hydroxy-4-oxo-4*H*-1-benzopyran-2-carboxylate was used instead of 2-cyano-7-hydroxy-4-oxo-4*H*-1-benzopyran in the synthesis of **7a**, **24** was obtained in 32% after recrystallization from EtOAc, mp 123-124 °C.

7-[[3-(2-Quinolinylmethoxy)phenyl]methoxy]-4-oxo-4*H*-1-benzopyran-2-carboxylic Acid (8). A mixture of 850 mg (1.77 mmol) of **24** and 773 mg (9.2 mmol) of sodium bicarbonate in 4 mL of water and 40 mL of EtOH was heated at 70 °C for 1 h and then stirred at room temperature overnight. The reaction mixture was poured into 100 mL of water and acidified to pH 3. The precipitated product was collected on a filter, triturated with methylene chloride, and filtered to give 350 mg (44%) of **8**, mp 201 °C dec.

Compounds **9**, **10**, **15**, **16**, and **19** were prepared according to the procedure for the synthesis of **8**.

Ethyl 3-(3,4-Dihydro-7-hydroxy-2-methyl-4-oxo-4*H*-1-benzopyran-2-yl)propanoate (23). A mixture of 2,4-dihydroxyacetophenone (10.0 g, 65.7 mmol) and pyrrolidine (6.6 mL, 79.1 mmol) in 75 mL of toluene were refluxed under a Dean-Stark trap for 2 h, cooled down to room temperature, and then ethyl levulinate (15 mL, 105.5 mmol) was added. The reaction was refluxed for 2 h and diluted with ethyl acetate. The organic solution was washed with 10% HCl solution, water, and a brine solution, dried, and evaporated to give an oil. Purification by chromatography (EtOAc-hexane = 3:7) gave 2.5 g (14%) of **23**: ^{1}H NMR ($CDCl_3$) δ 1.2 (t, 3 H), 1.4 (s, 3 H), 1.9-2.6 (m, 6 H), 4.1 (q, 2 H), 6.3 (d, 1 H), 6.5 (dd, 1 H), 7.7 (d, 1 H).

This compound was used for the synthesis of **14** without further purification.

5-[8-[4-(2-Quinolinylmethoxy)benzamido]-4-oxo-4*H*-1-benzopyran-2-yl]-1*H*-tetrazole (18). A mixture of 4-(quinolinyl-2-methoxy)benzoic acid[1] (1.28 g, 4.59 mmol) and oxalyl chloride (4.6 mL) in 50 mL of CH_2Cl_2 and 5 mL of DMF was refluxed for 30 min. After concentration of the solvent in vacuo, the residue in 10 mL of CH_2Cl_2 was added dropwise to a solution of 5-(8-amino-4-oxo-1*H*-1-benzopyran-2-yl)-1*H*-tetrazole[7] (1.05 g, 4.59 mmol) in 40 mL of CH_2Cl_2 and 14 mL of pyridine in an ice bath. After stirring at room temperature overnight, the reaction mixture was poured in to 1 N HCl solution and extracted with EtOAc. The organic solution was dried and evaporated to dryness, and the residue was recrystallized from methanol to give 130 mg (6%) of **18**: mp 245-247 °C; ^{1}H NMR (DMSO-d_6) δ 5.4 (s, 2 H), 7.0 (s, 1 H), 7.2 (d, 2 H), 7.4-8.0 (m, 10 H), 8.1 (d, 1 H), 8.4 (d, 1 H).

Biological Assays. All biological assays are described in the first paper of this series.[1]

Acknowledgment. We would like to thank Dr. R. Youssefyeh for the synthesis of compound **25**. We wish to thank the following individuals for their excellent technical skills: S. O'Rourke, G. Schuessler, D. Sweeney, and J. Travis. Members of the Analytical Department are also acknowledged for the analytical data.

Conformational Properties of Semirigid Antipsychotic Drugs: The Pharmacophore for Dopamine D-2 Antagonist Activity

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Conformational energy calculations using the MM2-87 program have been performed on the tetracyclic spiro amines **1** (A23887) and **2** (A31472) which have previously been shown to have considerable affinity for dopamine D-2 receptors. These compounds are important for defining the pharmacophore for D-2 antagonist activity due to their limited conformational freedom. Possible foldings of the multicyclic structure were energy minimized and the barriers for inversion and for rotation of the ammonium group were computed. The conformational properties of **1** and **2** are consistent with a pharmacophore recently proposed by Liljefors and Bøgesø. The greater affinity of (*S*)-octoclothepin for D-2 receptors as compared with its enantiomer was attributed to the latter having an incorrect orientation of the ammonium hydrogen despite the correct folding of the tricyclic structure. Other D-2 antagonists with limited conformational freedom such as butaclamol, isobutaclamol, loxapine, clozapine, and resolved cyproheptadine analogues were also found to be consistent with the pharmacophore. In addition, **1**, **2**, and their enantiomers were tested on radioligand binding assays for dopamine D-1, dopamine D-2, noradrenergic α-1, serotonergic 5-HT_2, muscarinic, and σ receptors. **1** and **2** have greater affinities than their enantiomers in the D-1, D-2, α-1, and 5-HT_2 assays though there was little difference between **2** and its enantiomer in the latter two assays. In the muscarinic assays, **2** and its enantiomer, which were approximately equipotent, had greater affinity than **1** and its enantiomer. None of the compounds had substantial affinity for σ receptors. Since the same enantiomers of **1**, **2**, butaclamol, and the resolved cyproheptadine analogues also have greater affinities for D-1 receptors, the conformational requirements of D-1 ligands appear to be quite similar to those of D-2 ligands.

Introduction

An implicit assumption made by most medicinal chemists is that compounds that interact with the same receptor site have some common three-dimensional structure (pharmacophore) that is responsible for their activity at the site. However, the structural flexibility present in virtually all pharmacologically active compounds makes it difficult to assign the biologically active conformer. In this work, we examine the conformational properties of antipsychotic drugs with limited conformational freedom in an effort to define a common pharmacophore.

The pharmacological property of antipsychotic drugs that is believed to be responsible for their clinical activity is their ability to antagonize the binding of dopamine to D-2 receptors.[1,2] Two series of conformationally restricted compounds with this property are tetracyclic spiro amines (Figures 1 and 2) that contain two asymmetric centers resulting in two pairs of enantiomers Only one of the four isomers in each series had significant affinity for D-2 receptors and activity in in vivo assays assumed to predict antipsychotic activity.[3-5] However, the X-ray structures[6,7]

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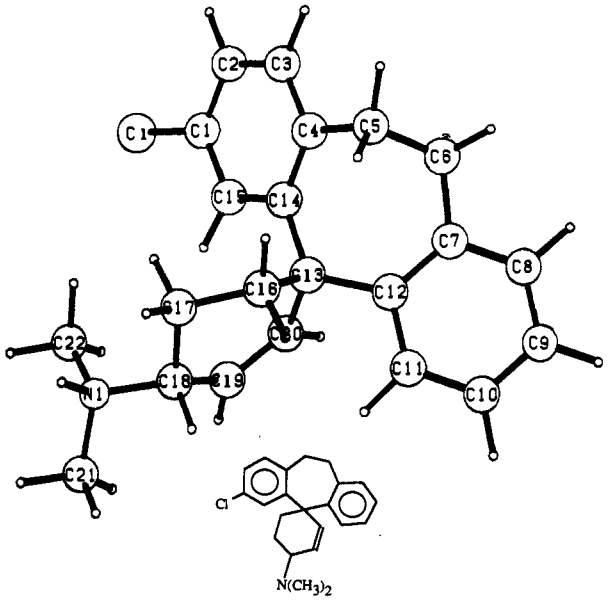

Figure 1. Proposed conformer of 1 that is responsible for activity at dopamine D-2 receptors. For the protonated compound, this conformer is 0.4 kcal/mol above the global minimum.

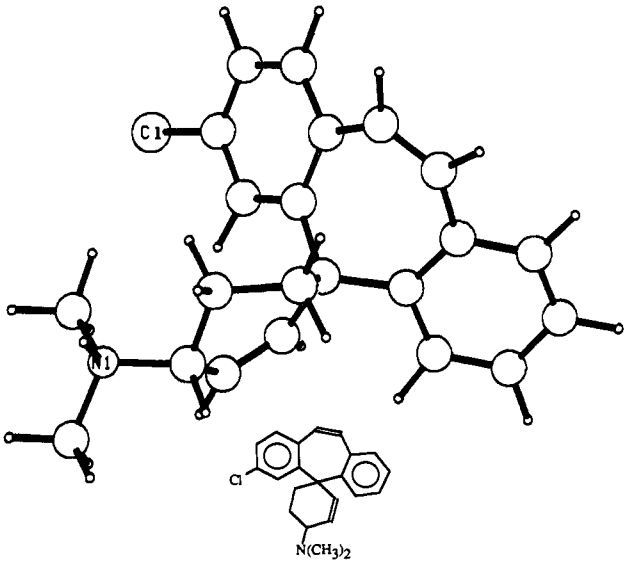

Figure 2. Proposed conformer of 2 that is responsible for activity at dopamine D-2 receptors. For the protonated compound, this conformer is 0.6 kcal/mol above the global minimum.

of the active enantiomers, 1 (A23887) (Figure 1) and 2 (A31472) (Figure 2), showed that the folding of the equivalent ring structures was the *opposite* of that found in the crystal structure of (*S*)-octoclothepin,[5,8] the enantiomer with greater antipsychotic activity.[9] To examine this inconsistency more closely, the conformational properties of 1, 2, and octoclothepin have been characterized. A brief description of some of these results has previously been reported.[10]

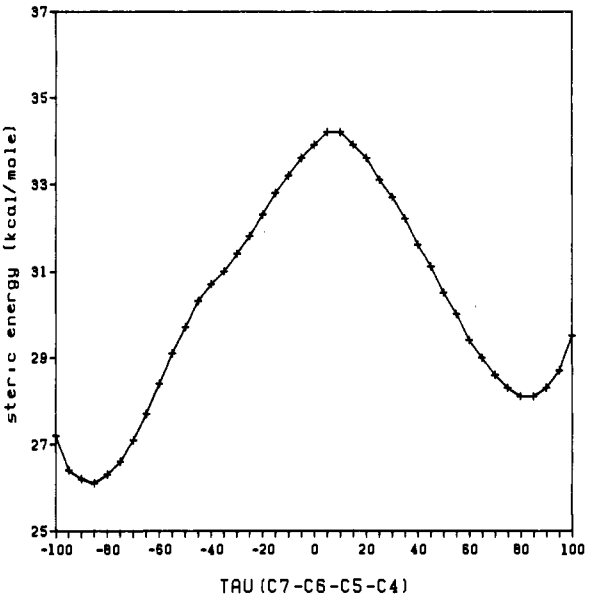

Figure 3. Energy barrier for inversion of 1.

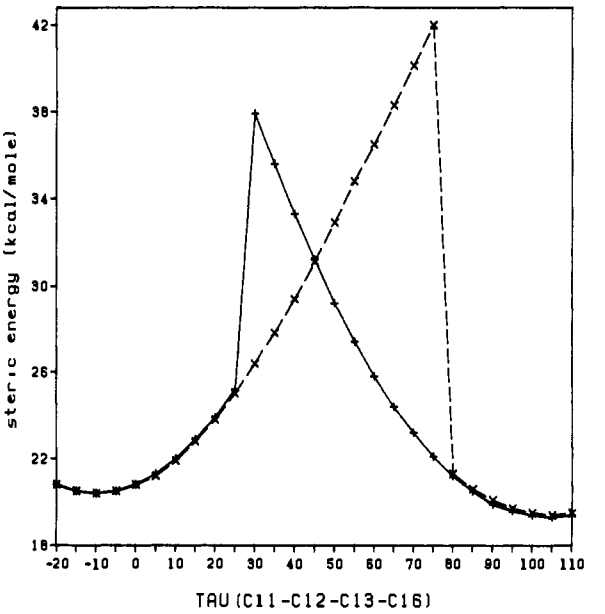

Figure 4. Energy barrier for inversion of 2: (+–+) low energy to high energy, (x–x) high energy to low energy.

Recently, a pharmacophore for D-2 receptor antagonism has been proposed by Liljefors and Bøgesø.[11] An inconsistency was also noted between the structure of the D-2 antagonist (1*R*,3*S*)-tefludazine and (*S*)-octoclothepin as represented by its crystal structure. The inconsistency could be resolved, however, if the tricyclic structure of (*S*)-octoclothepin was inverted. Using MM2-85 calculations to characterize the energies and geometries of possible conformers of (*S*)-octoclothepin, it was found that a conformer with the opposite folding of the tricyclic structure was only 1.2 kcal/mol higher in energy than the computed global minimum. This conformer was then found to correspond to one of the low energy conformers of (1*R*,3*S*)-tefludazine. As part of this study, we have confirmed the results for octoclothepin and tefludazine and examined the consistency of the proposed pharmacophore with the conformational properties of 1 and 2. We have also at-

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Table I. Dihedral Angles and Intramolecular Distances That Describe the Global Minima and Proposed Biologically Active Conformer of 1 and 2 (The steric energies are for the protonated form of the compounds. The X-ray crystallographic results are included for comparison purposes)

	1			2		
	global minimum	biol active	X-ray[a]	global minimum	biol active	X-ray[b]
C6–C5–C4–C3	−110	−110	−111, −113	−147	−147	−147
C7–C6–C5–C4	−87	−86	−84, −81	−1	−1	1
C8–C7–C6–C5	−142	−143	−144, −145	149	149	146
C13–C12–C11–C10	178	178	−179, 179	−178	−178	−177
C16–C13–C12–C11	69	69	76, 77	105	104	117
C17–C16–C13–C12	−154	−155	−154, −157	−169	−168	−167
C18–C17–C16–C13	63	63	62, 66	66	66	68
C19–C18–C17–C16	−49	−49	−52, −52	−45	−46	−50
C20–C19–C18–C17	18	20	24, 21	15	16	19
C13–C20–C19–C18	0	−1	−1, −1	−1	−2	−1
C16–C13–C20–C19	11	12	9, 12	19	18	14
C17–C16–C13–C20	−42	−43	−40, −44	−50	−49	−47
N1–C18–C17–C16	−174	−174	−177, −177	−170	−171	−173
N1–C18–C19–C20	146	142	152, 148	140	137	144
C21–N1–C18–C17	61	−161	61, 50	62	−160	66
C22–N1–C18–C17	−66	74	−68, −78	−65	75	−61
HN1–N1–C18–C17	177	−44	c	178	−43	c
N1-phenyl center, Å	5.7	5.6	5.8, 5.7	5.5	5.5	5.8
N1-phenyl plane, Å	2.7	2.8	3.0, 3.2	3.6	3.6	3.9
phenyl–phenyl angle, deg	142	142	143, 139	132	132	123
steric energy, kcal/mol	22.4	22.8		16.1	16.7	

[a] Computed from fractional coordinates in ref 6; two molecules in asymmetric unit cell. [b] Computed from fractional coordinates in ref 7. [c] Hydrogen atoms not given.

tempted to extend the proposed pharmacophore to other D-2 antagonists with limited conformational freedom including butaclamol, isobutaclamol, loxapine, clozapine, and resolved cyproheptadine analogues.

It was also thought worthwhile to examine the enantiomers of 1 and 2 in a series of receptor binding assays specific for various receptor subtypes. These included dopamine D-1, dopamine D-2, noradrenergic α-1, serotonergic 5-HT_2, muscarinic, and σ receptors. Given the limited conformational freedom of the compounds, this may provide useful information on the structural requirements for those receptor subtypes.

Results

Both 1 and 2 have limited conformational freedom with the major possibilities being (1) inversion of the tricyclic structure and (2) rotation of the amine (ammonium) group. The barriers for inversion are shown in Figures 3 and 4 while the barriers for rotation of the ammonium group are shown in Figure 5. The height of the barrier for inversion for 2 was found to vary depending on the direction of the inversion and both barriers are shown. The dihedral angles that describe the computed global minimum for the protonated molecule are listed in Table I. Both compounds prefer a similar conformation of the multicyclic structure with the energy difference for the different foldings of the ring being 2.0 kcal/mol for 1 (Figure 3) and 1.1 kcal/mol for 2 (Figure 4). Of the three conformers of the ammonium group (Figure 5), the difference in their steric energies is only 0.8 kcal/mol for 1 and 1.1 kcal/mol for 2 with a low barrier to rotation.

Another conformational possibility that was considered was whether the amine (ammonium) group was pseudoequatorial or pseudoaxial. Surprisingly, some of the conformers with a pseudoaxial arrangement proved to be relatively favorable when the dihedral angle HN1–N1–C18–C17 was in the vicinity of 60°. For 1, one of these conformers was only 0.1 kcal/mol above the global minimum for the protonated molecule and 0.9 kcal/mol for the free base. For 2, these conformers were less favorable with the best one being 1.7 kcal/mol above the global minimum for the protonated molecule and 1.6 kcal/mol for the free base. These conformers will not be considered further since they do not appear to be consistent with the structures of the remaining antipsychotic drugs examined in this study.

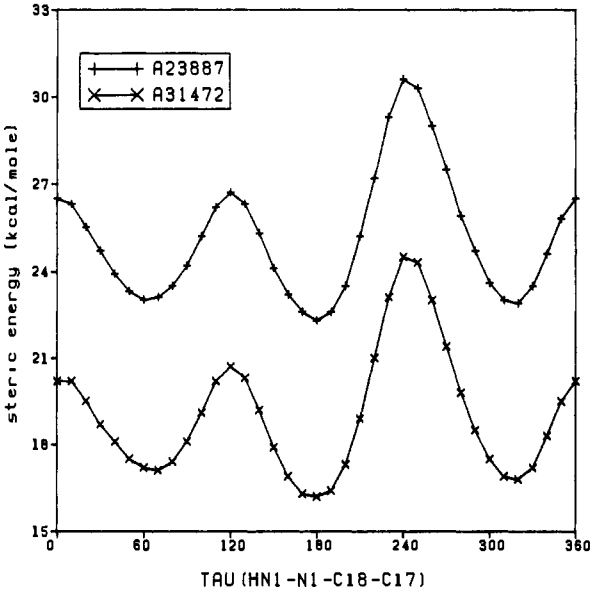

Figure 5. Energy barrier for rotation of ammonium group for 1 and 2.

The conformational energy results for unprotonated octoclothepin and tefludazine are essentially the same as found previously[11] with one minor exception (Table II). In this work, conformer 9, the one that is observed by X-ray crystallography,[8] is the computed global minimum. In contrast, conformer 10 was found to be the global minimum in the previous study and the crystal conformer was 0.4 kcal/mol higher in energy. This discrepancy appears to be solely due to differences with regard to the global minimum since all other conformational energy differences are the same. In this study, conformer 10 was found to be 0.6 kcal/mol above the crystallographic conformer and global minimum. With this change, the proposed biologically active conformer, conformer 1, is 0.8 kcal/mol above the global minimum rather than the 1.2 kcal/mol reported previously.

Table II. Computed Conformational Energies (kcal/mol) of Protonated and Unprotonated (*S*)-Octoclothepin for the Two Foldings of the Tricyclic Structure, Position of the Piperazine Ring, and Rotation of the Piperazine Ring (Conformer 1 is shown in Figure 6)

		pseudoequatorial			pseudoaxial		
		1	2	3	4	5	6
folding I	unprotonated	15.2	20.4	18.6	18.1	18.1	17.2
	protonated	9.6	15.5	12.1	9.1	9.2	10.4
		pseudoequatorial			pseudoaxial		
		7	8	9	10	11	12
folding II	unprotonated	16.9	16.3	14.4	15.0	23.4	
	protonated	11.2	10.5	7.7	7.3	18.9	

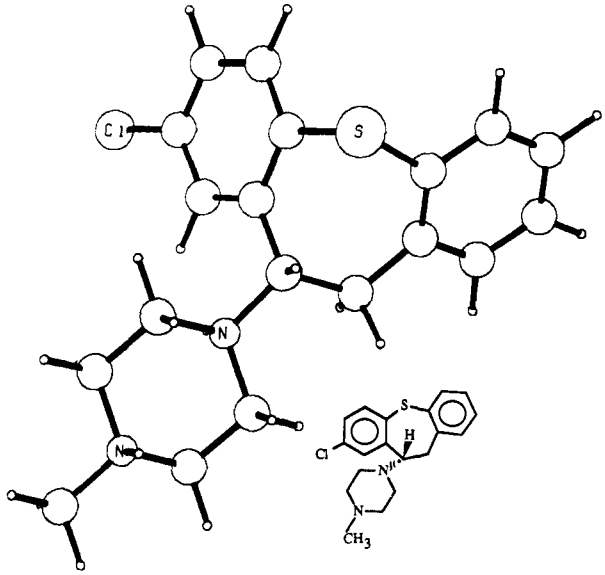

Figure 6. Conformer of (*S*)-octoclothepin proposed by Liljefors and Bøgesø for antipsychotic activity.[11] For the protonated compound, this conformer is 2.3 kcal/mol above the global minimum.

Calculations were also performed on protonated octoclothepin (Table II) unlike the previous study which only examined the unprotonated form.[11] The inclusion of these electrostatic forces affected the various conformers differently and caused some rearrangement of their relative favorabilities. The global minimum is now conformer 10 while conformer 1 (Figure 6), the proposed biologically active form, is now 2.3 kcal/mol above that. However, the lower affinity *R* enantiomer continues to favor the same folding of the tricyclic structure as the proposed pharmacophore while the higher affinity *S* enantiomer favors the opposite folding.

The results of the radioactive ligand binding assays for D-2 (raclopride), D-1 (SCH23390), α-1 (WB4101), 5-HT_2 (ketanserin), muscarinic (QNB), and σ (DTG) receptors are shown in Table III. Compounds 1 and 2 were found to have significantly greater affinities for the D-2 receptor than their respective enantiomers. A similar enantiomeric relationship was found in the D-1, α-1, and 5-HT_2 receptor assays though there appeared to be little difference between 2 and its enantiomer on the latter two assays. In the muscarinic assay, there was little difference within the pairs of enantiomer, but 2 and its enantiomer had considerably greater affinity than 1 and its enantiomer. All of the compounds had weak affinities for σ receptors.

Discussion

There is good agreement between the MM2-87 results and those of X-ray crystallography for 1 and 2[6,7] (Table I). The computed global minimum is also the conformer seen in the solid state and the dihedral angles that describe the conformers are also quite similar.

Table III. Binding Affinities of 1 and Its Enantiomer 1′ and 2 and Its Enantiomer 2′ for D-2 (Raclopride), D-1 (SCH23390), α-1 (WB4101), 5HT_2 (Ketanserin), Muscarinic (QNB), and σ (DTG) Receptors[a]

	K_i, nM			
	1	1′	2	2′
raclopride	2.0	200.	0.66	6.5
	1.7–2.4	190.–220.	0.54–0.84	5.8–7.5
SCH23390	0.33	68.	0.30	3.1
	0.28–0.38	60.–78.	0.27–0.34	2.7–3.7
WB4101	12.	680.	36.	61.
	8.3–20.	620.–770.	30.–45.	54.–72.
ketanserin	0.57	30.	1.0	1.3
	0.49–0.68	27.–33.	0.8–1.2	1.2–1.5
QNB	94.	43.	4.3	7.2
	86.–100.	39.–47.	3.8–5.0	5.4–11.
DTG	550.	1400.	1600.	800.
	500.–600.	1300.–1600.	1400.–1900.	740.–860.

[a] The K_i values were determined by using the nonlinear iterative program LIGAND. Since the K_i values were calculated (with standard errors) as the inverse of the K_i, the standard error has to be presented as the "standard error range of the estimate".

A nuclear magnetic resonance study of 2 and some of its analogues has been performed in solution.[12] The free energy difference between the two possible foldings of 2 was found to be 1.9 kcal/mol, which compares with a computed 1.1 kcal/mol energy difference between the lowest energy conformer for each folding of the tricyclic structure. The reported free energy barrier to inversion between the two foldings was found to be 16.1 kcal/mol for the N,N-demethylated analogue of 2, which compares with a computed energy barrier of 19.5 kcal/mol.

As was found previously[4] in a D-2 receptor assay using spiperone as the radioactive ligand, 1 and 2 have considerably greater affinities than their respective enantiomers (Table III). In the D-1 assay, both 1 and 2 have considerable affinity and similar enantiomeric ratios hold (Table III). The same also appears to be true for butaclamol and a cyproheptadine analogue where the same enantiomer has the greatest affinity for both D-1 and D-2 receptors.[2] Given the limited conformational flexibility of these compounds, this suggests that D-1 and D-2 receptor ligands have similar conformational requirements and that more subtle interactions between ligand and receptor are responsible for dopamine receptor subtype selectivity.

D-2 Pharmacophore. There are a number of tricyclic antipsychotic drugs including such well-known classes as the phenothiazines and thioxanthenes.[1] Other well-studied compounds include octoclothepin, loxapine, clozapine, and butaclamol. The tricyclic structure in these compounds is nonplanar and folded like a "V" or an upside down "V". There appears to be little or no energy difference between these two foldings for most antipsychotic drugs. For example, both are present in many crystal structures of

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Table IV. Computed Distance between the Ammonium (Amine) Nitrogen and the Relevant Ring Center for the Biologically Active Conformer of the Compounds[a]

	N-phenyl center, Å	N-phenyl plane, Å	phenyl-phenyl angle, deg
octoclothepin	6.3	2.7	102
loxapine	6.1	2.3	121
clozapine	7.8	0.2	128
butaclamol	3.8	0.5	122
isobutaclamol	3.7	0.8	121
cyproheptadine analogue	6.1	2.6	127

[a] Also included is the angle between the least squares planes of the phenyl rings. The geometrical parameters are for the energy minimized structures except for loxapine and clozapine where x-ray crystal structures were used.

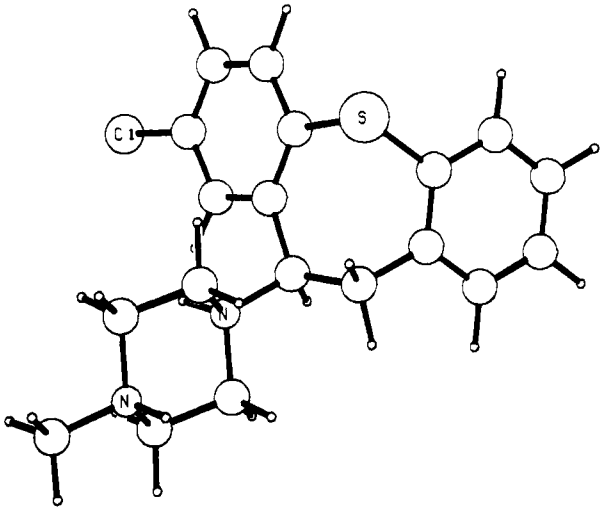

Figure 7. Low-energy conformer of (*R*)-octoclothepin that most resembles the proposed pharmacophore. For the protonated form, this conformer is 0.4 kcal/mol above the global minimum.

phenothiazines where the angle between the two phenyl rings is about 140°.[13] In thioxanthenes, the angle is about 145°.[14] In loxapine and clozapine, which lack asymmetric centers, the angle is about 115° and the two conformers are mirror images[15] and would, therefore, have identical energies. In crystal structures of octoclothepin and a related compound, the angle between the phenyl rings is 104° and 120°.[8,16] It was proposed[11] that the higher energy folding in (*S*)-octoclothepin (1.2 kcal/mol, 0.8 kcal/mol in this work for the unprotonated form) is responsible for antipsychotic activity. The conformational results for **1** and **2** are consistent with this proposal. As can been seen from Figures 3 and 4, this folding of the tricyclic structure is favored by 2.0 and 1.1 kcal/mol, respectively. These preferred foldings are shown in Figures 1 and 2.

Aside from the folding of the tricyclic structure, the global minimum and crystallographic conformation for **1** and **2** appear to have a different orientation of the ammonium hydrogen from the proposed pharmacophore (not shown). However, the ammonium group can be rotated to the right orientation (Figures 1 and 2) with an energy penalty of 0.4 and 0.6 kcal/mol for **1** and **2**, respectively (Figure 5). Computed intramolecular geometrical distances

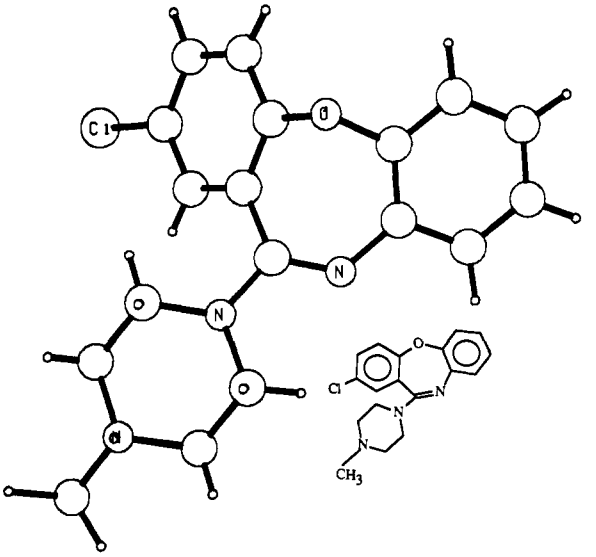

Figure 8. One of the two mirror image conformers of loxapine. Drawn from the crystal structure of loxapine succinate monohydrate.[22]

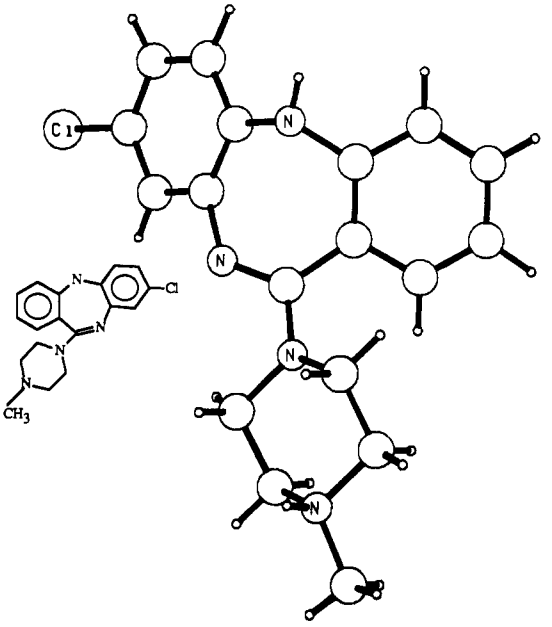

Figure 9. One of the two mirror image conformers of clozapine. Drawn from the crystal structure of clozapine dihydrobromide. A hydrogen atom attached to the double-bonded nitrogen atom appears to be an artifact of the crystal environment and has been omitted.[21]

for these conformers are presented in Table I while the equivalent distances in other compounds are presented in Table IV.

The importance of the orientation of the ammonium hydrogen in antipsychotic drugs is illustrated by the lessened potency of (*R*)-octoclothepin relative to (*S*)-octoclothepin despite the former preferring the correct folding of the tricyclic structure. It should be noted that the distal nitrogen atom in octoclothepin is the crucial one for antipsychotic activity.[17] The low-energy conformer for (*R*)-octoclothepin that most resembles the pharmacophore is shown in Figure 7. To achieve the orientation of the ammonium hydrogen shown in Figures 1, 2, and 6, the piperazine ring would have to be rotated about 60°,

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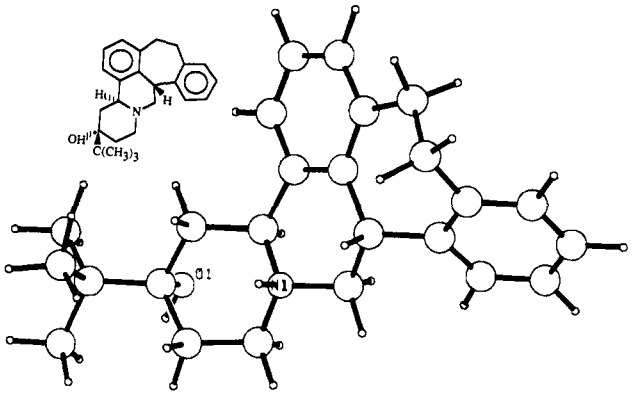

Figure 10. Trans conformer A of butaclamol. For the protonated compound, this conformer is the global minimum.[31]

which would require about 5 kcal/mol (Figure 5 in ref 11). Thus, the biologically active conformer is lower in energy for (*S*)-octoclothepin than it is in (*R*)-octoclothepin (0.8 kcal/mol versus 5 kcal/mol for the unprotonated form), which would account for the 11-fold potency advantage of the former. A similar conclusion was arrived at previously.[11]

Loxapine and Clozapine. The two tricyclic structure foldings are mirror images since these compounds lack an asymmetric center. For both loxapine and clozapine, one of these corresponds to the proposed pharmacophore (Figures 8 and 9). There has been an attempt to synthesize clozapine analogues in which the barrier to inversion is made sufficiently high to allow resolution of the two forms in an effort to separate the antipsychotic action from deleterious side effects.[18]

It is important to note that the conformation of the piperazine ring in loxapine and clozapine appears to be fixed since it is part of a resonance structure. This has been shown by semiempirical quantum mechanical PCILO calculations[19] and also appears as a shortening of the piperazine-tricyclic bond and sp^2 hybridization for the proximal nitrogen atom in the piperazine ring.[15] Additional evidence for a greatly preferred conformation of the piperazine ring for a given folding of the tricyclic structure is that, in 11 different crystal structures of loxapine, clozapine, and closely related analogues,[15,20-24] the piperazine ring has the same conformation (not shown). The only exception to this are two compounds in which a bulky group on the aromatic ring appears to sterically hinder the planar structure necessary for resonance.[23] Interestingly, this also results in a considerable weakening of the affinity for D-2 receptors.[23] For loxapine, the ammonium hydrogen appears to be fixed with the correct orientation of the ammonium group (Figure 8).

The major structural difference between clozapine and loxapine that appears to be related to the atypical properties of the former is that the chlorine substituent is on the other phenyl ring.[25] Superimposing the chlorine-containing phenyl rings of loxapine and clozapine and assuming that the same folding of the tricyclic structure is required, the biologically active conformer is shown in Figure 9. It should be noted that the distal nitrogen atom is crucial for D-2 receptor binding affinity.[25,26] In that conformer, the distal ammonium hydrogen appears to be pointing in a direction consistent with the proposed pharmacophore. However, the distance of the distal nitrogen atom is 7.8 Å from the chlorine-containing phenyl ring, which compares with a distance of 6.1 Å for loxapine (Table IV).

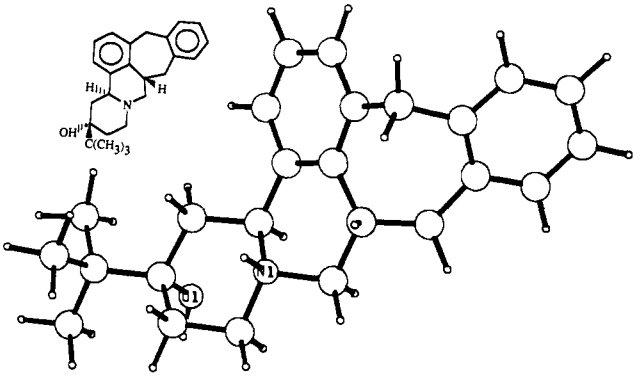

Figure 11. Trans conformer A of isobutaclamol. For the protonated compound, this conformer is 1.2 kcal/mol above global minimum.[31]

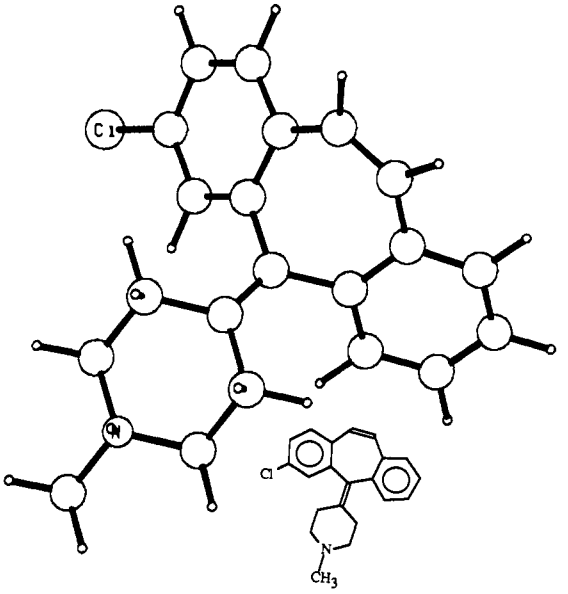

Figure 12. The resolved conformer for cyproheptadine analogues that has greater affinity for D-2 receptors.[40]

Butaclamol and Isobutaclamol. On the basis of a comparison of butaclamol with the dopamine agonist apomorphine, it was initially proposed that the biologically active form is "trans conformer B" and that the relevant phenyl ring is the one on the right (Figure 10).[27] However, initial MM2 calculations[28] found that the energy of "trans

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conformer B" was 2.7 kcal/mol above "trans conformer A" (Figure 10), the conformer observed by X-ray crystallography. Similar conformational results have been reported by others.[11,29] NMR studies of butaclamol in solution have proven to be consistent with the calculations in that only NOEs due to "trans conformer A" have been observed.[29,30] The unfavorability of "trans conformer B" tends to cast doubt as to its role as the biologically active conformer. It was also found that two conformers in which the ring junction of the bridgehead nitrogen is cis were preferred over "trans conformer A".[28] More recently, using the updated MM2-85 parameter set for ammonium nitrogen, it was found that "trans conformer A" was preferred by 0.3–0.5 kcal/mol over the cis conformers though the cis conformers are still preferred by about 1 kcal/mol for the unprotonated compound.[31] With use of NMR coupling constants, both cis and trans conformers have been observed for butaclamol in DMSO solution with a small preference for the latter.[30,32]

Considering the structures of the cis conformers, they appear to be incompatible with the proposed pharmacophore (not shown). However, "trans conformer A" (Figure 10) does seem to correspond to the pharmacophore. The pharmacophore, however, suggests that the phenyl ring to the left rather than the one to the right is the relevant one. Butaclamol appears to be anomalous with regard to chlorine substitutions of the phenyl ring which consistently results in significantly increased potencies in other classes of antipsychotic drugs.[1] In butaclamol, however, chlorine substituents in a variety of positions result in modestly decreased potencies at best.[33,34] Butaclamol is also anomalous in that it requires a *tert*-butyl or other bulky group which appear to bind to a lipophilic accessory site in the receptor.[27,35] The proposed pharmacophore, however, suggests that all but one of these chlorine-containing analogues may have had the substituent on the wrong ring. It should be noted, however, that the anomalous structure-activity relationships of butaclamol may be related to the shorter distance of the ammonium nitrogen atom to the center of the relevant phenyl ring which is 3.8 Å compared with values of 6.1–6.3 Å for the typical antipsychotic compounds studied here (Table IV). This suggests that butaclamol may be interacting with the D-2 receptor in a nonoptimal manner and, thus, may be a poor template in D-2 receptor mapping studies.[36,37]

With regard to isobutaclamol, a cis conformer is computed to be preferred by about 1 kcal/mol over the two trans conformers.[31] The cis conformer is also observed by X-ray crystallography.[38] As with butaclamol, only the trans conformers appear to be compatible with the proposed pharmacophore (Figure 11) and the distance of the ammonium nitrogen atom to the relevant phenyl ring is a relatively short 3.7 Å (Table IV). The proposal that the left phenyl ring of butaclamol and isobutaclamol is the relevant one for the pharmacophore is attractive since this portion of the molecule is invariant in the two compounds unlike the other phenyl ring.

Cyproheptadine Analogues. Another series of compounds with limited conformational freedom that are active as D-2 antagonists are cyproheptadine analogues with certain 3-substituents.[39] In these compounds, the inversion barrier for the tricyclic structure is sufficiently high that atropisomers (conformational enantiomers) can be resolved.[39] For the enantiomers with the higher affinity on D-2 receptor binding assays,[39] the folding of the tricyclic structure is consistent with the proposed pharmacophore (Figure 12).

In these molecules, the piperidine ring is held in place by the exocyclic double bond and the remaining major conformational possibility is inversion of the piperidine ring with the *N*-methyl group in the equatorial position. These two possible conformers were energy minimized with the MM2-87 program with the result that the conformer shown in Figure 12 is preferred by 0.4 kcal/mol for the protonated molecule though the two have essentially the same energy for the unprotonated form. In two crystal structures,[39,40] the observed conformer has the piperidine ring inverted from the conformer shown in Figure 12. In a recent study of cyproheptadine, these two conformers were also evaluated with the MM2-85 program for the unprotonated form with the result that the two conformers had essentially the same energies, which agrees with the results found here.[41] In an NMR study of protonated cyproheptadine,[41] the populations of the two conformers were found to be in a 4:1 ratio, which would correspond to a free energy difference of 0.8 kcal/mol. However, it was concluded that the conformational preferences are the opposite of those found in the present study. The computational and NMR studies indicate that the two conformers are close in energy and that both should be significantly populated with the conformer shown in Figure 12 corresponding to the proposed pharmacophore. The distances within this compound are also consistent with those of the other compounds examined in this study (Table IV).

Conclusions

The conformational properties of D-2 antagonists with limited conformational freedom have been examined for consistency with a common pharmacophore. While none of the compounds is conformationally homogeneous, all are found to contain a single conformer in common. Of the two possible foldings of the tricyclic structure, the same one is preferred by the active enantiomers of **1** (by 2.0 kcal/mol), **2** (by 1.1 kcal/mol), butaclamol, isobutaclamol, and the cyproheptadine analogues. For the more active (*S*)-octoclothepin, the other folding is preferred by 2.3 kcal/mol for the protonated molecule. However, for (*R*)-octoclothepin, which prefers the correct folding, the orientation of the ammonium hydrogen appears to be wrong. For a given folding on the tricyclic structure, the orientation of the ammonium hydrogen appears to be fixed

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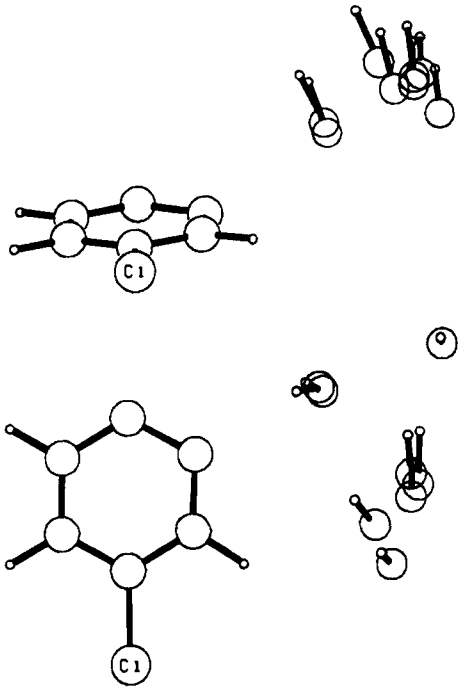

Figure 13. Two views of the proposed pharmacophore in which the relevant phenyl rings are superimposed and the resulting positions of the NH groups are plotted for 1, 2, (*S*)-octoclothepin, loxapine, clozapine, butaclamol, isobutaclamol, and the cycloheptadine analogue. To simplify the figures, only those portions of the molecules are shown. It is to be understood that the curvature of the tricyclic structures in every case is convex upwards.

in loxapine and clozapine. The same orientation of the ammonium hydrogen is also possible in all of the compounds with a maximum energy of 0.4–0.6 kcal/mol for 1 and 2. These results are summarized in Figure 13a, which shows the relevant phenyl rings superimposed and the resulting positions of the ammonium hydrogens. As can be seen, the ammonium groups are close to or above the plane of the relevant phenyl ring in each of the compounds (Table IV) and the NH groups are pointing in approximately the same direction. Thus, all of the compounds appear to be consistent with a common pharmacophore. The compounds that are most critical for the pharmacophore are loxapine, clozapine, and the resolved cycloheptadine analogues since they only contain two low-energy conformers that need to be considered. With regard to D-1 receptors, the same enantiomer is active for 1, 2, butaclamol, and a cycloheptadine analogue, which suggests that the conformational requirements of D-1 and D-2 ligands are similar.

The results presented here suggest that the distance between the ammonium group and the relevant phenyl ring may not be that crucial for D-2 antagonism since it appears to vary between 3.7 and 7.8 Å for compounds with significant affinity for D-2 receptors (Table IV). However, this distance may determine anomalous or atypical properties of antipsychotic drugs. Butaclamol, in which the distance is a relatively short 3.8 Å, may be anomalous with regard to chlorine substitution of the aromatic ring and the necessity of a bulky group to bind to a lipophilic accessory site. On the other hand, clozapine, which is an atypical antipyschotic drug due to its lack of extrapyramidal side effects, has a value of 7.8 Å for this intramolecular distance. The other, more typical antipsychotic drugs have values in the vicinity of 6.1–6.3 Å.

The results of this analysis also point out the advantage of performing quantitative conformational analysis with molecular mechanics calculations. Since there is no reason to assume that the conformer observed by X-ray crystallography or, for that matter, the computed global minimum is the one responsible for pharmacological activity, it is important to be able to assess the favorability of all possible low-energy conformers. In the present analysis, the crystallographic conformer and computed global minimum do not appear to be responsible for the D-2 antagonist activity in 1, 2, octoclothepin, and cycloheptadine analogues. Only by considering other low-energy conformers does one obtain a consistent picture of the pharmacophore for D-2 antagonists.

Methods

Computational. Energy-minimization studies were performed with respect to all internal coordinates using the MM2-87 program and parameter set.[42] Initial geometries were computed by a previously described program.[43] The numbering system for 1 and 2 is shown in Figure 1. For the computation of the inversion barrier of the tricyclic structure, it was discovered that the inversion could be driven with the C7–C6–C5–C4 dihedral angle for 1 and with C11–C12–C13–C16 for 2. Since these dihedral angles are internal to a ring structure, DRIVER option 1 was used with a fix to the FORTRAN code which allows the dihedral angle to pass through 0° and 180°.[44] These dihedral angles were varied with 5° increments. For the barrier to the rotation of the ammonium group, DRIVER option 2 was used with 10° increments. For the barrier calculations, energy minimization was with respect to all internal coordinates aside from the constrained dihedral angle.

Most of the calculations reported here were performed for both the protonated and unprotonated forms of the compounds. Unless specified otherwise, the reported results are for the protonated forms since it is likely that the amine group that is present in all antipsychotic drugs will be protonated at physiological pH. For octoclothepin, both nitrogen atoms of the piperazine ring were protonated. It should be noted that, for the first time, the 1987 version of the MM2 program contains explicit interactions between a point charge on the ammonium nitrogen and the remaining dipoles and charges in the molecule. For 1 and 2, the default dielectric constant of 1.5 was used since little difference was found for the protonated and unprotonated forms. For octoclothepin, however, a dielectric constant of 80 was used to approximate an aqueous solution.

Receptor-Binding Assays. The receptor-binding assays were performed in plastic test tubes using homogenates from rat striatum, rat cortex, or guinea pig brains. The incubations were terminated and the bound radioligands were separated from free using a cell harvester. Details of the methods have previously been published.[45,46]

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