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Crystallographic, theoretical and molecular modelling studies on the conformations of the salicylamide, raclopride, a selective dopamine-D_2 antagonist*

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The structure of the potent dopamine-D_2 antagonist, raclopride, (*S*)-3,5-dichloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamide (+)-tartrate, has been determined by X-ray crystallography. The benzamide moiety of raclopride is planar in accordance with other salicylamides (FLA 797 and eticlopride). The planar conformation is stabilized by two intramolecular hydrogen bonds, i.e. one between the amide hydrogen and the methoxy group and one between the phenol hydrogen and the carbonyl group. The side-chain of raclopride has an extended conformation in contrast to the solid state conformations of FLA 797 and eticlopride. The side-chain conformations were studied by rigid rotations followed by MM2PI relaxations of the eight local minima found. Small energy differences (<4·0 kcal mol^{-1}) exist between the various extended and folded conformations. Based on modelling studies with piquindone as template, it is suggested that the salicylamides with *N*-ethyl-2-pyrrolidinylmethyl side-chains interact with the dopamine-D_2 receptor in a folded or a half-folded conformation.

Two categories of central dopamine receptors, dopamine-D_1 and dopamine-D_2, have been identified (Stoof & Kebabian 1984). Dopamine-D_1 receptors are positively coupled to adenylate cyclase, whilst dopamine-D_2 receptors are either unconnected or negatively coupled to adenylate cyclase (Stoof & Kebabian 1981, 1984; Meunier & Labrie 1982). The substituted benzamides are a recently developed class of compounds, which preferentially block the dopamine-D_2 receptors (Jenner & Marsden 1981; Ögren et al 1984, 1986; de Paulis 1985; Hall et al 1986). The mode of action of antipsychotic agents has been suggested to be related to the ability to block dopamine-D_2 receptors (e.g. Seeman 1980).

There exist several main types of benzamide derivatives with different side chains and/or aromatic substituents. The common denominator is the 2-methoxybenzamide part often referred to as orthopramide. The side-chains are of four principal types, i.e. aminoethyl (metoclopramide), 2-pyrrolidinylmethyl (sulpiride), 3-pyrrolidinyl (YM 09151-2) and 4-piperidyl (clebopride) as shown in Fig. 1. Most of the first reported derivatives (e.g. sulpiride) are substituted in the 5-position and unsubstituted in the 6-position. Recently, a series of compounds containing 6-methoxy (remoxipride) or 6-hydroxy (eticlopride, raclopride, FLA 797) substituents have been reported from these laboratories (Fig. 2) (Florvall & Ögren 1982; Ögren et al 1984, 1986; de Paulis et al 1985a, b, 1986; Hall et al 1986). Remoxipride was shown to improve schizophrenic symptomatology in an open study (Lindström et al 1985). The salicylamides, [^{3}H]eticlopride and [^{3}H]raclopride, have proved to be potent and selective dopamine-D_2 receptor ligands under in-vitro as well as in-vivo conditions (Köhler et al 1985, 1986; Hall et al 1985; Hall & Wedel 1986; Köhler & Radesäter 1986). By virtue of these properties, [^{11}C]raclopride has been

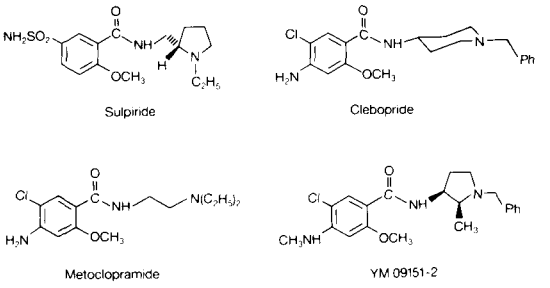

FIG. 1. Representative examples of antidopaminergic 2-methoxybenzamides containing the four main types of side-chains.

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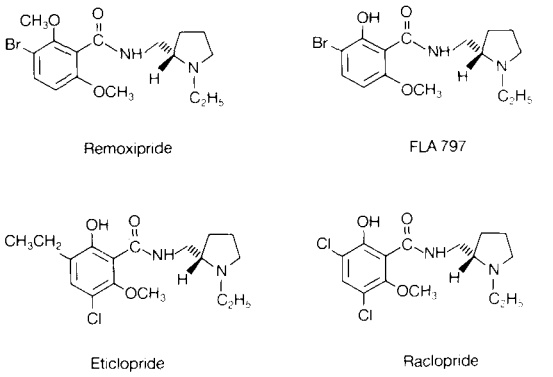

FIG. 2. Recently developed 2-methoxybenzamides with 6-methoxy or 6-hydroxy substituents.

used in positron emission tomography (PET) to visualize the dopamine-D_2 receptors in the living human brain (Farde et al 1985, 1986). Moreover, it has been suggested that raclopride is able to discriminate between various subclasses of functionally coupled dopamine-D_2 receptors (Ögren et al 1986).

Many experimental and theoretical studies have been done to elucidate how the substituted benzamides may interact with the dopamine receptor at the molecular level (e.g. Testa et al 1986). The 4-piperidyl derivatives (e.g. clebopride) exist exclusively in the extended form, whereas the 2-pyrrolidinylmethyl benzamides have considerable conformational flexibility (van de Waterbeemd & Testa 1983). By utilizing various topographical dopamine receptor models, both extended (Testa et al 1986) and folded (Högberg et al 1986) conformers of 2-pyrrolidinylmethyl derivatives have been proposed to be the conformation recognized by the receptor. Such receptor models have been derived from conformationally restricted compounds. Humber et al used butaclamol and isobutaclamol (Fig. 3) to define coordinates of a widely used 3-D receptor map including a naphthalene binding site (α and β regions), a nitrogen binding site and a lipophilic accessory binding site 4·5 Å away from the nitrogen site (Humber et al 1979b; Philipp et al 1979). Olson et al (1981) have used piquindone (Fig. 3) and other antipsychotics as model compounds. The pyrroloisoquinoline structure contains three recognition sites, i.e. a π-excessive pyrrole ring (π_1-site), a keto function (π_2-site) and an aliphatic nitrogen, which chemically relate to the 2-hydroxy-6-methoxyphenyl group, the amide carbonyl and the pyrrolidine nitrogen in the salicylamides. Contrary to the (iso)-butaclamol model, the piquindone model has no strict requirement for an accessory lipophilic binding.

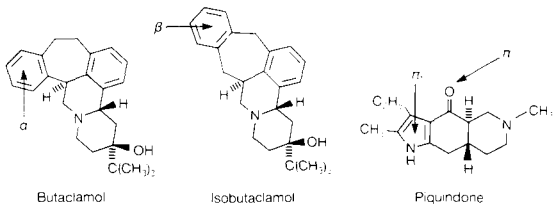

FIG. 3. Conformationally restricted neuroleptics used in dopamine receptor mapping. Pertinent pharmacophores are indicated according to original designations.

Theoretical and crystallographic investigations provide essential information about possible low-energy conformations likely to be involved in the receptor interaction. As a part of the structure-activity studies of the 6-oxygenated 2-methoxybenzamides (Fig. 2), the solid state conformations of remoxipride, FLA 797 (Högberg et al 1986) and eticlopride (Wägner et al 1985; de Paulis et al 1985b) have been determined.

In the present paper we report on the X-ray structure of raclopride tartrate. Furthermore, the conformational behaviour of raclopride, a representative model for the group of substituted salicylamides (including eticlopride and FLA 797), has been investigated using molecular mechanics (force field) calculations (cf. Burkert & Allinger 1982). Finally, the different solid state conformations and the theoretically derived conformations have been related to the preferred rigid template, i.e. piquindone, by means of distance parameters and molecular modelling.

MATERIALS AND METHODS

Crystallography

Single crystals of raclopride (+)-tartrate, FLA 870, $C_{15}H_{20}O_3N_2Cl_2 \times C_4H_6O_6$ were obtained from recrystallization in absolute ethanol. The crystal chosen for the data collection was 0·08 × 0·62 × 0·64 mm in size and oriented with the crystallographic b-axis parallel to the ϕ-axis of the goniostat. A Philips PW 1100 automated diffractometer, equipped with monochromatized CuK(α)-radiation was used to measure 2011 unique reflections, utilizing the ω-2θ scan technique. The θ-range was: 1–67°; scan width 1·4° and scan speed 0·035° s^{-1}. All observed reflections were corrected for Lorenz and polarization factors as well as for absorption. Least squared refined unit cell parameters were calculated from an X-ray powder diffraction photograph taken in a Guinier-Hägg focusing camera with monochro-

matized CuK(α_1)-radiation (λ = 1·54056 Å) and Si (a = 5·4309 Å) as internal standard (Malmros & Werner 1973). The crystal structure was elucidated by a combination of Patterson- and direct methods. From the Patterson synthesis the positions of the pseudo-related Cl-atoms were determined and the knowledge was exploited in the MULTAN 80 program (Main et al 1980). Many successive Fourier maps had to be calculated before a reasonable structural model was found.

The structure was completed and refined in the program system SHELX 76 (Sheldrick 1976). All non-H atoms were refined anisotropically except for C(12), which was suffering from abnormal thermal vibration or disorder. Most H-atom positions were calculated from known geometrical considerations (C–H = 1·08 Å) and supplied with four different isotropic thermal factors. Exceptions were the hydrogen-bonded H-atoms H(3), H(8) and H(14) of the raclopride molecule and the hydroxyl H-atom H(3″) of the tartrate, which were refined individually with isotopic thermal parameters. The two carboxyl H-atoms of the tartrate group could, however, never be located. In the last cycle of refinement the largest parameter shift was 0·022 of the non-H atoms. Five reflections (11 5 5, −3 0 1, 1 0 0, −15 4 6, −16 0 5) were omitted due to their abnormally high δ/σ ratio.

A summary of some selected crystal and experimental data is given in Table 1. Lists of atomic coordinates and anisotropic thermal parameters have been deposited with the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK. Any request should be accompanied by the complete literature citation of this paper. Structure factors and Bijvoet differences are available from the authors.

Table 1. Some selected crystal data for raclopride tartrate.

Formula	$C_{15}H_{20}O_9N_2Cl_2 \cdot C_2H_6O_6$
Space group	$P2_1$
Number of molecules in the unit cell	Z = 2
Cell dimensions	a = 16·214(7) Å b = 9·567(3) Å c = 7·581(2) Å β = 108·20(3)°
Calculated density	d_c = 1·422 g cm^{-3}
Linear absorption coefficient (CuKα)	μ = 30·2 cm^{-1}
Number of unique collected reflections	2011
Number of reflections for which $F \geq 6\sigma(F)$	1922
Number of parameters refined	309
Final linear R-value	R = 5·80%
Weighted R-value {w = 22·76 [σ^2(F) + 0·009(F)^2]$^{-1}$}	R_w = 5·91%

Calculations

We have used a molecular mechanics program MM2PI, which is similar to the MMPI-MM2 hybrid force field developed by Lipkowitz et al (1984). The MM2PI program uses the π-system routines of MMPI together with the MM2 equations and parameters. MM2PI provides geometries and energies that are comparable with those obtained by MMP2 (Norinder unpublished). The parent rigid trial structures of raclopride were obtained by using the MACCS program (Molecular Design Ltd, Hayward, California, USA) followed by MM2PI relaxation. The rigid rotations were carried out with a subroutine in the MIMIC program (Liljefors 1983). The molecular modelling was performed by comparison of the molecules with proper alignment according to the least-squares fitting routine in CHEM-X (developed and distributed by Chemical Design Ltd, Oxford, UK).

RESULTS

Crystallography

The molecular structure and atom numbering, shown in Fig. 4, refer to the correct absolute

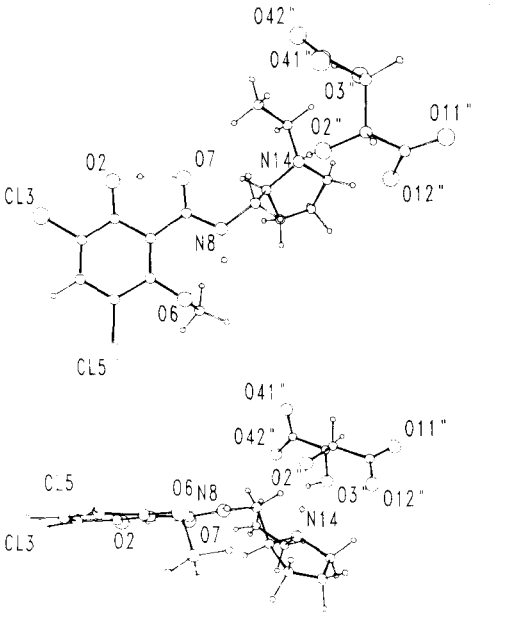

FIG. 4. Molecular structure and atom numbering of raclopride (+)-tartrate displayed from two different angles. Atoms with a prime refer to the tartrate anion.

configuration of raclopride (+)-(*R,R*)-tartrate. The pertinent possible intra- and intermolecular hydrogen bonds are shown in Table 2. The molecular packing arrangement is drawn in a mixed ball-and-stick/van der Waals style for clarity (Fig. 5). The absolute configuration, measured from Bijvoet differences of 14 reflections, entirely confirmed the (*S*)-enantiomeric form (Bijvoet et al 1951). The (+)-tartrate anion, being an asymmetric marker in itself, was determined to possess the (*R,R*)-configuration.

Table 2. Possible intra- and intermolecular hydrogen bonds in raclopride tartrate with estimated standard deviations in parentheses. Primed atoms refer to the tartrate molecule.

	Distances (Å)			Angle (°)
Donor-H · · · Acceptor	D · · · A	D–H	H · · · A	<D-H · · · A
O(2)-H(2) · · · O(7)	2·467(6)	0·88(5)	1·60(5)	169(6)
N(8)-H(8) · · · O(6)	2.704(6)	1·05(8)	1·94(7)	127(5)
N(14)–H(14) · · · O(2″)	2·880(7)[a]	0·63(9)	2·28(10)	163(11)

Intramolecular distances (Å)

Tartrate

O(12″) · · · O(2″)	2·630(5)
O(3″) · · · O(2″)	2·816(6)
O(3″) · · · O(42″)	2·626(6)

Intermolecular distances (Å)

Raclopride–Tartrate		Tartrate–Tartrate	
N(8) · · · O(11″)	3·009(6)[b]	O(2″) · · · O(42″)	2·637(6)[c]
O(6) · · · O(11″)	3·130(5)[b]	O(12″) · · · O(41″)	2·492(5)[d]

Symmetry operations: [a] x, y, z; [b] 2 − x, ½ + y, 2 − z; [c] 2 − x, ½ + y, 1 − z; [d] x, y, z + 1.

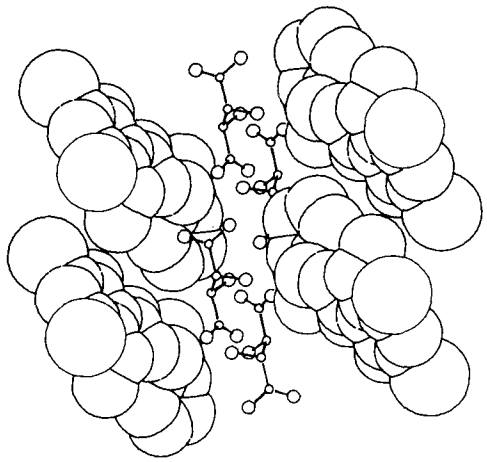

FIG. 5. Molecular packing arrangement of the raclopride (+)-tartrate, viewed along the crystallographic b-axis and drawn in a mixed ball-and-stick/van der Waals style. The four most closely packed molecules involved in short intermolecular contacts are shown. No H-atoms are included.

The benzamide part of the raclopride molecule adopts a rigid planar conformation stabilized by two intramolecular hydrogen bonds. Except for the common interaction from the amide nitrogen to the methoxy oxygen, N(8)–H(8) · · · O(6) = 2·704(6) Å, found in most *ortho*-methoxy benzamides, another hydrogen bond between the phenol and carbonyl oxygen atoms, O(2)–H(2) · · · O(7) = 2·467(6) Å, is also found. This observation is in agreement with other *ortho*-hydroxy substituted benzamides (salicylamides) (Wägner et al 1985; Högberg et al 1986). It is notable that the only component diverging from the plane is the methyl group of the methoxy moiety.

The side-chain in raclopride has an extended conformation in contrast to the other investigated salicylamides. The interchain torsion angles τ_3: C(7)N(8)–C(9)C(10) and τ_4: N(8)C(9)–C(10)-N(14), possessing the greatest conformational freedom, are −78° and 176°, respectively. The conformation of the pyrrolidine ring takes a half-chair form, with the two-fold axis through the C(12) atom and with a phase angle P = 88(5)° (Cremer & Pople 1975). The dihedral angle between the pyrrolidine and benzene ring least-squares planes is 41·8(3)°.

The distances between the centre of the benzene ring and the tertiary nitrogen N(14) is 7·32 Å and the out-of-plane distance is 0·84 Å. The distances to N(14) from the centres of the two pseudo-rings including N–H · · · O and O–H · · · O are 5·25 Å and 5·47 Å, respectively.

Bonds involving the tartrate carboxyl group C(1″)O(11″)O(12″) are normal with a separation of 0·1 Å of the C–O and C=O distances. The corresponding values of the deprotonated carboxyl group C(4″)O(41″)O(42″) are equal. Remarkably, the hydroxy oxygen O(2″), instead of the carboxylate oxygen in the tartrate, is involved in the hydrogen bonding to the protonated tertiary nitrogen N(14) of the raclopride molecule. Thus, the hydroxyl O(2″)H acts as a hydrogen bonding bridge between the two ionic centres. The short intermolecular interaction between the carboxyl oxygen O(11″) and the amine nitrogen N(8) of the raclopride molecule is also notable.

Calculations

To ascertain relevant basic structural parameters in the calculations, we have utilized salient features from the crystal structures (cf. Table 3). The benzamide moiety in the solid state conformations of all salicylamides examined so far (vide infra) has a planar arrangement, i.e. a torsion angle

Table 3. Selected topographical X-ray features of various antidopaminergic compounds.

Compound	Distances (Å)[a]		Torsion angles (°)[b]					Theor. min[c]	Ref.
	Ar-N	N-plane	τ_1	τ_2	τ_3	τ_4	τ_5		
Raclopride tartrate	7·3	−0·8	− 7	−173	− 78	176	−115	4	
Eticlopride HCl	6·2	2·6	− 5	−179	93	60	− 98	1	Wägner et al (1985)
FLA 797 (base)	6·0	0·5	− 4	177	−161	− 51	− 75	3	Högberg et al (1986)
(±)-Sulpiride (base)	7·4[d]	0·8[d]	−13	179	−124	171	− 69	5	Houttemane et al (1981)
(±)-Sulpiride HCl[e]	6·1[d]	2·3[d]	− 5	178	84	59	−103	1	Blaton et al (1981)
Piquindone HCl[e]	5·9	0·1							Olson et al (1981)
(±)-Butaclamol HBr	5·1[d]	0·5[d]							Bird et al (1976)
(+)-Isobutaclamol HBr	6·0[d]	1·1[d]							Humber et al (1979a, b)

[a] Distances from the nitrogen to the centre of the aromatic ring (Ar–N) and above the aromatic ring plane (N-plane).
[b] Positive sign for clockwise rotation. τ_1 to τ_5 corresponds to the string (from left to right): C(6)C(1)–C(7)–N(8)–C(9)–C(10)–N(14)C(15). Cf. Table 4.
[c] Corresponding conformation calculated in Fig. 6 (rigid rotation) and Table 4 (MM2PI).
[d] Distances calculated from the X-ray coordinates obtained from Cambridge Crystallographic Data File.
[e] Torsion angles refer to the (*s*)-enantiomer.

τ_1: C(6)C(1)–C(7)N(8) of about −5°. This torsion angle was held constant at −5° in the following calculations in order to mimic the intramolecular hydrogen bond. Likewise, the amide bond has a perfect *trans* orientation [τ_2: C(1)C(7)–N(8)C(9) being about 180°] in a variety of 2-methoxybenzamides (cf. references in Högberg et al 1986). Crystallographic data on various benzamides including sulpiride, remoxipride, eticlopride and raclopride have shown that the 1-ethyl group in the pyrrolidine ring is oriented *trans* to the 2-methylamino chain. For comparative purposes, both the *cis* and the *trans* configurations of raclopride were initially subjected to conformational analysis. The torsion angles τ_3 and τ_4, which consequently mainly determine the side-chain conformations, were changed by rigid rotation (10° increments) in the two trial structures obtained from MACCS–MM2PI. The final energy surface was calculated from the grid obtained by rigid rotation using spline-interpolation. The calculations showed consistently lower (>1·8 kcal mol^{-1}) energies for the local minima of the *trans* forms in comparison with the corresponding *cis* forms. The conformation τ_3 vs τ_4 energy surface of the *trans* configuration was found to contain eight local minima (Fig. 6), which subsequently were subjected to full MM2PI relaxation except for the torsion angle τ_1. Final energies and selected geometry data are presented in Table 4.

Our calculations indicate that a half-folded conformation (#8) is the global minimum and a folded conformation (#3) is less than 0·5 kcal mol^{-1} higher in energy. However, all local minima found in this conformational search are within 4 kcal mol^{-1} of each other and include various folded and extended forms. According to this calculation the distance between the centre of the aromatic ring and the nitrogen atom can easily range from 6 to about 7·5 Å (Table 4).

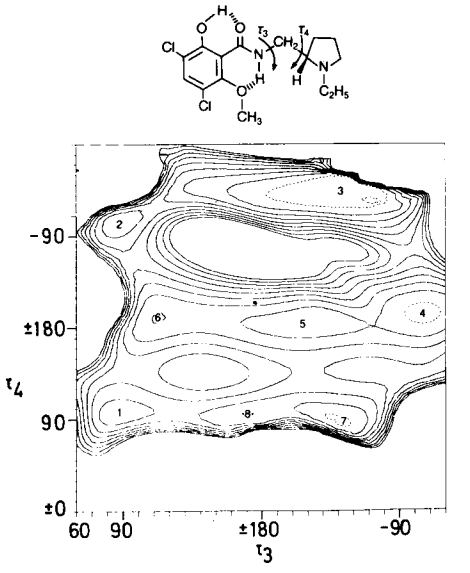

FIG. 6. Energy surface of raclopride generated by rigid rotations of τ_3 and τ_4 in 10° increments. The final energy surface was calculated from the grid using spline-interpolation.

Molecular modelling

As mentioned earlier, the most widely used conformationally restricted compounds for modelling of the dopamine receptor are (iso)butaclamol and piquindone shown in Fig. 3 (Humber et al 1979a, b; Philipp et al 1979; Olson et al 1981). The essential

distance parameters are the distance between the nitrogen atom and the centre of the aromatic ring and the height of the nitrogen atom above the aromatic ring plane. These parameters for the calculated conformations of raclopride and crystal conformations of different dopamine receptor blockers are shown in Tables 3 and 4.

Table 4. Prominent features of low-energy conformations of raclopride calculated by MM2PI.[a]

Minimum[b]	Energy[c] kcal/mol	Torsion angles (°)[d]		Distances (Å)[f]	
		τ_c[e]	τ_4[e]	Ar–N	N-plane
1	3·68	86	83	6·8	2·3
2	3·26	94	− 70	6·2	1·4
3	0·42	−118	− 49	6·0	−0·4
4	2·46	− 58	−168	7·1	−1·7
5	2·66	−164	−174	7·6	0·6
6	4·04	112	−173	6·5	1·3
7	2·16	−132	81	6·3	−2·2
8	0·00	−167	79	6·7	0·0

[a] The 1-ethyl group is oriented *trans* to the 2-methylamino chain of the pyrrolidine ring. Torsion angle τ_1C(6)C(1)–C(7)N(8) fixed at −5°.
[b] Calculated from the corresponding minimum in Fig. 6.
[c] Relative to minimum #8.
[d] Positive sign for clockwise rotation.
[e] τ_3 = C(7)N(8)–C(9)C(10), τ_4 = N(8)C(9)–C(10)N(14).
[f] Distances from the nitrogen to the centre of the aromatic ring (Ar–N) and above the aromatic ring plane (N-plane, positive sign when carbonyl is oriented upwards and nitrogen above plane of paper).

First, we compare the different solid-state conformations of the benzamides with the preferred template piquindone with respect to the distance parameters shown in Table 3. The salicylamide FLA 797 exhibits a remarkable fit both in terms of nitrogen distance from (6·0 Å) and height above (0·5 Å) the aromatic ring. The folded conformation found in eticlopride will place the nitrogen too high above the ring plane, but it might be argued that a π–π stacking or other type of interaction at the receptor can tolerate a smaller angular deviation which could bring the nitrogen within contact. However, the extended X-ray conformation of raclopride is inappropriate for an acceptable overlap. When the calculated conformations of raclopride in Table 4 are compared with piquindone, the closest match is obtained with #3, which is one of the energetically most favoured conformations.

Molecular modelling of the eight minimized raclopride conformations and the above mentioned X-ray structures, with piquindone as template, results in a more appropriate comparison than that by the simple distance parameters. The superimposition of the benzamide with piquindone was based on the three assumed recognition sites (π_1, π_2 and N) by minimization with respect to the aromatic ring atoms, carbonyl moieties and aliphatic nitrogens with attached lone-pairs. Four representative graphic overlays are shown from two different angles in Fig. 7 (a–d). The excess salicylamide volumes are contoured at the van der Waals radii.

The raclopride conformation #3, having ideal distance parameters, exhibits an excess volume primarily from the 3-, 4- and 5-pyrrolidine carbons and the 5-chloro substituent (Fig. 7a). The latter substituent, (5-Cl) in the *ortho* position with regard to the methoxy group, is not as crucial for activity as the substituent *para* to the methoxy group as mentioned earlier. Most antidopaminergic benzamides, e.g. sulpiride, clebopride and FLA 797, in fact lack a substituent in the position *ortho* to the essential methoxy group (de Paulis 1985; de Paulis et al 1985a). This is clearly seen in Fig. 7d having the solid state conformation of FLA 797 superimposed on piquindone. The overlays of these two structures (Fig. 7a, b) are similar with the exception of the excess volume generated by the less important chloro substituent in raclopride. This latter type of substituent *ortho* to the methoxy group can be tolerated by the receptor not only in the salicylamide class of compounds (de Paulis et al 1986) but also in other derivatives such as tropapride and veralipride (de Paulis 1985). It should be noted that the halogens in the 3-position of the salicylamides raclopride (Fig. 7a) and FLA 797 (Fig. 7d) are completely included in the van der Waals volume generated by piquindone. Thus, this particular type of superimposition with piquindone and *para*-substituted orthopramides is consistent with the observation that 2- and 3-alkyl substituents of limited size in the pyrrole part of piquindone related compounds are mandatory for high activity (Olson et al 1983).

The global minimum #8 of raclopride also shows a favourable overlap with piquindone (Fig. 7b), indicating that the slightly longer distance between the aromatic ring and the nitrogen in the half-folded conformation is more reasonable than originally anticipated. It should be noted that the *N*-ethyl group in conformation #8 occupies the same excess space as the pyrrolidine ring (i.e. carbons 3, 4 and 5) in conformation #3. However, conformation #2 is clearly less likely than might be expected on the basis of the distance parameters (Fig. 7c). The other structures showed less efficient overlays and are thus not depicted.

DISCUSSION

The crystal conformation of raclopride shows notable similarities with FLA 797 and eticlopride hydrochloride. The salicylamide part in all three

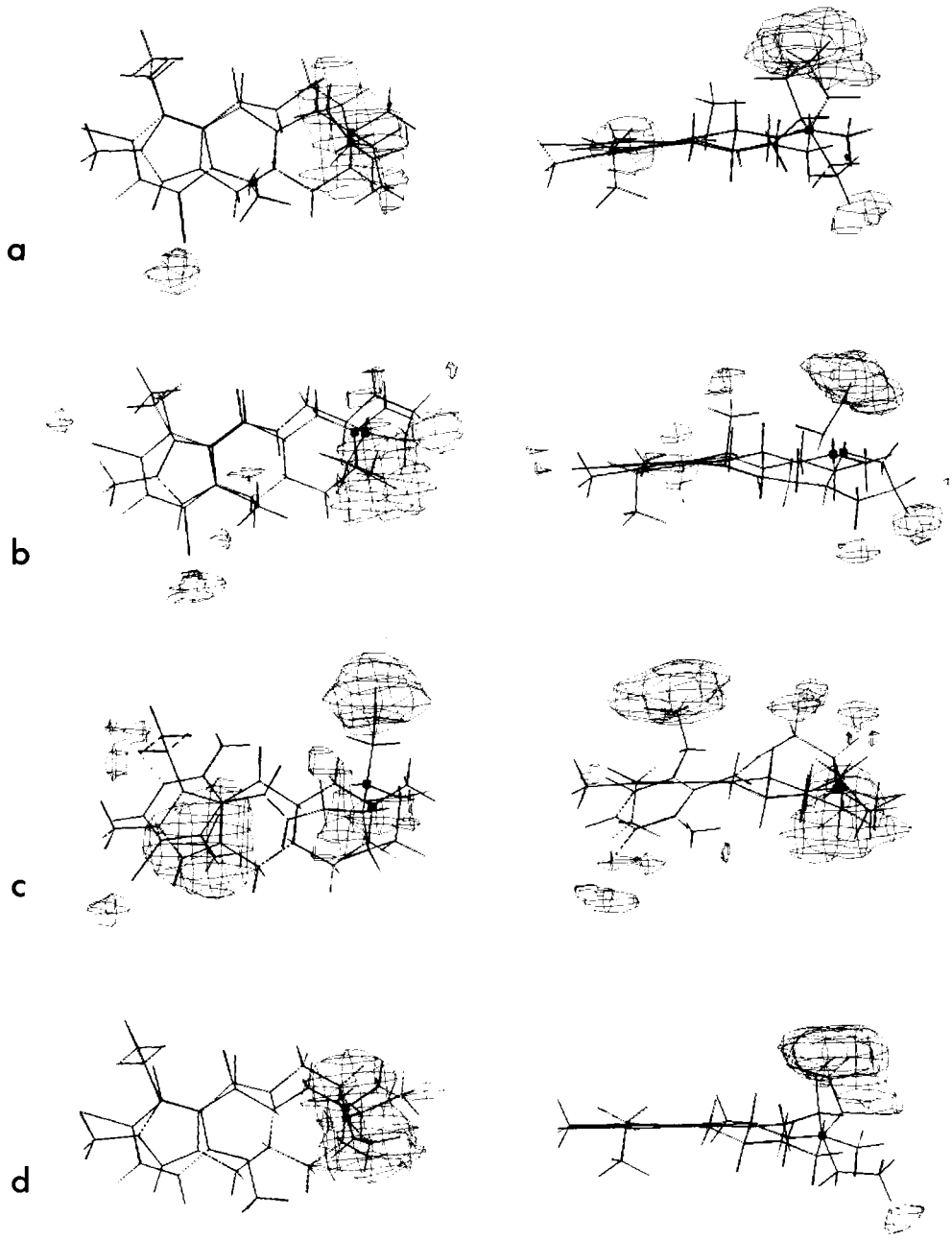

FIG. 7. Displays of two different views of the superimpositions of three pertinent calculated conformations of raclopride (a: #3, b: #8, c: #2) and the X-ray conformation of FLA 797 (d) with piquindone. The excess volume of the salicylamides is contoured at the van der Waals radii. The aliphatic nitrogens are marked.

compounds has a planar conformation which is stabilized by the two intramolecular hydrogen bonds from the amide hydrogen to the methoxy oxygen atom and from the phenol hydrogen to the carbonyl oxygen atom. This common feature, clearly shown in Table 3 by the practically identical values of τ_1 and τ_2, is thus not influenced by the differences in salt forms or aromatic substituents. It should be noted that this planar benzamide arrangement is present also in the solid state of other 2-methoxybenzamides lacking the 6-hydroxy substituent, e.g. sulpiride (Houttemane et al 1981; Blaton et al 1981), metoclopramide (Cesario et al 1981), YM-09151-2 (Furuya et al 1982) and its diastereomer YM-09151-1 (Furuya et al 1986).

The appearance of the side-chain in the crystal conformations of the salicylamides raclopride, eticlopride and FLA 797 differ considerably (τ_3, τ_4 and τ_5 shown in Table 3). The side-chain conformation is apparently independent of whether the pyrrolidine is in base or protonated form, which is underlined by comparison of the two sulpiride structures in Table 3. Thus, the extended conformation of raclopride is not necessarily due to the tartrate, which is involved in extensive hydrogen bonding in the crystal, since a similar conformation is found for sulpiride base. The X-ray studies indicate that the salicylamide moiety is also likely to be in a planar hydrogen bonded form when the compounds elicit the biological response. However, no conclusive information about which of the different side-chain conformations is preferred can be deduced on the basis of the crystal conformations alone.

The theoretical conformational analysis of the model salicylamide raclopride shows that there are a variety of folded and extended conformations possible within a small energetic span of 4 kcal mol^{-1}. In Table 3 it can be seen that the crystal conformations of raclopride, eticlopride, FLA 797 and sulpiride in fact correspond to four of the eight local minima found by the MM2PI calculations. An exact match of the torsion angles should not be expected due to the methodological differences. Yet, this fact underlines the relevance of the calculations since the X-ray analysis provides the three-dimensional arrangement of at least one of the minimum energy conformations, even if it is affected by crystal packing forces such as π–π stacking and hydrogen bonding (Kitaigorodsky 1973; Duax 1978; Högberg et al 1986).

A half-folded (#8) and a folded (#3) conformation are energetically most favoured, which partly is in line with the results obtained by van de Waterbeemd & Testa (1981). They investigated a model molecule [*N*-(1-methyl-2-pyrrolidinylmethyl)-2-methoxybenzamide] in the protonated form by the quantum mechanical PCILO method. The global minimum thus obtained was a folded conformer having a hydrogen bond between the carbonyl and the protonated nitrogen. This particular conformation stems from a *cis* orientation of the 1-methyl group and the 2-methylamino side-chain in the pyrrolidine ring. Consequently, this folded conformation has no corresponding minimum in our calculations, since we only refined the energetically more favoured *trans* forms of the free base by the MM2PI relaxation. Besides, the PCILO method is known to overestimate attractive interactions between non-bonded atoms, especially hydrogen bonds (Bendl & Pretsch 1982). However, the additional three local minima found by van de Waterbeemd & Testa (1981) are close to the minima #7, #4 and #6 obtained by the MM2PI calculation. It is important to note that low-energy folded conformations are found by force-field (MM2PI) calculations without any need for an internal hydrogen bond between the carbonyl and the pyrrolidinium group (cf. van de Waterbeemd & Testa 1983). Still, there are no conclusive arguments either for or against any of the possible side-chain conformations as being more relevant than the others in the receptor interaction. For this purpose one needs to study rigid analogues or to make comparisons with other geometrically defined antidopaminergic compounds as described in the preceding section (Fig. 7).

The 2-methoxybenzamides with *N*-ethyl-2-pyrrolidinylmethyl side-chains have several features that make piquindone the rigid template of choice, i.e. a reasonable chemical relationship, a flat aromatic/pseudo-ring arrangement and a small *N*-alkyl substituent without the requirement of accessory binding. The latter requirement is for example not met by 4-piperidyl derivatives such as clebopride which have the *N*-benzyl substituent as a prerequisite for activity (Prieto 1977). Besides, there are certain characteristics of the binding of piquindone that make it an attractive template for mapping of the benzamide conformations (Nakajima & Iwata 1984). Firstly, sulpiride, metoclopramide, molindone and domperidone exerted a more potent inhibition of [^{3}H]piquindone binding than of [^{3}H]spiperone binding, whereas other classical antipsychotics were almost equipotent at the two binding sites. Secondly, the binding of piquindone, sulpiride, metoclopramide and molindone to [^{3}H]spiperone binding sites was markedly sodium dependent in contrast to spiperone, chlor-

promazine and domperidone. Finally, pyrroloisoquinoline compounds having a nonlipophilic *N*-substituent (methyl, propyl, cyclopropylmethyl, allyl) were more potent at [^{3}H]piquindone binding sites whereas compounds with lipophilic *N*-substituents were equipotent at [^{3}H]piquindone and [^{3}H]spiperone binding sites. It has been argued that the *N*-substituents may influence the selectivity between dopamine-D_1 and -D_2 receptors (Olson et al 1983).

Based on the modelling studies of the salicylamides with piquindone shown in Fig. 7, it can be concluded that the benzamides with an *N*-ethyl-2-pyrrolidinylmethyl side-chain are likely to have a planar hydrogen-bonded pseudo-ring system and a folded or a half-folded side-chain conformation when interacting with the dopamine receptor. If this hypothesis is correct the distance between the centre of the aromatic ring and the nitrogen atom (ca 6 Å) is significantly longer than the corresponding distance in dopamine, having a fully extended *trans* side-chain (5 Å). An extended phenethylamine moiety has been proposed as an essential part of the pharmacophore in several dopamine agonists (Ernst 1967; Horn & Rodgers 1980; Cannon et al 1981; Cannon 1985; Seeman et al 1985) and antagonists (Horn & Snyder 1971; Humber et al 1975; Harbert et al 1980; van de Waterbeemd & Testa 1983) (cf. reviews by Seeman 1980; Andrews & Lloyd 1982; Kaiser & Jain 1985). For an agonist it is highly relevant to expect such a resemblance with the endogenous transmitter. However, for an antagonist this might be the case (Johansson et al 1986) but there are several other possible ways to block the receptor than merely by overlapping in a complementary fashion with the endogenous pharmacophore. Such explanations include binding to an allosteric site or binding to only one of the sites essential for the transmitter (e.g. aromatic or amine binding site) combined with one or several other accessory sites. This view lends support from the different structure–activity relationships found for agonists (e.g. Cannon 1983, 1985; Kaiser & Jain 1985) and antagonists (e.g. Kaiser et al 1980). Besides, dopamine agonists and antagonists exhibit certain different binding properties (e.g. Seeman 1980; Creese et al 1983; Seeman et al 1986; Creese & Leff 1986), which can make comparisons of their structures erroneous. This risk of using dopamine antagonists to probe the agonist binding site has been pointed out previously (e.g. Carnmalm et al 1979; Cannon et al 1981; McDermed 1983).

The phenethylamine moiety has been invoked in the pharmacophore of 2-methoxybenzamide antidopaminergics by regarding the hydrogen-bonded pseudo-ring as the aromatic counterpart (van de Waterbeemd & Testa 1983). By recent molecular electrostatic potential (MEP) studies, van de Waterbeemd et al (1986a, b) slightly revised this model due to an inadequate correspondence between regions of positive and negative potential generated by dopamine and orthopramides. The receptor topography thus proposed by van de Waterbeemd and Testa is based on an extended side-chain, contrasting with our own view. Further studies are needed to gain a deeper insight into which is the bioactive conformation. Different requirements might be involved for benzamides with a small alkyl nitrogen substituent (sulpiride, raclopride, eticlopride, etc) and for semirigidly extended benzamides which require lipophilic nitrogen substituents (clebopride, YM-09151-2, BRL 25594, etc).

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