Biologically Active Conformers of Phenothiazines and Thioxanthenes. Further Evidence for a Ligand Model of Dopamine D2 Receptor Antagonists

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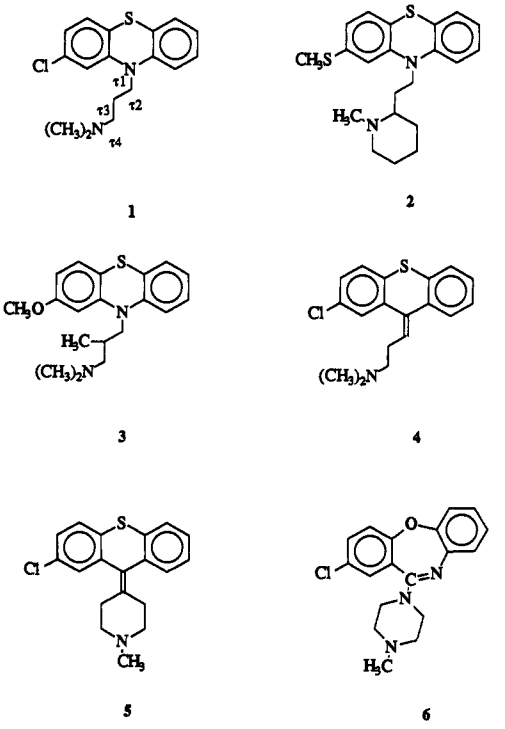
Conformational analyses have been performed on several phenothiazine and thioxanthene dopamine antagonists using the MM2-87 program and parameter set. The compounds that were examined are thioridazine (**2**), methotrimeprazine (**3**), *cis*- and *trans*-chlorprothixene, and a piperidylidene derivative of chlorprothixene. In addition, (+)-**2** and (−)-**3** were determined by X-ray crystallography to have the *R* absolute configuration. The above compounds were superimposed onto loxapine, which was used as a template for the previously proposed dopamine D2 receptor ligand model. The conformational properties and receptor affinities of these compounds were found to be entirely consistent with the ligand model. For example, a conformer of (+)-*R*-**2** that is consistent with the ligand model is lower in energy than a consistent conformer for (−)-*S*-**2**, which agrees with the higher D2 receptor affinity of the former. Similarly, in agreement with the much higher affinity of (−)-*R*-**3** relative to (+)-*S*-**3**, only the former contains a low energy conformer consistent with the ligand model. The ligand model is also consistent with the greater potency of *cis*-thioxanthenes over the trans isomers. These results emphasize the importance of the correct orientation of the ammonium hydrogen for high affinity at the D2 receptor. The pharmacophore for D2 receptor ligands is compared with a recently proposed pharmacophore for D1 ligands.

The serendiptitious discovery of the antipsychotic action of the phenothiazine chlorpromazine (**1**) in the early 1950s initiated a new era in the treatment of schizophrenia.[1] A large number of phenothiazines and related compounds have been synthesized and their structure–activity relationships elaborated in great detail.[1] The thioxanthenes are closely related to phenothiazines with which they share similar structure–activity relationships for antipsychotic activity.[1] The antipsychotic activity of these compounds appears to be due to their ability to antagonize the dopamine D2 receptor[2,3] or perhaps, as suggested more recently, the pharmacologically similar D4 receptor.[4] Despite this progress, the conformation that is responsible for the antipsychotic properties of the phenothiazines and thioxanthenes remains unclear due to their flexibility.[5-7] This work was initiated in an effort to perform a detailed conformational analysis of selected compounds in these classes of antipsychotic drugs in order to determine the three-dimensional structure that is responsible for their biological activity.

An early attempt to assign the biologically active form of the phenothiazines occurred when it was noted that the crystal structure of dopamine superimposes quite well onto the crystal structure of **1**.[8] Consistent with this model, it was also suggested that the 2-substituent that is vital for potent antipsychotic activity in phenothiazines and thioxanthenes may play a role in orienting their side chains toward the 2-substituent.[9] This model has been criticized, however, for not being consistent with more rigid compounds.[10] Thus, the good fit between the crystal structures of dopamine and chlorpromazine may be an unfortunate coincidence. It has been noted that there is a second way to superimpose dopamine onto the chlorpromazine structure.[11]

A detailed ligand model for D2 antagonists has recently been proposed.[12] By focusing on compounds with limited conformational freedom, it was possible to arrive at a number of conclusions. All of the active compounds were found to have a conformer in common and this was proposed as the biologically active form. The conformational features of the ligand model that were determined to be important were the curvature of the nonplanar tricyclic structure and the orientation of the ammonium hydrogen. The distance between the ammonium nitrogen and the substituted phenyl ring was found to be less important since it varied from 3.7 to 7.8 Å for active compounds. However, this distance was ~6.1 Å for typical D2 antagonists, and those compounds that deviated from

Scheme I



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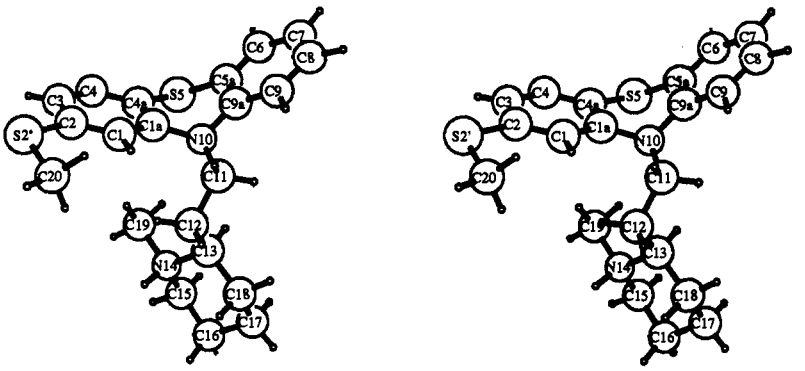

Figure 1. Conformation found for (+)-(*R*)-thioridazine (2) by X-ray crystallography. The conformer is [-143,73,-143,-174] II.

this distance appeared to have atypical properties or structure-activity relationships. It was also concluded that the ligand requirements for D1 and D2 receptors are quite similar in that all of these conformationally restricted compounds had similar affinities at both receptor types. A similar ligand model for dopamine antagonism has been proposed by Liljefors and Bøgesø.[13,14]

As indicated above, a phenothiazine such as 1 has considerable conformational flexibility. There are four torsion angles in 1 that can affect its three-dimensional structure. Each torsion angle can assume a trans and two gauche values, resulting in 81 possible combinations of the four dihedral angles. In addition, the nonplanar tricyclic phenothiazine structure can invert, doubling the number of distinct conformers to 162. To limit the possibilities, this work has focused on the chiral phenothiazines thioridazine (2) and methotrimeprazine (3). It has recently been shown that both enantiomers of 2 have considerable affinity for dopamine receptors but with different stereoselectivities.[15] The (+)-enantiomer has a 3-fold greater affinity for D2 receptors whereas the (-)-enantiomer has a 10-fold greater affinity for D1 receptors. For 3, there is a 45-fold difference in affinity for D2 receptors and a 14-fold difference in affinity for D1 receptors, both in favor of the (-)-enantiomer (also known as levomepromazine).[16,17] In thioxanthenes, the introduction of a double bond between the tricyclic structure and the side chain results in geometrical isomers and reduces the conformational freedom. It has been found that cis isomers (relative to a substituent on the tricyclic structure) are invariably more potent than their trans counterparts.[18] Calculations are also reported for *cis*-(4) and *trans*-chlorprothixene and a rigid analog of the thioxanthenes (5).[10] Also, as part of this project, the absolute configurations of the enantiomers of 2 and 3 have been determined by X-ray crystallography.

An important assumption that is made in this study is that the biologically active conformer of these compounds should have a relatively low energy although the binding energy of these ligands to dopamine receptors is generally much higher than the energy differences between conformers. However, requiring that a substantial amount of energy be put into a compound to achieve the biologically active conformer should result in a weakening of the ligand–receptor complex and much lower affinity for the receptor. Also, focusing on enantiomeric compounds such as 2 and 3 offers an important advantage in conformational studies of receptor ligands since enantiomers have identical physicochemical properties. Thus, any differences in receptor affinity are likely to be due to the ability of the different enantiomers to achieve the required biologically active form. This should provide a relatively stringent test of the previously proposed ligand model.

Results and Discussion

The atomic numbering scheme for 2 is shown in Figure 1 and similar numbering schemes are used for 3–5. The side chains of these compounds are a function of four torsion angles and different conformations of the side chain will be denoted as [τ1,τ2,τ3,τ4] (Scheme I). For 2 τ1 is defined as C12–C11–N10–C1a, τ2 as C13–C12–C11–N10, τ3 as N14–C13–C12–C11, and τ4 as C15–N14–C13–C12. For 3 and 4, which have equivalent *N*-methyl groups, τ4 will be given with respect to both *N*-methyl groups. In addition, the tricyclic structure in these compounds is nonplanar and a suffix of I or II will be appended to indicate which of the two mirror image conformations of the tricyclic structures is indicated. Folding I, in which the convex side of the tricyclic structure is upward when the substituted phenyl ring is on the left, is required by the ligand model for D2 antagonists.

The structure observed for (+)-2 crystallized as a fumarate salt is shown in Figure 1 and the final refined coordinates are given in Tables I and II. The conformation of the crystal structure is [60,73,-131,-174] II and the absolute configuration is *R*. Thus, the (+)-enantiomer preferred by D2 receptors has the *R* configuration while the (-)-enantiomer preferred by D1 receptors has the *S* configuration. These assignments are in agreement with their recent determination through the stereospecific synthesis of chemical intermediates.[19] The crystal conformation for (+)-2 differs from that observed for the racemic free base of 2 where the *R*-enantiomorph was found in the [-66,-170,51,179] I conformation and the *S*-enantiomorph in the [-128,169,-54,-179] II conformation.[20] These two conformers are closely related since the mirror image of the latter, which is also present in the crystal, is [-60,-169,54,179] I. The crystal structure for the free base of (-)-3 is [-70,165,-65,163 (-76)] I and is shown in Figure 2. This is essentially the same conformer as found previously[21,22] (coordinates are not given) and confirms the assignment of the *R* configuration to the (-)-enantiomer.[21]

The results of the conformational energy calculations for (+)-*R*-2, (-)-*R*-3, and *cis*- and *trans*-4 are listed in Tables III–V. More detailed conformational information on the global minima, the biologically active conformers (see below), and the crystal structures are given in Tables VI–VIII. The computed global minimum for (+)-*R*-2 is similar to its crystal conformation while the conformer for (-)-*R*-3 that is most similar to the crystal conformation is 0.8

Table I. Final Atom Coordinates ($\times 10^4$) and Isotropic Thermal Parameters ($\times 10^2$) for Non-Hydrogen Atoms in (+)-Thioridazine (2) Fumarate

atom	$x/a(\sigma)$	$y/b(\sigma)$	$z/c(\sigma)$	Biso(σ)	atom	$x/a(\sigma)$	$y/b(\sigma)$	$z/c(\sigma)$	Biso(σ)
C1	11022(2)	5568(1)	5779(3)	408(7)	N14	10361(2)	7789(1)	4874(2)	323(5)
C1a	10255(2)	5841(1)	6634(3)	389(6)	C15	9770(3)	8446(1)	4708(4)	432(7)
C2	10757(3)	5122(1)	4722(4)	517(8)	C16	10477(3)	9049(1)	4905(4)	538(9)
C3	9698(4)	4937(2)	4554(5)	633(11)	C17	10996(4)	9032(2)	6316(5)	636(11)
C4	8943(3)	5190(2)	5416(5)	605(10)	C18	11600(3)	8374(1)	6484(5)	540(10)
C4a	9191(2)	5647(1)	6456(4)	470(8)	C19	9604(2)	7217(1)	4677(4)	424(17)
S5	8220(1)	6015(1)	7500(1)	595(2)	S2′	11691(1)	4762(1)	3592(1)	729(3)
C5a	8939(2)	6045(1)	9052(4)	482(8)	C20	12845(4)	5241(2)	3910(7)	775(15)
C6	8461(3)	5941(2)	10361(6)	619(12)	O26	5318(2)	6943(1)	9695(2)	490(5)
C7	9049(4)	5977(2)	11559(6)	681(13)	C21	4295(2)	7071(1)	9514(3)	384(6)
C8	10102(4)	6110(2)	11481(4)	595(10)	C22	3692(2)	7011(1)	10816(3)	388(7)
C9	10593(3)	6211(1)	10214(4)	466(8)	C23	2808(2)	7344(1)	11028(3)	357(6)
C9a	10018(2)	6196(1)	8994(3)	402(7)	C24	2229(2)	7335(1)	12368(3)	388(6)
N10	10472(2)	6323(1)	7673(3)	375(5)	O27	1416(1)	7714(1)	12444(2)	437(5)
C11	11482(2)	6692(1)	7640(3)	355(6)	O28	2554(2)	6977(1)	13315(2)	631(7)
C12	11629(2)	7105(1)	6317(3)	350(6)	O29	3912(2)	7227(1)	8423(2)	522(6)
C13	10935(2)	7742(1)	6238(3)	347(6)					

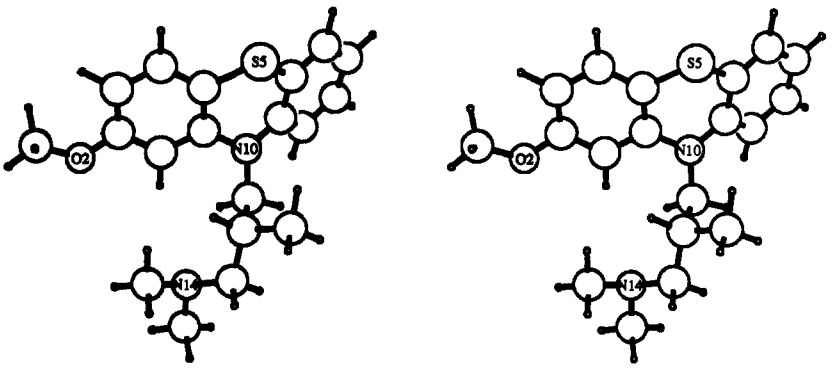

Figure 2. Conformation found for (−)-(*R*)-methotrimeprazine (also known as levomepromazine, 3) by X-ray crystallography. The conformer is [−70,165,−65,163 (−76)] I.

Table II. Atomic Coordinates ($\times 10^3$) for Hydrogen Atoms in (+)-Thioridazine (2) Fumarate

atom	x	y	z	atom	x	y	z
H1	1177	570	594	H16	1006	944	475
H3	959	467	385	H16	1097	910	413
H4	826	506	535	H17	1043	905	697
H6	763	583	1042	H17	1138	942	650
H7	871	585	1241	H18	1214	837	674
H8	1047	617	1241	H18	1183	835	745
H9	1136	633	1020	H19	909	722	549
H11	1147	702	844	H19	932	725	377
H11	1207	640	777	H19	999	678	472
H12	1233	725	638	H20	1279	574	373
H12	1150	684	551	H20	1336	509	330
H13	1039	771	693	H20	1325	514	502
HN14	1083	776	411	H26	582	713	882
H15	925	848	538	H28	393	673	1151
H15	944	844	377	H29	258	762	1039

kcal/mol above the global minimum. The computed conformer most similar to those observed by crystallography for the free bases of racemic 2[20] and *cis*-4[23] are 1.6 and 1.3 kcal/mol above the global minima, respectively. Thus, all of the crystal conformers are computed to have reasonably low energies.

Of the three compounds, 3 appears to have the most restricted conformational space, with only 10 conformers that are within 3 kcal/mol of the global minimum. Half of these have folding I while the other half have folding II. For 2, there are 10 conformers with folding I and 8 conformers with folding II that are within 3 kcal/mol of the global minimum. The side chain in *cis*- and *trans*-4 appears to have the most flexibility with 18 conformers within 3 kcal/mol for each folding of the tricyclic structure. It can be seen from Table V that the difference in the position of the 2-Cl between *cis*- and *trans*-4 has only a minor effect on the conformational energy differences. It should be noted that for *cis*- and *trans*-4, each conformer with folding I has a corresponding mirror image conformer with folding II. For 3, there are corresponding conformers with foldings I and II due to the minor effect of the 2-substituent on the computed conformational energy. This is less true for 2 due to the bulky nature of the piperidine ring, which interacts differently with the different foldings of the tricyclic structure.

With respect to $\tau 1$ for the phenothiazines, one of the minima in the energy surface is in the vicinity of 80° for folding I and −80° for folding II. However, these conformers are much less favorable, and none of the low-energy forms listed in Tables III and IV have this value. These values of $\tau 1$ are also associated with a flattening of the phenothiazine structure with the angle between the two phenyl planes going from about 135° for the low-energy conformers to about 150–160°. For the thioxanthenes, $\tau 1$ is restricted to the vicinity of 0° for the biologically active cis-compounds and to 180° for the less active trans compounds. Combining the results for the phenothiazines and thioxanthenes, it appears that the biologically active form of the phenothiazines must have $\tau 1 \approx -60°$ with the required folding I since conformers with $\tau 1 \approx 60°$ are unfavorable.

With regard to $\tau 3$, values of this dihedral angle appear to be restricted for 2 depending on the enantiomer. For (+)-*R*-2, values in the vicinity of −60° are less favorable. Conformers with this value of $\tau 3$, when they are stable, are consistently less favorable than other values and $\tau 3$ is

Table III. Energy-Minimized Conformers of (+)-*R*-**2** within 3 kcal/mol of the Global Minimum along with Their RMS Fit to the D2 Ligand Model

folding I [τ1,τ2,τ3,τ4]	steric energy (kcal/mol)	rms fit (Å)	folding II [τ1,τ2,τ3,τ4]	steric energy (kcal/mol)	rms fit (Å)[a]
[-144,48,171,179]	27.2	0.70	[50,46,173,179]	26.4	0.66
[-53,173,173,179][b]	27.7	0.31	[53,172,173,179]	27.6	0.33
[-52,-75,172,179]	27.9	0.55	[52,172,53,179]	28.1	0.15
[-59,-55,75,-177]	27.9	1.10	[148,-77,171,179]	28.3	0.67
[-54,-176,54,179]	28.0	0.23	[53,72,54,178]	28.4	0.50
[-148,171,174,179]	28.2	0.63	[148,-175,54,179]	28.5	0.14
[-149,72,54,178]	28.7	0.49	[143,-55,75,-177]	28.5	0.94
[-50,-52,-104,180]	28.7	0.56	[147,175,172,179]	28.6	0.62
[-148,171,52,179]	28.8	0.27	[53,179,-102,180][c]	29.6	0.30
[-52,-170,-100,180]	29.1	0.27			

[a] While all of the dihedral angles correspond to (+)-*R*-**2**, the rms values in this column correspond to the mirror image (-)-*S*-**2** with fold type I. See the text. [b] Conformer of (+)-*R*-**2** that is consistent with the D2 ligand model. [c] While this conformer is slightly more than 3 kcal/mol above the global minimum, the mirror image of this conformer may be responsible for the D2 activity of (-)-*S*-**2**. See the text.

Table IV. Energy-Minimized Conformers of (-)-**3** (Levomepromazine) within 3 kcal/mol of the Global Minimum along with Their RMS Fit to the D2 Ligand Model

folding I [τ1,τ2,τ3,τ4]	steric energy (kcal/mol)	rms fit (Å)	folding II [τ1,τ2,τ3,τ4]	steric energy (kcal/mol)	rms fit (Å)[a]
[-148,62,-68,162 (-75)]	23.4	0.93	[56,64,-67,162 (-76)]	23.3	1.04
[-147,56,179,65 (-172)]	24.1	0.88	[54,56,178,65 (-172)]	24.0	0.64
[-54,-177,-58,170 (-67)]	24.1	0.22	[148,-174,-55,171 (-66)]	24.3	0.26
[-54,178,178,64 (-173)][b]	24.5	0.29	[148,178,178,64 (-173)]	24.6	0.60
[-76,-53,-49,170 (-67)]	26.1	0.77	[81,-60,-50,171 (-65)]	25.8	1.04

[a] While all of the dihedral angles correspond to (-)-**3**, the rms values in this column correspond to the mirror image (+)-**3** with fold type I. See the text. [b] Conformer of (-)-*R*-**3** that is consistent with the D2 ligand model.

Table V. Energy-Minimized Conformations with Folding I That Are within 3 kcal/mol of the Global Minimum for *cis*-**4** and *trans*-Chlorprothixene

cis-**4** [τ1,τ2,τ3,τ4]	steric energy (kcal/mol)	rms fit (Å)	*trans*-**4** [τ1,τ2,τ3,τ4]	steric energy (kcal/mol)	rms fit (Å)
[-4,-123,53,66 (-171)]	7.3	0.89	[-173,127,-52,172 (-64)]	7.3	0.63
[-3,113,179,67 (-170)][a]	7.7	0.37	[-174,-114,-176,171 (-66)]	8.0	0.61
[-3,115,-169,173 (-64)]	7.9	0.41	[-174,-115,169,64 (-173)]	8.1	0.62
[-4,135,170,64 (-173)]	8.2	0.36	[-175,138,-171,173 (-63)]	8.3	0.65
[-2,113,54,65 (-172)]	8.3	0.16	[-174,-114,-55,172 (-65)]	8.4	0.16
[-3,114,-173,-59 (68)]	8.3	0.41	[-174,134,172,64 (-173)]	8.5	0.63
[-4,-134,-171,173 (-63)]	8.4	0.31	[-170,57,45,67 (-169)]	8.6	0.79
[-4,-55,-44,169 (-67)]	8.5	0.89	[-174,-114,175,-67 (60)]	8.7	0.62
[-3,-136,84,174 (-62)]	8.6	0.58	[-173,136,-84,62 (-174)]	8.7	0.61
[-4,-137,-58,175 (-62)]	8.7	0.26	[-174,132,57,62 (-174)]	8.8	0.21
[-2,-61,-173,171 (-66)]	8.7	0.65	[-172,65,174,66 (-171)]	9.0	0.77
[-3,-128,71,-70 (58)]	9.1	0.64	[-172,128,-69,-56 (72)]	9.2	0.64
[-3,-137,180,-64 (63)]	9.2	0.32	[-174,135,-177,-62 (65)]	9.3	0.65
[-3,132,-76,75 (-163)]	9.4	0.47	[-175,-133,76,165 (-73)]	9.5	0.40
[-4,133,-74,157 (-81)]	9.4	0.48	[-171,-132,74,81 (-157)]	9.8	0.40
[-2,-67,172,-68 (59)]	9.5	0.71	[-172,70,-173,-60 (67)]	9.9	0.79
[-4,-52,-78,66 (-171)]	9.8	0.70	[-172,52,78,171 (-66)]	9.9	0.68
[-2,105,99,174 (-63)]	10.2	0.15	[-175,-106,-98,62 (-174)][a]	10.3	0.32

[a] Conformer of *cis*- and *trans*-**4** that is consistent with the D2 ligand model.

displaced to about -100°. This appears to be due to steric interactions between the *N*-methyl group of the piperidine ring with the atoms of the side chain. For (-)-*S*-**2**, conformers with τ3 = +60° are less favorable and displaced to about +100° for the same reason. This conformational difference may be responsible for the enantiomeric difference in affinity for D2 receptors (see below).

With regard to τ4, there are some additional regularities for those compounds with two equivalent *N*-methyl groups (**3** and **4**). Conformers in which both *N*-methyl groups are gauche are somewhat less favorable. For **2**, τ4 is part of the piperidine ring and has a value of about 180° for the optimal conformer in which all of the groups attached to the piperidine ring are equatorial.

Some calculations were also performed on the semirigid thioxanthene analog **5**. For a given folding of the tricyclic structure, the piperidine ring can invert. For folding I, the conformer shown in Figure 3 is preferred by 0.2 kcal/mol over the conformer with the inverted piperidine ring. Dihedral angles for this conformer are also given in Table VIII.

Superposition Studies

One of the goals of this work is to explain the pharmacology of dopamine D2 receptor antagonists in terms of their molecular structures. Among the issues that will be addressed are the following: (1) Why does (+)-*R*-**2** have 3 times the affinity of (-)-*S*-**2** for D2 receptors? (2) Why does (-)-*R*-**3** have 45 times the affinity of (+)-*S*-**3** for D2 receptors? (3) Why does the activity of the thioxanthenes (**4**) mainly reside in the cis isomers rather than the trans isomers? The previously proposed D2 ligand model will be used to provide a framework, and the ability to answer these questions successfully will be a measure of its success. The crystal structure of the typical dopamine antagonist loxapine (**6**),[24] which has little conformational flexibility,[12]

Table VI. Important Torsion Angles and Geometrical Parameters for Selected Computed Conformers and Crystal Conformers of **2**

dihedral angle	global minimum	biologically active	X-ray[a]	X-ray[b]	X-ray[c]
C6-C5a-S5-C4a	−145	145	−144	149	142
C5a-S5-C4a-C4	145	−145	144	−149	−142
C11-N10-C1a-C1	29	−27	16	−28	−21
C11-N10-C9a-C9	−28	26	−20	26	23
C12-C11-N10-C1a	50	−53	60	−60	−66
C12-C11-N10-C9a	−149	149	−151	128	140
C13-C12-C11-N10	46	173	73	−169	−170
N14-C13-C12-C11	173	173	−131	54	51
C15-N14-C13-C12	179	179	−174	179	179
C16-C15-N14-C13	62	62	55	61	64
C17-C16-C15-N14	−59	−60	−58	−60	−61
C18-C17-C16-C15	57	57	57	56	59
C19-N14-C13-C12	56	57	64	61	60
C19-N14-C13-C18	177	178	−173	−177	−179
C19-N14-C15-C16	−173	−172	179	−178	−174
C20-S2′-C2-C3	110	−118	169	148	180
C20-S2′-C2-C1	−70	−61	−12	−32	−1
N-phenyl distance[d] (Å)	5.2	6.1	4.8	6.5	6.5
phenyl phenyl angle (deg)	136	135	137	146	134
energy (kcal/mol)	26.4	27.7			

[a] This work. [b] The mirror image of molecule I, ref 20. [c] Molecule II, ref 20. [d] Substituted phenyl ring.

Table VII. Important Torsion Angles and Geometrical Parameters for Selected Calculated Conformers and Crystal Conformer of **3**

dihedral angle	global minimum	biologically active	X-ray[a]
C6-C5a-C5-C4a	−145	145	145
C5a-C5-C4a-C4	145	−145	−145
C11-N10-C1a-C1	25	−28	−21
C11-N10-C9a-C9	26	26	27
C12-C11-N10-C1a	56	−54	−70
C12-C11-N10-C9a	−147	148	130
C13-C12-C11-N10	64	178	165
N14-C13-C12-C11	−67	178	−65
C15-N14-C13-C12	−76	−173	−76
C16-N14-C13-C12	162	64	163
C12′-C12-C11-N10	−175	−59	−71
C12′-C12-C13-N14	173	56	171
C18-O2-C2-C3	0	0	−12
C18-O2-C2-C1	180	180	168
N-phenyl distance[b] (Å)	4.0	6.2	5.1
phenyl-phenyl angle (deg)	135	134	139
energy (kcal/mol)	23.3	24.5	

[a] This work, similar to results in refs 21 and 22. [b] Substituted phenyl ring.

Table VIII. Important Torsion Angles and Geometrical Parameters for Selected Calculated Conformers and Crystal Conformer of *cis*-**4** and -**5**

dihedral angle	global minimum	biologically active of 4	X-ray of 4[a]	global minimum of 5
C6-C5a-C5-C4a	146	146	150	141
C5a-C5-C4a-C4	−146	−146	−148	−141
C11-C10-C1a-C1	−50	−48	−40	−56
C11-C10-C9a-C9	46	45	39	56
C12-C11-C10-C1a	−4	−3	−3	1
C12-C11-C10-C9a	172	174	176	176
C13-C12-C11-C10	−123	113	−151	120
N14-C13-C12-C11	53	179	173	59
C15-N14-C13-C12	−171	−170	−178	−60
C16-N14-C13-C12	66	67	61	176
N-phenyl distance[b] (Å)	5.0	5.7	6.2	6.2
phenyl-phenyl angle (deg)	137	137	142	129
energy (kcal/mol)	7.3	7.7		12.6

[a] Reference 23. [b] Substituted phenyl ring.

will be used as a template with each of the low-energy conformers being superimposed onto it in a least-squares sense. It should be noted that, in addition to this root mean square (rms) fit, an additional factor that will be important is the requirement that the ammonium hydrogens of the conformers point in the same general direction. Finally, as discussed above, the biologically active conformer should have low energy to maintain high affinity for the D2 receptor.

The low-energy conformers of (+)-*R*-**2** that are within 3 kcal/mol of the global minimum are listed in Table III. Since the ligand model requires folding I, only those with this fold will be considered. However, the mirror image of conformers of (+)-*R*-**2** with folding II are conformers of (−)-*S*-**2** with folding I with identical energies. While all of the conformers listed in the table correspond to (+)-*R*-**2**, the rms fits listed under folding II actually refer to the equivalent conformer of (−)-*S*-**2** with folding I. This has also been done for **3** in Table IV.

A number of low-energy conformers of (+)-*R*-**2** appear to be a good rms fit to **6**. However, upon visual inspection, only the conformer [−53,173,173,179] I (Figure 4a) preserves the correct orientation of the ammonium hydrogen and is, therefore, proposed as the biologically active form. This conformer is 1.3 kcal/mol above the global minimum and has a rms fit of 0.31 Å. With regard to the (−)-*S*-**2** conformers, none of those listed in the table is both a good fit and has the correct orientation of the ammonium hydrogen. One possibility for the biologically active form of (−)-*S*-**2** is the conformer [−53,176,102,180] I (Figure 4b). This conformer has a rms fit of 0.30 Å and does have the correct orientation of the ammonium hydrogen. However, due to steric interactions between the *N*-methyl and the side chain, the energy of this conformer is 1.9 kcal/mol above that of the conformer shown in Figure 4a. This could account for its lessened affinity for D2 receptors. An alternative is that another conformer, with lower energy but which does not fit the ligand model as well, is responsible for the biological activity.

For (−)-*R*-**3**, only the conformer [−54,178,178,64 (−173)] I (Figure 5) appears to meet the requirements of the ligand model. This conformer is 1.2 kcal/mol above the global minimum and has an rms fit of 0.29 Å. None of the low-energy conformers of (+)-*S*-**3** appear to meet the requirements of the ligand model. As can be seen from Figures

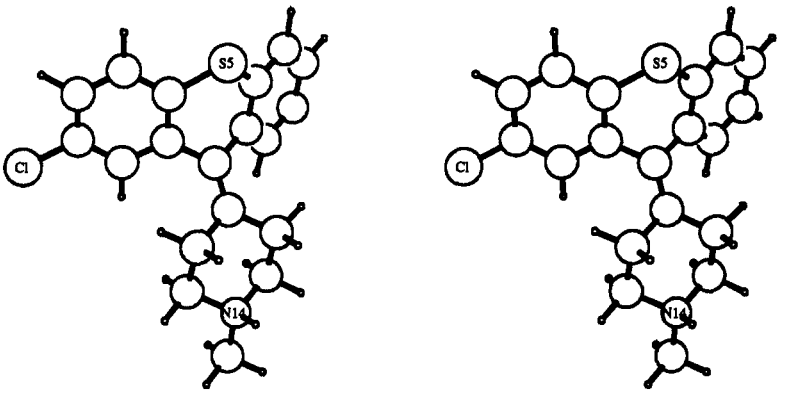

Figure 3. The energy-minimized structure of the semirigid thioxanthene analog **5**. The conformer is [1,120,59,176 (-60)] I.

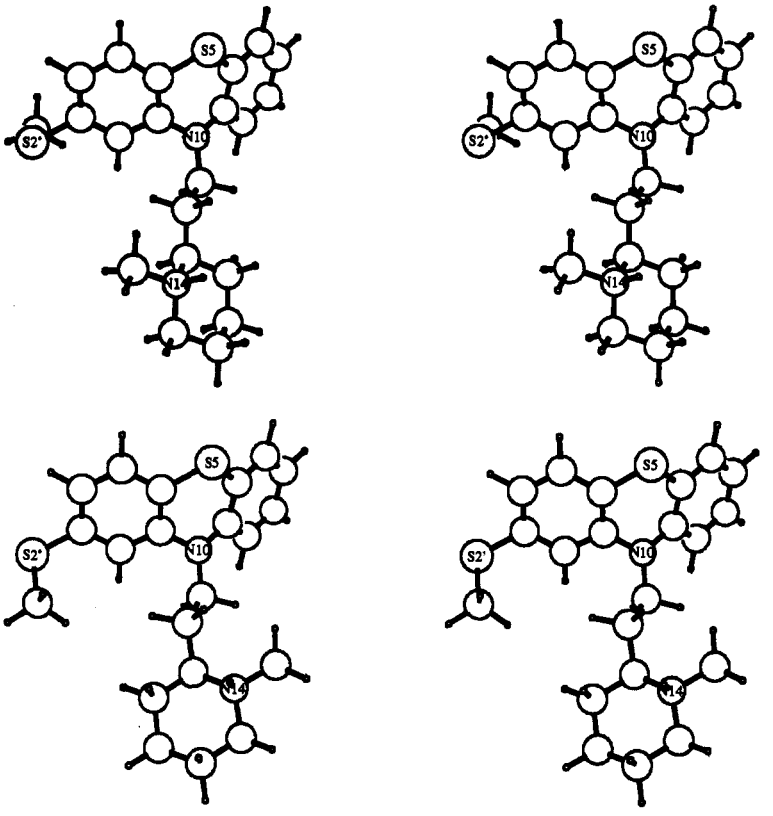

Figure 4. The conformers which appear to be the biologically active forms of (a) (+)-*R*-**2** and (b) (-)-*S*-**2**. The conformations are [-54,174,173,179] I and [-53,-176,102,180] I, respectively.

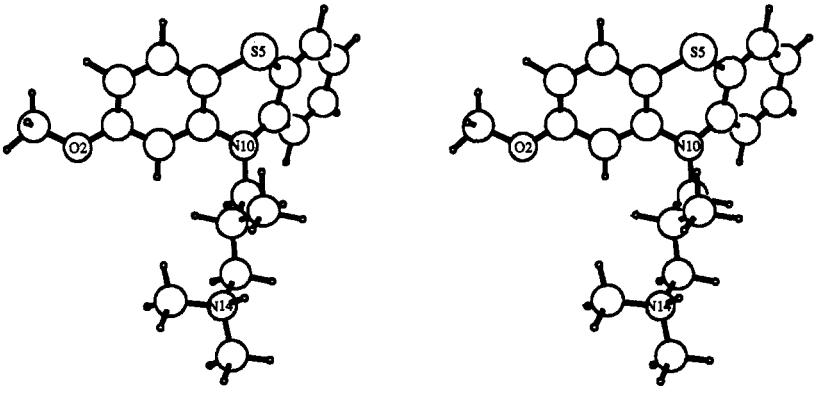

Figure 5. The conformer that appears to be the biologically active form of (-)-*R*-**3**. The conformer is [-54,178,178,64 (-173)] I.

4a and **5**, the proposed biologically active conformers of (+)-*R*-**2** and (-)-*R*-**3** are quite similar.

For *cis*-**4**, the conformer [-3,113,179,67 (-170)] I (Figure 6) appears to meet the criteria of the ligand model with an energy 0.4 kcal/mol above the global minimum, an rms fit of 0.37 Å, and the correct orientation of the ammonium hydrogen. For *trans*-**4**, none of the conformers appear to meet the criteria. A conformer such as [175,-106,-98, -174 (62)] I is a good fit to the ligand model but its energy is 3.0 kcal/mol above the global minimum. Thus, the factor

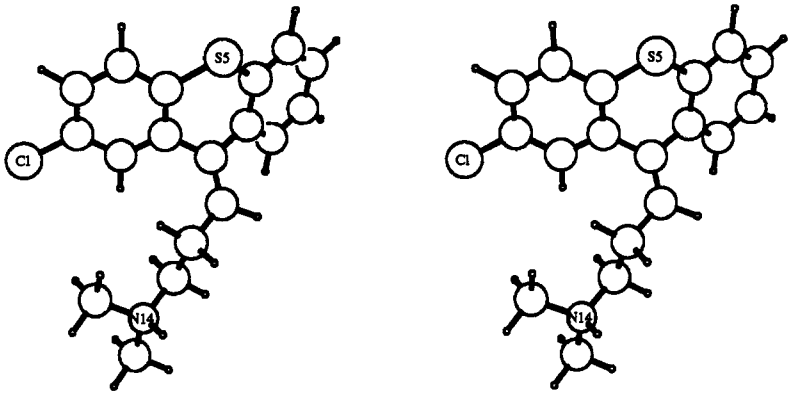

Figure 6. The conformer that appears to be the biologically active form for *cis*-**4**. The conformer is [-2,113,179,67 (-170)] I.

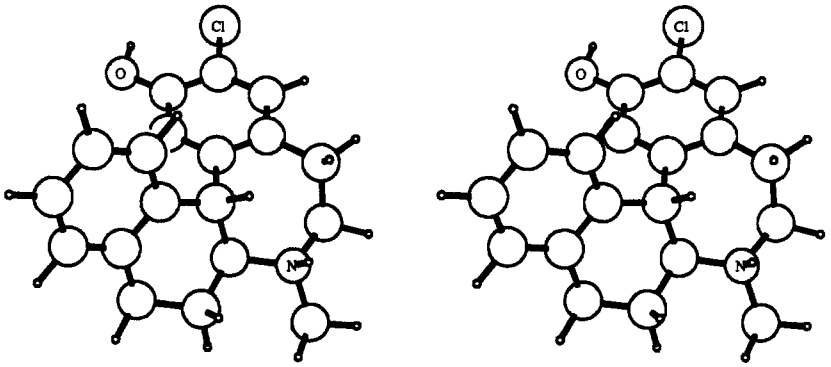

Figure 7. The conformation that appears to be responsible for the D1 receptor activity of benzazepine derivatives as represented by a semirigid derivative.[29,30]

that accounts for the much lower activity of the trans isomers is the inability to achieve the correct orientation of the ammonium hydrogen in a low-energy conformer.

The conformer shown in Figure 3 appears to be the biologically active form for **5** since the rms fit is 0.11 Å for superimposing it onto **6**. This indicates a very close fit and the orientation of the ammonium hydrogen is consistent with the ligand model. A question has been raised as to why *cis*-thioxanthenes are more active than trans isomers if both should be able to assume the conformation represented in Figure 3 equally well.[10] The apparent answer to this is that the biologically active form of *cis*-**4** does not appear to correspond exactly to the structure of **5**. For *cis*-**4**, the active form appears to be [-3,113,179,67 (-170)] I while that for **5** is [1,120,59,176 (-60)] I. The conformer for *cis*-**4** that most closely corresponds to **5** is 2.9 kcal/mol above the global minimum and 2.5 kcal/mol above the biologically active form. Thus, the effect of the piperidylidene ring is to force **5** into a conformation that is not easily attainable by open chain analogs like *cis*- or *trans*-**4**.

The affinity of a ligand for a receptor site can be directly related to its free energy of binding to the site. For dopamine antagonists with typical properties, the free energy of binding is primarily due to a large increase in entropy.[25-28] Nevertheless, the energy of the biologically active form relative to its global minimum should be an important factor in the enthalpy of binding. That is, everything else being taken as equal, the ligand–receptor complex should be weakened for a ligand in which several kcal/mol are required to assume the biologically active form. In this work, differences in receptor affinity between two pairs of enantiomers and one pair of close geometrical isomers have been related to their ability to assume the biologically active form as defined by the previously proposed ligand model.[12] For the D2 antagonists studied here, this analysis appears to be entirely successful. A reason for this success may be that one is comparing receptor affinities between pairs of enantiomers or close geometrical isomers since each pair will have either identical or very similar physicochemical properties. In addition, many, if not most, of the interactions that each ligand in the pair will make with the receptor site will be identical. Thus, one would not expect to be able to explain differences in receptor affinity between (-)-**2** and (-)-**3** in this way since the two compounds differ in the nature of the 2-substituent, among other things, and this is an important factor in D2 antagonist activity.[1]

Recently, a pharmacophore for D1 antagonist activity has been defined using a semirigid benzazepine that has considerably less conformational flexibility than the original D1 selective benzazepines.[29,30] The energy-minimized structure for this compound is shown in Figure 7. The distance between the ammonium nitrogen in this compound is considerably smaller (~4.3 Å) as compared with most D2 antagonists (~6.1 Å). However, this difference may not be crucial since all of the compounds used to define the D2 pharmacophore for which D1 receptor affinity has been measured also have considerable affinity for D1 receptors.[12] The structure of the benzazepines does appear to be uniquely favorable for binding to D1 receptors since the compounds generally have higher affinity for D1 receptors than other classes of dopamine antagonists.[17,31]

It would be of interest to compare the pharmacophore for D1 antagonist activity, as represented by Figure 7, with that for D2 antagonist activity. However, due to the much smaller distance between the ammonium nitrogen and the substituted phenyl ring in the benzazepines, none of the possible lease squares superpositions appears to be

entirely satisfactory. A plausible way to align the ligand models for D2 and D1 antagonist activity is in the orientations shown in Figures 3 and 7, since this preserve a similar orientation of the ammonium hydrogen, which does appear to be a crucial factor.[12] The D1 selectivity of the benzazepines then appears to be due to the steric interference of the unsubstituted phenyl ring which occupies considerable space that is unoccupied in D2 antagonists. The presence of this phenyl ring is crucial for the D1 selectivity of the benzazepines.[32]

One would also like to be able to explain the 10-fold higher affinity of (−)-*S*-**2** over (+)-*R*-**2** for D1 receptors. This favorability of (−)-*S*-**2** for D1 receptors appears to be due to intrinsic molecular factors for this enantiomer rather than just a decrease of D1 affinity by (+)-*R*-**2**.[15,17] This argues strongly against the energetically unfavorable conformer in Figure 4b being responsible for the D1 receptor affinity of (−)-*S*-**2**. However, it was not possible to decide which of the other low-energy conformers is responsible for its D1 receptor activity due to the differences in the geometries of the compounds discussed above.

Conclusions

The conformational and pharmacological properties of the enantiomers of **2** and **3** and the geometrical isomers of **4** appear to be entirely consistent with the previously proposed ligand model for D2 antagonists. For **2**, where the (+)-*R*-enantiomer has 3 times the affinity of the (−)-*S*-enantiomer, a low-energy conformer with the correct molecular geometry is more accessible for the former. For **3**, where the (−)-*R*-enantiomer has 45 times the affinity of the (+)-*S*-enantiomer, only the former has a low-energy conformer consistent with the ligand model. For *cis*-**4**, a low-energy conformer is consistent with the ligand model while for *trans*-**4**, the best conformer is 3.0 kcal/mol above the global minimum. As before, the crucial element for the ligand model appears to be the orientation of the ammonium hydrogen. These results also are consistent with the hypothesis that the relative energy of the biologically active conformer is an important factor in the activity of pharmacological compounds.

Experimental Section

Molecular Mechanics Studies. The calculations were performed with the MM2-87 program and parameter set.[33,34] Energy minimizations were performed with respect to all internal degrees of freedom. The initial Cartesian coordinates for the energy minimizations were generated by either a previously described program,[35] by the PCMODEL program,[36] or by utilizing the DRIVER option of the MM2-87 program. The convergence criterion was set at $^1/_8$ of its default value to ensure complete convergence.

There were a number of missing parameters for sulfur and nitrogen bonded to a phenyl ring, most of which were obtained from the literature.[37,38] There were three remaining missing torsional parameters. Those for C_{sp3}-C_{sp3}-N_{sp3}-C_{sp2} and H-C_{sp3}-N_{sp3}-C_{sp2} were approximated from the torsional parameters in which the C_{sp2} is replaced by C_{sp3}. For S-C_{sp2}-C_{sp2}-N_{sp3}, the V2 and V3 terms were set to 16.25 and 2.0, respectively.

The calculations were performed on the protonated forms of the molecules with a dielectric constant of 80 to approximate an aqueous solution. To minimize the interaction between the side chain and the flexible 2-substituents of **2** and **3**, the 2-substituents were rotated away from the side chain and they generally stayed in that conformation. There was a small preference of the thiomethyl group of **2** to be on the convex side of the phenothiazine structure. During the course of the energy minimizations, it was realized that some conformers were being stabilized by intramolecular hydrogen bonding between the protonated nitrogen and various other moities. However, as the calculations are performed in the absence of solvent, which precludes intermolecular hydrogen bonding, it appears that the intramolecular hydrogen bonding is unrealistic and it was turned off. This results in much better agreement between calculations and experimentally observed conformers for a number of molecules.[39]

The least-squares superposition studies of the various compounds was performed with the PCMODEL program. Superposition was with respect to the ammonium nitrogen and the six atoms of the substituted phenyl ring taking into account the position of the 2-substituent.

Crystallographic Studies. Suitable crystals of (+)-**2** fumarate were grown from methanol. The compound crystallized in the orthorhombic space group $P2_12_12_1$ with cell dimensions a = 12.679(3) Å, b = 19.728(5) Å, c = 9.65(2) Å, V = 2414.02(4) Å^3, Z = 4, ρ = 1.34 g/cm^3, $F(000)$ = 1040, T = 272 K, Cu Kα λ = 1.5418 Å, and extinction coefficient, μ = 22.34/cm. Suitable crystals of (−)-**3** (free base) were grown from methanol. It should be noted that (−)-**3** was dissolved as the HCl salt but only the free base crystallized out. The compound crystallized in the orthorhombic space group $P2_12_12_1$ with cell dimensions a = 12.779(1), b = 18.827(2), c = 7.5388(6), V = 1813.66(4), Z = 4, ρ = 1.152 g/cm^3, $F(000)$ = 672, T = 272 K, and Cu Kα λ = 1.5418.

A Nonius CAD4 automated diffractometer was used to collect the crystallographic data. The crystals were stable and showed no deterioration. Data were corrected for Lorentz, polarization, and extinction effects, but not for absorption. The structures were solved using the direct methods programs MULTAN and NQEST.[40,41] Anomalous scattering curves were taken from the International Tables.[42] For (+)-**2** fumarate, there were 2607 unique reflections, of which 2406 had $I > 2\sigma$. The R factor for the R-enantiomorph was 0.054 as compared with 0.060 for the S-enantiomorph. For the free base of (−)-**3**, there were 2166 unique reflections of which 1967 has $I > 2\sigma$. The R factor for the R-enantiomorph was 0.049 as compared with 0.060 for the S-enantiomorph.

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