A Study on the Contribution of the 1-Phenyl Substituent to the Molecular Electrostatic Potentials of Some Benzazepines in Relation to Selective Dopamine D-1 Receptor Activity

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The molecular electrostatic potentials for a selective dopamine D-1 receptor antagonist, 7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-methylbenzazepine (SCH 23390 (1)), and a selective dopamine D-1 receptor agonist, 7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SK&F 38393 (2)), have been calculated in order to obtain an understanding of the nature of the interactions between the phenyl ring and the receptor. Analogues of 1 with conformationally constrained phenyl rings have also been studied. Based on this study, the conclusion is drawn that an important part of the interaction between the phenyl ring in the benzazepines and the receptor is due to electrostatic forces, and that the phenyl ring interacts with the same receptor site as the oxygen atom of the 8-hydroxy group.

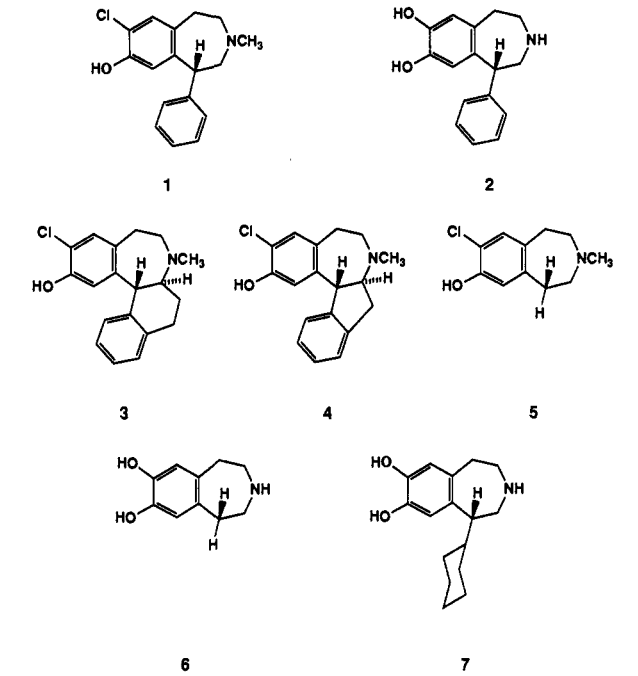
Introduction

Dopamine (DA) receptors are divided into three subpopulations, D-1, D-2, and D-3.[1,2] Selective agonists and antagonists are known for the DA D-1 and D-2 subtypes of receptors. 7-Chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-3-methyl-1*H*-3-benzazepine (SCH 23390 (1)) is a selective and potent DA D-1 receptor antagonist,[3,5-7] while 7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine, (SK&F 38393 (2)) is a selective and potent DA D-1 receptor agonist.[4,5] These compounds display a high degree of enantioselectivity, and the activities reside almost exclusively in the *R*-enantiomer.[4,5,7]

For these compounds it has been shown that the phenyl ring is of decisive importance for the interaction with the DA D-1 receptor.[1] For instance, if the phenyl ring in 2 is replaced by a hydrogen atom or a cyclohexane ring, the biological activity is markedly decreased.[8] The hydrophobic contribution of a phenyl ring and a cyclohexyl ring is similar.[9] Thus, if the phenyl ring interacts with the receptor only through hydrophobic interactions, the cyclohexyl analogue of 2 should show about the same biological activity as 2. As this is not the case, there are reasons to believe that the phenyl ring may interact with the receptor via electrostatic forces. The phenyl ring has a quite strong electrostatic potential field[10] and can take part in electrostatic interactions. Furthermore, these electrostatic interactions differ depending on the direction of the interaction between a phenyl group and another polar group. The interaction between a benzene ring and a positively charged species is most favorable if the positive species interacts with the benzene ring above or below the ring plane. On the other hand, the interaction between a benzene ring and a negatively charged species is most favorable if the negative species is located in the plane of the benzene ring.[10] In order to investigate possible interactions between the phenyl ring of the benzazepines and the DA D-1 receptor, the molecular electrostatic potentials for 1 and 2 and the analogues 3-7 (Chart I) in their probable biologically active conformations[11-13] have been calculated. The electrostatic potentials for different phenyl rotamers of 1 and 2 have been calculated, and possible relationships between the DA D-1 receptor affinities of the closely related compounds 1, 3, and 4 and the anisotropic electrostatic potential field of the phenyl ring have been investigated.

In a recent work, Charifson et al.[13] have found a correlation between the calculated molecular dipole moment

Chart I



orientations of 1 and some other selective D-1 receptor antagonists and their receptor affinities. However, their

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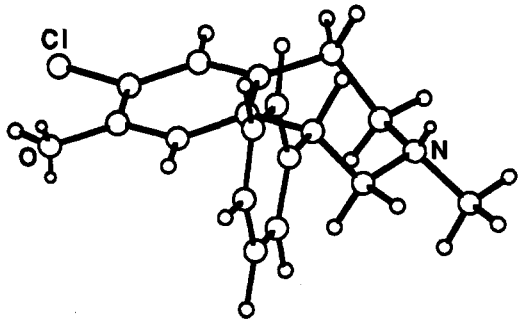


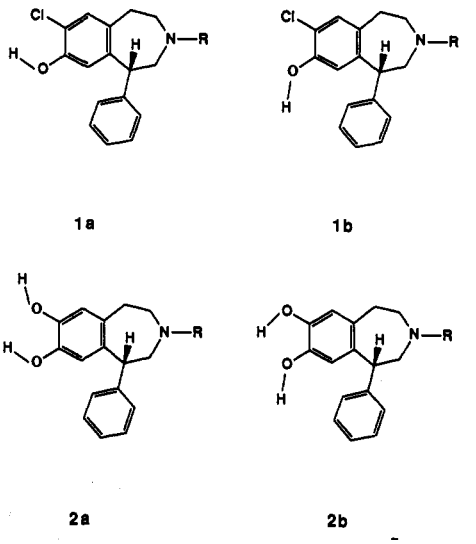
Figure 1. Proposed biologically active conformation of compound 1 with respect to the conformation of the tetrahydroazepine ring and the phenyl ring orientation.

study does not take the electrostatic potential field of the phenyl ring[10] into account and thus does not consider the possibility of electrostatic interactions between the 1-phenyl ring and the receptor.

Computational Methods

The molecular mechanics calculations were performed by using the MM2(85) program developed by Allinger and co-workers[14-18] including the full treatment of conjugated systems.[15] Our version of MM2(85) includes bond order dependent torsional constants[16] and hydrogen bonding potentials according to MM2(87).[18] Potential energy curves

Chart II



were generated by using the driver option in the MM2(85) program.

Electrostatic potential derived atomic point charges were obtained using the program GAUSSIAN 80 UCSF and a minimal basis set, STO-3G,[19] and the program MOPAC 5.0 ESP[20] using the AM1 method. Both methods generate point charges which reproduce the electrostatic potential calculated from the wave functions. The AM1 ESP method is used as this method generates point charges for a benzene ring which are very close to the point charges arrived at in our previous study on benzene–benzene interactions[10] in which the point charges were determined by fitting to experimental data and high quality ab initio calculations of geometries and energies for the benzene dimer. As the electrostatic potential derived charges depend on the conformation, the calculations have been done for all different conformations studied. The electrostatic potentials using the point charges are calculated as $\sum q_i/R_i$, where q_i is the point charge at the atom i, and R_i is the distance between a positive unit charge and atom i.

In the quantum mechanical calculations, geometries obtained by MM2(85) were used.

The calculations using GRID[21] (version 7) have been performed with the dielectric constant 80 and with two planes per angstrom. The hydroxy group of the target molecule may donate one and accept two hydrogen bonds (the hydroxy oxygen is treated as a sp^3-hybridized atom). The positively charged probe is a (spherical) sp^3-hybridized cationic NH_3 group. It has the charge of +0.66, and it can donate three hydrogen bonds. The negatively charged probe is a carboxy oxygen atom with the charge of 0.45 and accepts two hydrogen bonds.

Results and Discussion

Conformational Analysis of 1 and 2. Compounds 1 and 2 may adopt a number of different conformations.

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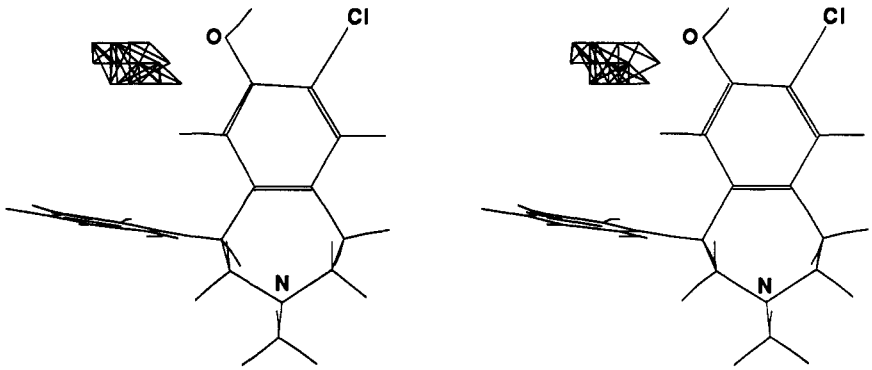

Figure 2. A stereoview of the interaction, calculated by the GRID program, between 1 in the global energy minimum and a (spherical) sp^3-hybridized cationic NH_3 probe. The isoenergy contours show the energy range from -5.0 to -4.0 kcal/mol.

Based on molecular mechanics calculations, it has been suggested by us[11] and others[12,13] that the conformation of 1 and 2 which is responsible for the biological effect ("the biologically active conformation") is a chair conformation with a pseudoequatorial phenyl ring, and for 1 an equatorial *N*-methyl group. This conformation is shown in Figure 1. We have also suggested that the orientation of the phenyl ring in the receptor-bound molecule does not deviate in terms of dihedral angles by more than ca. ±30 degrees from the preferred orientation, in which the two aromatic ring planes are essentially orthogonal.[11]

According to molecular mechanics calculations,[11] the hydroxy group prefers a conformation in which the hydrogen atom is located in the same plane as the benzene ring. For the antagonists 1, 3, and 4, the global energy minimum is the one in which the hydroxy hydrogen atom is pointing toward the chlorine atom, corresponding to conformation **1a** in Chart II. This was also found by Charifson et al.[13] According to MM2(85) calculations, the minimum in which the hydroxy hydrogen is pointing away from the chlorine atom, conformation **1b** in Chart II, is 0.9 kcal/mol higher in energy. This is in reasonable agreement with experimental data for *o*-chlorophenol.[22] Far-infrared spectroscopic investigations in gas phase and solution (cyclohexane) show that the intramolecularly hydrogen bonded conformation is preferred by 1.63 and 1.62 kcal/mol, respectively. According to molecular mechanics calculations, the barrier to rotation of the hydroxy group is 5 kcal/mol. On the basis of the high barrier to rotation, we have assumed that the hydroxy group interacts with the receptor in one of the energy minima or in a conformation close to them. For the agonists, the energy difference between conformer **2a** and **2b** in Chart II is small, 0.3 kcal/mol, with conformer **2b** as the more stable one.

Identification of Possible Receptor Sites. In order to identify possible receptor sites which can interact with the 1-phenyl and 8-hydroxy groups, calculations for the interaction between 1 and different probes simulating receptor sites have been performed. This has been done using the program GRID.[21] In Figure 2 the interactions according to calculations using the program GRID[21] between 1 in conformation **a** and a positively charged probe, a sp^3-hybridized cationic NH_3 group, are shown. The calculated interaction between 1 in conformation **b** and a negatively charged probe, an anionic carboxy oxygen atom, is shown in Figure 3. These two figures display favorable interaction sites located on the face of the phenyl ring and close to the oxygen atom (Figure 2) and the hydrogen atom (Figure 3) of the 8-hydroxy group.

Table I. Dopamine D-1 Receptor Binding Data for Compounds 1, 3, and 4

compd	config	[^{3}H]SCH 23390 displacement: K_i (nM)
1	*R*	0.3[a]
1	*S*	192[a]
3	6a*S*,13b*R*	1.9 ± 0.6[b]
4	racemic	7[c]

[a] Data from ref 7. [b] Data from ref 12. [c] Data from ref 25.

Table II. Pharmacological Data for Compounds 2, 6, and 7

compd[a]	adenylate cyclase stimulation: EC_{50} (M)
2	7.1×10^{-8}[a]
6	5.2×10^{-6}[a]
7	$>10^{-6}$[a]

[a] Racemates, ref 8.

Figures 2 and 3 suggest the possibility that the phenyl ring and the 8-hydroxy group may interact with the same receptor site via electrostatic interactions. In this situation the presence and orientation of the phenyl ring may either increase or decrease the electrostatic interactions with the receptor site. In conformation **a** (Chart II) the oxygen atom of the 8-hydroxy group and the phenyl ring can interact with the same receptor site. In this conformation the oxygen atom may interact as a hydrogen bond acceptor with this site. In conformation **b** the hydrogen atom of the 8-hydroxy group and the phenyl ring can interact with the same receptor site with the 8-OH group as a hydrogen bond donor.

In order to quantify the electrostatic interactions suggested above and to determine the contribution from the phenyl ring to the electrostatic potential in the region of favorable interactions displayed in Figure 2 and 3, the electrostatic potential was calculated at two points (p1 and p2) for conformation **a** and at one point (p3) for conformation **b**. The positions of points p1–p3 are defined in Figure 4a and b. In conformation **a**, p1 and p2 are located 2.8 Å from the oxygen atom and in the same direction as the oxygen lone pairs. In conformation **b**, p3 is located 2.8 Å from the oxygen atom and in the same direction as the OH bond. These points simulate receptor sites interacting with the hydroxy group.[23,24]

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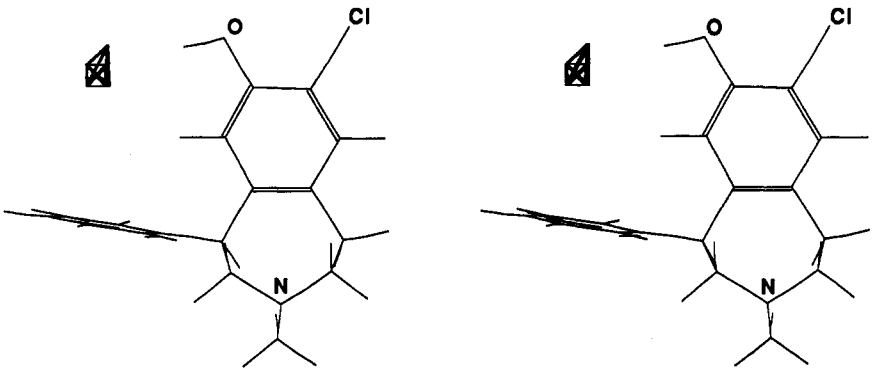

Figure 3. A stereoview of the interaction, calculated by the GRID program, between compound 1 in the global energy minimum and an anionic carboxy oxygen probe. The isoenergy contours show the energy range from −6.0 to −4.5 kcal/mol.

Compounds 3[12] and 4[25] are D-1 antagonists in which the phenyl ring is conformationally constrained. For 1 in the global energy minimum, the calculated angle between the two benzene ring planes is 86 deg. In 3 it is reduced to 74 deg and in 4 it is 55 deg. As can be seen in Table I, compound 1 has the highest affinity and 4 the lowest affinity for the D-1 receptor. The electrostatic potentials for 5–7 were calculated in order to get information about the contribution from the phenyl ring to the electrostatic potential.

Electrostatic Potentials for 1 and 3–5. The electrostatic potentials were calculated using two different methods. One is ab initio with a minimal basis set, STO-3G, in which atomic charges that reproduce the electrostatic potential based on the wave function are generated.[19] The other is a semiempirical method, AM1, from which point charges that reproduce the electrostatic potential are also calculated.[26] The point charges obtained for the atoms in benzene using the ab initio method with a minimal basis set are −0.06 for carbon and +0.06 for hydrogen. We have found that the size of the point charges necessary to treat the electrostatic properties in benzene correctly is ±0.15.[10] If a larger basis set, 3-21G or 6-31G*, is used, the size of the point charges is increased to ±0.16 and ±0.14, respectively. However, due to the size of the molecules it is not realistic to use these larger basis sets for the calculations discussed in this paper. Instead, a semiempirical method, AM1, was used. The size of the AM1 point charges for benzene is ±0.15.

The electrostatic potential at points p1 and p2 for 1 in conformation **a** and at point p3 for 1 in conformation **b** were calculated for six different phenyl ring rotamers. The results of these calculations for 1 and 3–5 are given in Table III. The results for p1 and p2 refer to interactions with a positive unit charge and those for p3 with a negative unit charge. This means that for p1 and p2 a negative electrostatic potential implies an energetically favorable interaction between the molecule and the simulated *positively* charged receptor site. For point p3, a negative potential implies a favorable electrostatic interaction between the molecule and a *negatively* charged receptor site.

As can be seen in Table III, the interaction between 1 in conformation **a** and a positively charged receptor site at points p1 and p2 is energetically favorable. The interaction is most favorable for the phenyl ring rotamer with τ = 90 degrees. This corresponds to the global energy minimum of 1. It can also be seen that the contribution from the phenyl ring to the electrostatic potential is substantial and is optimal for this rotamer (p1) or for a rotamer with τ = 120 degrees (p2). This corresponds to conformations for which the positive charge at p1 and p2 interacts with the face of the phenyl ring. According to the calculations, the contribution from the phenyl ring is largest at point p1. The contribution from the phenyl ring to the electrostatic potential calculated from AM1 charges is significantly larger than that obtained from STO-3G charges. The reason for this is the larger size of the point charges obtained by AM1 compared to STO-3G. As discussed above the AM1 charges seem to be realistic in this case.

The electrostatic potential decreases when the two phenyl rings approach coplanarity. If τ = 0 degrees, which corresponds to coplanar phenyl rings, the electrostatic potential at point p1 is decreased by 1.38 kcal/mol according to STO-3G charges and by 4.27 kcal/mol according to AM1 charges. In addition, for this rotamer the conformational energy is very high, 8.1 kcal/mol (Table III). The results for point p2 are very similar in this respect.

The interaction between 1 in conformation **b** and a negatively charged receptor site at point p3 is energetically favorable. At this point the most favorable interaction is found for the phenyl ring rotamer with τ = 0 degrees. This corresponds to the conformation in which the two phenyl rings are coplanar. However, for all phenyl rotamers the contribution from the phenyl ring to the electrostatic potential is positive. That is, the electrostatic interactions between the phenyl ring and the point p3 are always repulsive. The least repulsive situation is for τ = 0 degrees, but for this rotamer the conformational energy is very high, 8.1 kcal/mol (Table III).

To conclude, if the oxygen atom in the 8-hydroxy group of 1 interacts with a receptor site as a hydrogen bond acceptor (conformer **a**), the electrostatic potential as well as the conformational energies suggest that the most favorable interaction is found when the two phenyl ring planes are orthogonal. This corresponds to our previously proposed biologically active conformation of 1.[11] On the other hand, if the hydrogen atom in the 8-hydroxy group interacts in conformation **b** as a hydrogen bond donor, the electrostatic potentials suggest that the most favorable interaction is found when the two phenyl rings are coplanar. This corresponds to the high-energy transition state for phenyl ring rotation. However, if the hydrogen atom in the 8-hydroxy group interacts with the receptor in conformation **b**, an even better electrostatic interaction is obtained if the phenyl ring is removed (compound 5, Table III).

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Table III. Electrostatic Potentials (in kcal/mol) for Compounds 1, 3–5 in Conformation a (p1 and p2) and in Conformation b (p3)[a]

compd	τ[b]	conformational energy (kcal/mol)	electrostatic potential at point p1				electrostatic potential at point p2				electrostatic potential at point p3			
			STO-3G	contribution from Ph[c]	AM1	contribution from Ph[c]	STO-3G	contribution from Ph[c]	AM1	contribution from Ph[c]	STO-3G	contribution from Ph[c]	AM1	contribution from Ph[c]
1	0	8.1	-6.19	0.35	-5.53	1.18	-5.51	0.45	-6.30	0.21	-21.67	0.71	-17.03	1.39
1	30	5.2	-6.83	-0.29	-8.21	-1.50	-5.98	-0.02	-5.83	0.68	-20.47	1.91	-14.16	4.26
1	60	1.4	-7.22	-0.68	-9.32	-2.61	-6.26	-0.30	-7.10	-0.59	-20.33	2.05	-12.83	5.60
1	90	0.0	-7.57	-1.03	-9.80	-3.09	-6.46	-0.50	-8.17	-1.66	-20.09	2.29	-11.95	6.47
1	120	1.4	-7.40	-0.86	-9.09	-2.38	-6.50	-0.54	-8.56	-2.05	-20.21	2.17	-12.08	6.34
1	150	4.4	-6.99	-0.45	-7.85	-1.14	-6.24	-0.28	-8.09	-1.58	-20.53	1.85	-13.24	5.18
3	118		-7.96		-9.76		-6.25		-8.23		-20.36		-12.17	
4	135		-6.61		-6.75		-6.34		-8.63		-20.94		-14.27	
5			-6.54		-6.71		-5.96		-6.51		-22.38		-18.42	

[a] Points p1 and p2 have a positive unit charge. Point p3 has a negative unit charge. [b] τ is the $C_{sp}2$–$C_{sp}3$–$C_{sp}2$–$C_{sp}2$ dihedral angle connecting the seven-membered ring and the phenyl group. [c] The electrostatic potential for 1 with phenyl rotamer τ minus the electrostatic potential for 5.

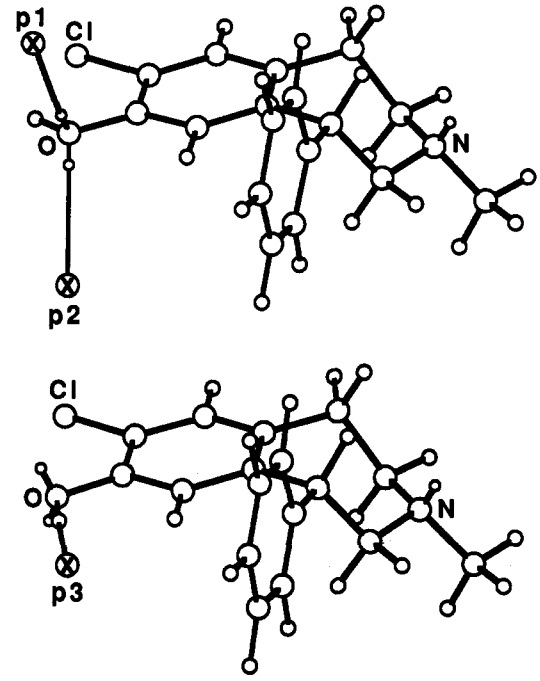

Figure 4. (a) Compound 1 in conformation **a** with the proposed receptor sites p1 and p2 shown. (b) Compound 1 in conformation **b** with the proposed receptor site p3 shown.

In 3 and 4 the torsional angles (τ in Table III) are 118 and 135 degrees, respectively. On comparing the electrostatic potentials at point p1 for 1 (in the global energy minimum), 3, and 4, the results in Table III show that the interaction between a positive charge and the molecules in conformation **a** is about equal for 3 and 1 and less favorable for 4. At point p2, the calculated electrostatic potentials show that the interaction energy is about the same for 1, 3, and 4. The relative affinities between 1 and 3 and between 3 and 4, respectively, are too small (Table I) to be used in a quantitative comparison. However, 1 has a higher affinity than 4 by a factor of 23 which should be significant in this context. The calculated electrostatic potentials at point p1 (Table III) imply that for conformation **a** (Figure 4a) 1 should display a higher affinity than 4, in agreement with experiment. The electrostatic potentials at point p2 are essentially identical for 1 and 4. In contrast, the calculated electrostatic potentials for conformation **b** (Figure 4b) at point p3 (Table III) predict that 4 should be significantly more active than 1, which do not agree with experimental affinities.

Electrostatic Potentials for 2, 6, and 7. The results of these calculations are given in Table IV. The conformational energies and the phenyl contributions to the electrostatic potential at points p1, p2, and p3 for different rotamers of 2 are virtually identical to those for 1, and thus only the calculated values for the lowest energy phenyl rotamer are shown in the table. According to Table IV, the electrostatic potentials for 6 and 7 in conformation **a** are less negative than that for 2. The main difference between 2 and 7 is the lack of possible electrostatic interactions between the cyclohexyl group and a receptor site. The calculated conformational energy of the cyclohexyl ring in 7 in an orientation corresponding to the proposed biologically active orientation of the 1-phenyl ring in 2 is only 0.1 kcal/mol above that of the most stable orientation. Thus, there is no conformational energy penalty for the cyclohexyl ring. Furthermore, since many 1-phenyl-substituted derivatives of 2 and its 6-chloro analogue retain potent activity,[8] the somewhat greater volume of a chair cyclohexyl ring compared to an unsub-

Table IV. Electrostatic Potentials (in kcal/mol) for Compound 2, 6, and 7 in Conformation a (p1 and P2) and in Conformation b (p3)[a]

	electrostatic potential at point p1				electrostatic potential at point p2				electrostatic potential at point p3			
compd	STO-3G	contribution from Ph[b]	AM1	contribution from Ph[b]	STO-3G	contribution from Ph[b]	AM1	contribution from Ph[b]	STO-3G	contribution from Ph[b]	AM1	contribution from Ph[b]
2	-11.30	-0.99	-10.60	-2.98	-10.12	-0.41	-8.79	-1.41	-19.59	2.21	-11.71	6.44
6	-10.31		-7.62		-9.71		-7.38		-21.80		-18.15	
7	-9.71		-7.16		-9.30		-7.34		-23.03		-18.02	

[a] Points p1 and p2 have a positive unit charge. Point p3 has a negative unit charge. [b] The electrostatic potential for 2 minus the electrostatic potential for 6.

stituted 1-phenyl is most probably not the cause of the low biological activity of 7.

The calculations suggest that the electrostatic interactions between 2 and receptor sites corresponding to p1 and p2 should be more favorable than the interactions between the same receptor sites and compounds 6 and 7. The calculations performed for 2, 6, and 7 in conformation b predict that 6 and 7 should have higher biological activity than 2 due to electrostatic interactions. Thus, the results obtained for conformation a, but not for b, are in agreement with observed relative biological activities for the D-1 receptor (Table II).

As shown above for 1, the phenyl rotamer of 2 giving the strongest electrostatic interaction with p1 and p2 is the one in which the two aromatic ring planes are orthogonal, corresponding to the global energy minimum of 2 and to the proposed biologically active conformation.[10] Since receptor binding data for the agonists are not available, the conclusions above should be treated with caution.

Conclusions

Calculations of molecular electrostatic potentials for the compounds studied in this work suggest that the phenyl ring interacts with the DA D-1 receptor by electrostatic forces. For antagonists as well as agonists, maximal electrostatic interactions with receptor sites at p1 and p2 are obtained for a phenyl ring rotamer in which the two phenyl ring planes are orthogonal. This corresponds to the global energy minimum of 1 and 2 and to our previously proposed biologically active conformation for these compounds.[11] Only in conformation a, in which the oxygen atom of the 8-hydroxy group is a hydrogen bond acceptor with respect to our proposed receptor sites, does the phenyl ring give a favorable contribution to the electrostatic interaction with these sites. In the alternative conformation b, in which the 8-hydroxy is a hydrogen bond donor with respect to our proposed receptor site (p3), the contribution from the phenyl ring to the electrostatic interaction and thus to the binding energy is repulsive.

The results above indicate that the electrostatic potential field of substituents in compounds related to 1 and 2 should be taken into account in the design of new DA D-1 agonists and antagonists.

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3-Carboxy-5-methyl-*N*-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide, a New Prodrug for the Antiarthritic Agent 2-Cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-2-butenamide[1]

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The title compound 3-carboxyisoxazole 3 was synthesized by cycloaddition of carbethoxyformonitrile oxide to *N*-[4-(trifluoromethyl)phenyl]-3-pyrrolidino-2-butenamide (6) with spontaneous elimination of pyrrolidine followed by hydrolysis of the ethyl ester. Compound 3 was shown to be absorbed intact after oral administration to rats. Over 24 h, the compound was metabolized to yield plasma concentrations of the antiinflammatory agent 2-cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-2-butenamide (2), similar to those obtained following an equivalent dose of the established prodrug of 5-methyl-*N*-[4-(trifluoromethyl)phenyl]isoxazole-4-carboxamide (1).

Over the past several decades many pharmaceutical agents have been devised using the concept of prodrugs. This strategy has been useful in overcoming a variety of problems which would have precluded the development of many parent compounds as medicinal agents. Some virtues ascribed in the literature to prodrugs include improved solubility and bioavailability, tissue-specific delivery, diminution of side effects, sustained release of metabolically unstable agents, and lengthened shelf life.[2] Although less abundant than rationally devised prodrugs, there are many examples of compounds shown to exhibit their particular biological effect only after some metabolic transformation[3] or where a metabolite has efficacy similar to that of the parent compound.

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(2) Reviews: (a) Armstrong, R. N. Mechanistic Aspects of Xenobiotic Metabolism as Related to Drug Design. *Annu. Rep. Med. Chem.* 1988, *23*, 315-324. (b) Bodor, N.; Kaminski, J. J. Prodrugs and Site-Specific Chemical Delivery Systems. *Annu. Rep. Med. Chem.* 1987, *22*, 303-313.

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