Preparation of Some 10-[3-(Dimethylamino)-1-propyl]-10*H*-pyrazino[2,3-*b*][1,4]benzothiazines as Potential Neuroleptics

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Chloro- and methyl-substituted 10*H*-pyrazino[2,3-*b*][1,4]benzothiazines were prepared and their structures determined by ^{13}C NMR and X-ray crystallographic analysis. Alkylation afforded the 10-[3-(dimethylamino)-1-propyl] derivatives, which were compared to chlorpromazine in receptor-binding assays, in vivo behavioral tests, and electrochemical oxidation studies. In this series, the 2-chloro compound, **4c**, proved to be the most effective derivative in displacing [^3H]siperone, [^3H]apomorphine, and [^3H]prazosin radioligands from binding sites, being approximately as potent as chlorpromazine in this respect. However, none of the 10*H*-pyrazino[2,3-*b*][1,4]benzothiazines of this study were as active as chlorpromazine in in vivo tests predictive of neuroleptic activity.

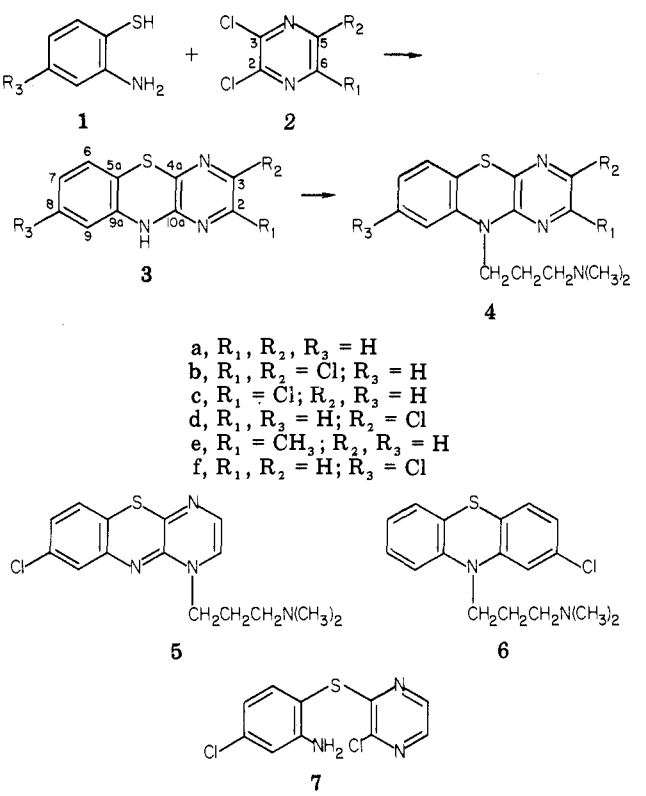
Previous reports from these laboratories have described the serotoninmimetic properties of some piperazinylquinoxalines[1] and piperazinylpyrazines.[2] In order to appraise further the utility of the pyrazine ring as an alternative to phenyl in the design of therapeutic agents, we have now prepared some 10-[3-(dimethylamino)propyl]-10*H*-pyrazino[2,3-*b*][1,4]benzothiazines. These heterocycles correspond to replacement of one of the phenylene rings of certain phenothiazine neuroleptics, i.e., chlorpromazine (**6**), by pyrazine.

Chemistry. The unsubstituted[3,4] and 2,3-dichloro-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine,[3,5] **3a** and **3b**, had been prepared previously by reaction of 2-aminothiophenol with the symmetrical dichloro- and tetrachloropyrazines **2a** and **2b** (Scheme I). However, it was not apparent at the outset of this work if the monochloro derivatives **3c** and **3d** could be obtained from condensation of 2-aminothiophenol with trichloropyrazine (**2c**).

Reaction of **1a** and **2c** in DMF with triethylamine as the base provided a mixture of **3c** and **3d**, which could be separated cleanly into the two isomeric chloro-10*H*-pyrazino[2,3-*b*][1,4]benzothiazines by silica gel chromatography. Although the 2-position of **2c** appears to be the most likely site for initial attack by the aminothiophenol,[6] the possibility of accompanying Smiles rearrangements and the fact that both chloro isomers were isolated from the reaction precluded any attempts at structural assignment through mechanistic considerations. However, structures could be assigned to these isomers through analysis of ^{13}C NMR chemical shifts and long-range couplings.

^{13}C resonances of carbons ortho, meta, and para to the pyrazine methine carbon, C-3 in **3c** and C-2 in **3d**, can be resolved through the use of ^{13}C–^1H couplings. The **4a** carbon resonance of both **3c** and **3d** was identified by the three-bond coupling of 5–7 Hz between C-4a and the N-10 proton.[7] In addition, resonances exhibiting couplings of 0 and 11 Hz can be assigned to pyrazine carbons para and meta to the pyrazine methine carbon, respectively.[7] Of the two chloropyrazine structures **3c** and **3d**, only **3c** contains a carbon atom, C-4a, coupled to the N-10 proton through three bonds and meta to the pyrazine methine carbon. In **3d**, a carbon atom, C-4a, is present that retains the same relationship to N-10 but is now para to the methine carbon. In addition, the pyrazine carbons substituted with chlorine and ortho to the methine carbon in both **3c** and **3d** exhibited characteristic couplings of 7–8 Hz.[7] On this basis, structures were assigned to the two chloro isomers as either **3c** or **3d** (Table I).

Scheme I



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(4) (a) Carter, S. D.; Cheeseman, G. W. H. *Tetrahedron* **1977**, *33*, 827. (b) Okafor, C. O. *J. Heterocycl. Chem.* **1981**, *18*, 405.
(5) Gulbenk, A. H.; Horne, D. J.; Johnston, H. U.S. Patent 3 746 707, 1973; *Chem. Abstr.* **1973**, *79*, 105301*h*. Gulbenk, A. H.; Horne, D. J.; Johnston, H. U.S. Patent 3 663 543, 1972; *Chem. Abstr.* **1972**, *77*, 48509*g*.
(6) The 2-chloro of 2,3,6-trichloropyrazine has been reported to undergo regioselective displacement by methoxide (Carter, D. R.; Boer, F. P. *J. Heterocycl. Chem.* **1972**, *9*, 335), by ammonia (Palamidessi, G.; Bernardi, L.; Leone, A. *Farmaco, Ed. Sci.* **1966**, *21*, 805), and by hydrazine (Cere, L.; Marchese, A.; Rossi, P. F. *Rass. Chim.* **1975**, *27*, 294; *Chem. Abstr.* **1977**, *87*, 23211*e*).
(7) Unpublished observations from these laboratories.

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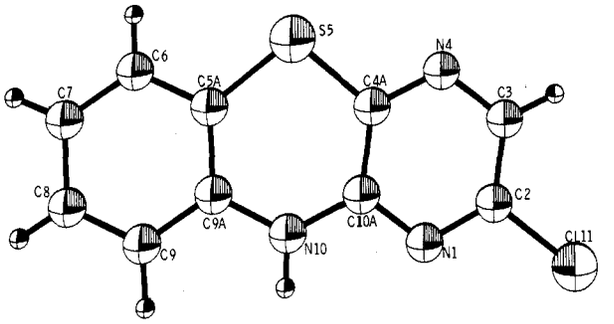

Figure 1. A computer-generated perspective drawing[19] of **3c** drawn from the X-ray coordinates.

The same structural assignments were obtained independently through an analysis of the ^{13}C NMR chemical shifts of the four pyrazine carbons. Arguments based on simple chemical-shift theory[8a] predict that C-10a of **3**, being bonded to nitrogen, the most electronegative of the four pyrazine substituents, should be the farthest downfield of the four pyrazine resonances. Therefore, the 148-ppm resonance is assigned to this carbon. Also, introduction of an amino group into the pyrazine ring would be expected to induce upfield shifts in ^{13}C resonances of the pyrazine carbons ortho and para to the substituted carbon.[7] Therefore, the pyrazine carbon substituted with chlorine, C-3, of **3d**, being para to the amino-bearing carbon, C-10a, would have a higher-field chemical shift than that of the chloro-containing carbon, C-2, of **3c**, which is meta to C-10a. A reverse order should be observed for the pyrazine methine carbons, C-2 and C-3, of **3d** and **3c**. All of these conditions are satisfied only if the structures and chemical shifts for **3c** and **3d** are as assigned in Table I.

Confirmation of these structural assignments was obtained through X-ray crystallographic analysis of the isomer with the highest R_f upon silica gel chromatography. Figure 1 is a computer-generated drawing of **3c** showing that the Cl atom is bonded to C-2 and not C-3. There are no abnormal bond distances or angles and no close intermolecular contacts. The two terminal six-membered rings of **3c** are planar to within 0.01 Å, while the slightly distorted central ring is planar to within 0.03 Å.

Condensation of 2,3-dichloro-6-methylpyrazine,[9] **2e**, with 2-aminothiophenol under conditions similar to that used for preparation of **3c** and **3d** afforded only a single 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine derivative. This product could be assigned the 2-methyl structure **3e** by similar analysis of the ^{13}C NMR spectrum, even though no C4a–NH coupling was observed in this case.

The 8-chloro-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (**3f**) was prepared unambiguously by heating the thioether **7** at 220 °C for 2 h. Thioether **7**, in turn, was prepared from condensation of **1f**[10] with the symmetrical 2,3-dichloropyrazine (**2a**).

Alkylation of the 10*H*-pyrazino[2,3-*b*][1,4]benzothiazines with 3-(dimethylamino)propyl chloride afforded **4** in good yield. In the case of the 8-chloro derivative **4f**, a 17% yield of an isomeric alkylation product, formulated as **5**, was also obtained.[11]

Table I. ^{13}C NMR Spectral Parameters[a]

compd	C-2	C-3	C-4a	C-5a	C-6	C-7	C-8	C-9	C-9a	C-10a	CH_3
3c[b,c]	143.1 (^2J = 8)	133.1 (^1J = 196)	137.8 ($^3J_{NH}$ = 7, ^3J = 11)	115.5	127.9[d]	123.3	126.0[d]	115.8	137.1	148.4 ($^4J \cong 0$)	
3d[b,c]	137.6 (^1J = 194)	137.4 (^2J = 7)	139.2 ($^3J_{NH}$ = 5, $^4J \cong 0$)	114.6	128.2[d]	123.0	126.0[d]	115.6	137.8	148.0 (^3J = 11)	
3e[c,e]	148.1 (^2J = 10, $^2J_{CH_3}$ = 7)	136.3 (^1J = 184, $^3J_{CH_3}$ = 5)	137.0 (^3J = 9)	117.4	127.6[d]	123.0	126.6[d]	115.0	137.9	147.8 ($^4J \cong 0$)	20.3
2e[c,e]	144.7 (^3J = 11)	146.5 (^4J = 1.5)		152.3[f] (^2J = 11, $^2J_{CH_3}$ = 6)	141.4 (^1J = 187, $^3J_{CH_3}$ = 5)						20.4

[a] Chemical shifts in parts per million relative to internal Me_4Si. Coupling constants (J) in hertz; superscript indicates number of bonds between coupled carbon and proton, and subscript indicates specific proton. No subscript indicates carbon is coupled to the pyrazine methine proton. [b] In Me_2SO-d_6. [c] See Scheme I for numbering system. [d] These values in any row may be interchanged. [e] In $CDCl_3$. [f] Carbon 5 of **2e**, Scheme I.

(8) (a) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; p 197. (b) *Ibid.*; p 199.
(9) Adachi, J.; Sato, N. *J. Org. Chem.* **1972**, *37*, 221.
(10) Collings, A. J.; Morgan, K. J. *Tetrahedron* **1964**, *20*, 2167.
(11) Methylation of 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine and 12*H*-quinoxalino[2,3-*b*][1,4]benzothiazine have been reported to give the corresponding 1- and 11-methyl derivatives as minor products.[4a]

Table II. Polarographic Results[a]

compd	$E_{1/2}$, V pH 6.36[b]	$E_{1/2}$, V pH 1.15[c]	i_d, μA pH 6.36[b]	i_d, μA pH 1.15[c]
6[d]	+0.44	+0.30	150	95
4c	+0.7[e]	+0.56	sharp rise	107
4f	+0.7[e]	+0.60	sharp rise	95

[a] Pt ribbon electrode vs. SCE. [b] 2-Morpholinoethanesulfonic acid (Mes) buffer. [c] 0.1 M H_2SO_4. [d] Chlorpromazine. [e] Estimated.

Electrochemistry. Since cation radicals have been implicated in the metabolism and pharmacological activity of the phenothiazine neuroleptics,[12] the electrochemical behavior of some of the 10*H*-pyrazino[2,3-*b*][1,4]benzothiazines was compared to that of chlorpromazine (**6**). Merkle and Discher[13] found that electrolytic oxidation of chlorpromazine in strong acid (9 N H_2SO_4) yielded two well-defined one-electron polarographic waves resulting from disproportionation of the cation radical. In dilute acid (0.1 N H_2SO_4), only a single two-electron wave appeared, which was attributed to a spontaneous disproportionation reaction. However, McCreery et al.[14] reported that at pH 2–7, the initially formed cation radical did not disproportionate but instead reacted directly with nucleophiles in the solvent/electrolyte solution.

Our cyclic voltammetric studies with chlorpromazine agree qualitatively with those of McCreery. We found in our polarographic studies of chlorpromazine at a platinum electrode that the well-defined anodic wave obtained at pH 1.15 (0.1 M H_2SO_4) resembled that at pH 6.36 (0.1 M Mes buffer) but with the half-wave potential being more positive and the wave height larger at the higher pH. At pH 6.36, closely following the first wave, the anodic current began to rise as if to form a second wave, but no plateau was detected. These results probably arise from the relative instability of the chlorpromazine cation radical at this pH and suggest that a series of chemical reactions are taking place after formation of the radical.

The polarographic wave forms and cyclic voltammograms generated at a platinum electrode from **4c** and **4f** in 0.1 M H_2SO_4 (pH 1.15) were quite similar to those of chlorpromazine at this pH, except that their half-wave potentials were more positive (Table II). However at pH 6.36, the polarographic wave forms of **4c** and **4f** differed significantly from chlorpromazine in that both **4c** and **4f** yielded a sharply rising current with ill-defined plateaus. Also, at pH 6.36, the cyclic voltammogram of chlorpromazine yielded an anodic peak potential of +0.75 V (vs. SCE) at which the peak current decreased rapidly in 20 min during the course of continuous scanning at 0.20 V/s. In contrast, **4c** and **4f** under these conditions yielded no definite oxidation peak except for generation of a large peak current in the region of the solvent/electrolyte.

Compounds **4c,e,f** and chlorpromazine all formed transient pink solutions during electrolysis in 0.1 M H_2SO_4. However, only chlorpromazine and the methyl derivative **4e** produced a light pink color in the electrolyzed solutions at pH 6.36. The two 10*H*-pyrazino[2,3-*b*][1,4]benzothiazines lacking the dimethylaminopropyl side chain, **3c** and **3f**, did not undergo electrochemical oxidation under these conditions. Therefore, it appears that 10*H*-pyrazino[2,3-*b*][1,4]benzothiazines containing a 3-(dimethylamino)propyl side chain in the 10-position are capable of forming cation radicals that are in some respects similar to those obtained in the phenothiazine series.

Biological Results and Discussion

Relative affinities of the dimethylaminopropyl derivatives **4c–f** for some receptors relevant to neuroleptic action were characterized by measuring the displacement of radioligands from membrane binding sites in mammalian brain. Interactions with rat caudate dopamine receptors were measured by using [^{3}H]spiperone and [^{3}H]apomorphine antagonist and agonist radioligands, respectively. [^{3}H]Spiperone binding was measured by the method of Burt, Creese, and Snyder.[15] Of the present series of compounds, the 2-chloro derivative **4c** proved to be the most potent in displacing the antagonist ligand from the rat caudate binding site, being nearly as effective as chlorpromazine (**6**, Table III). The 2-methyl (**4e**) and 3-chloro (**4d**) derivatives were less potent, while the 8-chloro compound (**4f**) was relatively inactive.

Displacement of the agonist ligand [^{3}H]apomorphine from rat striatal membranes by this series of compounds was determined by the procedure of Seeman et al.[16] The same order of activities was observed in this binding assay as in that for the [^{3}H]spiperone receptor. The 2-chloro derivative **4c** was the most potent 10*H*-pyrazino[2,3-*b*]-[1,4]benzothiazine tested, with **4d–f** being less effective displacers of radioligand (Table III). Again, **4c** was approximately equipotent with chlorpromazine (**6**).

A different order of activities was observed for binding to the [^{3}H]prazosin site of calf cerebral cortex. In this case, the 8-chloro derivative **4f** exhibited significant activity, being about 16 times less potent than the 2-chloro analogue **4c**. As in the previous examples, **4c** was approximately as potent as chlorpromazine.

The dimethylaminopropyl derivatives **4c–f** were compared with chlorpromazine in two in vivo tests that are predictive of neuroleptic activity. Both **4c** (2-chloro) and **4d** (3-chloro) were inactive at doses up to 15 mg/kg ip, in producing postural asymmetries in caudate-lesioned mice[17] and in antagonizing amphetamine-induced hyperactivity in mice. For comparison, the estimated ED_{50} values for chlorpromazine in these two test procedures were 3.5 and 0.9 mg/kg ip, respectively. At 10-fold higher dose levels, 150 mg/kg ip, **4c** and **4d** exhibited some activity in one or both tests, but neither were as active as chlorpromazine.

The 2-methyl analogue **4e** also produced postural asymmetries in caudate-lesioned mice and antagonized behavioral effects of amphetamine at 150 mg/kg ip similar to the 2-chloro derivative **4c**. However, the 8-chloro compound **4f**, in contrast to **4c**, did not produce these effects even at 150 mg/kg ip. This lack of behavioral activity is consistent with the observed poor affinity of this isomer for the [^{3}H]spiperone receptor site. No signs of sedation were observed with **4f** as might have been expected from the activity of this compound in the [^{3}H]-prazosin receptor-binding assay.

In so far as these results in mice can be extrapolated to clinical major tranquilizing activity, none of the 10-[3-(dimethylamino)propyl]-10*H*-pyrazino[2,3-*b*][1,4]benzothiazines, **4c–f**, described here would appear to be as efficacious as chlorpromazine.

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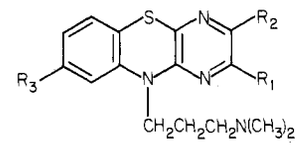
(16) Seeman, P.; Lee, T.; Chan-Wong, M.; Tedesio, J.; Wong, K. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4354.
(17) Lotti, V. J. *Life Sci.* **1971**, *10*, 781.

Table III. Displacement of Radioligand Binding by 10-[3-(Dimethylamino)propyl]-10*H*-pyrazino[2,3-*b*][1,4]benzothiazines, 4



compd	R_1	R_2	R_3	IC_{50}, nM [^{3}H]spiperone[a]	IC_{50}, nM [^{3}H]apomorphine[a]	K_i, nM, of [^{3}H]prazosin[b]
4c	Cl	H	H	62.1 ± 11.1	67.3 ± 8.9	5.6 ± 0.8
4d	H	Cl	H	553 ± 42	1222 ± 272	150 ± 40
4e	CH_3	H	H	382 ± 125	103 ± 19	
4f	H	H	Cl	9166 ± 602	5968 ± 1399	90 ± 9
6 (chlorpromazine)				9.88 ± 0.20	38.9 ± 13.0	12 ± 3

[a] Reported values are the mean plus or minus SD of three independent determinations. [b] Reported values are the mean of two independent determinations plus or minus the range.

Therefore, replacement of the chlorine-containing phenyl ring of chlorpromazine by pyrazine led to **4c**, which retained some neuroleptic activity and which proved to be similar to chlorpromazine in binding to the spiperone, apomorphine, and prazosin receptors. However, replacement of the nonhalogenated phenyl ring of chlorpromazine by pyrazine, **4f**, resulted in substantial loss of both neuroleptic activity and affinity for the spiperone and apomorphine binding sites. Since **4c** and **4f** exhibited almost identical electrochemical behavior, it would appear that in this series, polarographic and cyclic voltammetric studies cannot be used to predict neuroleptic activity.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus with open capillaries and are uncorrected. ^{1}H NMR spectra were recorded for all intermediates and final products on a Varian EM-90 instrument with Me_4Si as an internal standard. ^{13}C NMR spectra were determined on 0.5–2 M solutions with added Me_4Si by using a Varian CFT-20 spectrometer. Digital resolution was such as to yield accuracies of ±0.05 ppm and ±1.0 Hz for chemical shifts and coupling constants, respectively. TLC's were performed on Analtech fluorescent silica gel plates, and spots were detected by UV or exposure to I_2 vapor. Mass spectral data were acquired with an MS902 instrument, and UV data were determined on a Cary II spectrophotometer. HPLC's were run on a SP8000 HPLC fitted with a Dupont 25 × 0.4 cm Sil column. Where analyses are indicated only by symbols of the elements, analytical results for those elements were within ±0.4% of the theoretical values.

2-Chloro- (3c) and 3-Chloro-10*H*-pyrazino[2,3-*b*][1,4]-benzothiazine (3d). A solution of 2,3,6-trichloropyrazine (3.67 g, 20 mmol), 2-aminothiophenol (2.50 g, 20 mmol), and triethylamine (4.04 g, 40 mmol) in DMF (50 mL) was stirred at room temperature for 10 h, at 100 °C for 15 h, and then at 150 °C for 15 h. After the DMF was removed at 55 °C and 0.5 mm, H_2O was added and the crude product extracted into EtOAc. The EtOAc extract was washed with H_2O, dried (Na_2SO_4), filtered, and concentrated to give 5.3 g of a mixture consisting mainly of the 2-chloro and 3-chloro product isomers. The isomers could be separated cleanly by medium-pressure chromatography over silica gel 60 (E. Merck) with toluene as the elution solvent. The first isomer to be eluted from chromatography of 1.5 g of the isomer mixture proved to be 2-chloro-10*H*-pyrazino[2,3-*b*][1,4]-benzothiazine (**3c**; 350 mg, 26.3%): mp 172–176 °C with darkening at 165 °C; ^{1}H NMR ($CDCl_3$) δ 6.5 (d of d, 1 H, aromatic CH), 6.6 (br s, 1 H, NH, exchangeable), 6.8–7.1 (m, 3 H, aromatic CH), 7.7 (s, 1 H, N=CH); mass spectrum, *m/e* 235 (M^+ containing 1-Cl), 203 ($M^+ - S$), 200 ($M^+ - Cl$), 199 ($M^+ - HCl$); homogeneous upon TLC (toluene), R_f 0.31; homogeneous upon HPLC (hexane–CH_2Cl_2, 50:50). Anal. ($C_{10}H_6ClN_3S$) C, H, N.

The second isomer to be eluted was 3-chloro-10*H*-pyrazino-[2,3-*b*][1,4]benzothiazine (**3d**; 200 mg, 15%): mp 182–185 °C dec; ^{1}H NMR ($CDCl_3$) δ 6.5 (br d of d, 2 H, aromatic CH and NH, 1 H exchangeable), 6.8–7.1 (m, 3 H, aromatic CH), 7.6 (s, 1 H, N=CH); mass spectrum, *m/e* 235 (m^+ containing 1-Cl), 203 ($M^+ - S$), 200 ($M^+ - Cl$), 199 ($M^+ - HCl$); homogeneous upon TLC (toluene), R_f 0.23; homogeneous upon HPLC (hexane–CH_2Cl_2, 50:50). Anal. ($C_{10}H_6ClN_3S$) C, H, N.

2-Methyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (3e). A mixture of 2,3-dichloro-6-methylpyrazine[9] (650 mg, 3.99 mmol), 2-aminothiophenol (500 mg, 3.99 mmol), and triethylamine (480 mg, 4.74 mmol) in DMF (10 mL) was stirred at room temperature for 18 h and then at 150 °C for 1.5 h. After the DMF was removed at 50 °C and 0.5 mm, H_2O was added to the residue, and the crude product was extracted into EtOAc. The EtOAc extract was washed with H_2O, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to 800 mg of a greenish-yellow solid. Medium-pressure chromatography of this solid over silica gel 60 (E. Merck) and elution with a IPA–CH_2Cl_2, 1:99, mixture gave 570 mg (66.4%) of product, which was homogeneous uon TLC (IPA–CH_2Cl_2, 2:98) R_f 0.5. Recrystallization from a toluene–hexane mixture afforded an analytical sample: mp 155–160 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.3 (s, 3 H, CH_3), 6.4–7.1 (m, 5 H, one of which is exchangeable with D_2O, aromatics and NH), 7.7 (s, 1 H, N=CH; UV (EtOH) λ_{max} 246 nm (ε 31 500), 290 (1860), 322 (3610); mass spectrum, *m/e* 215 (M^+), 200 ($M - CH_3$), 188 (M – HCN) 183 ($M - S$). Anal. ($C_{11}H_9N_3S$) C, H, N.

2-[(2-Amino-4-chlorophenyl)thio]-3-chloropyrazine (7). A solution of 2-amino-4-chlorothiophenol hydrochloride[10] (2.65 g, 13.5 mmol), 2,3-dichloropyrazine (2.01 g, 13.5 mmol), and triethylamine (4.05 g, 40.0 mmol) in dry DMF (50 mL) was stirred at room temperature for 5 h and then at 150 °C for 6 h. After most of the DMF was removed at 50 °C and 0.5 mm, the solid residue was partitioned between H_2O and EtOAc. The organic extract was washed with H_2O, dried (Na_2SO_4), filtered, and concentrated. The remaining solid was chromatographed over 250 g of silica gel (E. Merck, 40–60 μm mesh) with CH_2Cl_2 elution to give 2.1 g (57.2%) of product, homogeneous upon TLC (CH_2Cl_2) R_f 0.3. Recrystallization from an EtOAc–hexane mixture afforded an analytical sample: mp 173–175 °C dec with resolidification to an orange solid, which decomposed at 200 °C; ^{1}H NMR ($CDCl_3$) δ 4.3 (br s, 2 H exchangeable with D_2O, NH_2), 6.7–6.9 (m, 2 H, aromatic CH), 7.3 (d, 1 H, aromatic CH), 8.1 (d, 1 H, N=CH), 8.3 (d, 1 H, N=CH). Anal. ($C_{10}H_7Cl_2N_3S$) C, H, N.

8-Chloro-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (3f). 2-[(2-Amino-4-chlorophenyl)thio]-3-chloropyrazine (**7f**; 1.34 g, 4.92 mmol) was heated neat to 220 °C over 2 h, at which time TLC (CH_2Cl_2) indicated that cyclization to the pyrazino[2,3-*b*][1,4]-benzothiazine was complete. After cooling, the residue was partitioned between EtOAc and saturated Na_2CO_3 solution. The EtOAc layer was washed with H_2O, dried (Na_2SO_4), filtered, and concentrated to 550 mg (47.4%) of product: homogeneous upon TLC (CH_2Cl_2), R_f 0.3. Recrystallization from EtOH–H_2O gave an analytical sample, mp 215–228 °C slow dec. Anal. ($C_{10}H_6ClN_3S$) C, H, N.

2-Chloro-10-[3-(dimethylamino)propyl]-10*H*-pyrazino-[2,3-*b*][1,4]benzothiazine Hydrochloride Hydrate (4c). To a solution of 2-chloro-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (400 mg, 1.70 mmol) in DMF (10 mL) was added 50% NaH in mineral oil (82 mg, 1.70 mmol) at room temperature under N_2. After the

mixture was stirred at room temperature for 15 min, a solution of 3-(dimethylamino)propyl chloride base (227 mg, 1.87 mmol) in DMF (5 mL) was added, and the reaction mixture was stirred at 100 °C for 90 min. DMF was removed at 0.2 mm and 60 °C, and the residue was partitioned between EtOAc and H_2O. The EtOAc layer was washed was water, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to 500 mg of a dark oil, homogeneous upon TLC (5% MeOH–95% $CHCl_3$), R_f 0.32. This product was treated with excess EtOH–HCl, and the resulting salt was recrystallized from an MeOH–EtOAc–hexane mixture to give 410 mg (64.3%) of the greenish yellow HCl hydrate: mp 127–132 °C dec turning yellow at 140 °C; ^1H NMR (D_2O) δ 1.9–2.2 (br t, 2 H, CH_2), 2.9 (s, 6 H, NCH_3), 3.2 (t, 2 H, CH_2), 3.6–3.9 (br t, 2 H, CH_2), 6.7–7.2 (m, 4 H, aromatic), 7.5 (s, 1 H, N=HC); homogeneous upon TLC (MeOH–$CHCl_3$, 5:95, saturated with concentrated NH_4OH), R_f 0.22. Anal. ($C_{15}H_{17}ClN_4S$·HCl·H_2O) C, H, N.

3-Chloro-10-[3-(dimethylamino)propyl]-10*H*-pyrazino-[2,3-*b*][1,4]benzothiazine hydrochloride hemihydrate (4d) was prepared in 54.3% yield from 3-chloro-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine and 3-(dimethylamino)propyl chloride base by the same procedure used to prepare the 2-chloro isomer: mp 210–220 °C dec with softening at 204 °C; ^1H NMR (D_2O) δ 1.9-2.2 (br, 2 H, CH_2), 2.9 (s, 6 H, NCH_3), 3.2 (t, 2 H, CH_2), 3.6–3.9 (br, 2 H, CH_2), 6.5–7.2 (m, 4 H, aromatic), 7.6 (s, 1 H, N=CH); homogeneous upon TLC (MeOH–$CHCl_3$, 5:95, saturated with concentrated NH_4OH), R_f 0.23. Anal. ($C_{15}H_{17}ClN_4S$·HCl·0.5H_2O) H, N; C: calcd, 49.18; found, 49.64.

2-Methyl-10-[3-(dimethylamino)propyl]-10*H*-pyrazino-[2,3-*b*][1,4]benzothiazine Dihydrochloride Hemihydrate (4e). To a solution of 2-methyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (660 mg, 3.07 mmol) in dry DMF (9 mL) was added 50% NaH in mineral oil (295 mg, 6.14 mmol), and the mixture was stirred at room temperature under N_2 for 15 min to complete formation of the sodium salt. After the addition of a solution of 3-(dimethylamino)propyl chloride hydrochloride hydrate in DMF (8 mL) which had been previously dried over 3A molecular sieves, the reaction mixture was stirred at 100 °C for 2 h under N_2. DMF was removed at 50 °C and 0.5 mm, and the residue was partitioned between H_2O and EtOAc. The EtOAc layer was washed with H_2O, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography of the residue over 200 g of silica gel (E. Merck, 40–60 μm mesh) and elution with a MeOH–$CHCl_3$, 5:95, mixture gave 400 mg (43.4%) of product as a yellow solid. This base was treated with excess EtOH–HCl, and the salt was recrystallized from MeOH–EtOAc to give 200 mg (17.1%) of the dihydrochloride hemihydrate of 4e: mp 166–168 °C; homogeneous upon TLC (MeOH–CH_2Cl_2, 10:90, saturated with concentrated NH_4OH), R_f 0.75. Anal. ($C_{16}H_{20}N_4S$·2HCl·0.5H_2O) C, H, N.

8-Chloro-10-[3-(dimethylamino)propyl]-10*H*-pyrazino-[2,3-*b*][1,4]benzothiazine Hydrochloride (4f). To a solution of 8-chloro-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (550 mg, 2.33 mmol) in DMF (9 mL) was added at room temperature, under N_2 50% NaH in mineral oil (224 mg, 4.67 mmol), and the mixture was stirred at room temperature for 15 min until formation of the Na salt was complete. A solution of 3-(dimethylamino)propyl chloride hydrochloride hydrate (451 mg, 2.56 mmol) in DMF (8 mL), which had been previously dried over 3A molecular sieves, was then added, and the mixture was stirred at 100 °C for 2 h. After the mixture was cooled, DMF was removed at 45 °C and 0.5 mm, and the residue was partitioned between H_2O and EtOAc. The EtOAc extract was washed with H_2O, dried (Na_2SO_4), filtered, and concentrated to 800 mg of a dark oil. Chromatography over 200 g of silica gel (E. Merck, 40–60 μm mesh) and elution with a MeOH–CH_2Cl_2, 10:90, mixture led to a clean separation of the two major components of the crude reaction mixture. The first compound to be eluted was converted to the HCl salt with excess EtOH–HCl and recrystallized from MeOH–EtOAc to give the 10-[3-(dimethylamino)propyl] derivative 4f as the orange 1.8HCl salt (400 mg, 46.6%): mp 212–217 °C dec turning yellow at 140 °C, after drying at 0.2 mm and 56 °C; ^1H NMR (D_2O) δ 1.8–2.1 (br m, 2 H, CH_2), 2.8 (s, 6 H, NCH_3), 3.1–3.3 (br t, 2 H, CH_2), 3.5–3.8 (br t, 2 H, CH_2), 6.4 (d, 1 H, aromatic CH), 6.6 (d, 1 H, aromatic CH), 6.7 (s, 1 H, aromatic CH), 7.5 (d, 1 H, N=CH), 7.6 (d, 1 H, N=CH); UV (EtOH–0.1 N HCl, 50:50) λ_{max} 250 nm (ε 33 700); homogeneous upon TLC (MeOH–$CHCl_3$, 10:90, saturated with concentrated NH_4OH), R_f 0.8. Anal. ($C_{15}H_{17}ClN_4S$·1.5HCl·0.5H_2O) C, H, N, Cl.

Further drying at 110 °C and 0.2 mm yielded the yellow monohydrochloride salt, mp 213–216 °C dec. Anal. ($C_{15}H_{17}ClN_4S$·HCl) C, H, N.

8-Chloro-1-[3-(dimethylamino)propyl]-1*H*-pyrazino[2,3-*b*][1,4]benzothiazine Dihydrochloride (5). The second compound to be eluted from the above chromatography was treated with excess EtOH–HCl and the HCl salt recrystallized from MeOH–EtOAc to give the 1-[3-(dimethylamino)propyl] derivative as the yellow dihydrochloride salt (160 mg, 17.4%): mp 219–225 °C dec turning orange at 140 °C; ^1H NMR (D_2O) δ 2.0–2.4 (m, 2 H, CH_2), 2.9 (s, 6 H, NCH_3), 3.2–3.4 (m, 2 H, CH_2), 4.0–4.2 (t, 2 H, CH_2), 6.9 (s, 1 H, aromatic CH), 7.0 (d, 1 H, N=CH), 7.2–7.3 (d of d, 2 H, aromatic CH), 7.5 (d, 1 H, N=CH); UV (EtOH–0.1 N HCl, 50:50) λ_{max} 252 nm (ε 34 700), shoulders at 246 and 258 nm; homogeneous upon TLC (MeOH–$CHCl_3$, 10:90, saturated with concentrated NH_4OH), R_f 0.6. Anal. ($C_{15}H_{17}ClN_4S$·2HCl) C, H, N.

X-ray Crystallography. Crystals of 3c formed from methanol as dark yellow prisms with symmetry $P2_1/c$ with a = 14.337 (3), b = 5.678 (1), c = 11.879 (2) Å and β = 97.92 (1)° for Z = 4. Of the 1283 unique reflections measured with Cu $K\alpha$ radiation, 1195 were observed [$I \geq 3(\sigma I)$] and corrected for Lorentz, polarization, and absorption effects. The structure was solved using a multisolution tangent formula[20] approach and refined by full-matrix least-squares analysis[21] by minimizing $\sum\omega(|F_o| - |F_c|)^2$ with $\omega = 1/(\sigma F_o)^2$. The final unweighted residual was 0.042. Tables IV, V, and VI (Supplementary Material) contain the fractional coordinates and temperature parameters, bond distances, and bond angles for 3c.

Electrochemistry. A Metrohm 261 dc polaricord (with an IR compensator) was used for polarographic experiments and a potentiostat/galvanostat (PAR 173) in conjunction with Universal Programmer (PAR 175) and an X–Y recorder (Houston 2000) were used for cyclic voltammetry. The three-electrode system was composed of a Pt ribbon or a carbon paste electrode (as working electrode), a platinum wire (as auxiliary), and SCE (as reference). Solvent/electrolytes included 0.1 M H_2SO_4 (pH 1.15), 2-morpholinoethanesulfonic acid (Mes) buffer, pH 6.36.

Receptor Binding Assays. Spiperone Binding. The procedure used to determine the extent of displacement of [^3H]-spiperone from a rat caudate preparation has been described previously.[18]

Apomorphine Binding. [^3H]Apomorphine binding was measured by the method of Seeman et al.[16] Rat striata were removed and homogenized in a Teflon–glass homogenizer (Type B, A. H. Thomas, Philadelphia, PA) with 20 passes at a constant speed of 500 pm in 20 volumes of buffer (15 mM Tris-HCl; 5 mM Na_2EDTA; 1.1 mM ascorbate; 12.5 μM mialamide, pH 7.4). This homogenate was then incubated directly in the homogenizing tube for 60 min at 37 °C and immediately flashed in an acetone–dry ice bath. The tissue was then thawed when ready for use or stored frozen overnight for use the following day.

When ready for use, the homogenate was thawed, resuspended in 10 volumes of buffer with 10 passes of the homogenizing pestle, and then centrifuged at 39000*g* for 15 min at 4 °C. The resultant supernatant was discarded, and the pellet was resuspended in 80 volumes of ice-cold buffer per original wet weight of tissue by using the Polytron (setting 5.3; 20 s).

Incubation assays were carried out in a final volume of 1 mL in polypropylene tubes. To each tube was added 0.1 mL of 3 mM [^3H]apomorphine (New England Nuclear Corp., Boston, MA;

(18) Randall, W. C.; Anderson, P. S.; Cresson, E. C.; Hunt, C. A.; Lyon, T. F.; Rittle, K. E.; Remy, D. C.; Springer, J. P.; Hirshfield, J. M.; Hoogsteen, K.; Williams, M.; Risely, E. A.; Totaro, J. A. *J. Med. Chem.* **1979**, *22*, 1222.
(19) Johnson, C. K., "ORTEP-II, A Fortran Thermal-Ellipsoid Plot Program"; Oak Ridge National Laboratory: Oak Ridge, TN, 1970.
(20) Main, P. "MULTAN, Program for the Automatic Solution of Crystal Structures from X-ray Diffraction Data"; University of York: England, 1980.
(21) Frenz, B. "Enraf-Nonius Structure Determination Package", V17.0, Delft: Holland, 1981.

specific activity 25–35 Ci/mmol) to give a final concentration of 0.3 nM; 0.1 mL of 100 μM (+)-butaclamol to determine nonspecific binding (to a final concentration of 10 μM), 0.1 mL of buffer containing the drug under test or 0.1 mL of buffer (total binding), and 0.8 mL of the caudate membrane suspension to give approximately 10 mg of tissue per assay tube. Incubation was continued at room temperature for 45 min, and ligand/receptor complexes were isolated by vacuum filtration on a Whatman GF/B glass-fiber filter, followed by 2 × 5 mL washes with buffer.

Prazosin Binding. [^{3}H]Prazosin [2-[4-(2-furoyl)piperazin-1-yl]-4-amino-6,7-dimethoxyquinazoline] was obtained from Amersham Corp. at a specific activity of 33 Ci/mmol and stored at −20 °C in EtOH containing 1% Et_2NH. The radiochemical purity of this ligand was periodically checked by TLC on E. Merck silica gel 60F-254 plates by using the systems EtOAc–MeOH–Et_2NH (80:20:1) and MeOH–H_2O–NH_4OH (30:20:5). Glass laboratoryware was used.

The radioligand displacement assay was conducted with homogenates of previously frozen (−75 °C) calf cerebral cortex. A Brinkmann Polytron PT-10 (setting 6, 10 s) was used to homogenize sections of calf cerebral cortex in 20 vol (w/v) of ice-cold 50 mM, pH 7.7, Tris-HCl buffer. The resulting homogenate was centrifuged twice at 48000*g* (Sorvall SS-34 rotor) for 10 min at 4 °C, with rehomogenization of the intermediate pellet in 20 vol of fresh buffer. The final pellet was resuspended in 50 vol of ice-cold buffer.

Triplicate assay tubes contained a final concentration of 0.14 nM [^{3}H]prazosin, 100 μL of various concentrations of the compound being tested, 1000 μL of tissue homogenate, and 50 mM, pH 7.7, Tris-HCl buffer to a final volume of 2000 μL. The reaction was initiated by the addition of tissue, and incubation continued for 30 min at 25 °C, at which time it was terminated by rapid filtration through Whatman GF/B glass-fiber filters under vacuum. Each filter was immediately rinsed with 3 × 5 aliquots of ice-cold buffer. The filters were removed into 10 mL of PCS (Amersham Corp.) and counted on a Packard Model 460C scintillation spectrometer at an efficiency of 35%. Specific binding was defined as the difference between samples with and without 1000 nM unlabeled prazosin.

Data Treatment. Nonlinear regression analysis was used on the sets of data of specific radioligand binding vs. concentration of test compounds to obtain IC_{50} concentrations. Apparent inhibition constants were then calculated by the following equation:

$$K_i = \frac{IC_{50}}{1 + [C]/K_d}$$

where [*C* is the concentration of [^{3}H]prazosin employed in the binding assay (0.14 nM) and K_d is its receptor dissociation constant.

Biological Methods. Compounds were tested in Carworth Farm mice (CF–1) strain, female, 18–22 g). Firstly, the abilities of the compounds to induce contralateral postural asymmetries in mice with unilateral caudate lesions[17] were examined 75 min after ip administration of various doses of the compounds. The second test employed antagonism of amphetamine-induced hyperactivity as the end point. In this procedure, the mice were administered various doses of the test compounds, ip, 2 h and 5 min prior to amphetamine (10 mg/kg sc). Forty-five minutes after amphetamine administration, the number of mice not exhibiting hyperactivity was recorded. The test compounds were administered at dose levels of 0.6, 3.0, and 15 mg/kg base weight (or in increments 10-fold higher in some cases) to groups of five mice per dose level. The number of animals responding at each dose level was used to approximate ED_{50} values.

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Supplementary Material Available: Fractional coordinates and temperature parameters (Table IV), bond distances (Table V), and bond angles (Table VI) of **3c** (3 pages). Ordering information is given on any current masthead page.