Conformational Analysis of Dopamine D-2 Receptor Antagonists of the Benzamide Series in Relation to a Recently Proposed D-2 Receptor-Interaction Model

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Conformational analysis using molecular mechanics calculations (MM2(87)) has been performed for four different types of benzamides which display high affinity for the dopamine D-2 receptor. In order to elucidate the conformation of the receptor-bound molecules, a previously described dopamine D-2 receptor-interaction model has been employed. We conclude that all four types of benzamides accommodated in the proposed receptor-interaction model are in low-energy conformations. An acyclic amide side chain is concluded to adopt an extended conformation in the receptor-bound benzamide. A phenylpyrrole analogue of the benzamides could similarily be fitted to the model. Using the receptor-interaction model, the enantioselectivity of benzamides with an *N*-ethyl-2-pyrrolidinylmethyl side chain could be rationalized in terms of different conformational energies of the receptor-bound enantiomers. Two different receptor sites for N-alkyl substituents are suggested.

Introduction

Selective antagonists are known for the dopamine (DA) D-1 and D-2 subtypes of receptors.[1] Among those, several types of substituted benzamides have been shown to be selective and potent DA D-2 receptor antagonists.[2-8] Four different types of benzamides which display high affinity for the DA D-2 receptor are shown in Chart I (1-4) as the parent molecules unsubstituted in the phenyl ring. Chart I also includes a phenylpyrrole analogue (5) of benzamide type 3. Specific examples of each of the four types of benzamides and the phenylpyrrole analogue are shown in Chart II, and their biological data are given in Table I.

In structure–activity and molecular modeling studies on benzamides, coplanarity between the phenyl ring and the amide group is generally assumed for the biologically active conformation of benzamides with respect to DA D-2 receptor affinity.[7-11] However, different conformations of the amide side chain in the receptor bound molecule have been proposed. Testa et al., employing PCILO calculations, have suggested that for benzamides of types 2 and

Chart I

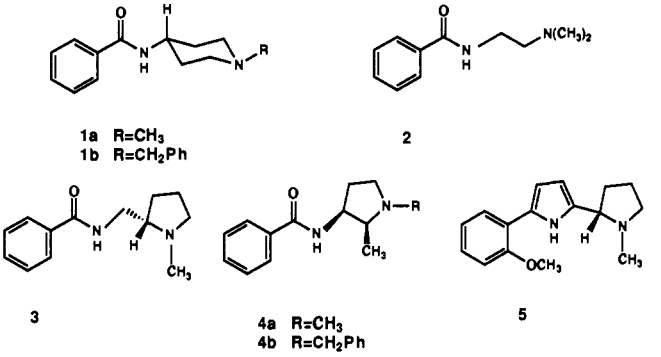
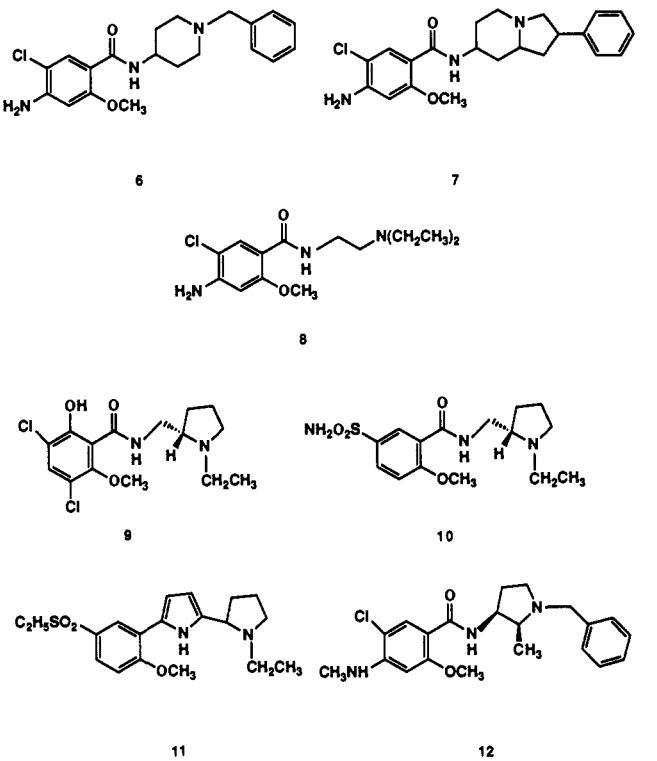


Chart II



3 (Chart I) an extended conformation of the amide nitrogen alkyl substituent is the most probable one for the receptor-bound molecule.[9,10] In contrast to this, a half-

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Table I. Biological Data for Compounds 6–12

compd	config	K_i (nM) [^{3}H]SPI	IC_{50} (nM) [^{3}H]SPI	ED_{50} (mg/kg)
6		2.2 ± 0.4[a]	17[b]	0.037[c]
7		–	–	0.024[c]
8		104 ± 2.1[a] 235[d]	820[b]	0.75[c]
9	*S*	9.5 ± 1.5[a]	32[d,f]	–
	R		2920[f]	
10	*S*	39.0 ± 7.0[e]	170[b]	–
	S		233[d]	
11	rac	29.0 ± 3.0[e]	–	–
12	cis	0.12 ± 0.04[a]	0.49[b]	

[a] Reference 2. [b] Reference 3. [c] Reference 4. [d] Reference 5. [e] Reference 6. [f] Reference 7.

folded conformation of the side chain in benzamides of type **3** has been proposed by Högberg et al.[7] This conclusion was based on molecular mechanics calculations and superimposition studies using piquindone as a template. Rognan et al. recently proposed an extended conformation for receptor-bound benzamides of types **3** and **4**.[11] Also in the work of Rognan et al., superimpositions with piquindone and molecular mechanics calculations were employed. Furthermore, in their work three different sites for *N*-alkyl substituents are proposed.

A DA D-2 *receptor-interaction model defining the spatial* relationships between pharmacophore elements required for DA D-2 receptor affinity has been proposed by Liljefors and Bøgesø.[12] This model is based on extensive conformational analysis and molecular superimposition studies of 1*R*,3*S*-4-[3-(4-fluorophenyl)-6-(trifluoromethyl)-indan-1-yl]-1-piperazineethanol (tefludazine) and (*S*)-octoclothepin. The model has successfully been used by Froimowitz in studies on tetracyclic spiroamines and cyproheptadine derivatives.[13,14] It has also recently been employed to rationalize the enantioselectivity of (*S*)- and (*R*)-octoclothepin.[15]

The present work was undertaken to investigate if the four different types of benzamides **1**–**4** and the analogous phenylpyrrole **5** shown in Chart I can be accommodated in the DA D-2 receptor-interaction model proposed by Liljefors and Bøgesø and thus give information on the conformation of the side chain in the biologically active conformation of benzamides and analogous compounds. Since the subject of the present study is the conformational

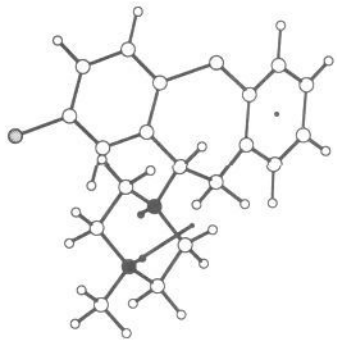

Figure 1. (*S*)-Octoclothepin in the biological active conformation suggested by Liljefors and Bøgesø.[12]

properties of the amide side chain and the overall accommodation of the different types of DA D-2 antagonistic benzamides in the Liljefors–Bøgesø receptor-interaction model, the study is restricted to the parent molecules **1**–**5**. Structure–activity relationships with respect to the substitution pattern of the phenyl ring have been extensively studied by Högberg et al.[7,8] and will not be discussed in this paper.

In order to find low-energy conformations for the parent molecules **1**–**5**, conformational analyses based on molecular mechanics calculations (MM2(87)) have been performed. Low-energy conformations of the compounds have then been least-squares fitted to the DA D-2 model proposed by Liljefors and Bøgesø. The spatial relationships defined by this model are represented by the deduced biologically active conformation of the potent DA D-2 receptor antagonist (*S*)-octoclothepin shown in Figure 1.[12]

Computational Methods

Conformational energies and energy-minimized molecular geometries were calculated using the molecular mechanics program MM2(87) developed by Allinger and coworkers.[16–19] Electrostatic interactions involving a phenyl ring were calculated using a $C(sp^2)$–H bond dipole of 0.7 D with the negative end at the carbon atom and a $C(sp^2)$–$C(sp^3)$ bond dipole of 1.0 D with the negative end at the sp^2-hybridized carbon atom.[20,21] The torsional force constants for the $N_{(sp^3)}$–$C_{(sp^3)}$–$C_{(sp^3)}$–$N_{(sp^2)}$ torsional angle have been set to V1 = 0.0, V2 = 0.0, V3 = 0.0, for the torsional angle $C_{(sp^2)}$–C(=O)–$N_{(sp^2)}$–H torsional angle V1 = 0.0, V2 = 5.0, V3 = 0.0, and for the C(=O)–$C_{(sp^2)}$–$C_{(sp^2)}$–$O_{(sp^3)}$ torsional angle V1 = 0.0, V2 = 15.0, V3 = 0.0. These parameters were chosen in analogy with

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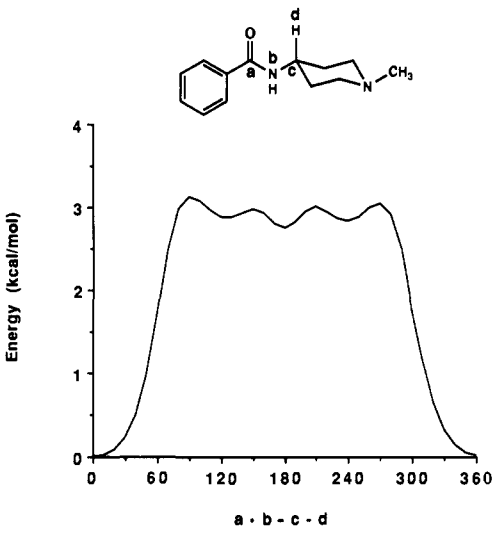

Figure 2. Calculated potential energy curve for rotation about the C(=O)–N–C–H torsional angle in **1a**.

parameters for similar combinations of atoms in the MM2(87) force-field parameter list.

As in our previous work, the calculations were performed for the unprotonated amines (with the unshared electron pair represented by a pseudoatom).[12] In the calculations on the conformational properties of the amide side chains, the phenyl ring and the amide group has been kept coplanar (using the driver option in MM2(87)) while the flexible side chains have been considered in different conformations. Input structures for the MM2(87) calculations were constructed by using the molecular modeling program MacMimic.[22]

To investigate the energetic consequences of small deviations from coplanarity of the phenyl ring and the amide moiety we have performed ab initio STO-3G calculations using GAUSSIAN 80 UCSF.[23] In these calculations *o*-methoxy-*N*-methylbenzamide with MM2(87) geometry has been employed as a model compound. MM2(87) is not suited for these calculations due to its less well developed hydrogen bonding potentials.

The benzamides and the phenylpyrrole have been least-squares fitted to the D-2 receptor-interaction model described by Liljefors and Bøgesø[12] by using (*S*)-octoclothepin, in its proposed biologically active conformation (Figure 1), as a template. Three different atoms or points in each of the molecules were used in the molecular least-squares fitting: (1) the center of the phenyl ring; in (*S*)-octoclothepin the phenyl ring to the right in Figure 1 has been employed; (2) the amine nitrogen atom; in (*S*)-octoclothepin the distal nitrogen atom has been used; and (3) a point 2.8 Å from the amine nitrogen atoms and in the same direction as the nitrogen lone-pair bond as indicated in Figure 1. This point simulates a hydrogen bonding site of the receptor. The superimpositions were performed with the MacMimic program.[22]

Results and Discussion

Benzamides of Type 1. The chair conformation of the piperidine ring with equatorial substituents is calculated to be significantly lower in energy than other alternative conformations. Since we are searching for low-energy conformations, the conformational analysis of **1a** was thus restricted to the rotation about the bond between the amide nitrogen and the piperidine ring in a chair conformation with equatorial substituents.

The calculated potential energy curve is shown in Figure 2. The lowest energy minimum is found for a C(=O)–N–C–H dihedral angle (a–b–c–d in Figure 2) close to 0° which corresponds to a conformation in which the C(c)–H(d) bond is eclipsing the amide bond. This is the only conformer of **1a** which is significantly populated. Three local energy minima are found for a–b–c–d torsional angles about ±120 and 180°. However, these local energy minima are higher in energy by ca. 3 kcal/mol.

Since the distance between the center of the phenyl ring and the amine nitrogen in **1a** cannot be significantly altered by low-energy conformational rearrangements, this compound may be used to identify the phenyl ring in the (*S*)-octoclothepin template to be employed in the superimposition studies. Only by using the phenyl ring of (*S*)-octoclothepin not substituted by the chlorine atom (the right phenyl ring in Figure 1) could a good superimposition between **1a** and the template molecule be achieved. The same phenyl ring of (*S*)-octoclothepin was used in all superimpositions in this study.

Compound **1a** displays an exceedingly good structural fit to the deduced biologically active conformation of (*S*)-octoclothepin, using the fitting points described above, provided that the torsional angle C(=O)–N–C–H is changed by −35° from the angle found in the lowest energy minimum. The calculated energy penalty for the rotation about the N–C (b–c) bond required for an optimal fit is only 0.4 kcal/mol (Figure 2). In this [illegible] (not shown) the superimposed phenyl ring planes deviate by ca. 20° from coplanarity. It is not clear if strict coplanarity of the phenyl rings in the superimposition is required for an optimal binding of the benzamide to the receptor. However, coplanarity of the phenyl rings may be achieved by a 20° rotation about the bond connecting the carbonyl group and the phenyl ring in the benzamide. The energy penalty for this conformational change in an *o*-methoxy-substituted phenyl ring as is present in **6** and **7** is calculated by ab initio STO-3G calculations (see Computational Methods) to be small, 0.4 kcal/mol. In the case of a requirement for coplanarity of the phenyl rings in the superimposition, this energy should be added to the conformational energy of the amide side chain given above. The fit after reorientation of the phenyl ring in **1a** is shown in Figure 3a. The rms value for this fit is very low, 0.08 Å.

Compound **1a** as well as the other benzamides in this study may be least-squares fitted to the template in two different orientations: with the amide carbonyl group pointing away from the sulfur atom of (*S*)-octoclothepin as in Figure 3a or towards the sulfur atom as shown in Figure 3b. In the superimposition shown in Figure 3b, the C(=O)–N–C–H torsional angle is 150°. The energy penalty for this conformational change is 3.0 kcal/mol and the rms value of the fit, 0.35 Å, is higher than for the fit shown in Figure 3a. In order to achieve coplanarity of the phenyl rings in this superimposition the phenyl ring in **1a** was rotated by 30°. The energy cost of such a reorientation of the phenyl ring in an *o*-methoxybenzamide is calculated, as described above, to be 0.8 kcal/mol.

If the carbonyl group of benzamides of type 1 is not involved in specific interactions with the receptor, the conformation in the superimposition in Figure 3a is the most probable biologically active conformation, due to the smaller energy penalty for this conformation.

In order to obtain a high affinity for the DA D-2 receptor, the nitrogen atom in the 4-piperidyl ring in com-

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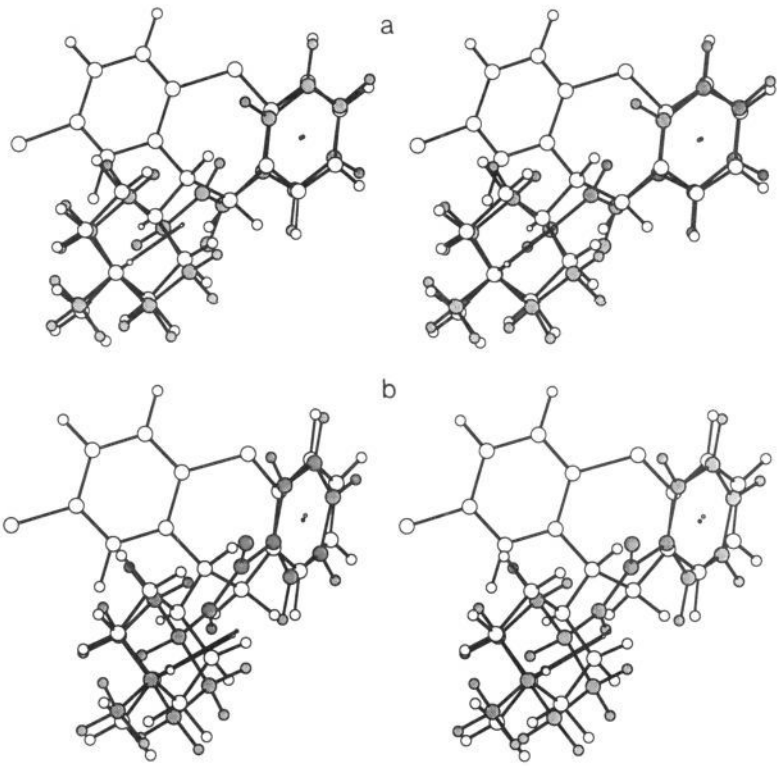

Figure 3. Least-squares superimpositions of **1a** (filled atoms) and (*S*)-octoclothepin illustrating the two alternative orientations of **1a**. In (a) the C(=O)–N–C–H torsional angle of **1a** is −35°. The rms value is 0.08 Å, in (b) the C(=O)–N–C–H torsional angle of **1a** is 150°. The rms value is 0.26 Å.

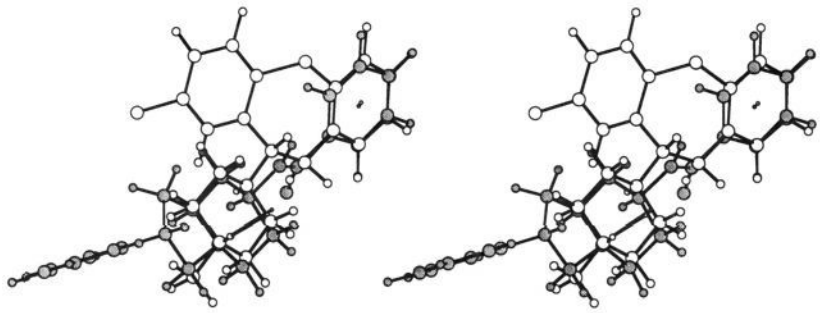

Figure 4. Molecular least-squares superimposition of **7** (filled atoms) and (*S*)-octoclothepin. The rms value is 0.24 Å.

pounds of type **1** has to be substituted with a benzyl group as in compound **6**, Chart II.[24] The analogous compound **7**, which is a potent D-2 antagonist, has restricted flexibility with respect to the orientation of the phenyl ring. Thus, the inclusion of compound **7** in this study may give information about the biologically active conformation of the phenyl ring in the *N*-benzyl group. The conformational properties of compound **7** with respect to rotation about the C(=O)–N–C–H dihedral is virtually identical to those of **1a**. The global energy minimum for compound **7** is found for a C(=O)–N–C–H angle of −5°. As for compound **1a** the C(=O)–N–C–H dihedral angle of **7** has to be rearranged by −35° in order to obtain an optimal fit to (*S*)-octoclothepin (Figure 4). The energy penalty for this conformational change is 0.4 kcal/mol. An additional 0.6 kcal/mol is calculated to be required for coplanarity of the phenyl rings in the superimposition.

An *N*-benzyl group in benzamides of type **1** (**1b**) can adopt three different staggered conformations. The 1p–N–C–C dihedral angle can be gauche+, gauche−, and anti. The energy differences between these conformers are calculated to be small, less than 0.5 kcal/mol. Superimposition studies of each these three conformations with compound **7** show that the conformation with a 1p–N–C–C dihedral angle in **1b** in a gauche+ arrangement is the only one which has the phenyl ring in the same area as that of compound **7** as shown in Figure 5.

Benzamides of Type 2. In order to find the low-energy minima for compound **2** various conformations were used as input trial structures for the energy minimization program. A primary alkyl group attached to a planar framework generally adopts a conformation in which the alkyl group is approximately perpendicular to the framework.[25] For this season the torsional angle a–b–c–d (Table

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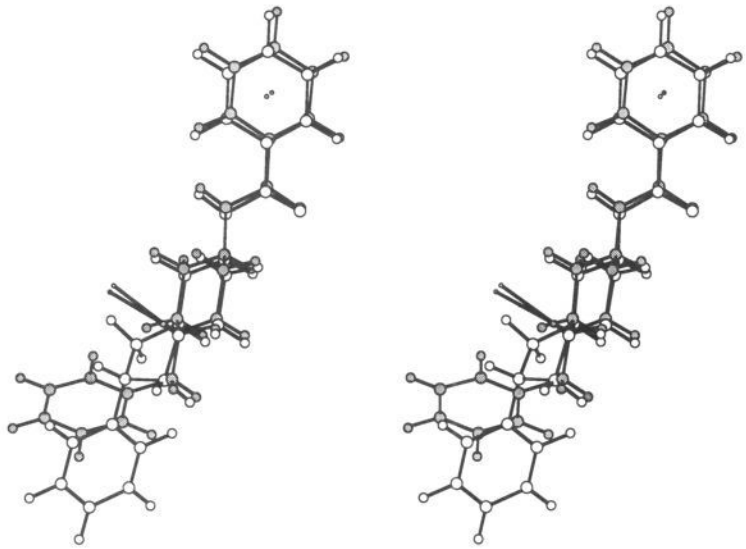


Figure 5. Least-squares superimposition of **1b** (filled atoms) with the *N*-benzyl group in gauche+ conformation and **7**. The rms value of this fit is 0.19 Å.

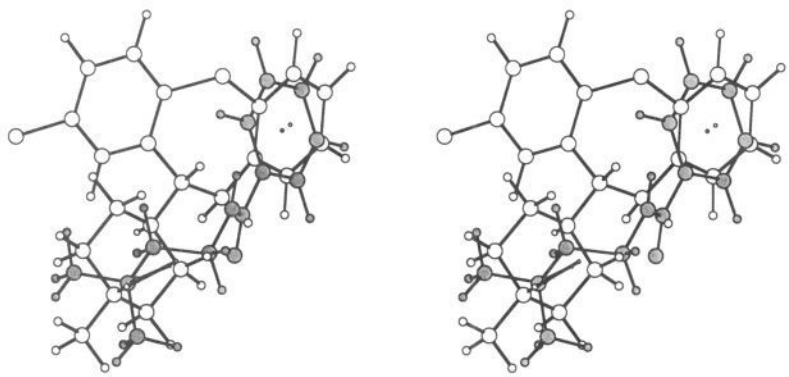
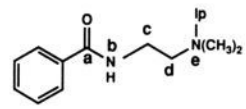


Figure 6. Molecular least-squares superimposition of **2** (filled atoms) in conformation 3 (Table II) and (*S*)-octoclothepin. The rms value is 0.39 Å.

II) was initially set to 90°. Rotations about the c–d and d–e bonds generate nine different staggered conformations. The results from the energy minimizations of these nine trial structures are shown in Table II. The lowest energy minimum is found to be a folded conformation, conformation 1 in Table II. For this conformation the N–C–C–N (b–c–d–e) torsional angle is −48°. Conformation 2 with a conformational energy of 0.7 kcal/mol is also a folded structure. Conformations 3 and 4 are 1.0 and 1.4 kcal/mol higher in energy than 1, respectively. These two conformations correspond to extended arrangements of the amide side chain. The results of these calculations are in good agreement with the results from a ^{1}H-NMR study in $CDCl_3$ solution.[26] This study indicates that the gauche conformation with respect to the N–C–C–N dihedral angle is the most stable one and that the trans (extended) conformation is 0.5 kcal/mol higher in energy.

Benzamides of type **2** can be well fitted to the active conformation of (*S*)-octoclothepin provided that the amide side chain adopts an extended (anti) conformation as in conformations 3, 4 and 7 in Table II. However, only conformations 3 and 7 have lone-pair directions which are compatible with that in (*S*)-octoclothepin. The least-squares superimposition of conformation 3 and (*S*)-octoclothepin, with the carbonyl group in the benzamide pointing away from the sulfur atom in (*S*)-octoclothepin, is shown in Figure 6. The conformational energy for the benzamide side chain in this fit is 1.0 kcal/mol (Table II). In the superimposition shown, the phenyl ring has been rotated by 35° at a calculated additional energy cost for an *o*-methoxybenzamide (as **8**) of 1.0 kcal/mol. The alternative superimposition between compound **2** in conformation 7 and (*S*)-octoclothepin (not shown) gives a fit with the carbonyl group of the benzamide pointing towards the sulfur atom. The conformational energy for the ben-

Table II. Molecular Mechanics Calculations on Compound **2**



conformation	torsional angle (deg) a–b–c–d	b–c–d–e	c–d–e–lp	conformational energy (kcal/mol)
1	−145	−48	56	0.0
2	−137	49	−58	0.7
3	−93	−170	57	1.0
4	−99	172	−58	1.4
5	−91	−81	−58	1.6
6	−139	83	59	1.9
7	−98	179	178	2.2
8	−127	70	175	2.4
9	−89	−68	−178	2.9

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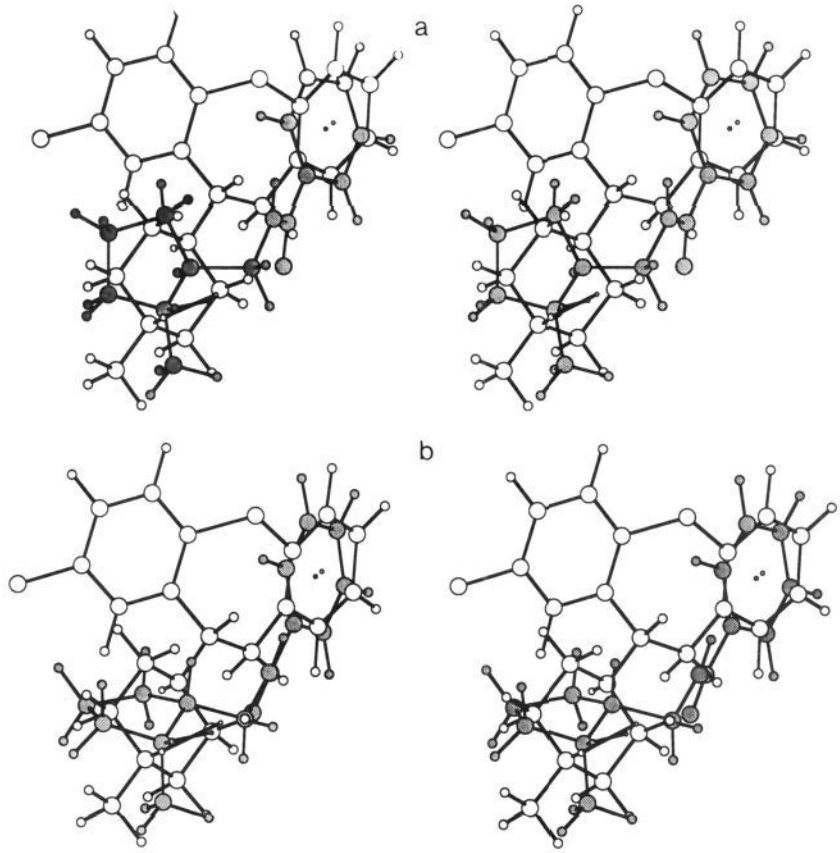


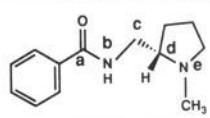
Figure 7. Least-squares superimpositions between (a) the *S*-enantiomer (rms = 0.41 Å) and (b) the *R*-enantiomer (rms = 0.45 Å) of **3** (filled atoms) and (*S*)-octoclothepin.

zamide side chain in this alternative fit is significantly higher, 2.2 kcal/mol. The additional energy penalty for coplanarity of the phenyl rings in the fit is calculated to be 1.0 kcal/mol.

The identification of an extended amide side chain conformation as the biologically active one in benzamides of type **2** is in agreement with the conclusion drawn by Testa et al.[9,10]

Benzamides of Type 3. For **3**, six different trial conformations were energy minimized. In these conformations the five-membered ring has its preferred conformation, which is an envelope conformation with the nitrogen atom out of the plane of the ring carbon atoms and with the *N*-alkyl substituent in an equatorial position. The C(=O)–N–C–C (a–b–c–d in Table III) torsional angle was set to ±100° in the trial structures, and the three staggered arrangements about the c–d bond were generated.[25] The results from the energy minimizations of the input structures are shown in Table III. As for **2**, the lowest energy minimum is found to be a folded conformation with a N–C–C–N torsional angle of −47°, conformation 1 in Table III. The second most stable conformation is an extended conformation with a N–C–C–N dihedral angle of −173°. Conformation 4 with a N–C–C–N torsional angle of 77° corresponds to a half-folded structure. This is calculated to be of 1.2 kcal/mol higher energy than the lowest energy one. Quantum chemistry calculations, using the PCILO method, on compound **3** with an *o*-methoxy group has been performed by Testa et al.[10] They find a folded conformation to be the most stable one with two extended conformations a few kcal/mol higher in energy. This is in reasonable agreement with our results. In contrast to these results, molecular mechanics calculations (MM2PI) on a substituted derivative of compound **3** performed by

Table III. Molecular Mechanics Calculations on Compound **3**



conformation	torsional angle (deg) a–b–c–d	torsional angle (deg) b–c–d–e	conformational energy (kcal/mol)
1	−119	−47	0.0
2	−91	−173	0.8
3	113	−54	1.0
4	87	77	1.2
5	105	−176	1.2
6	−164	76	1.8

Högberg et al. give a half-folded conformation as the most stable one and a folded conformation less than 0.5 kcal/mol higher in energy.[7] An X-ray analysis of the tartrate of **9** shows that the N–C–C–N torsional angle adopts an extended arrangement.[27] However, the X-ray structure of an analogue, FLA 797 (compound **10**), has a gauche N–C–C–N torsional angle, giving the molecule a half-folded conformation.[28]

For benzamides of type **3** the *S*-enantiomer is the most potent DA D-2 receptor antagonist (Table I). The best fit of (*S*)-**3** to (*S*)-octoclothepin is obtained for an extended conformation (conformation 2) of (*S*)-**3**. In this fit, shown in Figure 7a, the carbonyl group is pointing away from the sulfur atom in (*S*)-octoclothepin. The conformational energy for the benzamide side chain in this superimposition is 0.8 kcal/mol. In the fit shown, the phenyl ring has been rotated by 30°, which is calculated to require an additional 0.8 kcal/mol for an *o*-methoxy-substituted benzamide. Thus, as was the case for benzamides of type

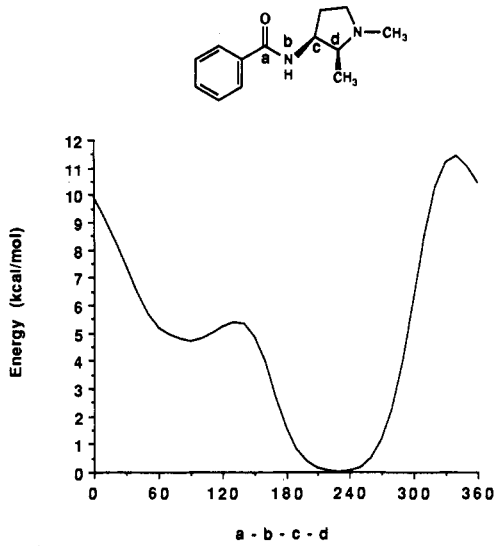


Figure 8. Calculated potential energy curve for rotation about the C(=O)–N–C–C(CH_3) dihedral angle in **4a**.

1 and 2 discussed above, an extended conformation of (*S*)-3 is the most likely biologically active conformation of this type of benzamides. The optimal orientation of the benzamide in the superimposition with (*S*)-octoclothepin is, as for 1 and 2, the one in which the amide carbonyl group is pointing away from the sulfur atom. The active conformation of (*S*)-3 deduced above is in agreement with Testa et al.[10] and Rognan et al.[11] but in disagreement with Högberg et al.[7] who suggested a half-folded conformation to be responsible for the biological activity.

In order to accommodate the *R*-enantiomer of 3 in the model with the amide carbonyl group pointing away from the sulfur atom in (*S*)-octoclothepin, an extended conformation with the *N*-alkyl substituent in an axial position has to be used as shown in Figure 7b. The conformational energy penalty of this conformation is calculated to be 3.3 kcal/mol. An additional 1.0 kcal/mol is calculated to be required to achieve the approximate coplanarity of the phenyl rings in this fit.

In order to fit the *R*-enantiomer of 3 to (*S*)-octoclothepin in the alternative orientation, a half-folded conformation with the *N*-alkyl substituent in an axial position must be employed (not shown). The energy penalty for such a conformation is calculated to be 4.7 kcal/mol. In order to achieve coplanarity of the phenyl rings in the fit an additional 0.6 kcal/mol is calculated to be required.

For the enantiomers of 3, the relative affinity for the DA D-2 receptor is approximately 100 (Table I). As a conformational free-energy increase of 1.4 kcal/mol at 300 K corresponds to a decrease of the affinity by a factor of 10, the calculated energy difference between the enantiomers of 3 in their deduced biologically active conformations (Figures 7a and 7b) corresponds well to their observed relative affinity. Thus, the DA D-2 receptor interaction employed in this study is also to rationalize the enantioselectivity of benzamides of type 3 in terms of the conformational energy difference of the deduced receptor-bound conformations.

Benzamides of Type 4. The calculated potential energy curve for rotation about the C(=O)–N–C–C(CH_3) (a–b–c–d) in **4a** is displayed in Figure 8. As for benzamides of type 3, the five-membered heterocyclic ring is calculated to prefer an envelope conformation with the nitrogen atom out of the plane of the other ring atoms and with the *N*-alkyl substituent in an pseudoequatorial position. The lowest energy minimum for **4a** is found for a torsional angle of 207°. This conformation is virtually identical to the one found by X-ray crystallography of compound 12 (Chart II).[29]

The N–C–C–N torsional angle in the calculated lowest energy structure of **4a** is −74°. In order to obtain an optimal structural fit between **4a** and (*S*)-octoclothepin the N–C–C–N torsional angle has to be changed to ca. −120° giving the five-membered ring an envelope conformation with the unsubstituted methylene group in an α position to the nitrogen atom out of the plane of the remaining ring atoms. The calculated energy penalty for this conformational rearrangement is 2.2 kcal/mol. The superimposition is shown in Figure 9. The alternative superimposition with the amide carbonyl group pointing towards the sulfur atom in (*S*)-octoclothepin may be achieved with a conformation of the five-membered ring in **4a** in which the N–C–C–N torsional angle is −164°. The rms value for this fit (not shown) is similar to that in Figure 9, but the energy penalty is somewhat higher, 3.3 kcal/mol. In this alternative superimposition an additional 0.8 kcal/mol is calculated to be required for coplanarity of the phenyl rings.

In the least-squares superimposition in Figure 9 the (*S*,*S*)-enantiomer of **4a** has been employed. The (*R*,*R*)-enantiomer may also be fitted to the template molecule with a similar rms value (0.40 Å) as for the fit in Figure 9 (0.45 Å). However, in the fit involving (*R*,*R*)-**4a** the calculated conformational energy for the benzamide is high, 5.1 kcal/mol. There are to our knowledge no reports on the D-2 receptor affinity of the pure enantiomers of benzamides of type 4.

As was the case for compound 1, the nitrogen atom in benzamides of type 4 has to be substituted with a benzyl group in order to obtain a high affinity for the DA D-2 receptor. If a benzyl group is substituted at the nitrogen atom (compound **4b**), this group can adopt three different staggered conformations with respect to the 1p–N–C–C torsional angle: gauche+, gauche−, and anti. The energy differences between the three staggered conformations with respect to the *N*–CH_2Ph bond in **4b** are calculated to be less than 0.7 kcal/mol. A structural comparison with compound 7 show that the conformation of **4b** in which the 1p–N–C–C torsional angle is in an anti conformation have the phenyl ring in the same area as compound 7.

Phenylpyrrole 5. Compound 5 has strong structural similarities to benzamides of type 3. The molecular mechanics calculations were performed with the pyrrolidine ring in the lowest energy envelope conformation. The potential energy curve for rotation about the N–C–C–N bond is shown in Figure 10. The N–C–C–N torsional angle is calculated to be 130° in the lowest energy minimum and about 330° in a shallow local energy minimum. The energy difference between the two minima is small, 0.1 kcal/mol.

As for the benzamides 1–4 the superimposition of 5 with the template molecule can be done with two different

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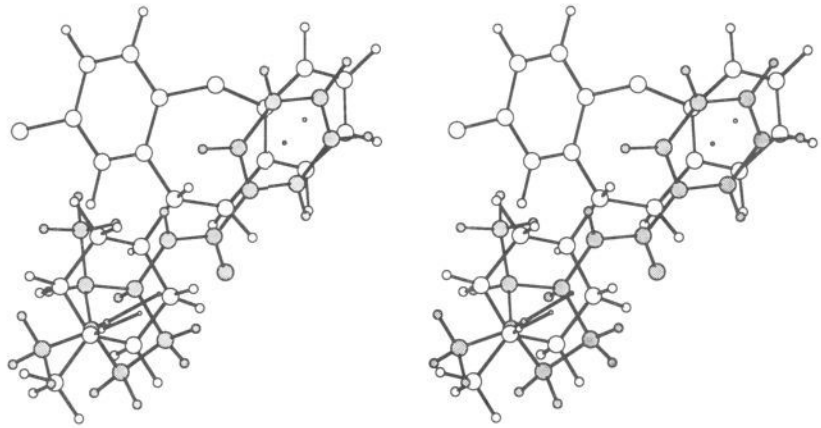

Figure 9. Least-squares superimposition of **4a** (filled atoms) and (*S*)-octoclothepin. The rms value of this fit is 0.74 Å.

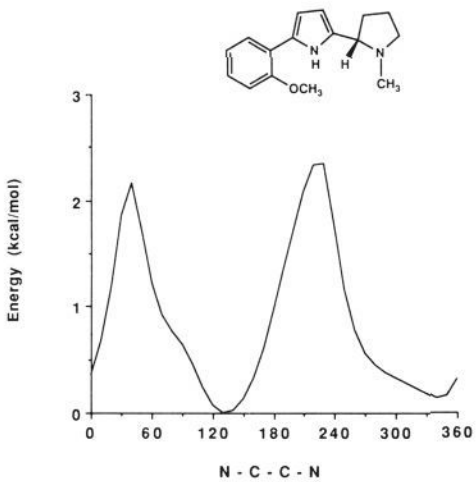

Figure 10. Calculated potential energy curve for rotation about the N–C–C–N dihedral angle in **5**.

orientations of **5**. The methoxy group of **5** may be directed toward or away from the sulfur atom in (*S*)-octoclothepin. If the superimposition is done with the methoxy group pointing towards the sulfur atom, the N–C–C–N torsional angle has to adopt a value of about 270°. The conformational energy penalty for such a conformation is 0.5 kcal/mol. The least squares fit is shown in Figure 11. In this fit the phenyl ring of **5** has been rotated by 10° to achieve an optimal coplanarity of the phenyl rings. The energy cost for this in an *o*-methoxy-substituted case is calculated to be only 0.2 kcal/mol. This fit corresponds to the one with the carbonyl group in the benzamides pointing away from the sulfur atom in the template molecule. The alternative superimposition (not shown) requires a conformation in which the N–C–C–N torsional angle is about 70°. The energy penalty for this conformation is 0.9 kcal/mol. Also in this case an additional 0.2 kcal/mol is required for an optimal coplanarity of the phenyl rings in the fit.

The molecular superimposition in Figure 11 was obtained by using the *S*-enantiomer of **5**. As for the analogous compound **3**, this enantiomer gives a better least squares fit to the template molecule and a lower conformational energy than the *R*-enantiomer.

The *N*-Alkyl Substituent. For the compounds discussed in this work, it can be seen in the molecular superimpositions in Figures 3, 7, 9, and 11 that the *N*-alkyl substituents are pointing in two different directions. These directions are denoted A and B and are schematically shown in Figure 12 in relation to the template molecule (*S*)-octoclothepin. Compounds **1** (Figure 3) and **4** (Figure 9) have their *N*-alkyl substituent pointing in direction B, while compounds **3** (Figure 7) and **5** (Figure 11) have their *N*-alkyl substituents pointing in direction A. Thus, it may be speculated that the *N*-alkyl substituent of different types of benzamides interacts with different receptor sites, rationalizing the requirement of a benzyl substituent in benzamides of types **1** and **4** for a high receptor affinity but not in benzamides of type **3**. This supports the conclusions drawn by Rognan et al.[11]

Conclusions

Low-energy conformations of the four different types of benzamides and a phenylpyrrole analogue shown in Chart I can be well accommodated in the DA D-2 receptor

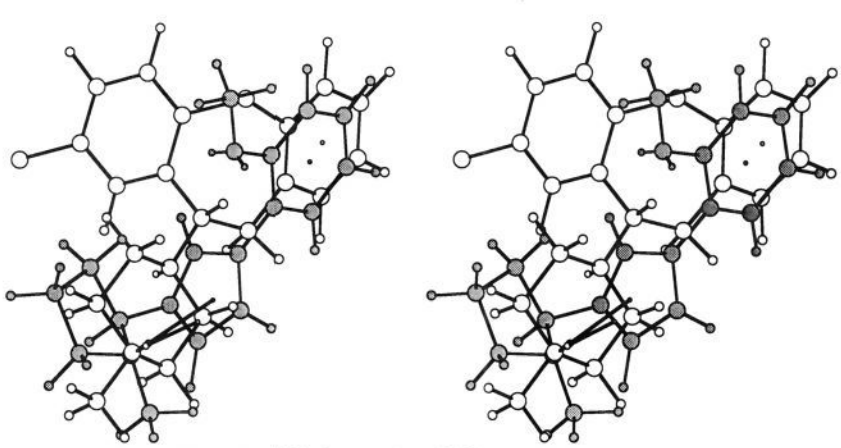

Figure 11. A least-squares superimposition of **5** (filled atoms) and (*S*)-octoclothepin. The rms value of this fit 0.76 Å.

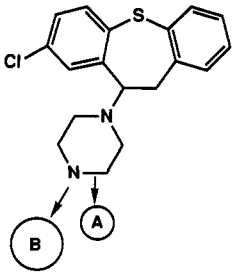

Figure 12. Schematic illustration showing the proposed *N*-alkyl interaction sites, A and B in relation to the structure of the template molecule (*S*)-octoclothepin. A is the site proposed to be used by the alkyl group in compounds with an *N*-ethyl substituent, while B is the proposed site for an *N*-benzyl substituent.

interaction model proposed by Liljefors and Bøgesø, extending this model to include the important benzamide class of DA D-2 receptor antagonists. For benzamides with an acyclic amide side chain, the most probable receptor-bound conformation is the one with an extended alkyl substituent. The enantioselectivity of the chiral benzamide of type **3** may be rationalized in terms of conformational energy differences for the receptor bound enantiomers. The *N*-alkyl substituent of the benzamides is proposed to be able to interact with two different sites for the *N*-alkyl substituent. For the benzamides studied in this work, the *N*-benzyl groups of compounds **6** and **12** are proposed to interact with one receptor site, while the alkyl group in benzamides with a *N*-ethyl group (compounds **9**, **10**, and **11**) may interact with the other site.

Acknowledgment. This work was supported by grants from the H. Lundbeck Foundation, Copenhagen, Denmark (to I.P.) and the Swedish Natural Science Research Council (to T.L.).

Registry No. **1a**, 1141-58-8; **1b**, 971-34-6; **2**, 63224-18-0; **3**, 140660-08-8; **4a**, 140660-09-9; **4b**, 140676-32-0; **5**, 140660-10-2; **6**, 55905-53-8; **7**, 140849-89-4; **8**, 364-62-5; (*R*)-**9**, 98527-07-2; (*S*)-**9**, 84225-95-6; **10**, 23672-07-3; **11**, 110319-89-6; **12**, 75272-39-8; dopamine, 51-61-6.

2-Alkynyl Derivatives of Adenosine and Adenosine-5′-*N*-ethyluronamide as Selective Agonists at A_2 Adenosine Receptors[1]

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In the search for more selective A_2-receptor agonists and on the basis that appropriate substitution at C2 is known to impart selectivity for A_2 receptors, 2-alkynyladenosines **2a–d** were resynthesized and evaluated in radioligand binding, adenylate cyclase, and platelet aggregation studies. Binding of [^{3}H]NECA to A_2 receptors of rat striatal membranes was inhibited by compounds **2a–d** with K_i values ranging from 2.8 to 16.4 nM. 2-Alkynyladenosines also exhibited high-affinity binding at solubilized A_2 receptors from human platelet membranes. Competition of 2-alkynyladenosines **2a–d** for the antagonist radioligand [^{3}H]DPCPX and for the agonist [^{3}H]CCPA gave K_i values in the nanomolar range, and the compounds showed moderate A_2 selectivity. In order to improve this selectivity, the corresponding 2-alkynyl derivatives of adenosine-5′-*N*-ethyluronamide **8a–d** were synthesized and tested. As expected, the 5′-*N*-ethyluronamide derivatives retained the A_2 affinity whereas the A_1 affinity was attenuated, resulting in an up to 10-fold increase in A_2 selectivity. A similar pattern was observed in adenylate cyclase assays and in platelet aggregation studies. A 30- to 45-fold selectivity for platelet A_2 receptors compared to A_1 receptors was found for compounds **8a–c** in adenylate cyclase studies.

Adenosine appears to mediate a wide variety of physiological functions including vasodilatation, vasoconstriction in the kidney, cardiac depression, inhibition of lipolysis, inhibition of platelet aggregation, inhibition of lymphocyte functions, inhibition of insulin release and potentiation of glucagon release in the pancreas, inhibition of neurotransmitter release from nerve endings, stimulation of steroidogenesis, and potentiation of histamine release from mast cells.[2] Many of its effects can be attributed to the action at receptors located on the cell surface, which are mediated by at least two extracellular receptors divided into two major subtypes, called A_1 and A_2.[3]

At A_1 receptors the most active analogues are N^6-substituted adenosines, whereas at A_2 receptors the most active compounds are adenosine-5′-*N*-alkyluronamides. We recently reported the synthesis of N^6-substituted 1-deazaadenosines,[4] and of 2-chloro-N^6-cyclopentyladenosine (CCPA) which proved to be an agonist with high affinity and approximately 10 000-fold selectivity for A_1 adenosine receptors.[5,6]

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