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Conformational preferences of the potent dopamine reuptake blocker BTCP and its analogs and their incorporation into a pharmacophore model

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Summary

Molecular mechanics calculations using MM3-92 and ab initio quantum mechanical calculations using SPARTAN 5.0 were performed on the structurally similar PCP and BTCP, in which only the latter has a cocaine-like pharmacological profile as a dopamine reuptake blocker. Calculations were also performed on BTCP analogs with a methyl group in various positions of the cyclohexane ring. The results for the cis-2-methyl compound, which retains good pharmacological activity, allowed us to determine that an aryl-axial conformer is the biologically active form for at least some of the compounds in this series. However, an aryl-equatorial conformer presents the identical pharmacophore, as shown by superposition of the two conformers. X-ray crystallographic structures were also obtained for BTCP and related compounds with a 2-methyl group on the cyclohexane ring, with reasonable agreement between the computational and experimental results. Superposition studies were performed with two rigid analogs of cocaine which illustrate the optimal orientations of the ammonium hydrogen for monoamine transporters. There is excellent agreement between a 'back-bridged' cocaine analog that is optimal as a dopamine reuptake blocker and the previously proposed biologically active conformer of methylphenidate. However, BTCP is found to be a better fit to the 'front-bridged' cocaine analog that is optimal for a serotonin reuptake blocker.

Introduction

BTCP (1-[1-(2-benzo[b]thienyl)cyclohexyl]piperidine) and PCP (phencyclidine) are close structural analogs (Scheme 1). PCP, in which the aryl group is phenyl, is a well-known psychotomimetic drug of abuse and the prototype for compounds with 'PCP-like' activity whose primary mode of action is through N-methyl-D-aspartate (NMDA) receptors [1]. The compound in which the phenyl ring is replaced by thienyl is known as TCP and has a pharmacological profile similar to PCP [2,3]. However, with a benzothienyl group, as in BTCP, there is a great weakening of PCP-like activity and the compound instead is a potent blocker of dopamine and norepinephrine neuronal uptake [4,5]. That is, the compound binds to the dopamine and norepinephrine transporters that remove those neurotransmitters from the neuronal synapse. Similarly, the substitution of a 2-naphthalene for the aryl group produces a potent reuptake blocker [6]. This pharmacological property, on the other hand, is relatively weak in PCP and TCP [7]. We have undertaken this study of BTCP and PCP to examine the possible structural bases for these differences in pharmacological activity.

From a conformational point of view, there are questions regarding the three dimensional structure responsible for the biological activity of BTCP and PCP. The aryl ring can be either axial or equatorial on the cyclohexane ring, in which case the piperi-

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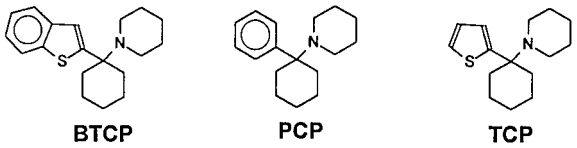


Scheme 1. Structures of BTCP, PCP and TCP.

dine ring would be equatorial or axial, respectively. Also, both the aryl and piperidine rings can rotate about the single bond by which they are attached to the cyclohexane ring. These possibilities have been examined by MM3-92 molecular mechanics and ab initio quantum mechanical calculations.

Numerous analogs of BTCP have been synthesized and assayed for pharmacological activity [6]. Among the analogs that are the most interesting from a structural point of view are those with various groups on the cyclohexane ring. Of the two isomers with a 2-methyl group, only the cis isomer has significant binding affinity for reuptake sites and ability to inhibit the reuptake of dopamine and norepinephrine. In contrast, both isomers with a 3-methyl group have greatly reduced affinities. In further contrast, both isomers with a 4-methyl group have affinities and activities similar to BTCP, though the trans-4-methyl compound appears to be somewhat less potent. Both isomers with a sterically bulky 4-tert-butyl group appear to be essentially devoid of activity as reuptake blockers. The conformational preferences of a number of these compounds have also been examined to see if there are any significant conformational tendencies associated with either activity or its lack.

A model has been developed for dopamine reuptake blockers of diverse structural classes [8]. Starting from cocaine and the related tropane CFT, the model encompasses other classes of compounds including 3-phenyl-1-indamines, 1-amino-4-phenyltetralins, and hexahydropyrrolo[2,1-a]isoquinolines. More recently, the model has been extended to include methylphenidate [9] and correctly predicted the decrease in potency of N-methyl derivatives of its analogs [10,11]. The model has now also been extended to bupropion in which both enantiomers were found to fit the model equally well [12] and this is consistent with the finding that the enantiomers have similar potencies as dopamine reuptake blockers [13]. We now attempt to incorporate BTCP and its analogs into this pharmacophore model.

The pharmacophore model [8] suggests that the orientation of the ammonium hydrogen is an important feature for a dopamine reuptake blocker and this has recently been found to be the case in cocaine analogs in which the ammonium hydrogen has a defined orientation due to additional rigidification [14,15]. It should be noted, however, that recent results have muddied the structural requirements for dopamine reuptake blockers. While reducing the basicity of the ammonium nitrogen by converting it into a simple amide greatly reduces the activity of cocaine [16], it has been found that sulfonamide analogs are still capable of potent activity, despite their reduced basicity and suggesting that an ammonium nitrogen may not always be needed [17]. Indeed, it now appears that cocaine analogs in which the nitrogen is replaced by oxygen or even carbon can also be quite potent as reuptake blockers [18–21]. These apparently discrepant results suggest that a wider variety of structures are capable of being potent dopamine reuptake blockers. While it appears that cocaine itself requires a basic nitrogen and that particular orientations of the ammonium hydrogens are optimal for different transporters in the rigid cocaine analogs [14,15], it is still possible to obtain potent compounds in the absence of these features if the structure is otherwise optimal.

Methods

Molecular mechanics studies

Energy minimizations of possible conformations of PCP, BTCP, and analogs of BTCP were performed with the MM3-92 program and parameter set [22] and all internal degrees of freedom were allowed to vary. Calculations were for the protonated form of the molecule. For the BTCP analogs with an asymmetric methyl group on the cyclohexane ring, the methyl group was always placed on the same edge to facilitate comparisons. There were missing torsional parameters for the atomic sequences C_{sp3}-C_{sp3}-C_{sp2}-S, N^+-C_{sp3}-C_{sp2}-S, and C_{sp2}-C_{sp3}-N^+-H which were approximated by the substitution of a similar atom into the atomic sequence. Specifically, C_{sp3}-C_{sp3}-C_{sp2}-C_{sp2} was available for the first missing parameter, N_{sp3}-C_{sp3}-C_{sp2}-C_{sp2} was available for the second, and C_{sp2}-C_{sp3}-N_{sp3}-H for the third. The latter two torsional parameters contained only zeros. The reported calculations are with a dielectric constant of 80 to damp out intramolecular electrostatic interactions. However, calculations performed with the default dielectric constant of 1.5 did not produce significantly different conformational results. Initial Cartesian coordinates

for the calculations were generated by the PCMODEL program [23] or the DRIVER option of the MM3-92 program. Energy minimization consisted of the diagonal Newton–Raphson optimization method. The conformation of each structure was checked after energy minimization to confirm that it had not changed into another conformer and all expected conformations were found to be stable. In addition to the aryl group or the piperidine ring being either axial or equatorial on the cyclohexane ring, both of these groups can also rotate. For PCP, a 180° rotation of the phenyl ring results in an equivalent conformation but this is not true for rotation of the asymmetric benzothienyl group. Significant rotation can occur about the piperidine–cyclohexane and the benzothienyl–cyclohexane bonds and these are reported with respect to both edges of the cyclohexane ring.

Ab initio calculations

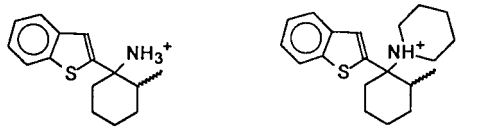
Ab initio quantum mechanical calculations were performed with the SPARTAN package, Version 5.0 [24]. Conformers that were previously energy minimized with MM3-92 were imported into SPARTAN and subjected to additional full energy minimization with the ab initio methods. Starting structures for the 6-31G* energy minimizations began with the 3-21G* energy minimized structures. On a Silicon Graphics O2 with an R10000 195 MHz processor, energy minimizations at the 3-21G* level took between 210–380 CPU minutes for PCP and 450–800 CPU minutes for BTCP and its analogs, though some of the latter took as long as 2600 CPU minutes. At the 6-31G* level, energy minimizations took between 4200–8500 CPU minutes.

Superposition studies

Least squares superpositions of BTCP and methylphenidate with two rigid cocaine analogs were performed with the PCMODEL program [23]. The atoms that were superimposed were the six carbon atoms of the phenyl ring (or the six-carbon portion of the benzothienyl ring) and the ammonium nitrogen. The coordinates for the two rigid cocaine analogs that were used as templates were obtained from the crystal structures [15] and subjected to energy minimization by the MM3-92 program to eliminate crystal packing effects.

X-ray crystallography

Crystal structures were obtained for BTCP and related compounds **1**–**4** (Scheme 2) with a 2-methyl group. BTCP was available in the Cocaine Treatment



1 = trans-2-methyl
2 = cis-2-methyl
3 = trans-2-methyl
4 = cis-2-methyl

Scheme 2. Structures of compounds related to BTCP.

Discovery Program of the National Institute on Drug Abuse and a suitable crystal was found in the received sample. Compounds **1**–**4** were synthesized by a procedure similar to one in the literature [6]. Crystals of **1** were obtained by solvent evaporation from a mixture of acetone, dichloromethane, and water. Crystals of **2** were obtained by solvent evaporation from a mixture of methanol and nitromethane. Crystals of **3** were grown by vapor diffusion of water into a saturated acetone solution. Crystals of **4** were grown from solvent evaporation from a mixture of nitromethane and ethanol.

Data for BTCP, **1**, **3**, and **4** were collected on a computer-controlled diffractometer with an incident beam graphite monochromator (Bruker P4 with Cu Kα radiation, $\lambda = 1.54178$ Å, T = 295 K). A least-squares refinement using 25 to 50 centered reflections was used to determine the cells. Reflections were measured in the θ–2θ mode to 2θ max = 115°. Corrections were applied for Lorentz and polarization effects. Face indexed numerical absorption corrections were applied, and maximum and minimum transmission were 0.88 and 0.58, 0.90 and 0.55, 0.91 and 0.63, and 0.87 and 0.50, respectively, for BTCP, **1**, **3** and **4**. All of the structures were solved by direct methods and refined on F^2 with the full matrix least-squares SHELTXL97 program [25]. The least-squares parameters refined include the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms bonded to nitrogen were refined while hydrogen atoms bonded to carbon utilized a riding model in which coordinate shifts of carbons were applied to the attached hydrogens with bond lengths of 0.96 Å, idealized bond angles, and U_{iso} (H) set to 1.2 U_{eq} (C) or 1.5 U_{eq} (C) for methyl groups.

The data for **2** were collected using a Bruker PLATFORM diffractometer with a Rigaku rotating anode source and Göbel mirrors (Cu Kα radiation, $\lambda = 1.54178$ Å) and SMART 1K CCD area detector. The data collection nominally covered a hemisphere in diffraction space by a combination of seven sets of exposures at different φ and 2θ angles. Each 30 s ex-

posure covered 0.3° in ω. Coverage of the unique set was over 93% to θ of 57°. This data for the small and weakly diffracting crystal yielded a structure with four ion anion pairs in the asymmetric unit which could only be refined to an R value of 0.13. However, there are indications that the crystal is twinned. Resolution of the twin law or elimination of twinning will require better data.

Additional data collection and refinement parameters and tables of atomic coordinates, bond distances, bond angles, and anisotropic thermal parameters have been deposited with the Cambridge Crystallographic Data Centre.

Results and discussion

Conformational energy results

The results of the MM3-92 calculations on PCP, BTCP and the BTCP analogs with methyl groups in various positions of the cyclohexane ring are shown in Table 1. The conformational energies of the same conformers as calculated by the ab initio methods are shown in Table 2. Most of the ab initio results are with the 3-21G* basis set, though some calculations were also performed with the 6-31G* basis set to examine basis set effects. The key dihedral angles of the various energy minimized conformers of BTCP are listed in Table 3. Conformers in which the aryl ring is flipped over are designated with primes. In PCP, conformers **1b** and **1c** and **2a** and **2b** are mirror images and have the same energies. In both PCP and BTCP, conformers in which the aryl ring is flipped over are mirror images. For example, **1b** and **1c**′ and **1c** and **1b**′ of BTCP are pairs of mirror images. This also occurs for the BTCP analogs with a symmetrical 4-methyl group. However, in the BTCP analogs with 2- and 3-methyl groups, molecular symmetry is disrupted and the corresponding conformers are no longer mirror images.

For BTCP, the global minima found by MM3-92 are conformers **1a** and **1a**′ in which the aryl group is axial on the cyclohexane ring. In this conformer (Figure 1), the aryl group is perpendicular to the approximate plane formed by the cyclohexane and piperidine rings. The preferred position of the piperidine ring is to place the ammonium hydrogen gauche to both edges of the cyclohexane ring, though rotating the piperidine ring results in only small increases in steric energy. The ab initio results agree with the MM3-92 results with regard to the relative favorability of the aryl axial conformers, but indicates a 0.8 kcal/mol preference for the conformer in which the piperidine ring is rotated 120°. For the aryl equatorial conformers, both the MM3-92 and ab initio results indicate a strong preference for the conformers in which the aryl group is parallel to the approximate plane of the cyclohexane ring and is perpendicular to the approximate plane of the piperidine ring (not shown).

Very similar results were found for PCP and phenyl-axial conformer **1a** is the global minimum by both the MM3-92 and ab initio calculations. However, the MM3-92 results indicate that this conformer is preferred by 2.7 kcal/mol over the best phenyl-equatorial conformer **2c** whereas the ab initio results indicate that this difference is only 1.0–1.1 kcal/mol. Both of these results differ somewhat from previous studies using molecular modeling which found the phenyl-equatorial conformer to be preferred. Using the MM2 program with a rigid phenyl ring, the phenyl-equatorial conformer was preferred by 0.2 kcal/mol [26] whereas using SYBYL, this difference was 0.3 kcal/mol [27]. It should be noted, based on extensive experimental results, that this conformational equilibrium is sensitive to the molecular environment and both conformers have been observed experimentally [27–30]. A phenyl-axial conformer has been proposed as the biologically active conformer for PCP based on an NMR conformational study [31].

In general, there is reasonable agreement between the MM3-92 and ab initio results in that both methods give the same low energy conformers. However, there appear to be some small but systematic differences between the conformational results of the two methods. The ab initio calculations appear to consistently provide somewhat less stabilization of aryl-axial relative to aryl-equatorial conformers as compared with the MM3-92 results. For BTCP, for example, the best aryl-equatorial conformer is 1.5 kcal/mol above the best aryl-axial conformer by the ab initio calculations, whereas this difference is 2.4 kcal/mol with MM3-92. For the trans-2-methyl analog, the MM3-92 calculations indicate that the best aryl-axial conformer is favored by 0.1 kcal/mol over the best aryl-equatorial conformer, while the ab initio results show a preference of 0.7 kcal/mol for the best aryl-equatorial conformer. Another small but systematic difference between the MM3-92 and ab initio results concerns the orientation of the piperidine ring in aryl-axial conformations. The MM3-92 results indicate that conformer **1a** of BTCP is the preferred orientation by 0.9 kcal/mol whereas the ab initio results suggest that

Table 1. Relative conformational energies[a] (kcal/mol) calculated by MM3-92 for PCP, BTCP and BTCP analogs

Conformer	PCP	BTCP	(2*S*)-cis-2-methyl	(2*R*)-trans-2-methyl	(3*S*)-cis-3-methyl	(3*R*)-trans-3-methyl	Cis-4-methyl	Trans-4-methyl
Aryl-axial								
1a	0.0	0.0	0.0	5.8	0.0	1.3	0.0	0.0
1b	1.0	0.9	5.0	0.5	0.9	2.1	1.0	1.0
1c	1.0	1.4	4.0	2.6	1.5	4.6	1.4	1.5
1a′		0.0	0.0	5.2	0.0	0.6	0.0	0.0
1b′		1.4	5.4	0.0	1.3	2.1	1.4	1.5
1c′		0.9	3.7	2.6	1.0	2.9	1.0	1.0
Aryl-equatorial								
2a	8.7	8.8	8.9	7.6	17.0	6.6	7.0	10.7
2b	8.7	8.7	13.0	9.2	16.2	6.2	6.9	10.6
2c	2.7	2.4	8.3	1.0	7.4	0.0	0.6	4.4
2a′		8.7	8.7	6.9	16.9	6.5	6.9	10.6
2b′		8.8	10.5	8.1	16.1	6.3	7.0	10.7
2c′		2.4	6.6	0.1	7.3	0.0	0.6	4.4

[a]Steric energies (kcal/mol) of global minima are: PCP, 31.1; BTCP, 38.0; cis-2-methyl, 44.2; trans-2-methyl, 45.2; cis-3-methyl, 38.8; trans-3-methyl, 41.4; cis-4-methyl, 40.7; trans-4-methyl, 38.8.

1b is preferred by 0.8 kcal/mol. This is also true for the analogs with a 4-methyl group which is too far away from the piperidine ring to directly affect its preferred orientation. However, significantly, both methods agree that conformer **1a** is greatly preferred in the cis-2-methyl analog. There is little difference in the ab initio results using the 3-21G* and 6-31G* basis sets.

Crystal structures

The crystal structures of BTCP and compounds **1**–**4** (Scheme 2) are shown in Figure 2. Experimental and refinement parameters are summarized in Table 4 and key dihedral angles of these structures are shown in Table 5. The structures of BTCP and compounds **1**, **3**, and **4** are disordered in a similar manner. The benzo[b]thiophene group occupies two overlapping positions that differ by a rotation of 180° about the bond to the cyclohexane ring and these have occupancy ratios of 86:14, 57:43, 70:30 and 55:45, respectively, for BTCP, **1**, **3**, and **4**. In **4**, there is an additional disorder involving the cis-2-methyl substituent. This disorder has the same independently determined 55:45 occupancy ratio as the benzo[b]thiophene, with the higher occupancy methyl position trans to the higher occupancy sulfur position. The degree of correlation of these two independently refined disorders cannot be determined by the diffraction analysis.

There are a few surprises in the crystal structures given the conformational results obtained by the MM3-92 and ab initio methods. For BTCP, the crystal structure is aryl-equatorial conformer **2c**, which is 2.4 and 1.5 kcal/mol above the global minimum by MM3-92 and ab initio, respectively. In our experience, crystal structures are usually closer to the global minimum than this. The structure of the cis-2-methyl analog **4** is in the expected aryl axial conformer **1a′**. However, the corresponding primary amine **2** is unexpectedly in an aryl-equatorial conformer despite the high unfavorabilities of these conformers. However, it should be noted that there are close 2.1 and 2.2 Å contacts between some of the ammonium hydrogens and some of the hydrogens of the cyclohexane ring and the 2-methyl group. In the corresponding compound with a piperidine ring, these close contacts would preclude the favorability of aryl-equatorial conformers. For the trans-2-methyl compounds **1** and **3**, these are also in aryl-equatorial conformers which, in these cases, are favorable structures.

Biologically active conformers

There is little difference in the conformational preferences of PCP and BTCP, so that one must look elsewhere for an explanation for the divergence in their pharmacological activities. An obvious explanation is that dopamine reuptake blockers require an aromatic ring that protrudes further than in PCP-like

Table 2. Relative conformational energies[a] (kcal/mol) as calculated by ab initio quantum mechanical calculations for PCP, BTCP and BTCP analogs with 3-21G* and 6-31G* basis sets

Conformer	PCP		BTCP		(2*S*)-cis-2-methyl 3-21G*	(2*R*)-trans-2-methyl 3-21G*	(3*S*)-cis-3-methyl 3-21G*	(3*R*)-trans-3-methyl 3-21G*	Cis-4-methyl 3-21G*	Trans-4-methyl 3-21G*
	3-21G*	6-31G*	3-21G*	6-31G*						
Aryl-axial										
1a	0.0	0.0	0.8	0.6	0.0	7.5	0.9	3.6	1.2	0.8
1b	0.1	0.3	0.0	0.0	4.3	0.7	0.0	3.1	0.4	0.0
1c	0.1		2.0	2.3	3.9	5.2	2.1	6.7	2.4	2.0
1a′			0.8		0.1	8.7	0.9	3.9	1.2	0.8
1b′			2.0		5.4	2.5	2.0	4.9	2.4	2.0
1c′			0.0		2.8	3.7	0.1	4.1	0.4	0.0
Aryl-equatorial										
2a	5.7		5.9		7.7	5.1	13.4	4.5	4.4	7.7
2b	5.7	5.1	7.9		9.3	8.4	14.0	6.3	6.4	9.7
2c	1.1	1.1	1.5		7.8	0.0	6.2	0.0	0.0	3.2
2a′			7.9	7.1	10.3	7.6	15.5	6.4	6.4	9.7
2b′			5.9	4.9	9.3	8.0	12.2	4.3	4.4	7.7
2c′			1.5	1.5	5.1	1.1	5.6	0.0	0.0	3.2

[a]Electronic energies (hartrees) of global minima are: PCP, −709.2330346 (3-21G*) and −713.1650722 (6-31G*); BTCP, −1180.2173568 (3-21G*) and −1186.4167294 (6-31G*); cis-2-methyl, −1219.0312413; trans-2-methyl, −1219.0306471; cis-3-methyl, −1219.0391073; trans-3-methyl, −1219.0367122; cis-4-methyl, −1219.0367532; trans-4-methyl, −1219.0390011.

Table 3. Dihedral angles (in degrees) that describe the conformations of various conformers of BTCP as found by the MM3-92 and 3-21G* calculations. Dihedral angles are with respect to the two edges of the cyclohexane ring

Conformer	MM3-92		3-21G*	
	H-N-$C_{cyclohex}$-$C_{cyclohex}$	S-C-$C_{cyclohex}$-$C_{cyclohex}$	H-N-$C_{cyclohex}$-$C_{cyclohex}$	S-C-$C_{cyclohex}$-$C_{cyclohex}$
Aryl-axial				
1a	55, −58	−147, −28	46, −70	−161, −37
1b	177, 61	−161, −43	−175, 66	−170, −46
1c	−61, −177	−134, −16	−65, 176	−142, −18
1a′	58, −55	28, 147	69, −46	37, 160
1b′	177, 61	16, 134	−176, 65	17, 142
1c′	−61, −177	43, 161	−66, 175	46, 170
Aryl-equatorial				
2a	78, −161	−42, −158	79, −156	−50, −171
2b	160, −78	−13, −129	155, −81	−17, −139
2c	−57, 59	−24, −139	−73, 44	−44, −165
2a′	78, −160	129, 13	81, −155	139, 17
2b′	161, −78	158, 42	156, −79	171, 50
2c′	−59, 56	139, 24	−44, 73	165, 44

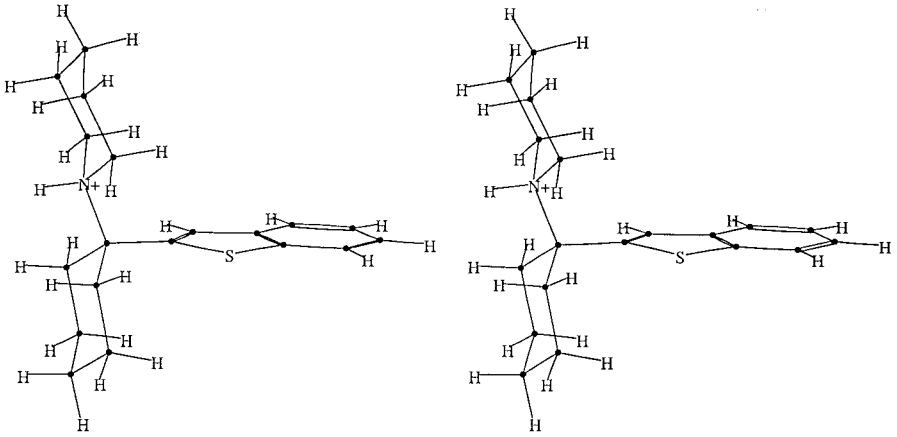


Figure 1. Stereo image of the energy minimized conformer **1a**, which is the global minimum (along with **1a**′) by the MM3-92 program.

Table 4. X-ray experimental and refinement parameters for BTCP and compounds **1**, **3**, and **4** (Scheme 2)[a]

	BTCP	Compound **1**	Compound **3**	Compound **4**
Empirical formula	$C_{19}H_{25}NS$	$C_{15}H_{20}N^{+}S\ Cl^{-}$	$C_{20}H_{27}NS$	$C_{20}H_{27}NS$
Crystal system	orthorhombic	monoclinic	monoclinic	monoclinic
Space group	$Pca2_1$	$P2_1/c$	$P2_1/c$	$P2_1/n$
a, Å	11.671(1)	16.480(2)	18.089(3)	6.583(1)
b, Å	15.504(1)	6.269(1)	6.494(1)	14.595(1)
c, Å	9.150(1)	14.486(2)	14.994(3)	18.156(2)
α, degrees	90	90	90	90
β, degrees	90	108.11(1)	98.30(2)	96.40(1)
γ, degrees	90	90	90	90
Volume, $Å^3$	1655.6(2)	1422.6(4)	1743.0(6)	1733.6(3)
Z	4	4	4	4
Formula weight	299.5	281.8	313.5	313.5
ρ (calculated), g cm^{-3}	1.20	1.32	1.20	1.20
Crystal dimension, mm	0.46 × 0.38 × 0.08	0.60 × 0.18 × 0.03	0.72 × 0.22 × 0.06	0.86 × 0.18 × 0.08
μ absorp. coeff., mm^{-1}	1.66	3.58	1.60	1.61
h, k, l collected	$-12\leq1$, $-16\leq1$, $-10\leq2$	$-18\leq0$, $0\leq6$, $-15\leq15$	$-19\leq19$, $-7\leq0$, $-16\leq4$	$-1\leq7$, $-1\leq15$, $-19\leq19$
Data collected	1721	2097	2789	3412
R_{int}, merge equivalents	0.017	0.014	0.027	0.034
Unique data	1382	1945	2380	2323
Unique data, $I_o>2\sigma I_o$	1224	1618	1777	1946
Refined parameters	205	186	219	235
R1[b], wR2[c] (obs. data)	0.034, 0.084	0.069, 0.177	0.050, 0.117	0.051, 0.127
R1[b], wR2[c], S[d](all data)	0.041, 0.089, 1.08	0.082, 0.186, 1.19	0.072, 0.129, 1.04	0.061, 0.133, 1.07
Fourier differences, $eÅ^{-3}$	0.12, −0.11	0.72, −0.34	0.25, −0.14	0.17, −0.18

[a]Compound **2** has not been included in the table because of uncertainty about the quality of the results (see text).
[b]$R1 = \Sigma(||F_o| - |F_c||)/\Sigma|F_o|$.
[c]$wR2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$.
[d]$S = [\Sigma w(F_o^2 - F_c^2)^2/(n-p)]^{1/2}$.

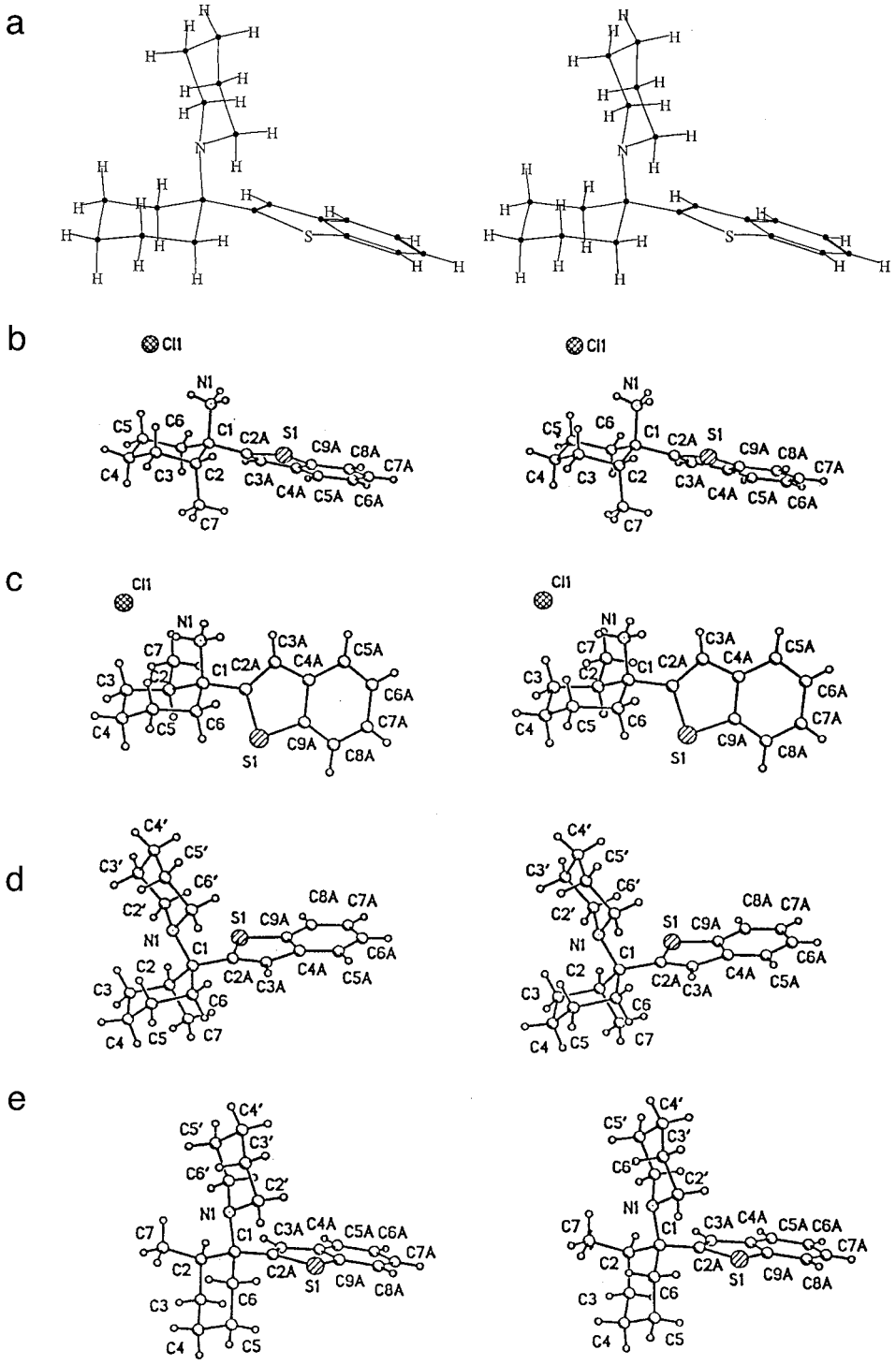


Figure 2. Crystal structures of (a) BTCP, (b) the HCl salt of the trans-2-methyl, primary amine analog of BTCP, (c) the HCl salt of the cis-2-methyl, primary amine analog of BTCP, (d) the trans-2-methyl analog of BTCP, and (e) the cis-2-methyl analog of BTCP.

Table 5. Conformations found by X-ray crystallography for BTCP and compounds **1**–**4** (Scheme 2). For BTCP, **3** and **4**, which crystallized as free bases, the dihedral angles (in degrees) are with respect to a hypothetical lone pair on the nitrogen. The conformers with the aryl ring flipped over are also present in the crystal

	Conformer	H-N-$C_{cyclohex}$-$C_{cyclohex}$	S-C-$C_{cyclohex}$-$C_{cyclohex}$
BTCP	**2c**	−55, 60	−27, −146
Compound **2**	Aryl-equatorial		−56, 68
Compound **4**	**1a**′	60, −57	37, 156
Compound **1**	Aryl-equatorial		−48, −173
Compound **3**	**2c**	−60, 55	−48, −165

compounds. As shown below, the phenyl part of the benzothienyl ring matches quite closely the aromatic ring of other dopamine reuptake blockers.

As indicated above, the aryl ring can be either axial or equatorial and both the aryl and piperidine rings can rotate about single bonds. BTCP itself is of little usefulness in deciding the conformational issue of the biologically active form, since a variety of conformers have low energies when the aryl ring is axial and a pair of conformers have plausible energies (2.4 kcal/mol by MM3-92 and 1.5 kcal/mol by ab initio) when the aryl ring is equatorial. However, the cis-2-methyl analog, which has about the same activity as BTCP, appears to provide conclusive proof regarding the biologically active conformer. First, aryl-equatorial conformers have considerably higher energies than is consistent with its potent activity with the best aryl-equatorial conformer being 6.6 kcal/mol above the global minimum by MM3-92 and 5.1 kcal/mol by ab initio. Secondly, the cis-2-methyl group is sufficiently close to the piperidine ring to sterically impact possible conformers of the piperidine ring and only a single pair of conformers, **1a** and **1a**′, have energies consistent with potent activity. The hypothesis that **1a** and/or **1a**′ are the biologically active forms is also consistent with the relative lack of activity of the trans-2-methyl analog in which these conformers are unfavorable by 5.2–5.8 kcal/mol by MM3-92 and 5.2–7.5 kcal/mol by ab initio. However, this result for the trans-2-methyl analog is only consistent with the hypothesis and not conclusive, since steric effects of the group at the dopamine transporter may be the cause of lack of activity. This appears to be the case for the cis- and trans-3-methyl analogs which have greatly reduced activity. Given that the cis-3-methyl group does not have an appreciable conformational effect on the relative favorabilities of the various conformers of BTCP, this suggests that the 3-methyl group is having a direct steric effect that interferes with binding to the dopamine transporter. Similarly, the trans-3-methyl analog has similar conformational preferences to the trans-4-methyl analog, which also suggests that this group is making a direct steric interaction which prevents binding to the transporter. Finally, this hypothesis is also consistent with the potent activity of the cis- and trans-4-methyl analogs, since **1a** and **1a**′ are the global minima for the former by MM3-92 and only 1.2 and 0.8 kcal/mol above the global minima by ab initio.

While **1a** and/or **1a**′ are clearly the biologically active forms for the pharmacologically active cis-2-methyl and trans-4-methyl analogs, it should be noted that it is still possible for an aryl-equatorial conformer to be responsible for the pharmacological activity of the cis-4-methyl analog, since the aryl-equatorial conformers **2c** and **2c**′ present essentially identical pharmacophores as the aryl-axial conformers **1a** and **1a**′ (Figure 3). As can be seen, the aryl and piperidine rings can be superimposed in an exact manner and the only difference is in the steric placement of their cyclohexane rings, which may or may not be important. The somewhat higher potency of the cis-4-methyl analog over the trans-4-methyl analog and its equipotency with BTCP [6] suggests that it is not necessary for the former to change its preferred conformation in order to interact with dopamine transporters. A similar superposition of aryl-axial and aryl-equatorial conformers has been performed for PCP [27] and can also be inferred from the activity of a PCP analog with an adamantyl group in place of the cyclohexane ring [26].

Dopamine reuptake pharmacophore

As indicated in the Introduction, a dopamine reuptake pharmacophore has been proposed which encompasses a number of compounds with this

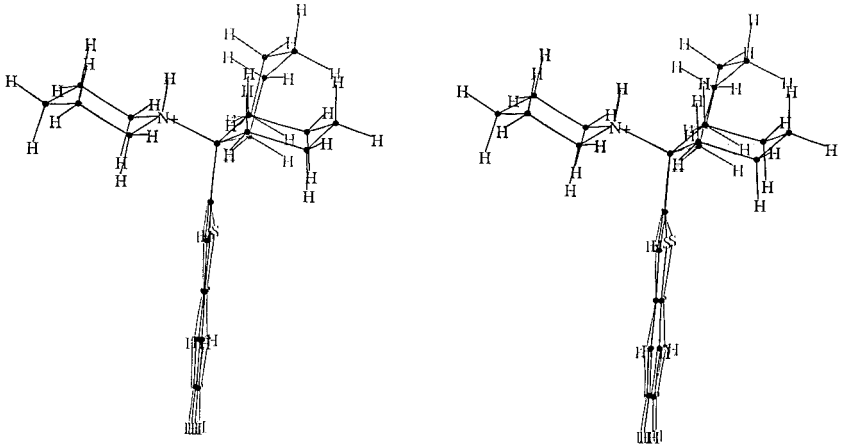
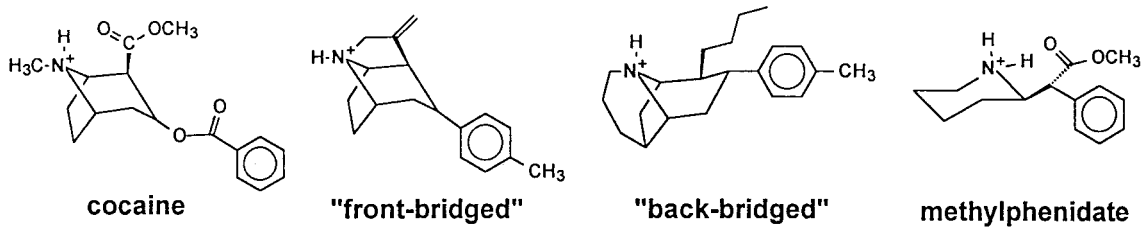


Figure 3. Stereo image of the superposition of the preferred aryl-axial (dark line) and aryl-equatorial (light line) conformers of BTCP, showing that they present identical pharmacophores except for the steric bulk of the cyclohexane ring.



Scheme 3. Additional dopamine reuptake blockers.

pharmacological property including cocaine and methylphenidate (Scheme 3). A key feature of the model is the direction of the ammonium hydrogen (or lone pair for the free base). The importance of this feature was recently shown by the synthesis and testing of cocaine analogs in which additional rigidification produced defined directions of the ammonium hydrogen (or amine lone pair) (Scheme 3) [15]. It was found that 'back-bridged' analogs, in which the ammonium hydrogen was consistent with the pharmacophore model, had higher affinities for the dopamine transporter and were more selective for it relative to the serotonin transporter. In contrast, 'front-bridged' analogs had higher affinities for the serotonin transporter and were more selective for it relative to the dopamine transporter. (Both classes of compounds had approximately equal high affinities for the norepinephrine transporter, suggesting that the differences in orientation of the ammonium hydrogen did not play a major role for the latter.) It should be noted, however, that these preferences were not absolute and that 'front-bridged' analogs still had considerable affinity at the dopamine transporter. Given the relative rigidity of these 'front-' and 'back-bridged' analogs, we decided to utilize them as model compounds with which to superimpose additional dopamine reuptake blockers.

Methylphenidate is an important dopamine reuptake blocker in clinical use and also was important in the development of the pharmacophore model. It shares the methyl ester group of cocaine and CFT and it was found that the atoms from the nitrogen through the ester group can be perfectly aligned [9]. However, aligning this sequence of atoms means that the phenyl rings of methylphenidate, cocaine, and CFT will clearly occupy different positions in space. Based on this alignment, it was predicted that the addition of an N-methyl group to the secondary amine of methylphenidate and its analogs would result in a reduction of their activities and this prediction proved to be correct and provided additional evidence for the model [10,11]. We would now like to superimpose what was proposed as the biologically active form of methylphenidate with one of the 'back-bridged' cocaine analogs that have optimal activity as dopamine reuptake blockers. However, as these compounds do not have the methyl ester of methylphenidate, cocaine, or CFT, we tried to superimpose simultaneously the phenyl rings and the ammonium group.

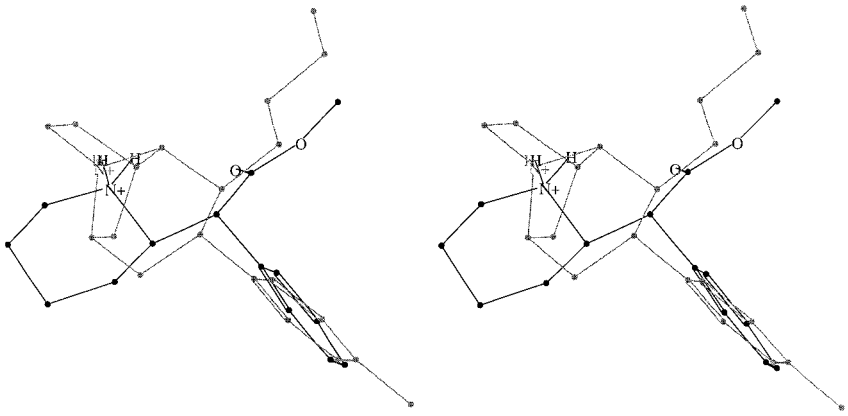


Figure 4. Stereo image of the superposition of the proposed biological active conformer of methylphenidate (dark line) with a 'back-bridged' cocaine analog (light line).

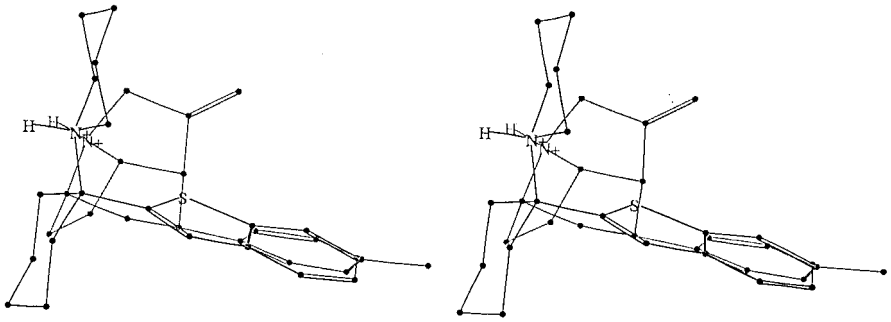


Figure 5. Stereo image of the superposition of the global minimum of BTCP (dark line) with a 'front-bridged' cocaine analog (light line).

Somewhat surprisingly, these can be aligned with an rms fit of 0.26 Å and one can also obtain a similar orientation of the ammonium hydrogens of the two compounds (Figure 4). However, it is now the axial ammonium hydrogen of methylphenidate that is correctly aligned rather than the equatorial one as found previously. Thus, it seems that, depending on what is superimposed, either one of the ammonium hydrogens of methylphenidate can appear to be the crucial one. However, the consistent decrease found in N-methyl derivatives of methylphenidate analogs suggests that the first alignment is the appropriate one, at least most of the time. However, the superposition of methylphenidate with the 'back-bridged' cocaine analog suggests that the second alignment may also be appropriate under some conditions.

Now that we have determined the biologically active conformer for BTCP and its analogs, we can address whether this class of compounds is consistent with the previously defined pharmacophore for dopamine reuptake blockers. To that end, we attempted to superimpose the putative biologically active conformer of BTCP with the 'back-bridged' analog. However, while the superposition can be fit to an rms of 0.44 Å, it appeared to be impossible to align the ammonium hydrogens in the same manner as was done with methylphenidate (not shown). However, one can fit the biologically active conformer to the 'front-bridged' analog to an rms of 0.33 Å with a good alignment of the ammonium hydrogens (Figure 5). Thus, the structure of BTCP appears to be a better fit to serotonin reuptake blockers than dopamine reuptake blockers. Nevertheless, as indicated above, even the 'front-bridged' cocaine analogs have considerable activity as dopamine reuptake blockers.

Conclusions

Molecular mechanics calculations using MM3-92 and ab initio quantum mechanical calculations using SPARTAN 5.0 indicate that an aryl-axial conformer is the biologically active form for at least some compounds in the BTCP series, with the key compound

being the cis-2-methyl analog which retains good activity. However, an aryl-equatorial conformer presents the identical pharmacophore and may be responsible for the activity of the cis-4-methyl analog. Using rigidified cocaine analogs with defined orientations of the ammonium hydrogen as templates, it was found that the previously proposed biologically active conformer of methylphenidate is an excellent fit to the 'back-bridged' cocaine analog which is optimal for dopamine reuptake blockers. In this superposition of the phenyl rings and the ammonium nitrogen, however, the axial ammonium hydrogen is the crucial one rather than the equatorial one found previously. The orientation of the ammonium hydrogen in BTCP, however, is a better fit to the 'front-bridged' cocaine analog which is optimal for a serotonin reuptake blocker. Nevertheless, even the latter can have considerable activity as a dopamine reuptake blocker.

Acknowledgements

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