stretching and bending are possible in the substrate and receptor as well. Lumping all phenyl axial conformers together, the geometry would then be characterized as having a nitrogen–phenyl center distance of 4.3–5.1 Å and a nitrogen–phenyl plane distance of 0.7–2.8 Å.

The 1,3,4-trimethyl-4-phenylpiperidines are somewhat different from the above compounds in that the phenyl ring was occasionally found to prefer a distinctly different conformation with τ(C4′–C4–C13–C14) $\simeq$ 180°. This did not significantly affect the nitrogen–phenyl center distance but does have an appreciable effect on the nitrogen–phenyl plane distance (~0.0 Å).

The results of X-ray crystallographic studies of α- and β-prodine have been included in Table I to facilitate a detailed comparison between the computed and observed geometries. The crystal structure of meperidine was not included, since it appears to be very approximate with a rather high disagreement factor.[8] The observed structures of α- and β-prodine in the crystal state are in reasonably good agreement with the computed geometries. The HCl salt of β-prodine was found to have a gauche value of 80° for τ(C12–C11–C10–O1). Our results indicate that this conformation would only be 0.5 kcal/mol above the global minimum. Another feature in which there is some variability is in the dihedral angle, which describes the tilt of the phenyl ring. There is very good agreement for α-prodine with a computed value of τ(O1–C4–C13–C14) = 151° as opposed to 152°. For β-prodine, there is more of a discrepancy with computed values of 128 and 127° as opposed to 138 and 147°.

Acknowledgment. This work was supported by Grant DA-02193 from the National Institute of Drug Abuse. The author acknowledges stimulating discussions with Professors Gil Hite, Steven Matthysse, and Philip Portoghese.

Effects of Conformationally Restricted 4-Piperazinyl-10*H*-thienobenzodiazepine Neuroleptics on Central Dopaminergic and Cholinergic Systems[1]

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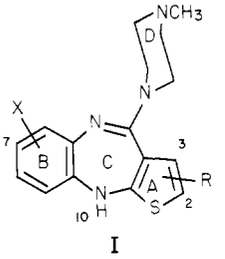
The levels of antidopaminergic and anticholinergic activities of neuroleptics, 4-piperazinyl-10*H*-thienobenzodiazepines, are modulated by imposing steric impedence to the piperazine ring. The optimum situation in favor of the anticholinergic action is reached in compound **5**, 2,3-dimethyl-7-fluoro-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine, where a maximum activity (equivalent to hyoscine), as measured by the [^{3}H]QNB receptor binding assay, is obtained. The structure–activity relationships found highlight the importance of certain spatial dispositions of the distal piperazine nitrogen (electron lone pair) with respect to the tricyclic system. The evidence for molecular topography of these compounds is presented from X-ray, NMR, and other physical data. The conformational aspects for correspondence to the relevant receptors are discussed.

The antipsychotic activity, as well as the extrapyramidal side effects (EPS), of neuroleptic drugs is correlated with their ability to block central dopaminergic transmission. Most of the biochemical and pharmacological tests have been designed to recognize agents that produce such antidopaminergic effects. For example, neuroleptics typically increase dopamine (DA) turnover, compete with DA receptor ligands for membrane binding sites, induce catalepsy, and block a conditioned avoidance response (CAR) in trained animals. In the striatum, the dopaminergic neurons from the substantia nigra form inhibitory synapses with cholinergic interneurons. Thus, a reduction in the dopaminergic input to these neurons results in an increased release of acetylcholine.[2] This leads, in turn, to catalepsy in animals and extrapyramidal symptoms (drug-induced Parkinsonism) in man.[3] Support for this view is derived from the fact that centrally acting anticholinergic agents alleviate these symptoms without interfering with the antipsychotic actions of the neuroleptics. In fact, neuroleptics, which possess anticholinergic properties (clozapine, thioridazine), produce a reduced incidence of EPS in the clinic.[4] Several lines of evidence[5] indicate that neuroleptics produce their antipsychotic action by blocking DA receptors in the mesolimbic area of the brain. Neuroleptics differ widely in their ability to block central cholinergic muscarinic receptors. Thus, in order to achieve a maximum reduction in side effects, it is important to obtain a correct balance of the antidopaminergic and anticholinergic activities.

Recently, we have reported[6] a series of thienobenzodiazepines (I) possessing neuroleptic activity, as demonstrated by their ability to inhibit a CAR and to produce



I

[†] Lilly Research Centre Ltd.
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(1) Part 5 of the series Heteroarenobenzodiazepines. For Part 4, see J. K. Chakrabarti, J. F. Fairhurst, N. J. A. Gutteridge, L. Horsman, I. A. Pullar, C. W. Smith, D. J. Steggles, D. E. Tupper, and F. C. Wright, *J. Med. Chem.*, **23**, 884 (1980).
(2) P. L. McGeer, E. C. McGeer, and T. Hattori, *Biochem. Psychopharmacol.*, **16**, 397 (1977).
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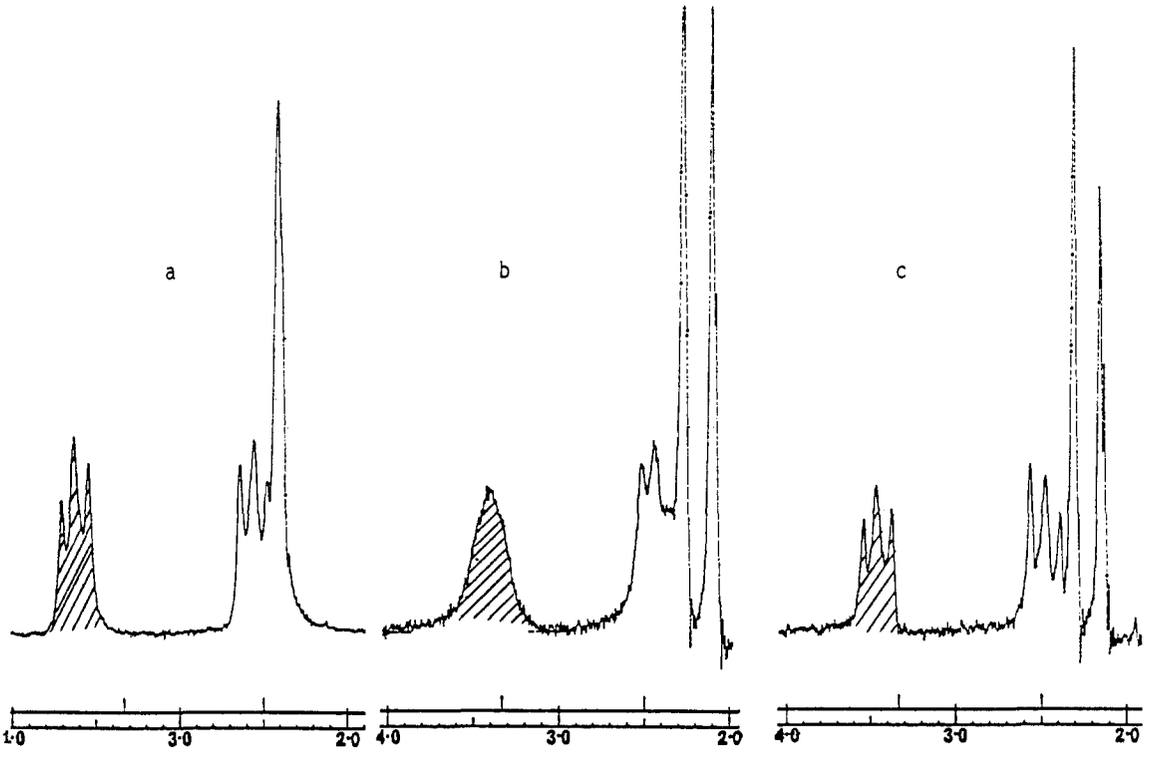

Figure 1. NMR 60-MHz spectra of compound **2** at 40 °C (a) and compound 4 at 40 °C (b) and 60 °C (c). The proximal piperazine methylene signals are shaded.

catalepsy in rats. As well as having antidopaminergic activity, a number of these compounds show potent anticholinergic activity in terms of their oxotremorine antagonism. Our results indicate that a piperazine ring attached to the tricyclic system is essential for activity. A small alkyl substitution in the 2-position of the thiophene ring enhances the antidopaminergic activity, but a similar substitution in the 3-position considerably reduces the antidopaminergic activity, while retaining a good level of anticholinergic potency. Such a shift in the profile of activity is significant, since a substitution at position 3 would be expected to impose a considerable steric impedence on the piperazine ring. Thus, it was of interest to study the influence of various alternative structures bearing a sterically hindered piperazine ring on the level of these two antagonistic activities. These conformationally restricted molecules, due to the altered spatial disposition of the piperazine ring with respect to the tricyclic system, may also provide information regarding any steric specificity required for the respective activities.

In this paper, we examined some thienobenzodiazepines with conformationally restricted piperazine rings. The preparation of these derivatives has been effected by imposition of steric factors by alkyl substitution in the 3-position of the thiophene ring and also by introducing *C*-methyl substituted piperazines to the tricyclic system. Receptor binding techniques provide a sensitive in vitro method for determining the receptor affinities. [^{3}H]-Spiroperidol is the ligand of choice for the screening of neuroleptics. Similarly, [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) is widely used to study muscarinic cholinergic receptors. The synthesis and evaluation of antidopaminergic and anticholinergic activities of these derivatives in terms of their ability to compete with these respective ligands for specific binding sites in rat brain membranes are described. The in vivo anticholinergic activity of these compounds has also been studied in terms of their ability to protect mice from a lethal dose of physostigmine. The ability to protect mice from such lethality is an indication of central, rather than peripheral, anticholinergic action, as it has been shown that anticholinergic compounds that do not pass the blood–brain barrier are inactive in this test.[7] The evidence for the relevant structural conformations is presented from the X-ray crystallographic studies, NMR, and other physical data.

Chemistry. The novel amidines (Table I) were prepared by reaction of the appropriate diamino ester (II) with *N*-methylpiperazine in the presence of $TiCl_4$ in anisole (route A, method C in ref[6]) or by a similar reaction at a lower temperature of diazepinone (III) with an appropriately substituted piperazine diluted with triethylamine (route B) (Scheme I).

^{1}H NMR Spectra. The ^{1}H NMR spectra of 1–9 at 40 °C and 60 MHz were clearly separated into two classes. Compound **2** (and other 3-unsubstituted thiophenes) showed a normal, unbroadened pattern for the piperazine ring protons near δ 3.6 and 2.5 (see Figure 1a). The same piperazine pattern was seen for clozapine. By contrast, compound **4** and other 3-alkyl substituted thiophenes (**3** and **5**–**8**) showed a severely broadened absorption for the piperazine ring protons, the effect being most marked at the proximal methylene groups (see Figure 1b) for the large alkyl substituents. A guide to the ability of increasingly bulky alkyl groups to broaden the proximal piperazine methylene (PM) signals is given by the width (hertz) of this signal at half-height ($W_{1/2}$, Table II).

X-ray analysis of clozapine[8] has shown that the piperazine ring closely approaches phenyl ring A in the solid state. In our series, we have been able to show a positive nuclear Overhauser enhancement (NOE)[9] of the PM sig-

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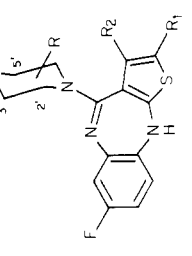
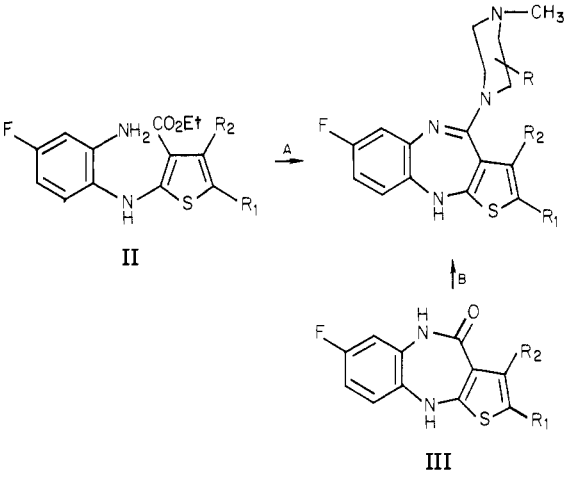
Table I. Activities of Compounds 1-12 Compared with Standard Drugs



compd	R	R_1	R_2	% yield (method)	mp, °C	recrystn solvent	formula [b]	IC_{50}, [c] nM [3H]spiroperidol [g]	IC_{50}, [c] nM [3H]QNB [g]	physostigmine lethality: [c] % protection (dose, mg/kg po)
1	H	H	H	*a*				199 ± 48 (3)	574 ± 130 (3)	NT
2	H	CH_3	H	*a*				20 ± 9 (4)	74 ± 21 (3)	75 (10), [d] 64 (5), [d] 50 (2.5) [d]
3	H	-$(CH_2)_4$-		*a*				77 ± 30 (3)	34 ± 13 (3)	71 (25), [d] 46 (10), [d] 46 (5) [d]
4	H	H	CH_3	*a*				624 ± 236 (3)	101 ± 31 (3)	67 (10), [d] 65 (5), [d] 5 (2.5)
5	H	CH_3	CH_3	39 (A)	242	CH_3CN	$C_{18}H_{21}FN_4S$	223 ± 39 (3)	9 ± 0.3 (3)	74 (10), [d] 33 (5), [e] 5 (2.5)
6	H	CH_3	C_2H_5	22 (A)	161	$CHCl_3$-hexane	$C_{19}H_{23}FN_4S$	172 ± 66 (3)	26 ± 11 (3)	53 (20), [d] 75 (10), [d] 60 (5) [d]
7	H	H	*i*-C_3H_7	46 (A)	265	CH_3CN-EtOAc	$C_{19}H_{23}FN_4S$	180 ± 61 (3)	26 ± 11 (3)	35 (50) [e]
8	H	CH_3	*i*-C_3H_7	37 (A)	212	EtOAc-hexane	$C_{20}H_{25}FN_4S$	75 ± 23 (3)	64 ± 9 (3)	35 (50) [e]
9	H	C_2H_5	H	*a*				11 ± 4 (3)	116 ± 28 (3)	75 (12), [d] 33 (10), [e] 18 (8)
10	3'-CH_3	C_2H_5	H	44 (B)	110	cyclohexane	$C_{19}H_{23}FN_4S$	31 ± 10 (3)	97 ± 32 (3)	42 (20), [e] 17 (10)
11	3',5'-$(CH_3)_2$	C_2H_5	H	16 (B)	184-186	cyclohexane	$C_{20}H_{25}FN_4S$	91 ± 25 (3)	258 ± 55 (3)	24 (50)
12	2,5'-$(CH_3)_2$	C_2H_5	H	16 (B)	181-184	cyclohexane	$C_{20}H_{25}FN_4S$	93 ± 31 (3)	247 ± 47 (3)	NT
clozapine								247 ± 20 (4)	179 ± 5 (5)	53 (15) [d]
haloperidol								11 ± 6 (3)	>10 000 (3)	0 (5)
hyoscine								NT	10 ± 5 (3)	68 (1) [d,f]

[a] See ref 6. [b] All compounds were analyzed for C, H, N, and S. [c] See pharmacological methods. [d] Significantly different from controls, $p < 0.001$. [e] Significantly different from controls, $p < 0.02$. [f] Intraperitoneal administration. [g] Mean ± SD (n). NT = not tested.

Scheme I



II

III

Table II. Physical Data

compd	NMR signal width ($w_{1/2}$), Hz	coalescence temp, °C	$\Delta G^{\ddagger}$, kcal mol^{-1}	pK_a
1	11.5			7.40
2	11.5	<-40	<11.1	7.50
3	13.0			7.25
4	13.5	30	14.7	7.30
5	16.0	37	15.0	7.20
6	34.5 [a]			
7	>20			6.90
8	>30 [b]	55	15.9	7.05
9	11.5			7.40
10 [c]				7.50
clozapine	11.5			7.25

[a] Extrapolated values from measurements at 27 °C. [b] PM separates into two signals. [c] No piperazine ring inversion, C-CH_3 shown to be equatorial by coupling constant measurements.

nals when irradiating one of the two isopropyl methyl doublet signals in **7**. This indicates close spatial approach of one of the isopropyl methyl groups and the PM's. No such enhancement (and, hence, approach) was seen for the thiophene 3-proton and the methylene signals in **2**. A rationale for the observed broadening of the piperazine signals described above involves the hindrance to rotation of the piperazine ring about the inter-ring C4–N bond caused by the steric bulk of the 3-alkyl substituent. As expected, the effect of raising the temperature of the NMR experiment is to accelerate this rotational process. Thus, compound **4** gives a spectrum at 60 °C in which the PM signals lose all of their broadening (see Figure 1c).

A very similar explanation has been given for a series of *N*-amidino and *N*-amido substituted piperidines,[10] which show line broadening for the proximal piperidine carbon signals (which is eliminated by raising the temperature). It has been concluded that rotation around the central amidino C–N bond of >N–C=N– is the slowest stereochemical process and, thus, is the first to be halted on cooling. Two other processes were considered, viz., piperidine chair/chair interconversion and nitrogen inversion. The latter was thought to be the fastest of the three processes. The evidence from X-ray analysis points to the existence of two types of amidines corresponding to axial and equatorial alignment of the piperidine with

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respect to the amidine –C=N– bond. This difference is not maintained in solution at normal temperatures and is directly comparable with our NMR and X-ray results (see later) on **2**, **4**, and **8** (which can be regarded as *N*-amidinopiperazines).

The ^{1}H NMR spectra of the methyl isomers **2** and **4** were examined at 360 MHz for temperatures down to –60 °C. The unhindered isomer **2** shows piperazine ring proton broadening when cooled from ambient to –30 °C. This is greater for the proximal than the distal methylenes, suggesting that piperazine ring rotation is slowing. Between –30 and –60 °C there are at least five separate piperazine ring proton resonances δ 4.32 (1 H), 3.74 (1 H), 3.01 and 2.90 (4 H), and 2.23 (2 H). Thus, a second stereochemical process (<200 s^{-1}) is now contributing and causing differences between the axial and equatorial methylene protons, probably the chair/chair ring interconversion as noted in the case of *N*-amidinopiperidines.[10]

The behavior of the hindered methyl isomer **4** is considerably more complicated, and even at +20 °C, as many as eight signals are observed. Analysis of the spectra on cooling below –20 °C infers that a third process is involved. Considering the X-ray data (see later) this can probably be attributed to inversion at the proximal nitrogen atom, i.e., an axial/equatorial inversion of the piperazine ring about the inter-ring C4–N bond. We cannot eliminate the possibility that ring flapping of the tricycle, via inversion at the bridge N–H, can contribute to the complex behavior for **4**.

It was not possible to use the same broadening criteria for rate processes in the piperazine *C*-methyl derivatives (**10**–**12**), since the introduction of the extra methyl group caused the piperazine ring to adopt a single conformation. Thus, the equatorial protons of the proximal methylene groups were strongly deshielded by the tricyclic ring and spin-decoupling experiments showed that the 3′-*C*-methyl group was equatorially orientated.

^{13}C NMR Spectra. The absence of complicated multiplet patterns in the ^{1}H broad band decoupled ^{13}C NMR spectra of compounds **1**–**9** makes this technique more suitable than ^{1}H NMR for the measurement of coalescence temperatures.[11] The spectrum of the most hindered compound (**8**) showed two separate carbon signals for the PM at ambient temperatures, whereas a single sharp line was obtained for **2** under the same conditions. Compound **4** exhibited an intermediate situation, i.e., a single broadened resonance. By varying the temperature (–42 to +115 °C, C_5D_5N), we could measure the point at which the two lines coalesced to one signal (T_c). From these values we have calculated the free energies of activation ($\Delta G^{\ddagger}$) for the piperazine ring rotation about the C4–N bond for four compounds (Table II). As expected, there are greater barriers to ring rotation for the larger alkyl group (e.g., **4** < **5** < **8**). For solubility reasons, it was not possible to cool the solution of unhindered compound **2** below –40 °C. At this temperature, the coalescence point had still not been reached; hence, $\Delta G^{\ddagger}$ must be less than 11.1 kcal mol^{-1}. The amidinopiperidines[10] gave $\Delta G^{\ddagger}$ values, calculated in the same way, that were from 8.0 to 10.9 kcal mol^{-1}. Thus, the hindered members of the current series of thiobenzodiazepines have very much higher barriers to piperazine ring rotation. This is also consistent with the partial double-bond character of the C–N bond as revealed by X-ray analysis of **2**, **4**, and **8**, similar to the analogous bond in clozapine.[8] PCILO (Perturbative Configuration Interaction using Localized Orbitals) calculation done for the rotation around this bond in clozapine also indicated steric hindrance between the proximal methylene pair of the piperazine ring and the C_1 hydrogen atom.[12]

Basicity (pK_a) Data. The basicity of the distal piperazine nitrogen atom in this series of thienobenzodiazepines and in clozapine can readily be measured by potentiometric titration in 60% aqueous DMF solvent. The pK_a's are given in Table II and are accurate to 0.05 unit. It was found that the hindered members of the series were reproducibly 0.1 to 0.3 unit less basic than the unhindered compounds.

The pK_a's of both the distal and proximal nitrogen of the piperazine ring in several piperazinyldibenzo-epines have been reported.[13] We were, however, unable to titrate the proximal nitrogen atom of the piperazine ring on our series of compounds. This nitrogen, which forms the part of the amidine function, is relatively less basic. The higher proportion of organic solvent needed to aid solubility may have further weakened this nitrogen to less than the detection limit of the glass electrode.

Ultraviolet and Infrared Spectra. Ultraviolet absorption maxima (λ_{max}) were measured for **2** and **4** in methanol solvent. The hindered 3-methyl isomer **4** had λ_{max} at 267 nm, which was 5 nm lower than the unhindered 2-methyl isomer **2**. An analogous difference of 5 nm was seen in the spectra of **7** and 2-isopropyl isomer.[6] This hypsochromic shift is best understood in terms of the decreased inter-ring conjugation in **4** and **7** caused by the hindering alkyl groups.

The infrared stretching frequencies of CH_2–N groups in saturated nitrogen heterocycles (the Bohlmann bands) depend upon the equatorial/axial distribution of substituents.[14] A comparison of **2** and **4** was made for both solid (KBr) and solution ($CHCl_3$) states in the region 2500–3000 cm^{-1}. It was found that the two compounds gave very different spectra in this region as solids but that their spectra in solution were superimposable. Further, the strong solution bands seen at 2865 and 2820 cm^{-1} in both were identical in frequencies to those in solid **2** but unlike solid **4** (only one band seen at 2830 cm^{-1}). This would agree with a change in conformation for **4** from axial to equatorial piperazine when changing from the solid (known to be axial by X-ray analysis) to solution. Compound **2** (already equatorial by X-ray) does not undergo this conformational change; hence, the infrared bands are unperturbed.

Results and Discussion

The various clinically effective psychoactive tricyclics exhibit a variety of pharmacological properties related to antidepressant, anticholinergic (antimuscarinic), antihistaminic, and antipsychotic actions. The majority of these molecules are flexible or semirigid, consisting of a central six or seven-membered ring linearly fused to two benzene rings on either side and a basic nitrogen connected to the central ring by a three-carbon (or equivalent) chain. It is also shown that a slight alteration in the basic side chain may profoundly influence the pharmacological profile. For example, if one of the alkyl substitutions of chlorpromazine is incorporated into a ring, the anticholinergic action is enhanced (e.g., thioridazine). Loxapine is a powerful neuroleptic agent, while its demethyl analogue amoxapine

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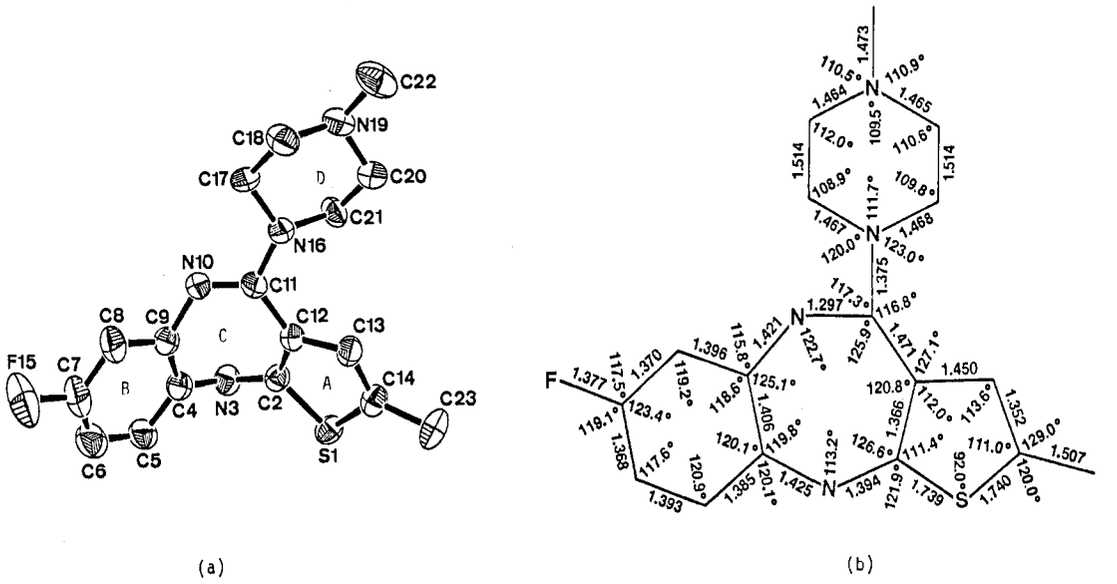

Figure 2. Compound **2**: (a) ORTEP plot (water molecule not shown) with thermal ellipsoids drawn at 50% probability level; (b) bond distances (σ = ±0.005–0.014 Å) and angles (σ = ±0.2–0.4°).

is a clinically effective antidepressant drug. It is thus reasonable to postulate that the diverse pharmacological and clinical responses of these compounds may partly result from different receptors according to the different spatial disposition of the basic nitrogen (or its protonated form) with respect to the tricyclic system.

X-ray crystallographic studies[8,15] on several tricyclic neuroleptics indicate that the basic side chain is orientated asymmetrically toward one of the aromatic rings. It is also apparent that a flexible propylamino side chain is required for increased neuroleptic activity in tricyclics like phenothiazines, where the 6-6-6 membered ring system is relatively flat. On the other hand, neuroleptics of the dibenzo-epine class with a 6-7-6 membered tricyclic system, which is in a boat form, require a basic piperazine ring,[6,13,16] where the conformational freedom of this ring is less than an open-chain alkylamine. It is thus evident that a certain orientation of this nitrogen or its lone pair of electrons with respect to the tricyclic system is a determinant factor for neuroleptic activity.

It has been suggested that the blockade of central dopamine receptors by the tricyclic neuroleptics results from a conformational complementarity between certain parts of these molecules and dopamine at receptors.[12,15,17] It is known from both X-ray and NMR studies that the trans form of dopamine is the preferred conformation,[18] and the distance of the nitrogen atom from the center of the catechol ring is 5.1 Å. The corresponding distance of the β-phenethylamine moiety in the stereospecific DA antagonist (+)-butaclamol (hydrochloride) is 5.1 Å,[19] suggesting that this compound binds to the DA receptors in a *trans*-dopamine overlapping conformation. It is also indicated that this part of the molecule is nearly coplanar, similar to the corresponding parts in the rigid DA agonists ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) and (*R*)-apomorphine. On the other hand, crystallographic studies[8,15] of the semirigid neuroleptics of the dibenzo-epine class show that the distance from the distal piperazine nitrogen atom to the center of the nearest benzene ring is in the range of 5.7 to 6.2 Å, slightly greater than the analogous distance in dopamine, and the similar distance to the other benzene ring is even greater ~7.7 Å. This latter conformational relationship has been considered important in postulated receptor interaction. In these cases, the mean plane of the piperazine ring is nearly parallel to the plane of this benzene ring.[13]

The results of our investigation are shown in Table I. The data represent the in vitro antidopaminergic activity as measured from the [^{3}H]spiroperidol binding assay. The in vivo activity of a number of compounds in this series, in terms of their ability to block a CAR and produce catalepsy, has been reported in our previous paper.[6] It will also be seen that, in addition to competing with the [^{3}H]QNB for muscarinic receptors, these compounds demonstrate central anticholinergic activity in vivo as determined against physostigmine lethality. The lack of correlation between the in vitro and in vivo data is probably due to differences in the absorption and/or metabolism of the compounds.

It is clearly indicated that compounds **2** and **9**, where the rotational barrier of the piperazine ring is relatively small, demonstrate potent antidopaminergic, as well as anticholinergic, activity. On the other hand, the compounds where this rotation is severely hindered show reduced antidopaminergic effect but retain anticholinergic activity. The compounds (**10**–**12**) with a piperazine ring substituted with a methyl group at the 3′-, 3′,5′- and 2′,5′-positions produce a reduced effect in both the parameters. This could be, in part, due to some steric hindrance on the neighboring nitrogen atom.

The molecular topography of **2** has been found by X-ray

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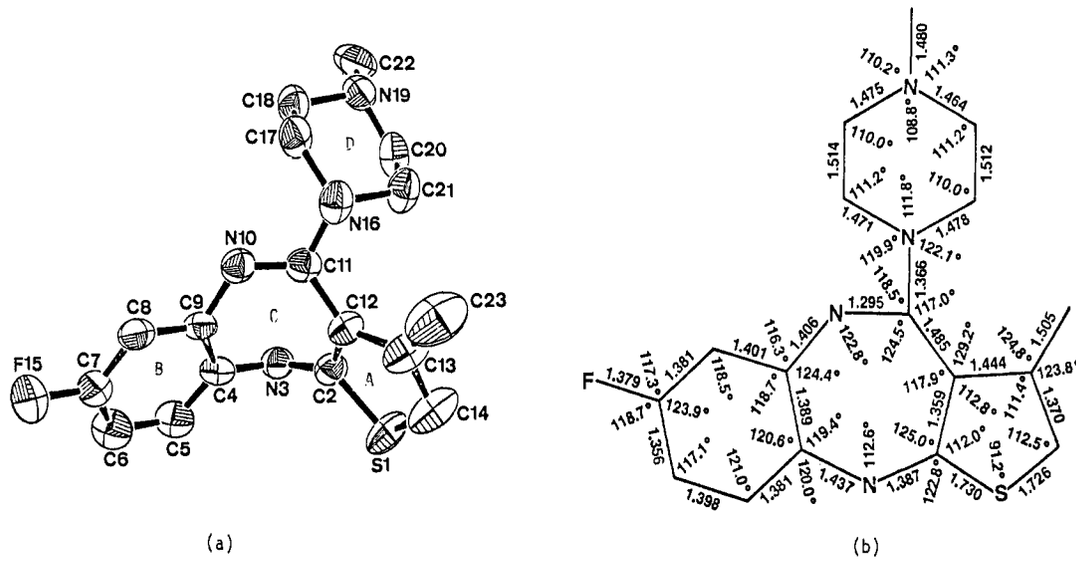


Figure 3. Compound 4: (a) ORTEP plot with thermal ellipsoids drawn at 50% probability level; (b) bond distances (σ = ±0.004–0.007 Å) and angles (σ = ±0.2–0.4°).

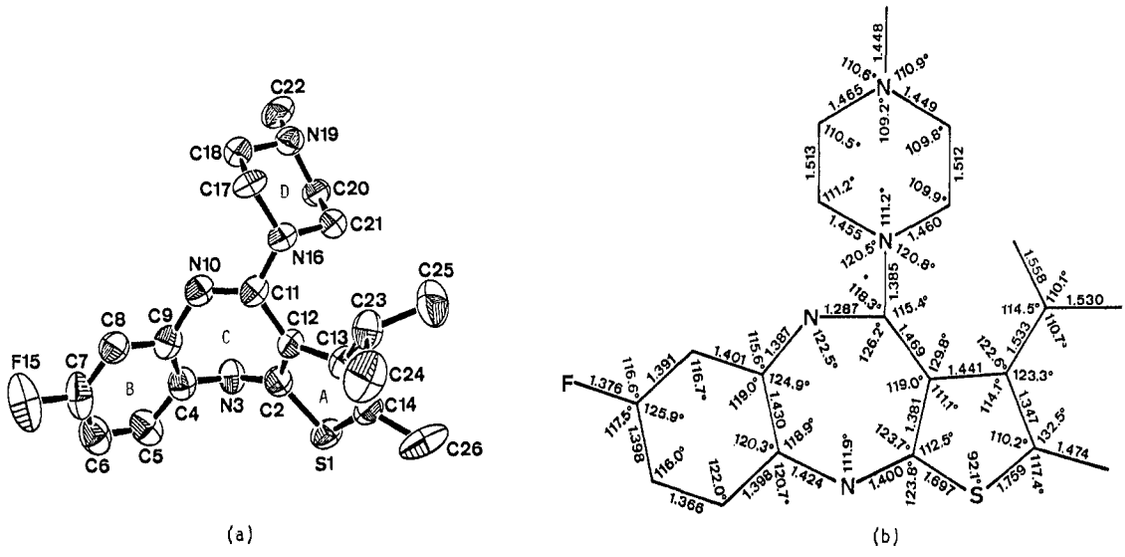
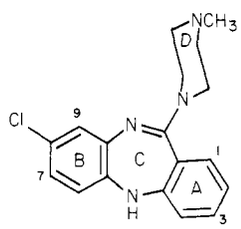
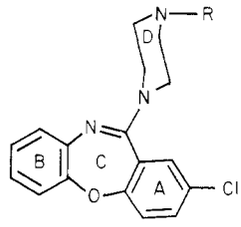


Figure 4. Compound 8: (a) ORTEP plot with thermal ellipsoids drawn at 50% probability level; (b) bond distances (σ = ±0.007–0.013 Å) and angles (σ = ±0.3–0.7°).

analysis to be very similar to the related dibenzo-epines, clozapine and loxapine.[8] The dihedral angles between the



clozapine



loxapine, R = CH_3
amoxapine, R = H

planes of the thiophene and benzene (A–B) rings in **2**, **4**, and **8** are 122.7, 118.2, and 120.6°, respectively. In all these structures, the tricyclic ring system is in a boat or butterfly conformation. The conformation of the piperazine ring is in almost a chair form with its distal N_{19} bearing an equatorially placed methyl group. The proximal N_{16} is nearly planar, and the bond angles about this N would suggest a trigonal rather than a tetrahedral atom, indicating a partial double-bond character to C_{11}–N_{16} (bond lengths 1.366 to 1.385 Å slightly shorter than the normal bond length 1.47 Å). Similar partial double-bond character of this bond is also found in clozapine and loxapine. The distances from N_{19} to the respective aromatic centroid and also to the bridge atom N_3 in **2** are similar to the corresponding distances in clozapine and loxapine (Table III). The distance from N_{19} to the thiophene centroid in each case is similar, but in **4** and **8** the distances from N_{19} to the center of the benzene ring and N_3 are slightly shorter (~1 Å). The most distinctive difference, however, is noted in the attachment of the C_{11}–N_{16} bond to the tricyclic system in the three compounds. In **2** this link is pseudoequatorial with respect to the piperazine ring, whereas in **4** and **8** this bond is pseudoaxial. This has altered the mean plane of the piperazine ring, which is nearly parallel (B–D dihedral angle = 19.7°) to the plane of the benzene ring in **2** similar to clozapine, to a steeply inclined position (almost perpendicular) in the latter compounds (B–D dihedral angles 72.7 and 68.0°). Such an axial form, probably thermodynamically more stable in the crystal structure, is not distinguishable in solution at ordinary temperatures as indicated in NMR and IR studies. The marked difference, however, is noticed in the rotational

Table III. Interatomic Distances and Interplanar Dihedral Angles for Rings A, B and D

compd	distance,[a] Å			dihedral angles, deg		
	N_{19}-X_3	N_{19}-A ring centroid	N_{19}-B ring centroid	A-B	A-D	B-D
2	7.028	5.942	7.743	122.7	37.6	19.7
4	5.762	5.789	7.090	118.2	78.9	72.7
8	5.787	5.828	6.970	120.6	76.1	68.0
clozapine	6.90	5.97	7.72	115	38	27
loxapine	6.74	6.19	7.73	114	43	27

[a] $\sigma \cong \pm 0.007$ Å for compounds **2**, **4**, and **8**.

barrier of the piperazine ring in these compounds due to the alkyl substitution at the C_{13} position. (See X-ray structures for numbering of atoms.)

By altering the substitution at this position, we have been able to dissociate the anticholinergic activity from the antidopaminergic effect. The 2,3-dimethyl substituted compound **5** is the most potent anticholinergic agent. In receptor binding capacity, it is equivalent to hyoscine. It is also clearly indicated that an optimal conformational state of the piperazine ring with certain orientation of its distal nitrogen with respect to the tricyclic system is necessary for maximum activity, since compounds **6**–**8**, where the steric impedence is greater than **5**, show reduced anticholinergic action. It is, however, interesting to note that these compounds also exhibit moderate antidopaminergic activity. This would suggest that a solution-state conformation of these compounds is relevant to the receptors.

On the other hand, receptor-bound molecules may assume a completely different conformation (induced fit). The binding site can vary from a situation in which the best model can be considered as a molecule held on a rigid solid state to one in which the active compound has partitioned from water into an organic phase. Such a model could better accomodate the solid-state conformation, where the piperazine ring is in an axial disposition, for the cholinergic receptor. The characteristic feature in potent anticholinergics has been found to be the nitrogen to the center of the ring (aromatic or saturated) vector generally steeply inclined to the mean plane of the ring.[20] The hyoscyamine cation has a "clawlike" shape with the distance between the extremities of the claw, the nitrogen to the center of the phenyl ring being 6.1 Å.[21] A similar feature can be derived from the crystal structure of compounds **4** and **8**, where the mean plane of the piperazine ring is almost perpendicularly inclined to the aromatic planes.

Our investigation points to the view that the spatial orientation of the electron lone pair of the distal nitrogen of the piperazine ring with respect to the tricyclic system is important for correspondence to the respective dopaminergic and cholinergic receptors. The comparison, in related structures, of interfeature distance only without consideration of the three-dimensional aspect of the drug–receptor interaction seems not to be warranted. It has been demonstrated recently that the stereo conformational specificity of the pyrroleethylamine rather than phenethylamine moeity in the rigid ergolines is important for dopaminergic action. This is further delineated by the fact that the rigid tricyclic structures containing pyrroleethylamine and pyrazoleethylamine moieties also display potent DA agonist activity.[22] The recognition of the multiple nature of DA receptors[23] and a specific site (mesolimbic) in the brain for antipsychotic activity[5] makes it debatable whether potent neuroleptics should possess a DA-like conformation. Recent PCILO analysis on two *o*-anisamide models (sulpiride group) has shown that the distance between the center of the aromatic ring at the terminal N is 6 Å, i.e., almost 1 Å longer than in the fully extended DA, suggesting a topographical dissimilarity of this class of antagonists to DA agonists at the receptor level.[29]

Experimental Section

Chemistry. Melting points were determined with a Köfler hot-stage apparatus and are uncorrected. All compounds were characterized by physical methods using IR, UV, and NMR. $MgSO_4$ was used as a drying agent. Microanalyses were within ±0.4% of the calculated values.

2,3-Dimethyl-7-fluoro-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine (5). Method A. Ethyl 4,5-dimethyl-2-(4-fluoro-2-nitroanilino)thiophene-3-carboxylate was prepared as in ref 24 from ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate[30] and 2,5-difluoronitrobenzene in Me_2SO with K_2CO_3 as a base in 57% yield, mp 144 °C (EtOH). The nitro ester (10 g, 0.03 mol) was hydrogenated in EtOAc at 60 psi with 10% Pd/C (0.5 g). After removal of the catalyst and solvent, the crude diamino ester was dissolved in a mixture of *N*-methylpiperazine (31.5 mL) and anisole (125 mL). A solution of $TiCl_4$ (8 mL) in anisole (40 mL) was added under N_2 and the stirred mixture was heated at 120 °C for 4 h and then under reflux for 20 h. After the mixture was cooled to 70 °C and the titanium salts were precipitated with 2-propanol (30 mL) and ammonia solution (20 M, 30 mL), the crude product was obtained from the solution and crystallized from acetonitrile to give 4.0 g (39%), mp 242 °C (CH_3CN).

4-(3,4-Dimethyl-1-piperazinyl)-2-ethyl-7-fluoro-10*H*-thieno[2,3-*b*][1,5]benzodiazepine (10). Method B. To a stirred solution of 2-ethyl-7-fluoro-5,10-dihydro-4*H*-thieno[2,3-*b*][1,5]-benzodiazepin-4-one[24] (1.5 g 0.0057 mol), 1,2-dimethylpiperazine[25]

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(2.7 g, 0.024 mol), and triethylamine (1.4 mL) in dry anisole (20 mL) was added a solution of $TiCl_4$ (1.75 mL) in anisole (5 mL). The mixture was stirred under N_2 at 140 °C for 3 h and then worked up as in method A to give 0.9 g (44%), mp 110 °C (cyclohexane).

Physical Methods. ^1H NMR spectra were recorded at 60 and 360 MHz on Varian A-60A and Bruker WH 360 spectrometers using $CDCl_3$ (99.8%) as solvent. ^{13}C NMR spectra were recorded at 20.0 MHz on a FT-80A spectrometer using $CDCl_3$ as the solvent for the spectra at ambient temperature and C_5D_5N for some of the variable-temperature work. All chemical shifts were referred to Me_4Si at δ 0.00. ^1H nuclear Overhauser effects were measured by integrating both coupled and homonuclear proton-decoupled spectra.

pK_a values were calculated by potentiometric titration of the piperazine distal nitrogen of the hydrochloride salts with 1.0 N NaOH in 60% dimethylformamide/water at 21 °C.

X-ray Crystallographic Studies. The X-ray crystal structures of compounds **2** monohydrate, 4, and 8 have been determined. The crystal data for these compounds are given in Table IV (see paragraph at the end of paper concerning Supplementary Material). The intensity data were collected on an automated four-angle X-ray diffractometer using monochromatic copper radiation. The structures were solved using the direct-methods program, MULTAN, and they were refined by the least-squares method to *R* factors of 0.055, 0.060, and 0.052, respectively. In the final refinement of each structure, all non-hydrogen atoms were included with anisotropic temperature factors, and all hydrogen atoms were included at assumed positions with isotropic temperature factors. The atomic coordinates for the non-hydrogen atoms are given in Table V (Supplementary Material), and the structures are shown in Figures 2-4. (The crystallographic numbering of atoms is cited in the relevant part of discussion.)

Pharmacology. Dopaminergic Receptor Binding ([^{3}H]-Spiroperidol). The assay was carried out in the striatum of the rat brain using the method described previously.[26]

Muscarinic Cholinergic Receptor Binding ([^{3}H]QNB). The method used was based on that described by Yamamura and Snyder.[27]

Male, Lilly Wistar rats (250-350 g), fed and watered ad libitum, were killed by cervical dislocation, the brains were rapidly removed, and the cerebellum was discarded. As each brain was dissected out, it was rapidly homogenized in 10 vol of ice-cold sucrose (0.32 M) in a Teflon/glass homogenizer (0.05-0.10 mm clearance, 25 strokes by hand). The homogenates were combined and rehomogenized in the same Teflon/glass homogenizer (25 strokes by hand). The combined homogenate was centrifuged at 1500*g* for 5 min at 0-4 °C, and the supernatant was used for the assay. After determination of the protein concentration,[28] the tissue was divided into 5-mL aliquots, which were stored at -50 °C for up to 3 months.

For each binding assay the tissue preparation was diluted in Krebs-Hensleit buffer, pH 7.4 (118.5 mM NaCl, 4.75 mM KCl, 2.52 mM $CaCl_2$, 1.17 mM KH_2PO_4, 1.18 mM $MgSO_4 \cdot 7H_2O$, and 2.5 mM $NaHCO_3$, gassed with 5% CO_2 in oxygen at 37 °C) to a concentration of 0.44 mg/mL. Incubations were carried out in 2.0 mL of Krebs-Hensleit buffer, pH 7.4, containing 0.5 mg of protein, 0.75 nM *dl*-[^{3}H]QNB, and varying concentrations of test compound. After incubation for 25 min at 37 °C in an atmosphere of 5% CO_2 in oxygen, the reaction was stopped by rapid centrifugation at 8000*g* for 45 min. The supernatant was aspirated off, the tissue pellet was digested in 1 mL of Soluene-350, and the radioactivity was determined. In every experiment, each concentration of test compound was assayed in quadruplicate.

Physostigmine Lethality. The method used was essentially that described by Collier et al.[7] Groups of 10 CFW mice (19-26 g) were dosed with the test compound, dissolved in distilled water or suspended in 0.5% carboxymethylcellulose, 1 h (0.5 h for hyoscine) prior to the administration of physostigmine (1 mg/kg ip). Mice were scored for tremor or death at 10, 20, and 60 min after the administration of physostigmine. The following scoring system was used: dead at 10 min reading (4), dead at subsequent reading (3), marked tremor (2), slight tremor (1), no effect (0). The results are expressed as the percent reduction in group score from the appropriate control group. The significance levels (Student's *t* test) refer to the difference in mean score between the treated and control groups.

Acknowledgment. We thank Dr. Douglas Dorman for obtaining the 360-MHz ^1H NMR spectra of compounds **2** and 4, David Smith for computer analyses of the X-ray structures, Fiona Crutchley for measuring the pK_a of clozapine, and Joy Howard and Margaret Baverstock for their technical assistance.

Supplementary Material Available: Crystal data and atomic coordinates for compounds **2**, 4, and 8 (2 pages). Ordering information is given on any current masthead page.

Nitrogen Bridgehead Compounds. 18.[1] New Antiallergic 4*H*-Pyrido[1,2-*a*]pyrimidin-4-ones. 1

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A new type of antiallergic agent, 9-hydrazono-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-ones, was synthesized and evaluated for inhibitory effects in the rat reagenic passive cutaneous anaphylaxis (PCA) screen. Several racemic 6-methyl derivatives were found to be more potent than disodium chromoglycate intravenously and some were also active orally. Structure-activity relationships are discussed. High stereospecificity was observed in the 6-methyl series between the enantiomers with 6*S* and 6*R* absolute configuration, the former being more active. Compound **17**, (+)-6(*S*)-methyl-9-(phenylhydrazono)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid [Chinoin-1045; UCB L140], has an ED_{50} value of 1.0 μmol/kg po and is now under clinical investigation.

The discovery of the mediator release inhibitor disodium chromoglycate 1 (DSCG) has provided a new approach to the therapy of bronchial asthma in man.[2]

In the past 10 years, since the introduction of DSCG for the treatment of asthma and allergic diseases,[3] there have

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