, *4*, 233.
(27) Meek, J. L.; Neff, N. H. *Br. J. Pharmacol.* **1972**, *45*, 435.
(28) Psychoyos, S. *Biochem. Pharmacol.* **1981**, *30*, 2182.
(29) Porsolt, R. D.; Anton, G.; Blavet, N.; Jalfre, M. *Eur. J. Pharmacol.* **1978**, *47*, 378. Gorka, Z.; Wojtasik, E. *Pol. J. Pharmacol. Pharm.* **1980**, *32*, 463.

The tricyclic analogue **13** demonstrated no biological activity in the above test systems.

Discussion

As might be anticipated from the structural relationship of **2** and **12** to mianserin, the three substances exhibited generally similar profiles of activity in the test systems employed. However, the two thiophene compounds appear to be more active as central presynaptic α-receptors than does mianserin. They exhibited greater affinity for high-affinity clonidine site A than did mianserin, and in the case of thiophene analogue **2** this was reflected in greater potency in the noradrenergic single unit activity experiments and in the determinations of MHPG levels following ip administration of the test substances. In contrast, binding to high-affinity clonidine site B, [^{3}H]rauwolscine binding, and antagonism of clonidine-induced analgesia were similar for all three compounds. There is also an indication that mianserin is a more potent antagonist of H_2 receptor mediated adenylate cyclase than thiophene analogue **2**. These differences could be of significance in view of two currently held hypotheses. A considerable body of evidence suggests that the therapeutic effects of antidepressant drugs may be due to the development of subsensitivity of the norepinephrine receptor coupled adenylate cyclase system.[30] Such an effect has been demonstrated for mianserin in rat brain,[30] and could be explained by the α_2-adrenoreceptor blocking properties of this drug.[5] An alternative hypothesis[7] suggests that the therapeutic effects of antidepressants may be associated with central antihistamine (H_2) effects. The present study indicates that **2**, **12**, and mianserin all have high affinity for the H_2 receptor, while mianserin has also been shown to be highly active in the system employed by Kanof and Greengard.[7] Thus, further study of **2** and **12** should provide useful information on the validity of these hypotheses.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. NMR spectra were obtained on a Varian EM 390, XL-100, XL-200, or CFT-20 instrument. The iterative spin simulation was performed via a LAOCOON program with magnetic equivalence as implemented on the XL-200. IR spectra were recorded on a Perkin-Elmer 137 spectrophotometer, and mass spectra were recorded on an A.E.I. MS 902 spectrometer.

2-(2-Thienyl)piperazine (5). A solution of ethylenediamine (4.1 g, 0.068 mol) in EtOH (25 mL) was added dropwise to a solution of 2-thienylglyoxal hydrate[10] (10.0 g, 0.063 mol) in EtOH (250 mL) at 0 °C under an atmosphere of dry N_2. The cooling bath was removed, and the reaction mixture was stirred for an additional 1.5 h. Sodium borohydride (4.8 g, 0.126 mol) was added, all at once, and a cooling bath used to control the exotherm. Stirring was continued for an additional 18 h. Water (25 mL) was added, and the EtOH was removed under reduced pressure. The remaining aqueous solution was extracted with CH_2Cl_2 (3 × 100 mL), and the extracts were dried (K_2CO_3). Removal of the solvent under reduced pressure gave **5** as an off-white solid (5.2 g, 52%). An analytical sample was obtained by addition of an Et_2O solution of **5** to an Et_2O solution of oxalic acid dihydrate. The resulting precipitate was washed with Et_2O and MeOH and the free base (mp 81–83 °C) was obtained with saturated K_2CO_3: ^{1}H NMR δ 7.27, 7.04 (m, 1 H; m, 2 H; thiophene protons), 4.05 (d of d, 1 H; methine proton), 2.90 (m, 6 H), 1.87 (s, 2 H; NH); IR 3250 (NH) cm^{-1}; MS, *m/e* (relative intensity) 168 (M$^+$, 15), 138 (15), 125 (100), 110 (70). Anal. ($C_8H_{12}N_2S$) C, H, N.

Ethyl 3-(2-Thienyl)piperazine-1-carboxylate (6). A solution of ethyl chloroformate (20.4 g, 0.19 mol) in HOAc (150 mL) was added dropwise with stirring to a solution of **5** (31.6 g, 0.19 mol) in HOAc (150 mL) at 65 °C under an atmosphere of dry N_2. After 1 h, the solution was allowed to cool to room temperature, and stirring was maintained for an additional 16 h. The reaction mixture was added to chilled 10 N NaOH (750 mL), and ice was added to control the exotherm. The mixture was extracted with CH_2Cl_2 (4 × 300 mL). The organic solution was dried (K_2CO_3), and the solvent was removed under reduced pressure to give **6** (30.0 g) as a light brown oil. This material was dissolved in MeOH (250 mL), and the solution was added to a solution of maleic acid (18.6 g, 1 equiv) in MeOH (250 mL). The mixture was treated with charcoal, and the solvent was removed under reduced pressure to give an oil, which crystallized on trituration with EtOAc. A yield of 44.0 g (65%) of **6** maleate, mp 165–166 °C, was obtained: ^{1}H NMR δ 7,68, 7.41, 7.16 (d, 1 H; d, 1 H; d of d, 1 H; thiophene protons), 6.18 (s, 2 H; maleic acid vinyl protons), 4.76 (d of d, 1 H; methine proton), 4.16 (m, 4 H), 3.36 (m, 4 H), 1.25 (t, 3 H); MS, *m/e* (relative intensity) 240 (M$^+$, 45), 116 (100). Anal. ($C_{11}H_{16}N_2O_2S \cdot C_4H_4O_4$) C, H, N.

1-Methyl-3-(2-thienyl)piperazine (4). A solution of **6** (27.2 g, 0.11 mol) in Et_2O (300 mL) was added to a stirred suspension of $LiAlH_4$ (10.8 g, 0.28 mol) in Et_2O (1300 mL). The mixture was refluxed for 12 **h**, cooled to room temperature, and worked up.[31] The Et_2O solution was dried (K_2CO_3) and evaporated to give **4** (18.4 g, 89%) as a light yellow oil, which was converted to the maleate salt as described above. A yield of 28 g of **4** maleate, mp 123–124.5 °C, was obtained: ^{1}H NMR δ 7.50, 7.32, 7.08 (d of d, 1 H; d, 1 H; d of d, 1 H; thiophene protons), 6.2 (s, 2 H; maleic acid vinyl protons), 4.65 (d of d, 1 H; methine proton), 2.8 [m, 9 H, including δ 2.50 (s, CH_3)]; MS, *m/e* (relative intensity) 182 (M$^+$, 80), 138 (35), 110 (100). Anal. ($C_9H_{14}N_2S \cdot C_4H_4O_4$) C, H, N.

1-[2-(4,4-Dimethyl-1,3-oxazolin-2-yl)phenyl]-4-methyl-2-(2-thienyl)piperazine (8). A solution of **4** (8.5 g, 0.046 mol) in dry THF (60 mL) was cooled to −78 °C under an atmosphere of dry N_2. *n*-Butyllithium in hexane (18 mL of 2.6 N) was added to the stirred solution during 5 min. The temperature of the resulting suspension was raised to 0 °C, and stirring was maintained for 0.5 h. A solution of **7**[13] (9.5 g, 0.046 mol) in dry THF (90 mL) was added during 45 min. The reaction mixture became dark. Stirring was continued for an additional 1 h at 0 °C and then at room temperature for 18 h. Water (15 mL) was added, and the THF was removed under reduced pressure. The residue was partitioned between Et_2O (150 mL) and H_2O (75 mL). The aqueous phase was extracted with Et_2O (2 × 50 mL), and the combined organic solutions were washed with saturated aqueous K_2CO_3 (75 mL) and dried (K_2CO_3). The solvent was removed under reduced pressure, and the residue was washed with petroleum ether (100 mL) to give **8** (11.8 g, 72%). An analytical sample was obtained by recrystallization from petroleum ether: mp 114–115 °C; ^{1}H NMR δ 7.25 (m, 7 H; aromatic protons), 4.80 (d of d, 1 H; methine proton), 4.16 (s, 2 H; oxazoline CH_2), 3.53 (m, 1 H), 2.75 [m, 8 H, including δ 2.37 (s, CH_3)], 1.43 (s, 6 H; oxazoline CH_3's); IR 1660 (C=N) cm^{-1}; MS, *m/e* (relative intensity) 355 (M$^+$, 30), 285 (100), 203 (85). Anal. ($C_{20}H_{25}N_3OS$) C, H, N.

1-(2-Carbethoxyphenyl)-4-methyl-2-(2-thienyl)piperazine (10). A solution of **8** (33.1 g, 0.090 mol) in ethanolic sulfuric acid (2.2 L of 1.5 N) was refluxed for 12 h. Approximately 75% of the EtOH was removed under reduced pressure, and the remaining solution was poured into saturated K_2CO_3 (1000 mL). This solution was extracted with CH_2Cl_2 (4 × 350 mL), and the extracts were dried (K_2CO_3) and evaporated. The resulting oil was purified by column chromatography with silica gel (600 g, solvent EtOAc). The ester **10** was isolated as a major component (30 g). The maleate salt was prepared as described above to give **10** maleate (28.2 g, 68%): mp 145–147 °C; ^{1}H NMR δ 7.72, 7.20, 6.78 (d, 1 H; m, 5 H; d of d, 1 H; aromatic protons), 6.41 (s, 2 H; maleic acid vinyl protons), 5.12 (d of d, 1 H; methine proton), 4.42 (q, 2 H; OCH_2 protons), 3.33 [m, 9 H, including δ 2.9 (s, 3 H, N-CH_3)], 1.43 (t, 3 H, C-CH_3); IR 1720 (C=O) cm^{-1}; MS, *m/e* (relative intensity) 300 (M$^+$, 30), 286 (30), 274 (100). Anal. ($C_{18}H_{22}N_2O_2S \cdot C_4H_4O_4$) C, H, N.

1-[2-(Hydroxymethyl)phenyl]-4-methyl-2-(2-thienyl)-

(30) Mishra, R.; Janowsky, A.; Sulser, F. *Neuropharmacology* **1980**, *19*, 983.

(31) In ref 15, p 583.

piperazine (3). A solution of **10** (10.7 g, 0.032 mol) in Et_2O (225 mL) was added to a stirred suspension of $LiAlH_4$ (3.9 g, 0.097 mol) in Et_2O (150 mL). The reaction mixture was refluxed for 12 h, cooled to room temperature, and worked up.[31] The resulting colorless oil crystallized to give analytically pure **3** (9.3 g, 98%): mp 81–83 °C; ^1H NMR δ 7.13, 6.80 (m, 5 H; m, 2 H; aromatic protons), 4.80 (m, 4 H; methine proton; CH_2OH protons), 2.90 (m, 6 H), 2.40 (s, 3 H, N-CH_3); MS, *m/e* (relative intensity) 288 (M^+, 60), 135 (45), 110 (85), 97 (95), 71 (100). Anal. ($C_{16}H_{20}N_2OS$) C, H, N.

1,2,3,4,10,13b-Hexahydro-2-methylpiperazino[1,2-*a*]thieno[2,3-*c*][1]benzazepine (2) and the Corresponding [3,2-*c*] Isomer (12). A solution of **3** (4.7 g, 0.016 mol) and polyphosphate ester[15] (93 g) in CH_2Cl_2 (930 mL) was refluxed for 12 h. The solution was cooled to room temperature, and the precipitated solid was filtered off. This material was partitioned between CH_2Cl_2 (300 mL) and saturated NH_4OH (300 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 75 mL), and the combined organic solutions were washed with saturated NaCl (100 mL) and dried (K_2CO_3). Evaporation of the solvent gave 3.1 g of an oil, which was chromatographed on silica gel (75 g). Elution with EtOAc gave two discrete fractions. Fraction 1 (**2**, 2.4 g, 45% yield) had an R_f of 0.2 on TLC; fraction 2 (**12**, 500 mg, 9.3% yield) had an R_f of 0.08 (solvent EtOAc). **2**: ^1H NMR δ 7.17 (m, 4 H, H_E, H_F, H_G, H_H), 6.88 (d, 1 H, H_B), 6.66 (d, J_{AB} = 5 Hz, 1 H, H_A), 4.71 (d, 1 H, $H_{D'}$), 4.08 (d of d of d, $J_{C_{ax}J_{ax}}$ = 10.2 Hz, $J_{C_{ax}J_{eq}}$ = 2.7 Hz, $J_{C_{ax}D'}$ = 0.8 Hz, 1 H, H_C), 3.41 (d, $J_{DD'}$ = 14 Hz, 1 H, H_D), 2.36 (s, 3 H, CH_{3_L}), 2.16–3.67 (6 H, CH_{2_J}, CH_{2_K}, CH_{2_L}); ^{13}C NMR 32.79 (t, C_J), 45.55 (q, C_{16}), 51.36 (t, C_8), 55.19 (t, C_7), 62.00 (d, C_6), 65.57 (t, C_9), 119.90 (d, C_{15}), 121.52 (d, C_{13}), 122.54 (d, C_3), 126.54 (d, C_{12}), 127.02 (d, C_2), 127.81 (d, C_{14}), 136.29 (s, C_{11}), 136.99 (s, C_4), 140.41 (s, C_5), 149.14 (s, C_{10}) ppm. Anal. for **2** maleate (CGS 11049A), mp 187–188.5 °C, ($C_{16}H_{18}N_2S \cdot C_4H_4O_4$) C, H, N. **12**: ^1H NMR δ 7.06 (m, 4 H, H_E, H_F, H_G, H_H), 6.96 (d, 1 H, H_B), 6.71 (d, *J* = 5 Hz, 1 H, H_A), 4.44 (d, 1 H, $H_{D'}$), 4.18 (d of d of d, $J_{C_{ax}J_{ax}}$ = 2.7 Hz, $J_{C_{ax}D'}$ = 0.8 Hz, 1 H, H_C), 3.38 (d, $J_{DD'}$ = 13.5 Hz, 1 H, H_D), 2.36 (s, 3 H, CH_{3_L}), 2.16–3.68 (m, 6 H, CH_{2_J}, CH_{2_K}, CH_{2_L}); ^{13}C NMR 31.64 (t, C_L), 45.66 (q, C_{16}), 51.34 (t, C_8), 55.42 (t, C_7), 63.23 (d, C_6), 63.32 (t, C_9), 119.94 (d, C_{15}), 120.56 (d, C_{23}), 122.46 (d, C_3), 126.41 (d, C_{12}), 127.34 (d, C_2), 127.83 (d, C_{14}), 135.09 (s, C_{11}), 136.32 (s, C_4), 139.72 (s, C_5), 149.51 (s, C_{10}) ppm. Anal. for **12** fumarate (CGS 15413A), mp 198–201 °C, ($C_{16}H_{18}N_2S \cdot C_4H_4O_4$) C, H, N. The mass spectra of the two isomers were essentially identical: *m/e* (relative intensity) 270 (M^+, 100), 226 (22), 199 (63), 72 (70).

1-(2-Formylphenyl)-4-methylpiperazine (9a). Anhydrous K_2CO_3 (21.6 g, 0.016 mol) was added to a solution of *N*-methylpiperazine (8.0 g, 0.08 mol) and 2-fluorobenzaldehyde (10.0 g, 0.08 mol) in Me_2SO (100 mL). The mixture was maintained at 95 °C with stirring for 24 h and then cooled and poured into H_2O (400 mL). The solution was acidified with 6 N HCl and extracted with CH_2Cl_2 (3 × 200 mL). The aqueous solution was made basic with 10% NaOH and extracted with CH_2Cl_2 (3 × 200 mL), and the extracts were dried (K_2CO_3). The solvent was removed under reduced pressure, and the residue was distilled to give analytically pure **9a** (7.4 g, 45%) as a pale yellow oil: bp 132 °C (0.2 mm); ^1H NMR δ 10.36 (s, 1 H; CHO), 7.33 (m, 4 H; aromatic protons), 3.10 (m, 4 H), 2.57 (m, 4 H), 2.33 (s, 3 H; N-CH_3); IR 1675 (C=O) cm^{-1}. Anal. ($C_{12}H_{16}N_2O$) C, H, N.

1-(2-Cyanophenyl)-4-methylpiperazine (9b). This substance was obtained by the method used for the preparation of **9a**. Use of 2-fluorobenzonitrile (9.7 g, 0.08 mol) gave analytically pure **9b** (6.9 g, 41%) as a pale yellow oil: bp 123–124 °C (0.1 mm); ^1H NMR δ 7.43, 6.95 (m, 2 H; m, 2 H; aromatic protons), 3.2 (m, 4 H), 2.6 (m, 4 H), 2.33 (s, 3 H, N-CH_3); IR 2200 (C≡N) cm^{-1}. Anal. ($C_{12}H_{15}N_3$) C, H, N.

2-[Methyl(2-thienylmethyl)amino]benzaldehyde (15). Following the procedure used for the preparation of **9a**, amine **14**[18] (21.2 g, 0.17 mol) gave analytically pure **15** (23.3 g, 61%) as a pale yellow oil: bp 126–128 °C (0.035 mm); ^1H NMR δ 10.52 (s, 1 H, CHO), 7.90, 7.53, 7.10 (m, 1 H; m, 1 H, m, 5 H; aromatic protons), 4.50 (s, 2 H), 2.88 (s, 3 H). Anal. ($C_{13}H_{13}NOS$) C, H, N.

2-[Methyl(2-thienylmethyl)amino]benzyl Alcohol (16). A solution of **15** (11.6 g, 0.05 mol) and sodium borohydride (3 g, 0.08 mol) in *i*-PrOH (150 mL) was refluxed for 18 h. The solvent was evaporated, and the residue was partitioned between Et_2O (150 mL and H_2O (100 mL). The Et_2O solution was washed with saturated K_2CO_3 (3 × 100 mL) and saturated NaCl (100 mL) and dried (K_2CO_3). The solution was evaporated, and the residue was distilled to give analytically pure **16** (10.7 g, 91%) as a pale yellow oil: bp 132 °C (0.02 mm); ^1H NHR δ 7.0 (m, 7 H; aromatic protons), 4.82 (s, 2 H; benzyl CH_2), 4.57 (s, 1 H; OH), 4.26 (s, 2 H), 2.67 (s, 3 H; N-CH_3). Anal. ($C_{13}H_{15}NOS$) C, H, N.

4,10-Dihydro-9-methyl-9*H*-thieno[2,3-*c*][1]benzazepine (13) and the Corresponding [3,2-*c*] Isomer (17). A solution of **16** (10.0 g) and polyphosphate ester[15] (200 g) in CH_2Cl_2 (1 L) was refluxed for 18 h. The solvent was removed under reduced pressure, and the residue was stirred with H_2O (2 L) for 3 days. The aqueous solution was made basic with 10% KOH and extracted with CH_2Cl_2 (3 × 250 mL). The CH_2Cl_2 extracts were dried (K_2CO_3) and evaporated under reduced pressure to give 9.3 g of a yellow solid. The two isomers were separated by HPLC with a Waters Prep 500A machine with silica gel columns and cyclohexane/toluene (1:1) as the solvent system. **13**: ^1H NMR δ 7.32 (t of m, J_{GH} = 7.96 Hz, 1 H, H_G), 7.23 (d of m, J_{EG} = 1.66 Hz, J_{EH} = 0.37 Hz, 1 H, H_E), 7.21 (d, $J_{HI} \approx$ 0.2 Hz, 1 H, H_H), 7.09 (d of m, 1 H, H_B), 7.06 (t of m, J_{FG} = 7.44 Hz, J_{FE} = 7.41 Hz, J_{FH} = 1.21 Hz, 1 H, H_F), 6.86 (d of m, J_{AB} = 5.02 Hz, J_{AC} = 0.43 Hz, J_{AD} = 0.40 Hz, 1 H, H_A), 4.26 (m, J_{CA} = 0.43 Hz, J_{CB} = 0.76 Hz, 2 H, CH_{2_C}), 4.06 (m, J_{DE} = 0.64 Hz, J_{DA} = 0.40 Hz, J_{DG} = 0.36 Hz, J_{DB} = 0.31 Hz, J_{DF} = 0.26 Hz, 2 H, CH_{2_D}), 3.01 (s, 3 H, CH_{3_I}); ^{13}C NMR 33.90 (t, C_I), 43.14 (q, C_{13}), 56.47 (t, C_6), 118.71 (d, C_{12}), 121.40 (d, C_{10}), 123.02 (d, C_3), 127.27 (d, C_9), 128.05 (d, C_2), 128.57 (d, C_{11}), 134.41 (s, C_8), 134.81 (s, C_4), 137.36 (s, C_5), 150.91 (s, C_7) ppm. Anal. ($C_{13}H_{13}NS$) C, H, N. **17**: ^1H NMR δ 7.15 (t of m, 1 H, H_G), 7.01 (d of m, J_{EG} = 1.59 Hz, 1 H, H_E), 7.00 (d of m, J_{HG} = 7.99 Hz, J_{HE} = 0.37 Hz, $J_{HI} \approx$ 0.2 Hz, 1 H, H_H), 6.88 (t of m, J_{FG} = 7.48 Hz, J_{FE} = 7.42 Hz, J_{FH} = 1.24 Hz, 1 H, H_F), 6.76 (d of m, 1 H, H_B), 6.45 (d of m, J_{AB} = 5.22 Hz, 1 H, H_A), 3.98 (m, J_{DC} = 1.26 Hz, J_{DE} = 0.64 Hz, J_{DG} = 0.36 Hz, J_{DA} = 0.37 Hz, J_{DB} = 0.30 Hz, J_{DF} = 0.14 Hz, 2 H, CH_{2_D}), 3.88 (m, J_{CA} = 0.37 Hz, 2 H, CH_{2_C}), 2.73 (s, 3 H, CH_{3_I}). T^1 measurements on all protons of **13** and **17** were performed and were entirely consistent with the indicated assignments: ^{13}C NMR 32.59 (t, C_1), 43.32 (q, C_{13}), 58.08 (t, C_6), 118.83 (d, C_{12}), 121.01 (d, C_{10}), 122.96 (d, C_3), 127.12 (d, C_9), 127.60 (d, C_2) 127.72 (d, C_{11}), 134.01 (s, C_8, C_4), 137.0 (s, C_5), 152.46 (s, C_7) ppm. The mass spectra of the two isomers were essentially identical: *m/e* (relative intensity) 215 (M^+, 100), 200 (30), 199 (22).

Biological Test Procedures. The [^3H]clonidine binding assay was performed by the method of Braunwalder et al.[20] and [^3H]prazosin binding by the procedure described by Greengrass and Bremner,[22] omitting ascorbic acid from the incubation medium. Serotonin receptor binding was determined by a modification of the procedure described by Bennett and Snyder,[23] homogenization being conducted at pH 7.7 at 25 °C using a medium containing 10 mM dithiothreitol. The final pellet was homogenized in a medium containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM dithiothreitol in addition to the components originally specified.[23] The [^3H]rauwolscine binding assay was performed by the method of Granat et al.[24] For the antagonism of clonidine-induced analgesia assay, a modification of the phenylquinone-writhing procedure described by Fielding et al.[25] was employed. Thirty minutes after ip injection of the test substance, male mice (18–22 g) were injected po with clonidine. Twenty minutes later, phenylquinone was injected ip, and the number of mice that writhed was counted 5–15 min after injection. Any animal writhing was considered a reactor. Noradrenergic single unit activity was determined by using procedures described by Engberg and Svensson.[26] Changes in MHPG levels were determined by the procedure of Meek and Neff.[27] Inhibition of histamine-activated adenylate cyclase was determined by the procedures of Psychoyos.[28] Swim test results were obtained by using the procedure of Porsolt et al.[29]

Acknowledgment. We thank Dr. H. B. Renfroe for his encouragement and support of this work. We are also grateful for valuable discussions of the biological test results with Drs. W. D. Cash, R. A. Lovell, S. Psychoyos, and G. P. Quinn. We thank L. Raabis for IR and NMR spectra (EM 390), C. Shimanskas for the mass spectra, and G.

Robertson and R. Oekinghaus for microanalytical data. We also thank the following for providing the biological test results: C. Atkins, B. Barbaz, A. Braunwalder, F. R. Granat, D. Johnson, H. J. Kalinsky, Dr. S. Psychoyos, M. B. Reid, G. A. Stone, and J. J. Welch. We are grateful to M. Makar and D. Vivian for preparing the typescript.

Registry No. 2, 85803-58-3; 2 maleate, 85803-59-4; 3, 85803-57-2; 4, 85803-52-7; 4 maleate, 85803-53-8; 5, 85803-49-2; 6, 85803-50-5; 6 maleate, 85803-51-6; 7, 57598-33-1; 8, 85803-54-9; 9a, 85803-62-9; 9b, 85803-63-0; 10, 85803-55-0; 10 maleate, 85803-56-1; 12, 85803-60-7; 12 fumarate, 85803-61-8; 13, 85803-66-3; 14, 58255-18-8; 15, 85803-64-1; 16, 85803-65-2; 17, 85803-67-4; 2-thienylglyoxal, 51445-63-7; ethylenediamine, 107-15-3; *N*-methylpiperazine, 109-01-3; 2-fluorobenzaldehyde, 446-52-6; 2-fluorobenzonitrile, 394-47-8.

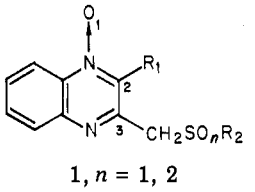
Synthesis and Antibacterial Activity of Some 3-[(Alkylthio)methyl]quinoxaline 1-Oxide Derivatives

John P. Dirlam,*[†] Joseph E. Presslitz,[†] and B. J. Williams[‡]

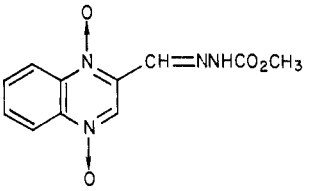
Pfizer Central Research, Groton, Connecticut 06340, and Pfizer Animal Health Research Department, Terre Haute, Indiana 47808. *Received September 1, 1982*

Some 3-[(alkylthio)methyl]quinoxaline 1-oxide derivatives (1) have been synthesized and screened for antibacterial activity. 2-Acetyl-3-[(methylsulfonyl)methyl]quinoxaline 1-oxide (**7a**) was found to possess good in vitro activity against some pathogens important to veterinary medicine including *Treponema hyodysenteriae*, a causative agent in swine dysentery. In an in vivo experiment, this compound (**7a**) completely protected pigs against a swine dysentery challenge over a 21-day period.

A wide variety of quinoxaline 1,4-dioxides have been described of value as antibacterial agents, animal growth promotants, and as agents for improving feed efficiency of animals.[1-3] Although a number of reports deal with the chemistry of quinoxaline 1-oxides,[4] only a few describe useful in vitro antibacterial activity.[5] Furthermore, to our knowledge, no quinoxaline 1-oxides have been reported with in vivo antibacterial activity. This paper describes the synthesis of certain 3-[(alkylthio)methyl]quinoxaline 1-oxide derivatives (1) having in vitro antibacterial activity against some pathogens important to veterinary medicine, i.e., *Escherichia coli*, *Pasteurella multocida*, *Salmonella choleraesuis*, and *Treponema hyodysenteriae*, as well as in vivo activity against swine dysentery.



1, n = 1, 2



2 (carbadox)

Synthesis. Until recently there were few methods available for the selective synthesis of suitable 2,3-disubstituted quinoxaline 1-oxide precursors required for this study, but it has been demonstrated in these laboratories that certain quinoxaline 1,4-dioxides bearing an electron-withdrawing group in the 2-position can be selectively monodeoxygenated to afford good yields of the desired starting materials.[6] In particular, the 3-[(alkylthio)-methyl]quinoxaline 1-oxide compounds (1) were prepared in the manner shown in Scheme I. Several 2-substituted 3-methylquinoxaline 1-oxides (**3a–c**)[6] were converted to the corresponding 3-bromomethyl derivatives (**4a–c**) via bromination with bromine–methanol. Alternatively, the chloromethyl intermediate **4d** was obtained from a 3-methylquinoxaline 1,4-dioxide by selective deoxygenation and simultaneous chlorination of the methyl group with *p*-toluenesulfonyl chloride.[7] Various alkylthio side chains were added to intermediate **4** to afford **5** by procedures developed previously for the synthesis of 2(3)-[(alkylthio)methyl]quinoxaline 1,4-dioxides (method A).[8] Oxidation of the sulfur with 1 or 2 equiv of *m*-chloroperbenzoic acid (MCPBA), method B or C, gave rise to the corresponding sulfoxides **6** or sulfones **7**, respectively. Alternatively, **7** could be synthesized from **4** in a one-step process by a displacement reaction[9] with sodium alkylsulfinates (method D). The aminolysis[8] of **6** or **7**, where $R_1 = CO_2CH_3$, allowed the synthesis of some carboxamides ($R_1 = CONH_2$, $CONHCH_3$; method E). The deacylation of **6** or **7**, where $R_1 = COCH_3$, afforded analogues unsubstituted in the 2-position (R_1 = H; method F).

[†] Pfizer Central Research.
[‡] Pfizer Animal Health Research Department.

(1) (a) J. Francis, J. K. Landquist, A. A. Levi, J. S. Silk, and J. M. Thorp, *Biochem. J.*, **63**, 455 (1956). (b) J. D. Johnston, M. Abu-el-Haj, E. Abushanab, B. W. Dominy, J. W. McFarland, C. H. Issidorides, and M. J. Haddadin, IUPAC Meeting, London, July 1968, Abstracts, H 4, p 437; "Abstrcts of Papers", 156th National Meeting of the American Chemical Society, Atlantic City, Sept 1968, American Chemical Society, Washington, DC, 1968, Abstr MEDI 015. (c) G. W. Thrasher, J. E. Shively, C. E. Askelson, W. E. Babcock, and R. R. Chalquest, *J. Anim. Sci.*, **31**, 333 (1970). (d) K. Ley and F. Seng, *Synthesis*, 415 (1975), and references cited therein.
(2) J. P. Dirlam and J. E. Presslitz, *J. Med. Chem.*, 21, 483 (1978).
(3) J. P. Dirlam, L. J. Czuba, B. W. Dominy, R. B. James, R. M. Pezzullo, J. E. Presslitz, and W. W. Windisch, *J. Med. Chem.*, **22**, 1118 (1979).
(4) (a) M. J. Haddadin, H. N. Alkaysi, and S. E. Saheb, *Tetrahedron*, **26**, 1115 (1970). (b) M. M. El Abadelah, S. S. Sabri, and H. I. Tashtoush, *Tetrahedron*, **35**, 2571 (1979). (c) A. F. Kluge, M. L. Maddox, and G. S. Lewis, *J. Org. Chem.*, **45**, 1909 (1980), and references cited therein.
(5) (a) M. L. Douglass, U.S. Patent 3 733 323 (1973); *Chem. Abstr.*, **79**, 42557y (1973). (b) J. M. Cox and R. A. Burrell, U.S. Patent 3 928 608 (1975).
(6) J. P. Dirlam and J. W. McFarland, *J. Org. Chem.*, **42**, 1360 (1977).
(7) M. Harmana and M. Yamazaki, *Chem. Pharm. Bull.*, 11, 415 (1963).
(8) J. P. Dirlam, U.S. Patent 4 039 540 (1977); *Chem. Abstr.*, **87**, 152275c (1977).
(9) B. B. Jarvis and B. A. Marien, *J. Org. Chem.*, **42**, 2676 (1977).

0022-2623/83/1826-1122$01.50/0