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Comparative structure–activity relationships of benztropine analogues at the dopamine transporter and histamine H_1 receptors

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Abstract—Benztropine (BZT) and its analogues inhibit dopamine uptake and bind with moderate to high affinity to the dopamine transporter (DAT). However, many of these compounds, in contrast to other monoamine uptake inhibitors, lack cocaine-like behavioral effects and fail to potentiate the effects of cocaine. The BZT analogues also exhibit varied binding affinities for muscarinic M_1 and histamine H_1 receptors. In this study, a comparative analysis was conducted of pharmacophoric features with respect to the activities of BZT analogues at the DAT and at the histamine H_1 receptor. The BZT analogues showed a wide range of histamine H_1 receptor (K_i = 16–37,600 nM) and DAT (K_i = 8.5–6370 nM) binding affinities. A stereoselective histamine H_1-antagonist pharmacophore, using a five-point superimposition of classical antagonists on the template, cyproheptadine, was developed. A series of superimpositions and comparisons were performed with various analogues of BZT. In general, smaller substituents were well tolerated on the aromatic rings of the diphenyl methoxy group for both the DAT and H_1 receptor, however, for the H_1 receptor, substitution at only one of the aromatic rings was preferred. The substituents at the 2- and N-positions of the tropane ring were preferred for DAT, however, these groups seem to overlap receptor essential regions in the histamine H_1 receptor. Molecular models at the DAT and the histamine H_1 receptor provide further insight into the structural requirements for binding affinity and selectivity that can be implemented in future drug design.

1. Introduction

Cocaine is a powerful psychostimulant, which produces its action through inhibition of dopamine reuptake at the dopamine transporter (DAT).[1,2] Extensive research over the past 15 years to discover medications for cocaine abuse has identified compounds from different structural classes that bind to the DAT and have varying pharmacological profiles.[3,4] Benztropine (BZT) and its analogues share some structural features with cocaine, bind to the DAT, and inhibit the uptake of dopamine.[5,6] Unlike cocaine, BZT is a clinically used agent and is not subjected to significant abuse. In general, novel analogues of BZT are not as effective as cocaine in producing pre-clinical indications of abuse.[7,8] The distinctive pharmacological profile of BZT analogues has been the subject of many molecular and pharmacological investigations.[9–11]

In addition to affinity for the DAT, the BZT analogues also have affinity for muscarinic M_1 and histamine H_1 receptors.[5,12–15] Structure–activity relationship (SAR) studies have identified compounds, which are selective for the DAT over muscarinic M_1 receptors.[16,17] In general, muscarinic receptor binding affinities were significantly reduced when the *N*-methyl group in BZT was replaced with substituents with greater steric bulk. However, these compounds did not produce stimulation of locomotor activity or cocaine-like discriminative stimulus effects in animal models of cocaine abuse thus ruling out a significant role of the muscarinic M_1 receptor on the behavioral actions of the BZT analogues.[8]

Keywords: Dopamine transporter; Benztropines; Histamine H_1 receptor; Molecular modeling; Cocaine; Pharmacophore.
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Recently, the role of histamine H_1 receptors in the behavioral actions of the BZT analogues was investigated.[18] In this study, a direct relationship between the histamine H_1 receptor binding to behavioral activity was not observed with the BZT analogues. Nevertheless, the interpretation of the biological bases for the actions of the BZT analogues could be better assessed if confounding affinity at the histamine receptor could be eliminated. Hence, in the present study, a comparison of SAR for the BZT analogues at the DAT to their SAR at histamine H_1 receptors was performed. The basic consideration in this work was to analyze the structural features of the BZT analogues which might contribute to histamine H_1 receptor activity and to identify regions in the molecule where better selectivity toward the DAT might be achieved through structural modification. To this end, a pharmacophore model for the histamine H_1-antagonist activity was derived based on the conformationally rigid classical antagonist cyproheptadine. This model was then used for further analysis of the BZT analogues for their histamine H_1-receptor activity.

2. Results and discussion

The classical histamine H_1 receptor antagonists have a general structure of two aromatic/hydrophobic rings and a side chain with nitrogen for cationic interactions.[19] These structural features are also observed in many CNS active agents.[20] The receptor selectivity is achieved either by structural or conformational preferences. Thus in this study, initially a pharmacophore at the histamine H_1 receptor was derived, which was then used to compare the structural and conformational requirements for the BZT analogues at the histamine H_1 receptor. Classical antagonists at the histamine H_1 receptor, with varying degrees of structural rigidities, were chosen (see Fig. 1) to derive the histamine H_1 pharmacophore. The present set of histamine H_1 antagonists is potent in in vitro and in vivo assays. However, the activity of these compounds was obtained from different sources, hence no quantitative comparisons could be made. This set also contained a subset of stereoisomeric pairs of antagonists. As the receptor interactions at the histamine H_1 receptor are stereoselective,[21] the activity data of these compounds were utilized qualitatively to evaluate different pharmacophore models.

The binding affinities of the BZT analogues were measured in a radioligand binding assay in a whole rat brain preparation using [^{3}H]mepyramine as the radioligand, where they exhibited binding affinities from 16 to 37,600 nM (Table 1).[18] There was no correlation between the activity of the BZT analogues at the DAT and histamine H_1 receptor (Fig. 2), suggesting that there are different structural requirements for binding at both the receptor sites.

2.1. The histamine H_1 antagonist pharmacophore

The first step in deriving the pharmacophore for histamine H_1 receptor involved the conformational analysis of the compounds. A conformationally rigid and potent antagonist cyproheptadine served as a template for our studies. Extensive molecular modeling with experimental data from NMR studies has provided strong support for the so-called 'chair2eq' conformation of cyproheptadine, as the active conformation.[22–24] Thus, this conformation was derived from the crystal structure as described in Section 4. A conformational search of the other antagonists (Fig. 1) yielded many low energy conformations with an intramolecular H-bond between the side-chain N–H and either the pyridine N or the O atom of the side chain. There are no reports on the existence of such H-bonded conformation, hence the conformational ensemble was analyzed after eliminating such potentially false low energy conformations. Similarly

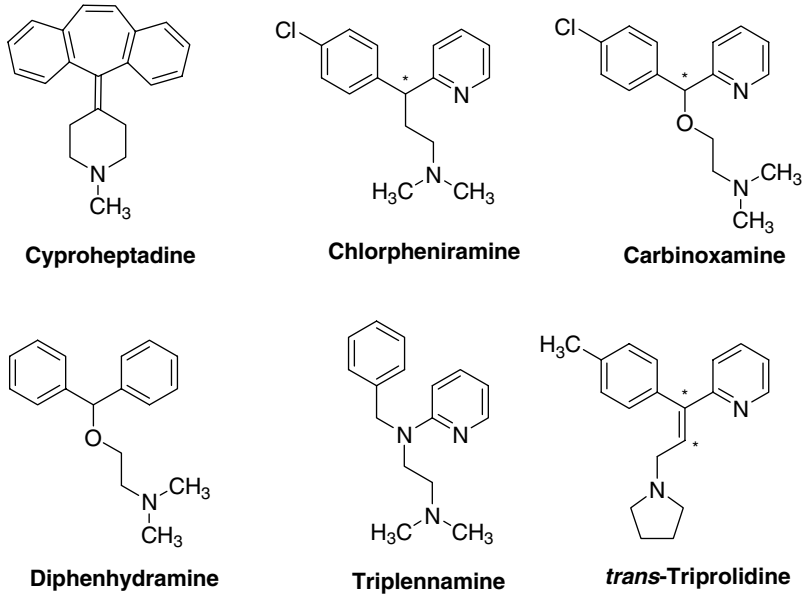
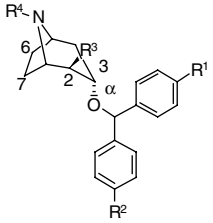


Figure 1. Structures of the classic histamine H_1 antagonist, the stereoisomeric pairs are indicated with '*' on the structures.

Table 1. Structures and binding affinities of the BZT analogues at DAT and histamine H_1 receptors



Compound	R^1	R^2	R^3	R^4	Binding affinity $K_i \pm$ SEM (nM)		Selectivity ratio H_1/DAT
					DAT	H_1	
1[a]	H	H	H	Me	118 ± 10.6	15.7 ± 2.13	0.1
2[a]	F	F	H	Me	11.8 ± 1.3	19.7 ± 1.32	1.7
3[a]	F	H	H	Me	32.2 ± 3.2	17.7 ± 1.55	0.6
4[a]	OMe	OMe	H	Me	2000 ± 140	157 ± 2.09	0.1
5[a]	OMe	H	H	Me	78.4 ± 6.3	28.6 ± 1.88	0.4
6[a]	Cl	Cl	H	Me	20 ± 2.8	122 ± 4.55	6.1
7[a]	Cl	H	H	Me	30 ± 3.6	39.3 ± 1.57	1.3
8[a]	Cl	H	H	Me, 3-β-isomer	854 ± 60	2200 ± 373	2.6
9[a]	CH_3	H	H	Me	187 ± 9.4	23.1 ± 2.02	0.1
10[b]	F	F	H	H	11.2 ± 1.2	65.4 ± 7.28	5.8
11[b]	F	F	H	Allyl	29.9 ± 3.0	24.9 ± 1.16	0.8
12[b]	F	F	H	Aminoethyl	14 ± 1.7	240 ± 32.6	17
13[b]	F	F	H	Indol-3′-yl ethyl	44.6 ± 4.9	333 ± 22.6	11
14[b]	F	F	H	*n*-Butylphenyl	8.51 ± 1.2	141 ± 6.72	17
15[c]	F	F	H	Phenylpropionamide	27.6 ± 1.9	140 ± 17.3	5.1
16[d]	F	F	COOMe	Me	12.9 ± 1.8	4440 ± 592	340
17[d]	F	F	COOEt	Me	16.8 ± .04	6710 ± 670	400
18[e]	F	F	H	Me, 6-β-OCOMe	376 ± 34.3	232 ± 31.4	0.6
19[e]	F	F	H	Me, 6-β-OCOPh	341 ± 44.3	2540 ± 99.8	7.5
Cocaine[f]	—	—	—	—	187 ± 18.7	1040 ± 43	5.6
Chlorpheniramine[f]	—	—	—	—	222 ± 28.5	—	—
Mepyramine[f]	—	—	—	—	645 ± 63	12.2 ± 1.19	0.02

[a] DAT activities were obtained from Ref. 7.
[b] DAT activities were obtained from Ref. 17.
[c] DAT activities were obtained from Ref. 30.
[d] DAT activities were obtained from Ref. 31.
[e] DAT activities were obtained from Ref. 33.
[f] DAT activities were obtained from Ref. 18.

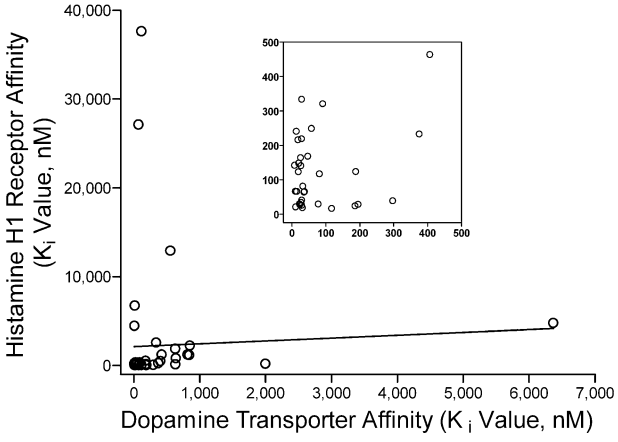


Figure 2. Linear regression of histamine H_1 receptor affinities and DAT affinities among BZT analogues. The r^2 value of the linear regression was 0.0020, which was not significant ($P = 0.7587$). The inset in the figure shows the distribution of the points when low affinity outliers are excluded.

the conformational analysis on the BZT analogues was performed as reported earlier wherein the conformational data for a starting ligand were obtained from the crystal structure.[25]

The low energy conformations were then used to compute the pharmacophoric features (Table 2) as defined in Figure 3. The comparison of molecular shapes and active conformation for a series of compounds has provided qualitative information on the pharmacophore responsible for activity and receptor binding.[26] Similarly, the crystallographic and conformational studies have been used to analyze the stereochemical requirements for binding at the histamine H_1 receptor.[27] The pharmacophoric distances were measured on the low energy conformation (see Fig. 3). Similarly the angle between the planes of the aromatic rings provided another structural measure for comparison with the activity. The average distances for the more active histamine H_1 antagonists were for DI 5.669 Å, DII 5.486 Å, and DIII of 5.091 Å,

Table 2. The pharmacophoric features of the histamine H_1 antagonist and the BZT analogues

Compound	Pharmacophoric features[a]			
	DI	DII	DIII	Angle
Cyproheptadine	6.128	6.128	4.918	54.61
Chlorpheniramine(S)	6.294	5.401	4.750	90.01
Chlorpheniramine(R)	5.278	6.303	4.772	80.01
Carbinoxamine(S)	5.594	5.062	4.771	96.18
Carbinoxamine(R)	5.536	6.038	4.719	77.09
Diphenhydramine	5.543	4.999	4.784	102.04
Triplennamine	5.852	5.349	6.306	13.44
Triprolidine(T)	4.604	5.9777	5.019	58.48
Triprolidine(C)	4.467	5.950	4.945	102.81
1	7.176	7.000	4.706	75.82
2	7.174	7.004	4.703	75.92
8	7.773	6.611	4.707	83.34

[a] See Figure 3 for the definition of the pharmacophoric features.

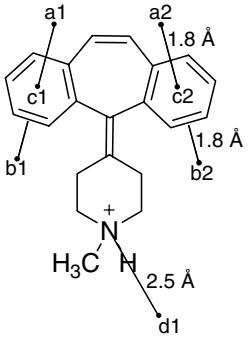


Figure 3. Definition of the receptor interaction points (dummy atoms). The atoms for superimposition A (c1, c2, and N), B (a1, b1, a2, b2, and N), C (c1, c2, and d1), and D (a1, b1, a2, b2, and d1) were used. The pharmacophoric distances DI (c1–N), DII (c2–N), and DIII (c1–c2) with angle between the planes of the aromatic rings were measured.

whereas less active antagonists had these distances 5.093 Å, 6.097 Å, and 4.812 Å, respectively. The BZT analogues, however, exhibited slightly greater DI and DII distances, with similar DIII distance. There was a wide variation in the angle between the planes of the aromatic rings across different antagonists (Table 2). However, the active isomers (S) of chlorpheniramine and carbinoxamine exhibited a value of 90°–96°, whereas the corresponding less active isomers (R) had values from 77° to 80°. The BZT analogues had this angle in a constant range of 75°–76°, except for the less active β-isomer, compound **8** which had a value of 83°. To summarize, the BZT analogues have slightly greater pharmacophoric distances with a fixed angle between the planes of the aromatic rings. This overlap in the general features of classical histamine H_1 antagonists might explain the observed affinity of the BZT analogues toward the histamine H_1 receptor.

The next step in deriving the pharmacophore is superimposition of the active conformation of the compounds on the template. The aromatic rings of the histamine H_1 antagonists have been called 'cis' or 'trans' based on their comparison with the rigid analogue triprolidine.[28] The effects of substitution on these aromatic rings are varied, which is evident in the activity differences of the stereoisomers that differ in the relative placement of the aromatic rings. It is essential to account for the substitution and orientation of the aromatic rings. Thus, two dummy atoms for each of the aromatic rings were defined (Fig. 3), which superimposed not only the centroids but also the planes of the aromatic rings. Similarly a dummy atom located tetrahedrally from the nitrogen was defined to account for the possible ionic interactions. Four different superimpositions were derived in which the 'cis' ring of the antagonist was superimposed on the 'cis' ring of the template (C–C orientation). To validate this orientation, a reversed superimposition (i.e., the 'cis' ring of the antagonist on the 'trans' ring of the template) was derived. Ideally the more active stereoisomers should provide better superimposition in the C–C orientation than in the C–T orientation, and a particular superimposition method should correctly discriminate between the true (C–C) and false (C–T) orientations.

Each superimposition was evaluated based on the root-mean-squared deviation (RMSD) values and the difference in the volume of superimposition, defined as ΔVs (Table 3). The superimposition A showed a large variation in RMSD for each histamine H_1 antagonist, however, there was no difference in the RMSD values between the correct (C–C) and incorrect (C–T) orientations of the aromatic rings. The superimposition B using the dummy atoms for the aromatic rings provided a better discrimination of the correct and incorrect orientations. For example, the active S isomer of carbinoxamine had a RMSD of 0.788 and 1.322 and the ΔVs of 71.5 and 104.2 with the rings in the C–C and C–T orientations, respectively. In contrast, the less active R isomer of carbinoxamine had a RMSD of 1.844 and 1.214 and the ΔVs of 111.5 and 97.4 with the rings in the C–C and C–T orientations, respectively. The superimpositions C and D using the dummy atom at the side-chain nitrogen provided essentially similar results as superimpositions A and B. There was no clear advantage in using superimposition C as no difference in the RMSD values between the C–C and C–T orientations was observed. The RMSD and the ΔVs were not consistent with the observed activity at the histamine H_1 receptor for superimposition D. A large difference between RMSD and the ΔVs for both ring orientations (C–C and C–T) provided a greater confidence in using the superimposition B for further interpretation of the SAR. Thus, it is more likely that the interactions at the aromatic rings are important for activity at the histamine H_1 receptor. The superimposition of the side-chain nitrogen atom further enhances the activity of these compounds.

A graphic visualization of the pharmacophore is depicted in Figure 4, left. The combined volume of the active compounds provided a measure of the receptor-excluded surface, which the active compounds tend to occupy. The less active compounds either have functional groups protruding outside this region or have less than optimal superimposition of the pharmacophore elements in this region. The less active R isomer of the carbinoxamine

Table 3. Superimposition of the histamine H_1 antagonist on the 'chair2eq' conformation of cyproheptadine

Compound		Superimposition							
		A		B		C		D	
		RMSD	ΔVs	RMSD	ΔVs	RMSD	ΔVs	RMSD	ΔVs
Chlorpheniramine(S)	C–C[a]	0.352	94.3	1.061	96.3	0.465	114.7	1.040	110.2
	C–T[b]	0.352	99.6	1.288	107.5	0.465	126.8	1.049	126.5
Chlorpheniramine(R)	C–C	0.404	104.1	1.560	107.8	0.414	128.4	1.227	131.1
	C–T	0.404	97.2	1.205	100.7	0.414	133.3	1.085	115.1
Carbinoxamine(S)	C–C	0.164	65.8	0.788	71.5	0.410	69.9	0.783	71.1
	C–T	0.164	97.9	1.322	104.2	0.410	126.1	0.760	127.8
Carbinoxamine(R)	C–C	0.415	110.1	1.844	111.5	1.246	141.8	2.131	138.2
	C–T	0.415	110.7	1.214	97.4	1.245	161.8	1.880	141.0
Diphenhydramine	C–C	0.161	59.2	0.610	62.8	0.108	62.1	0.596	63.4
	C–T	0.161	103.9	1.322	108.2	0.108	119.3	0.671	121.5
Triplennamine	C–C	0.750	99.4	1.042	95.8	1.296	144.2	1.521	133.7
	C–T	0.750	118.4	1.741	117.2	1.296	138.4	1.888	136.0
Triprolidine(T)	C–C	0.384	88.4	0.880	89.1	0.846	96.3	1.047	95.8
	C–T	0.384	107.7	2.232	122.6	0.846	164.0	2.963	155.6
Triprolidine(C)	C–C	0.361	110.3	1.972	102.3	0.833	158.6	2.635	152.6
	C–T	0.361	91.5	1.449	102.0	0.833	101.4	1.556	110.1

[a] Superimposition of the 'cis' ring of the compound on the 'cis' ring of the template.
[b] Superimposition of the 'cis' ring of the compound on the 'trans' ring of the template.

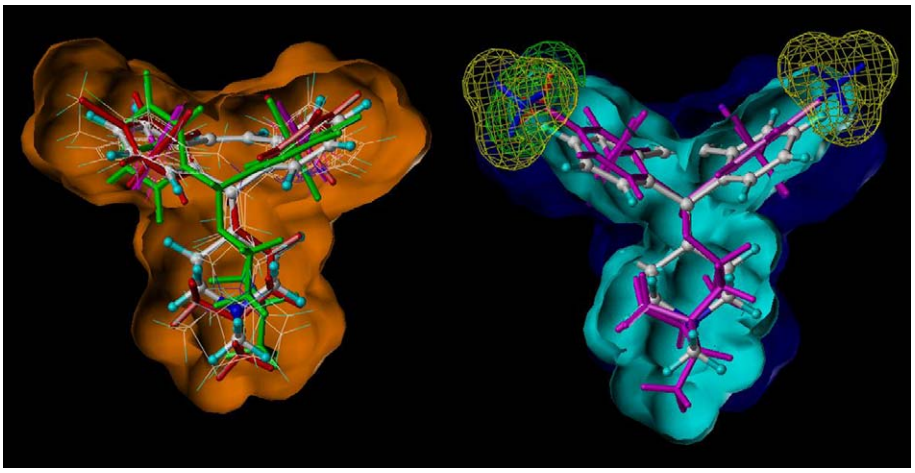


Figure 4. (Left) Superimposition of histamine H_1 antagonists ('cis' and 'trans' rings are shown on left and right sides of the molecules), with receptor-excluded volume (orange surface). The cyproheptadine (ball and stick; colored by atom type), carbinoxamine S (capped stick, red), and carbinoxamine R (capped stick; green) are shown. (Right) Superimposition of the BZT analogues on histamine H_1 pharmacophore. Receptor-excluded region for the DAT (cyan) and H_1 receptor (blue) with cyproheptadine (ball and stick, colored by atom type) and compound **2** (capped stick, magenta) are depicted. The receptor essential volume, green and yellow contours are shown for compounds **9** (capped stick; red) and **4** (capped stick; blue), respectively.

has the 'cis' ring almost perpendicular to that of the template, it also has the side-chain nitrogen oriented away from the template. A similar analysis of all other analogues fits well with the present pharmacophore, which could be used for comparison of the SAR of the BZT analogues.

2.2. Comparison of the SAR of BZT analogues at histamine H_1 receptor and DAT

The BZT analogues were superimposed using the five-point superimposition B on the histamine H_1 pharmacophore. The superimposition measures are presented in Table 4 along with the distances between each of the centroids of the aromatic rings and tropane nitrogen. These distances provide a measure of superimposition in different regions of the molecules. Overall the BZT analogues had better overlap in the C–C than C–T orientation, with a near constant RMSD of 0.75. The aromatic rings tend to overlap better than the tropane nitrogen as evident from the distances between the centroids (0.24–0.55 Å) and the nitrogen atom (0.97–1.31 Å). The calculation of the ΔVs corresponded to the activity of the BZT analogues at the histamine H_1 receptor. In general, the 3-substituted BZT analogues with the larger ΔVs values had poorer activity at the histamine H_1 receptor. Thus, the rank order for activity at the histamine H_1 receptor for the compounds is **1 > 3 > 2 > 7 > 6**, which is in line with the ΔVs, compound **1 < 3 < 2 < 7 < 6**.

Table 4. Superimposition of the BZT analogues on 'chair2eq' conformation of cyproheptadine using superimposition B

Compound	cis–cis (C–C)					cis–trans (C–T)				
	RMSD[a]	ΔVs[b]	C1C1[c]	C2C2[d]	NN[e]	RMSD	ΔVs	C1C1	C2C2	NN
1	0.763	83.3	0.317	0.241	0.980	1.292	110.1	0.449	0.295	1.359
2	0.765	86.4	0.316	0.243	0.981	1.291	113.3	0.449	0.294	1.358
3	0.763	85.1	0.317	0.241	0.980	1.292	112.0	0.449	0.295	1.359
6	0.765	104.5	0.316	0.243	0.980	1.291	131.2	0.449	0.294	1.357
7	0.764	94.2	0.316	0.242	0.980	1.291	120.5	0.449	0.294	1.357
8	1.095	122.9	0.552	0.327	1.312	1.389	134.1	0.630	0.527	1.518
14	0.776	200.1	0.321	0.245	0.991	1.290	227.5	0.453	0.294	1.358
15	0.784	195.1	0.325	0.245	0.992	1.291	221.3	0.457	0.292	1.356
16	0.750	123.3	0.312	0.243	0.977	1.302	148.6	0.447	0.298	1.370
19	0.757	173.5	0.309	0.242	0.973	1.278	200.5	0.440	0.291	1.348

[a] Root-mean-square difference for five-point superimposition B.
[b] Difference in volume of superimposition.
[c] Distance between centroids of 'cis' ring.
[d] Distance between centroids of 'trans' ring.
[e] Distance between nitrogen atoms.

Further comparison of the SAR of H_1 receptor and DAT binding for BZT analogues indicates that substitution at only one of the aromatic rings is preferred for H_1 binding, whereas for the DAT, small halogen substitution at both the aromatic rings improves binding affinity. This is evident from the selectivity ratios for disubstituted compounds **2** and **6**, which preferentially bind to DAT, as compared to monosubstituted **3**, **7**, or **9**, which bind with higher affinity to the histamine H_1 receptor than to the DAT. As depicted in Figure 4, right the disubstituted compound **2** (4,4-diF-BZT) is well within the receptor fields of both the DAT and H_1 receptor, whereas the 4-CH_3-BZT analogue compound **9** now protrudes away from the DAT receptor map, but is well within the histamine H_1 receptor, thus showing preference for the histamine H_1 receptor. Nevertheless, disubstitution with large bulky groups on the aromatic rings reduces binding affinity at both the DAT and the histamine H_1 receptor. For example, in compound **4**, the bulky methoxy group protrudes outside the receptor fields of the DAT and the histamine H_1 receptor, hence reducing its activity at both the receptors. In further analysis of the SAR, substitution with small electron-withdrawing groups like CN or CF_3, on only one aromatic ring, provides compounds more active at the histamine H_1 receptors with almost 5–7 times loss in affinity at the DAT. However, disubstitution with halogens (3,4-diCl, 3,4-diF) in one aromatic ring provides more DAT selective compounds (data not shown). The spatial displacement of aromatic rings from the nitrogen is important for both binding sites, as the β-isomer compound **8** was less active than the α-isomer compound **7**. This analysis provided further

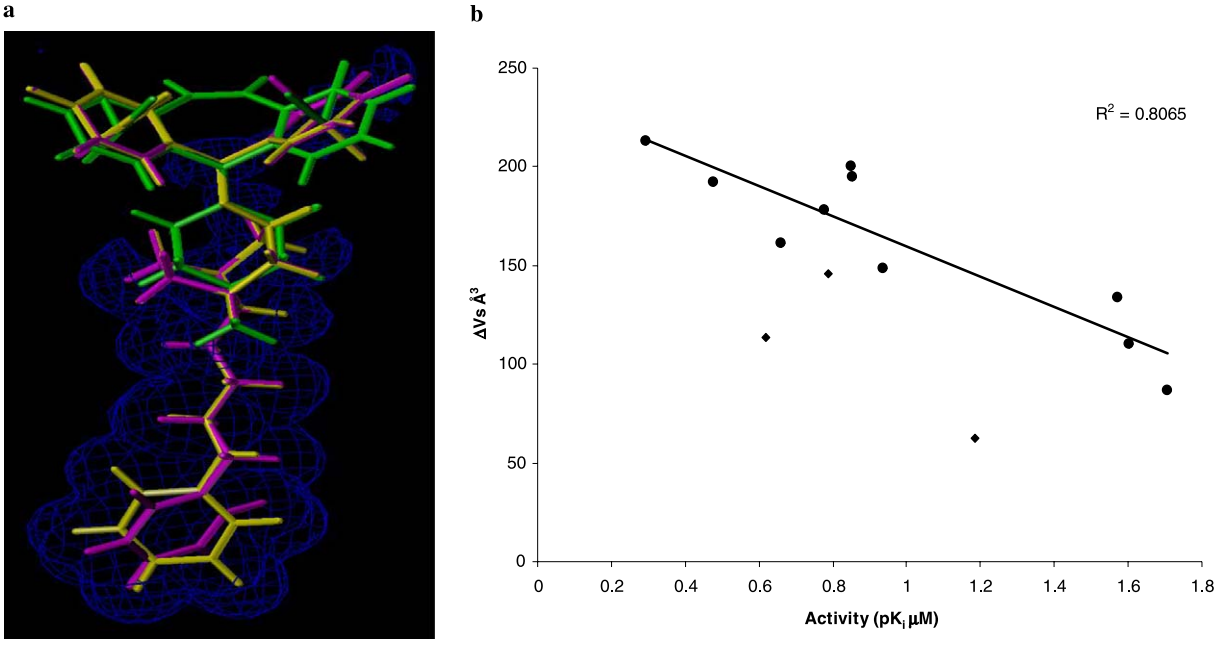


Figure 5. (a) A difference in volume of N-8-substituted BZT analogues **14** (magenta) and **15** (yellow) from the template cyproheptadine (green) is shown as a blue contour. (b) A plot of the ΔVs with the activity of BZT analogues ($N = 10$) at the histamine H_1 receptor, excluded compounds (♦) are shown in the graph.

support for the important role of substituents on the aromatic rings, as small changes in substitution pattern provided large differences in the activity at the DAT and histamine H_1 receptor.

Similarly the tropane N 8-substituted BZT analogues were superimposed on the histamine H_1 pharmacophore. These BZT analogues had similar DAT-optimized substitutions (4,4′-diF) on the aromatic rings, thus variation in the activity at the histamine H_1 receptor is solely dependent on the tropane N-8 substituent. There was no significant difference in the RMSD values among the compounds in this series (data not shown). There seemed to be a good correlation between the activity at the histamine H_1 receptor and the ΔVs (Figs. 5a and b). A correlation coefficient of $R^2 = 0.806$ was obtained after elimination of three compounds that contained a cationic N-8 substitution. The N substitution has been varied in histamine H_1 antagonists wherein introduction of a carboxylic acid group has improved their pharmacokinetic and pharmacodynamic properties leading to well-tolerated clinically used medications such as Cetirizine (Zyrtec®) and Fexofenadine (Allegra®). Site-directed mutagenesis studies have shown that this anionic group also acts as a 'second anchor' point by interacting with the Lys200 in transmembrane V of the histamine H_1 receptor.[29] Recently, in the BZT series, variations in the tropane N-8 substitution with sterically bulky groups and heteroatom linkers have provided potent DAT ligands.[30] Thus, the histamine H_1 antagonist tolerates substituents with anionic groups, whereas for the DAT sterically bulky groups (compounds **14** and **15**) improve activity. These distinct structural requirements for the DAT versus the histamine H_1 receptor at the N-8 position provide another avenue for further design of selective ligands.

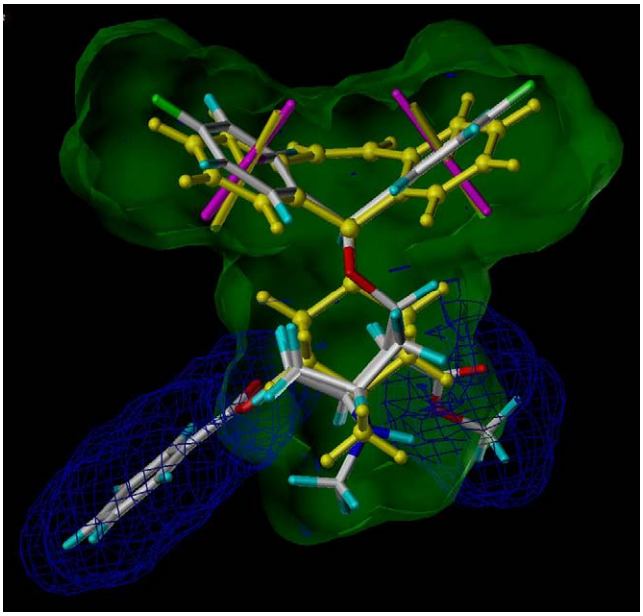


Figure 6. Superimposition of compounds **16** (capped stick; colored by atom type) and **19** (capped stick; colored by atom type) on cyproheptadine (ball and stick; yellow) with receptor essential volume for the histamine H_1 receptor in blue contours at the C-2 and C-6 positions of the tropane ring.

Substitutions at the 2- and 6-positions of the tropane ring have yielded potent ligands at the DAT.[31–33] The 2- and 6-substituted BZT analogues were by far the least active BZT analogues at the histamine H_1 receptor. For example, 2-substitution with an ester group in the (S)-stereochemistry (compounds **16** and **17**) provided potent DAT ligands with poor affinity at the histamine H_1 receptor. Moreover, large bulky substitutions at the 6-position of the tropane ring (compound **19**) are less well tolerated at both the DAT and the histamine H_1 receptors, with small substituents (compound **18**) providing moderately active compounds at the DAT. Mapping of the ΔVs on the receptor-excluded surface of the histamine H_1 receptor provides a measure of the receptor essential regions (Fig. 6), where the 2- and 6-substituents protrude outside the regions of the histamine H_1 receptor pharmacophore. Thus based on this study, one can depict the structural preferences for the DAT and the histamine H_1 receptor as shown in Figure 7. There are distinct binding requirements for the BZT analogues at the DAT as compared to the histamine H_1 receptor, and these differences can be exploited for further design of DAT selective compounds.

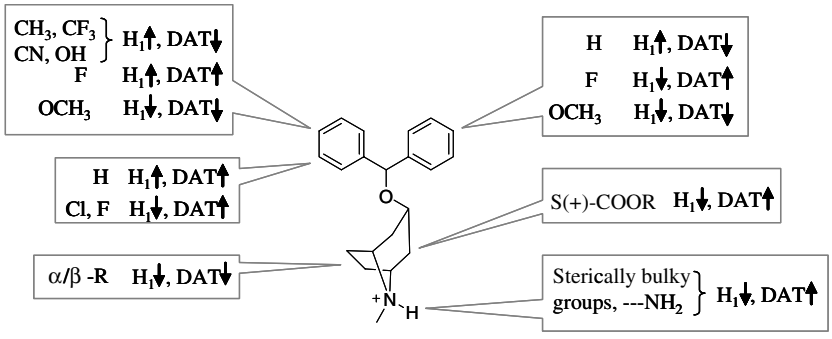


Figure 7. Summary of SAR of the BZT analogues at the DAT and histamine H_1 receptors, with effect of substitution on increase (↑) and decrease (↓) in activities.

3. Conclusions

The BZT analogues are DAT selective ligands, which have been extensively evaluated in animal models as potential cocaine abuse therapeutic agents. Some of these BZT analogues also exhibit varied binding affinities at the histamine H_1 receptor. A molecular modeling study was initiated to analyze the structural features which might explain their activity at the histamine H_1 receptor. A five-point pharmacophore model was derived that can predict the activity and stereoselectivity of the classical histamine H_1 antagonists qualitatively. A pharmacophoric comparison of classical H_1 antagonists with the BZT analogues showed that the substitution pattern on the aromatic rings of the diphenyl methoxy group has unique preferences suggesting different binding environments on the histamine H_1 receptor as compared to the DAT. Larger substitutions in the tropane N-8 position provide DAT selective compounds. Substitution at the 2- and 6-positions of the tropane ring generally resulted in DAT selectivity, however, with a slight reduction in affinity at the DAT for the 6-substituted compounds. These differential receptor maps provide further insight into the SAR of the BZT analogues at the DAT and histamine H_1 receptor that can now be used to design novel DAT selective compounds as molecular tools and potential medications for the treatment of cocaine abuse.

4. Experimental methods

4.1. Biological assays

The binding affinities of the BZT analogues at the DAT and histamine H_1 receptor were obtained from displacement of the radioligand [^{3}H]WIN 35,428 and [^{3}H]mepyramine from whole rat brain preparations, respectively, the details of which are published elsewhere.[18]

4.2. Molecular modeling

The molecular modeling studies were performed using the Sybyl 7.0 (Tripos Inc.)[34] software running on Silicon Graphics Octane workstation. The superimpositions and comparisons were performed using different molecular modeling routines as implemented in Sybyl. The structures of histamine H_1 antagonists and BZT analogues were obtained from the Cambridge Structural Database (CSD).[35] The molecular modeling study presented in this paper involved three steps. (1) Definition of the histamine H_1 pharmacophore. (2) Superimposition of the BZT analogues on the histamine H_1 pharmacophore. (3) A comparative analysis of the pharmacophoric features of BZT analogues with histamine H_1 antagonist.

4.2.1. Definition of the histamine H_1-antagonist pharmacophore. The pharmacophoric features of histamine H_1 antagonists have been extensively investigated. In this work, we generated a stereoselective pharmacophore with superimpositions on the structurally rigid template cyproheptadine. The steps involved in this modeling protocol are described in brief.

4.2.1.1. Step A: Selection of the histamine H_1 antagonist. The classical histamine H_1 antagonists have common structural features containing hydrophobic aromatic or cyclic aliphatic rings and a basic side chain at certain distances. A set of antagonists was chosen based on the known potency of its members at the histamine H_1 receptor. For example, cyproheptadine had a pK_i of 9.27 in a guinea pig cerebellum membrane preparation.[36] Similarly in a mammalian brain preparation diphenhydramine and triplennamine had K_i values of 17 and 35 nM, respectively,[37] and (S) chlorpheniramine was about 85 times more potent than the (R) chlorpheniramine, with K_i values of 8.0 and 700 nM, respectively.[37] In an in vivo guinea pig ileum preparation (S), carbinoxamine had an ED_{50} value of 6.4 nM as compared to 185 nM for the (R) carbinoxamine.[38,39] Further, *trans*-triprolidine had a pA_2 value of 9.94, whereas the *cis*-triprolidine had a pA_2 value of 6.88.[40] This dataset of antagonists contained semi-rigid analogues, like cyproheptadine and triprolidine. We also used stereoisomeric pairs of flexible antagonists to define and validate the pharmacophore models. The structures of these antagonists are depicted in Figure 1. The activities of these compounds were measured under different assay conditions hence no efforts were made to quantitatively compare the activities with those of the models generated. The activities were used as a qualitative guide for evaluating the pharmacophore models.

4.2.1.2. Step B: Conformational analysis of the histamine H_1 antagonist. The conformational search of the histamine H_1 antagonists was performed using the systematic search routine with default parameters as implemented in Sybyl 7.0. The starting geometries of the antagonists were derived from the crystal structures obtained from the CSD. The structurally rigid antagonist cyproheptadine has been the subject of extensive conformational studies which have suggested the so-called 'chair2eq' as a bioactive conformation.[22–24] We derived this geometry from the crystal structure (code: CYPHEP) by readjusting the conformation. The more active S isomer of chlorpheniramine (code: CPHMAL10) and the less active R isomer (code: JEGWUN) were constructed from the crystallographic information. Similarly the active S isomer of carbinoxamine (code: CARMAL10) and *trans*-triprolidine (code: TPROLC) were used to obtain the geometry of the less active isomers. The starting geometries of diphenhydramine (code: JEMJOA) and triplennamine (code: FIMNIY) were obtained after extensive evaluation of other geometries which were also observed in the crystal structure. The compounds were assigned Tripos forcefield potentials with all side-chain nitrogens modeled as protonated species. The rotatable bonds were searched systematically by rotating from 0° to 359° in a 15° increment. All the conformations within a 10 kcal/mol cutoff from the lowest energy conformation were extensively evaluated. The potential energy and the propensity to form an intramolecular H-bond between the proton on the side-chain nitrogen and the oxygen or pyridine nitrogen were used

to evaluate the conformational ensemble. The conformations were selected and energy minimized using the conjugate gradient method until a gradient of 0.001 kcal/mol/Å was achieved. The partial atomic charges were assigned by the Gasteiger–Marsilli method with a default dielectric of 1.

4.2.1.3. Step C: Superimposition of the histamine H_1 antagonists. In this study, RMS fitting of the superimposition points on the template was performed. The superimposition points were defined with the centroid of the aromatic rings and side-chain nitrogen. We also defined pseudoreceptor interaction points on the aromatic rings as two dummy atoms 1.8 Å above and below the plane from the centroid. In another variation, an interaction point at the cationic nitrogen 2.5 Å away in the direction of the proton was defined (see Fig. 2). Various superimpositions were performed as defined in Figure 2 to derive the pharmacophore. The aromatic rings can be superimposed in two different orientations. In the C–C orientation, the so-called 'cis' ring of the compounds was superimposed on the 'cis' ring of the template. Similarly in the C–T orientation, the 'cis' ring of the compounds was superimposed on the 'trans' ring of the template.

4.2.1.4. Step D: Pharmacophore of histamine H_1 antagonist. The superimpositions were evaluated by the quality of the fit as predicted from the RMSD and differences in the van der Waals volume of each compound from the template, defined as ΔVs. Also the correct orientations of the pharmacophore were evaluated based on the qualitative activity data of the stereoisomeric pairs of the histamine H_1 antagonist. The best superimposition was then used to define the pharmacophore.

4.2.2. Superimposition of the BZT analogues on the histamine H_1-antagonist pharmacophore. The low energy conformations of the BZT analogues were generated as described earlier.[25] The histamine H_1 pharmacophore was then used for superimposition of the BZT analogues. The quality of the superimpositions was evaluated based on the RMSD values and the ΔVs as defined earlier.

4.2.3. Comparative analysis of pharmacophoric features of the BZT analogues with the histamine H_1 antagonists. The distinctive structural features of the BZT analogues were analyzed by computing the distances between the pharmacophoric features as well as by receptor mapping. The effect of substitution at different positions of the tropane ring was evaluated in order to identify regions of specificity. The molecular volume of the compounds is computed using the MVOLUME routine. A union of molecular volume of the active compounds defines the receptor-excluded volume and extra volume that is filled by the receptor, and is unavailable for ligand binding is called as receptor essential volume. The distinctive features were then utilized in conjunction with biological activity of the compounds to analyze the structure–activity relationship at the DAT and the histamine H_1 receptor.

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