Potential Antipsychotic Agents. 9.† Synthesis and Stereoselective Dopamine D-2 Receptor Blockade of a Potent Class of Substituted (*R*)-*N*-[(1-Benzyl-2-pyrrolidinyl)methyl]benzamides. Relations to Other Side Chain Congeners‡

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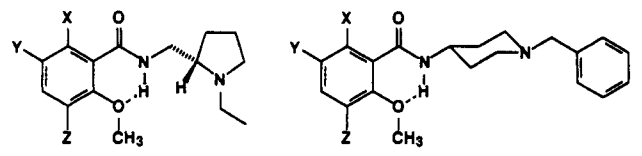
A number of substituted *N*-[(1-benzyl-2-pyrrolidinyl)methyl]benzamides and -salicylamides have been prepared and investigated as dopamine D-2 receptor antagonists in vitro and in vivo. The affinity was found to be confined to the *R* enantiomer, in contrast to the corresponding *N*-ethyl or *N*-allyl derivatives. The X-ray structure of one of the compounds (**15**) confirmed the *R* stereochemistry. This compound (**15**) was found to adopt a solid-state conformation in which the 4-fluorobenzyl group is folded over the salicylamide moiety. Benzamides having a 2,3-dimethoxy substitution pattern (**24** and **26**) or salicylamides with a 5,6-dimethoxy grouping (**21** and **22**) were especially potent, in that they inhibited [^{3}H]spiperone binding to rat striatal dopamine D-2 receptors in vitro with IC_{50} values of about 1 nM. The new compounds' ability to block apomorphine-induced stereotypies correlated with the affinity for the [^{3}H]spiperone binding site. Higher dose levels were necessary to induce catalepsy than to block the apomorphine-induced responses. The influence of the aromatic substituents on the potency of substituted benzamides with three types of side chains, i.e. (*R*)-(1-benzyl-2-pyrrolidinyl)methyl, (*S*)-(1-ethyl-2-pyrrolidinyl)methyl and 1-benzyl-4-piperidinyl, was compared. The 3-bromo-5,6-dimethoxysalicylamide substitution pattern was found to be the most general since it gave very potent compounds in all series. The substituted (*R*)-*N*-[(1-(4-fluorobenzyl)-2-pyrrolidinyl)methyl]benzamides (**26**) and -salicylamides (**22**) are suitable for development into ^{18}F radioligands without altering the parent structure.

The substituted 6-methoxy- and 5,6-dimethoxysalicylamides, e.g. **1**-**4**,[1-5] and related benzamides (**5**)[6] having 2-pyrrolidinylmethyl side chains are highly potent and selective dopamine D-2 receptor antagonists with a stereoselective affinity for the receptor and low nonspecific binding (Chart I). Since they also have a good penetration through the blood–brain barrier and a high in vivo activity, they are suitable tools for pharmacological investigations of dopaminergic functions. Different types of radioligands have also been prepared, e.g. ^{3}H-, ^{11}C-, ^{18}F-, ^{125}I-, and ^{123}I-labeled salicylamides, and successfully used in investigations of dopamine D-2 receptors in vitro and in vivo.[7-11] Animal studies indicate that several of the compounds have a low cataleptogenic profile which lend them potential to be effective drugs in the treatment of schizophrenia with a low tendency to induce extrapyramidal side effects (EPS).[5,11] Raclopride (**2**) is thus currently being investigated in clinical trials.[12]

The recently described 5,6-dimethoxysalicylamides (e.g. **4**) were found to be an extremely potent class of dopamine D-2 receptor antagonists in vitro as well as in vivo.[4] This substitution pattern (**9**) was also predicted by quantitative structure–activity relationship (QSAR) calculations to be highly favorable for benzamides with 4-piperidinyl side chains (Chart I).[13] This class of benzamides (e.g. **6**-**10**, tropapride and clebopride) require a lipophilic nitrogen substituent, such as a benzyl group, to possess high affinity for the dopamine D-2 receptor.[14-16] This prerequisite is

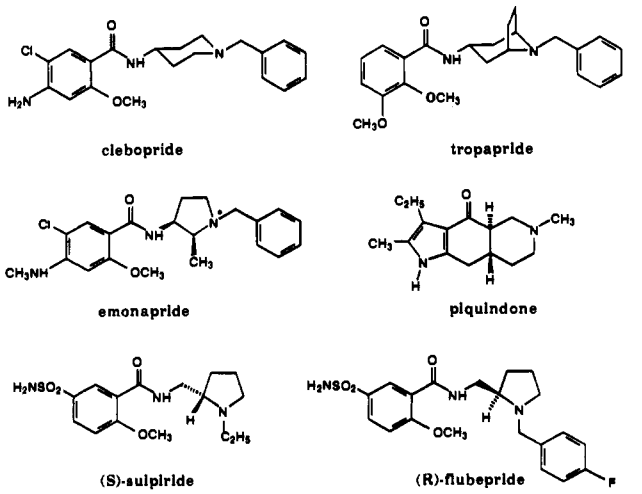
Chart I



A B

X	Y	Z	A	B
OH	Br	H	1 (FLA 797)	6
OH	Cl	Cl	2 (raclopride)	7
OH	Et	Cl	3 (eticlopride)	8
OH	Br	OMe	4 (FLB 463)	9
H	Br	OMe	5 (FLB 457)	10

Chart II



clebopride tropapride

emonapride piquindone

(S)-sulpiride (R)-flubepride

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also found in a series of 3-pyrrolidinyl derivatives (emonapride, Chart II).[17] Furthermore, the stereochemical requirements on the benzyl group in clebopride-related compounds having conformationally restricted side chains (indolizidine derivatives) were found to be strict.[18]

The substituted benzamides (remoxipride[19,20] and sulpiride[21,22]) and salicylamides[1,2,4,23] with (*N*-ethyl-2-pyrrolidinyl)methyl side chains display a stereoselective binding to central dopamine D-2 receptors, the *S* enantiomers being considerably more active than the corresponding *R* enantiomers. In contrast, flubepride, the *N*-(4-fluorobenzyl) analogue of sulpiride, is reported to have its antidopaminergic activity residing in the *R* enantiomer.[24] Thus, the change from a small *N*-ethyl group to a large lipophilic *N*-benzyl group results in a change in the stereochemical requirement on the 2-position of the pyrrolidine ring.

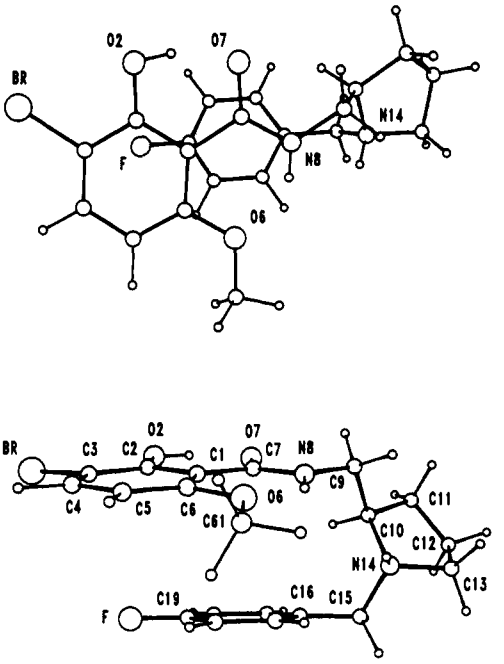

Figure 1. A perspective view of the molecular cation of **15**·HCl with a guide of the atomic numbering system adopted in the X-ray investigation. The molecule is viewed from two directions: perpendicular to the benzene-ring plane (top) and along benzene-ring plane (bottom).

We have previously suggested, on the basis of comparisons with piquindone, that the conformationally restricted salicylamides,[25] e.g. **1**–**4**, exert their action with a folded or half-folded side chain conformation in contrast to the clebopride type of benzamides.[5,11,20,23] The side chain of the latter type of compounds adopts an extended conformation and it has accordingly been proposed to be the common conformer for all types of benzamides.[26,27] Therefore, it is of interest to further investigate the importance of the nitrogen substituent and the chirality for the activity of (2-pyrrolidinylmethyl)salicylamides and -benzamides. Besides, it should be possible to obtain highly potent dopamine D-2 receptor antagonists related

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(25) The salicylamides have one intramolecular hydrogen bond (OH to CO) in addition to the one common for all 2-methoxybenzamides or orthopramides (NH to OMe).[5,20,23]

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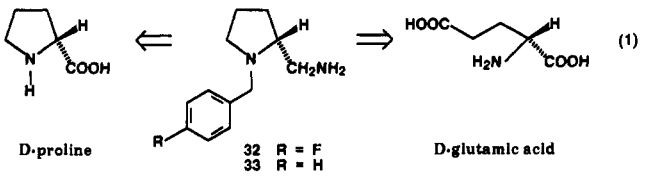
to flubepride by taking advantage of the structure–activity knowledge from previous work.[1-6,13,14]

This paper describes the synthesis and antidopaminergic properties of a number of *N*-benzyl-substituted benzamides related to the potent derivatives **1–10**. The stereoselective affinity for the dopamine D-2 receptor was ascertained in two cases. The solid-state conformation was determined for **15** and it is discussed in relation to other salicylamides.

Chemistry

The syntheses of the benzamides and the salicylamides were made in analogy with previously published methods for the corresponding compounds **1–10**.[1,2,4,6,14] The different benzoic and salicylic acids were converted to acyl chlorides and reacted with the appropriate 2-(aminomethyl)-1-pyrrolidine derivative. (*R*)-2-(Aminomethyl)-1-(4-fluorobenzyl)pyrrolidine (**32**) was conveniently obtained by a recently developed stereoconservative process involving dialkylation of D-proline followed by a cyanide-catalyzed aminolysis of the *N*-alkyl proline ester[28] and a final reduction of the primary amide (eq 1).[29] Alternatively, pyrrolidines **32** and **33** were prepared from D-glutamic acid.[30] Benzamides **16**, **19**, **20**, **24**, and **26** and salicylamides **11**, **12**, **15**, and **18** were obtained in good yields by reacting the acyl chlorides with the amines (method A, Table I). Salicylamides **14**, **17**, **21–23** were prepared by demethylation of the corresponding 2,6-dimethoxy derivative with boron tribromide as described earlier[1,2,4,6] (method B, Table I). In the case of demethylation of **20** both the major (**22**) and minor (**23**) isomers were isolated by radial chromatography. The *S* enantiomers **13** (precursor for **14**) and **27** were obtained by alkylation of the known[4,19] secondary pyrrolidines in excellent yields (method C, Table I). The desbromo compound **25** was isolated as an impurity in an early experiment in synthesizing the bromo compound **24** from a contaminated acid. It was included for comparative purposes with the clebopride series and with tropapride which are highly potent without having any 5-substituent.



(1)

X-ray Structure Determination of 15

Single crystals of **15** (hydrochloride) were obtained by recrystallization from ethanol and found to be orthorhombic with the space group $P2_12_12_1$ (M_r = 473.77; cell dimensions, a = 13.082 (8) Å, b = 21.005 (6) Å, c = 7.623 (4) Å; V = 2095 (2) Å^3; number of molecules in the unit cell, Z = 4; D_x = 1.502 g cm^{-3}; λ(CuKα) = 1.5418 Å, T = 294 K, μ = 41.67 cm^{-1}; $F(000)$ = 968). The final refinement converged to R = 0.0349 and R_w = 0.0383 for 261 parameters and 1699 reflections. Details are given in the Experimental Section. The absolute configuration, unambiguously confirming the *R* stereochemistry, was determined by Bijvoet ratio measurements of selected Friedel-paired reflections.[31]

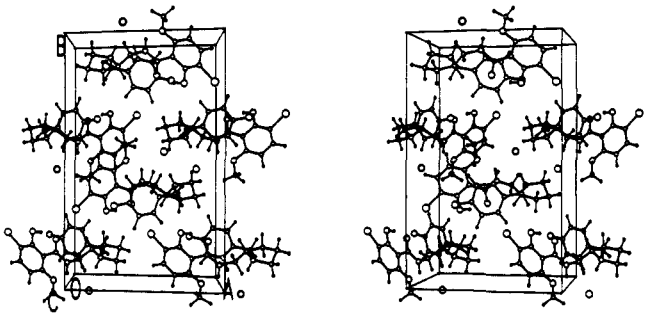


Figure 2. Stereoscopic view of **15**·HCl illustrating the crystal-packing arrangement down the *c* axis. The Cl atoms are marked as large open circles.

The atom labeling scheme of **15**·HCl adopted in the crystal structure analysis is shown in Figure 1, illustrating the sandwichlike folding of the fluorobenzyl group over the salicylamide moiety. In Figure 2 the crystal packing down the *c* axis is presented. Lists of refined fractional coordinates, equivalent isotropic thermal factors, bond lengths, bond angles, and torsion angles are deposited as supplementary material.

Bond distances and angles are all in reasonable ranges and in good agreement with those found in previously reported crystal structures of *o*-methoxybenzamides and -salicylamides, e.g. **1**·base,[20] **2**·tartrate,[23] and **3**·HCl.[32] Thus, the carboxamide moiety and the substituted benzene ring in **15**·HCl are coplanar [τ_1 = C(6)C(1)–C(7)N(8) = –0.5 (9)° and τ_2 = C(1)C(7)–N(8)C(9) = –177.0 (5)°] and stabilized by two intramolecular H bonds; one between the hydroxyl and the carbonyl oxygen [O(2)-H···O(7) = 2.458 (6) Å] and the other one between the amide nitrogen and the methoxy oxygen [N(8)-H···O(6) = 2.642 (6) Å]. The largest deviation from the benzene plane involve the atoms O(7) [0.064 (9) Å], O(6) [0.086 (4) Å], and C(61) [0.231 (8) Å]. Apart from the salt bridging between the chloride anion and the protonated tertiary nitrogen [N(14)-H···Cl = 3.064 (4) Å], the Cl atom also participates in a short interaction with the amide nitrogen N(8). Exact numerical values of the H-bonding scheme and some short intermolecular contacts are found as supplementary material.

The most prominent structural feature of **15**·HCl is the bent side chain conformation. The torsion angles invariably associated with the folding [τ_3 = C(7)N(8)–C(9)C(10) = –82.7 (7)°, τ_4 = N(8)C(9)–C(10)N(14) = –66.4 (5)°, τ_5 = C(9)C(10)–N(14)C(15) = 129.2 (5)°] describe the turn of the chain as a quasi-trans gauche eclipsed conformation. The torsion angles of the side chains in (2-pyrrolidinylmethyl)benzamides and -salicylamides are highly variable in the solid state.[5,23] The τ_3, τ_4, and τ_5 torsion angles in **15**·HCl are consistent with the values found in the racemic form of sulpiride hydrochloride[33] and in the mirror-imaged *R* form of eticlopride hydrochloride.[32] The only conformational difference is found in the final side chain angle τ_6 = C(10)N(14)–C(15)C(16), which is gauche in **15**·HCl (τ_6 = –62.1 (6)°) and trans in the other two structures. However, the gauche conformation of τ_6 brings the fluorobenzyl moiety over the benzene ring. The small dihedral angle 17.6 (2)° between the two aromatic ring planes is

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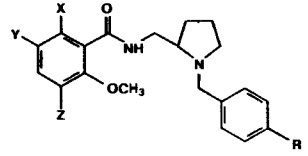
Table I. Structure, Physical Data, and Inhibition of [^{3}H]Spiperone Binding (IC_{50}) of *N*-Benzyl-Substituted Salicylamides and Benzamides



no.	X	Y	Z	R	stereochem	mp, °C	solvent	% yield[a]	methods[b]	$[\alpha]_D$, deg (c, solvent)[c]	formula	anal.[d]	[^{3}H]spiperone[e] IC_{50}, nM
11	OH	Br	H	H	*R/S*	177–181	acetone	64	A	–	$C_{20}H_{23}BrN_2O_3$·HCl	C,H,N	36
12	OH	Br	H	H	*R*	195–196	acetone	35	A	+103 (0.18, acetone)	$C_{20}H_{23}BrN_2O_3$·HCl	C,H,Br,Cl,N	18
13	OMe	Br	H	H	*S*	76–78		99	C	−53 (0.22, acetone)	$C_{21}H_{25}BrN_2O_3$	C,H,N	16300
14	OH	Br	H	H	*S*	205–206	MeOH/EtOH	58	B	−103 (0.21, acetone)	$C_{20}H_{23}BrN_2O_3$·HCl	C,H,Br,Cl,N	2680
15	OH	Br	H	F	*R*	219 (dec)	EtOH/MeOH	69	A	+96 (0.25, acetone)	$C_{20}H_{22}BrFN_2O_3$·HCl	C,H,Br,Cl,N	28
16	OMe	Cl	Cl	F	*R*	126–128	*i*-Pr_2O/Et_2O	78	A	+58 (0.23, acetone)	$C_{21}H_{23}Cl_2FN_2O_3$	C,H,N	2380
17	OH	Cl	Cl	F	*R*	165–167	EtOH/*i*-Pr_2O/H_2O	67	B	+96 (0.40, acetone)	$C_{20}H_{21}Cl_2FN_2O_3$·HCl	C,H,N	21
18	OH	Et	Cl	F	*R*	157–162	acetone	61	A	+62 (0.64, $CHCl_3$)	$C_{22}H_{26}ClFN_2O_3$·CH_3SO_3H	C,H,N,O	29
19	OMe	Br	OMe	H	R	112–114	*i*-Pr_2O	54	A	+57 (0.52, acetone)	$C_{22}H_{27}BrN_2O_4$	C,H,N	1860
20	OMe	Br	OMe	F	*R*	97–99	Et_2O/hexane	68	A	+53 (0.59, acetone)	$C_{22}H_{26}BrFN_2O_4$	C,H,N	2210
21	OH	Br	OMe	H	*R*	oil[f]		49	B	+94 (0.52, acetone)	$C_{21}H_{25}BrN_2O_4$	C,H,Br,N	2.4
22	OH	Br	OMe	F	*R*	188 (dec)	EtOH/Et_2O	62	B	+80 (0.64, acetone)	$C_{21}H_{24}BrFN_2O_4$·HCl	C,H,N	1.3[g]
23	OH	OMe	Br	F	*R*	oil[f]		19	B	+81 (1.1, acetone)	$C_{21}H_{24}BrFN_2O_4$		124
24	H	Br	OMe	H	*R*	oil[f]		65	A	+91 (0.68, acetone)	$C_{21}H_{25}BrN_2O_3$	C,H,Br,N	1.4
25[i]	H	H	OMe	H	*R*	oil[f]				+91 (0.32, acetone)	$C_{21}H_{26}N_2O_3$		21[h]
26	H	Br	OMe	F	*R*	158–159	acetone	78	A	+85 (0.96, acetone)	$C_{21}H_{24}BrFN_2O_3$·HCl	C,H,N	0.65
27	H	Br	OMe	F	*S*	158–159	acetone	84	C	−88 (0.17, acetone)	$C_{21}H_{24}BrFN_2O_3$·HCl·H_2O	C,H,N	126

[a] Isolated and nonoptimized yields. [b] See the Experimental Section. [c] Obtained at 20–25 °C. [d] Elemental analysis are within ±0.4% of the theoretical values. [e] Correlation coefficients $r > 0.98$ unless otherwise indicated. [f] Characterized by NMR and mass spectrometry. Purity ascertained by TLC and capillary GC. [g] $r = 0.93$. [h] $r = 0.90$. [i] Isolated as an impurity in an early experiment in synthesizing **24** from a contaminated acid.

Table II. Inhibition of [^{3}H]Raclopride Binding and in Vivo Activities in the Rat (ED_{50}, μmol/kg ip) of Substituted *N*-Benzyl and Known [(*N*-Ethyl-2-pyrrolidinyl)methyl]benzamides

	[^{3}H]raclopride[a]		block of apomorphine-induced responses			catalepsy,[d]
compd	K_i, nM	*n*	hyperactivity[b]	stereotypies[b]	hypothermia[c]	bar test
12	0.80 (0.64–1.08)	4	5.6 (3.4–12.3)	18.6 (14.9–25.0)	nt	nt
14	nt[g]		>80	>80	nt	nt
15	0.43 (0.31–0.68)	3	2[f]	13.2 (9.6–21.8)	1.7 (1.0–2.9)[e]	27[f]
22	0.19 (0.13–0.32)	4	1.5 (1.1–2.1)	2.4 (1.9–3.5)	1.3 (0.8–2.3)	13 (5)
24	0.024 (0.021–0.029)	3	1.2 (0.8–1.7)	1.4 (1.2–1.8)	nt	nt
26	0.15 (0.12–0.19)	3	0.6 (0.1–1.2)	1.6 (1.3–2.1)	0.3 (0.02–0.7)[e]	18[f]
1	0.55 (0.45–0.71)	2	0.054 (0.03–0.10)	0.32 (0.29–0.36)	0.6 (0.3–1.2)	1[f]
2	1.30 (1.08–1.63)	3	0.13 (0.05–0.23)	1.80 (1.57–2.13)	0.20[f]	10 (2.4)
4	0.064 (0.041–0.138)	2	0.009 (0.004–0.017)	0.049 (0.042–0.057)	0.005 (0.003–0.087)	0.13 (0.04)
5	0.018 (0.014–0.024)	2	0.002 (0.0002–0.006)	0.14 (0.12–0.17)	0.005[f]	0.39 (0.1)

[a] The constants were determined with the LIGAND program with data from all experiments calculated together. The estimates of K_i and the standard error range of K_i (in parentheses) were determined from the inverse of the standard error of the estimate of K_i. [b] The compounds were injected ip 60 min prior to apomorphine hydrochloride (1 mg/kg sc). The hyperactivity and stereotypies were scored and calculated as described previously.[3] The ED_{50} values were calculated by regression analysis using Fieller's theorem for estimates of the 95% confidence limits. [c] The compounds were injected ip 30 min prior to apomorphine hydrochloride (1 mg/kg sc). The body temperature was measured by a rectal probe 15 min following apomorphine as described previously.[36] The ED_{50} values were calculated by regression analysis using Fieller's theorem for estimates of the 95% confidence limits. [d] Catalepsy was measured by the bar test 20, 40, 60, 90, 120, and 240 min after injection of the compound. The rat was placed with both forepaws on a 7 cm high horizontal bar, and the catalepsy interval was defined as the time required for both forelimbs to be removed from the bar. A rat was considered to reach catalepsy if it showed a mean time of at least 15 s from the three test occasions showing the largest descent latencies. The ED_{50} (SEM) was calculated by probit analysis. [e] Pergolide (0.1 mg/kg sc) was used as the dopamine agonist. [f] Estimated value interpolated from log dose–response curves. [g] Not tested.

consistent to the almost parallel arrangement of the rings, parted by a distance in the region of the sum of ordinary van der Waals radii. In the pyrrolidine ring the maximum deviation from planarity is 0.26 Å and involves the atoms C(12) and C(13) equally displaced on each side of the plane. The asymmetry parameters ΔC_2(C12) = 0.09° and ΔC_s(C10) = 0.09° of the pyrrolidine ring, directly relating the deviation of the torsion angles from ideal symmetry, describe the ring conformation as midway between half-chair and envelope.[34]

The distance between the commonly used pharmacophoric groups, the aliphatic nitrogen N(14) and the center of the aromatic benzamide ring, is 6.34 Å and the displacement of N(14) from the least-squares plane of the aromatic ring is 2.25 Å.

Results and Discussion

Methods. The pharmacological evaluation was made according to previously described procedures.[3,4,35,36] The affinity for the dopamine D-2 receptor of the compounds was determined from the inhibition of [^{3}H]spiperone binding and/or [^{3}H]raclopride binding in rat striatal membranes in vitro (Tables I and II).[35] (+)-Butaclamol was used for determination of nonspecific binding. Some compounds were also studied in vivo for their ability to block apomorphine-induced oral stereotypies, hyperactivity, and hypothermia in the rat (Table II).[3,4,36] The tendency of the compounds to induce catalepsy was also determined in the rat (Table II).[3,4]

Benzamide Conformations. In accordance with findings from benzamides having other types of side chains,[1,2,4,14] 2,6-dimethoxy-substituted compounds **13**, **16**, **19**, and **20** have a very modest affinity for the dopamine D-2 receptor (Table I). The low affinity in this class is attributed to the steric crowding of the carbonyl group which prevents the formation of a coplanar conformation. On the other hand, the salicylamides which can adopt the essential planar conformation display a pronounced affinity for the dopamine D-2 receptor. Thus, R isomers **12**, **15**, **17**, and **18** and especially 5,6-dimethoxy-substituted salicylamides **21** and **22** are very active in blocking the [^{3}H]spiperone binding (Table I). The energy to bring a twisted (τ = 73°) carbonyl into planarity (τ = 15°) has been estimated by ab initio calculations to be 2.5 kcal mol^{-1} for 2,6-dimethoxy-*N*-methylbenzamide.[5] This energy barrier corresponds reasonably well with the observed at least 100-fold difference (ΔG = −2.8 kcal mol^{-1}) in activity between the 2,6-dimethoxybenzamides and the corresponding 6-methoxysalicylamides, i.e. **16** vs **17**, **19** vs **21**, and **20** vs **22**. This supports our previously suggested explanation for the difference in in vitro potency for remoxipride and the corresponding salicylamide **1**[20] and shows that the same general conformational requirements for the benzamide moiety are in operation also in this series.

Side Chain Conformations. The highly potent *N*-benzyl-substituted pyrrolidine benzamides and salicylamides of this type display a marked stereoselective affinity for the dopamine D-2 receptor. Thus, *S* isomers **14** and **27** are considerably less potent than the corresponding *R* isomers (Table I). This finding is in line with the early observation in the sulpiride series.[24] The investigated (*S*)-*N*-ethyl-2-pyrrolidines (**1–5**, **30**) and (*R*)-*N*-benzyl-2-pyrrolidines (**15**, **17**, **18**, **22**, **26**, **25**) with identical aromatic substituents are equipotent with one exception only, i.e. **3** and **18** being significantly different (Table III). Whether this otherwise notable parallelism reflects very similar pharmacophores is unclear since the switch in stereochemical requirement would imply that different side chain conformations are involved in the receptor interaction. If the (*S*)-(*N*-ethyl-2-pyrrolidinylmethyl)benzamides (e.g. **1–5**) would adopt an extended side chain conformation in analogy with the (*N*-benzyl-4-piperidinyl)benzamides (e.g. **6–10** and clebopride), as proposed by others,[26,27] this would be the case also for the *N*-benzyl-2-pyrrolidinyl derivatives described herein. However, the different stereochemical requirements for the *N*-ethyl- and *N*-benzylpyrrolidines are not consistent with this assumption. It rather underlines the previous objection in utilizing the clebopride type of benzamides in the modeling of (*N*-ethyl-2-pyrrolidinylmethyl)benzamides.[5,14,20,23] Notably, the 4-piperidinyl and (*R*)-(2-pyrrolidinylmethyl)benzamides display identical requirements with respect to the *N*-substituent, i.e. alkyl and allyl groups lead to inactive compounds[4,6] and benzyl groups to active compounds, which

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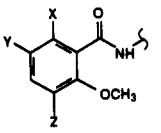
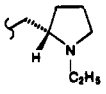
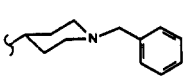
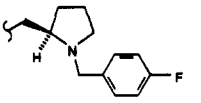
Table III. Influence of the Aromatic Substituents in the Three Types of Substituted Benzamides on the Affinity for the Spiperone Binding Site (IC_{50}, nM)[1,2,4,6,14]



X	Y	Z	no.			
OMe	Cl	Cl	**28**	>10000		
			29		3450	
			16			2380
OH	Br	H	**1**	12		
			6		41	
			15			28
OH	Cl	Cl	**2**	32		
			7		5.4	
			17			21
OH	Et	Cl	**3**	0.92		
			8		9.4	
			18			29
OH	Br	OMe	**4**	1.4		
			9		0.36	
			22			1.3
H	Br	OMe	**5**	1.2		
			10		5.0	
			26			0.65
H	H	OMe	**30**	52		
			31		6.3	
			25			21[a]

[a] *N*-Benzylpyrrolidine side chain since not prepared as 4-fluorobenzyl derivative. However, the benzyl and (4-fluorobenzyl)pyrrolidines are generally equipotent.

argue in favor of a common pharmacophore and conformation in these two series and not with the (*S*)-(2-pyrrolidinylmethyl)benzamides.

A third alternative side chain conformation of the benzamides (extended for 4-piperidinylbenzamides[20,27] and half-folded or folded for (*S*)-(2-pyrrolidinylmethyl)benzamides[5,11,23]) was found in the X-ray analysis of **15**·HCl (Figure 1). In this solid-state structure the benzyl group is folded backward over the internally hydrogen-bonded salicylamide moiety. This conformation can be the result of packing forces in the crystal. However, the existence of an internally stacked sandwich conformation is conceivable also in aqueous solution since it would minimize the lipophilic surface and it could be a factor contributing to the reversal in stereochemical requirements in the pyrrolidinyl series. The somewhat varying influence of the aromatic substituents on the activity in the three series may also be indicative of different 3-D pharmacophores. Further studies, such as molecular modeling with a G protein coupled dopamine D-2 receptor model and investigations of conformationally constrained analogues, are required to determine which (*R*)-*N*-benzyl-2-pyrrolidinyl side chain conformation is present during the receptor interaction.

Structure–Activity Relationships. The related compounds having a benzyl or *p*-fluorobenzyl group in the pyrrolidine ring are about equipotent in vitro (cf. **12** with **15**, **21** with **22**, and **24** with **26**). As mentioned above, the salicylamides having an additional 5-methoxy group, i.e. **21** and **22**, are the most potent compounds in this series. Regioisomer **23** is 2 orders of magnitude less active than **22**. Non-salicylamides **24** and **26** are about as active as the corresponding salicylamides **21** and **22**. This is consistent with data on *N*-ethyl derivatives also having two adjacent methoxy groups.[4,6b]

The present limited series of (*R*)-*N*-benzyl benzamides is not suitable for treatment in a QSAR analysis as we have done with the (*S*)-(*N*-ethyl-2-pyrrolidinylmethyl)- and the (*N*-benzyl-4-piperidinyl)benzamides.[13,14] However, it is interesting to compare a number of representative examples in the three series having identical aromatic substitution patterns (Table III). The 2,6-dimethoxy-3,5-dichloro substituents result in weakly potent or practically inactive compounds in all series. Both the salicylamide and the 6-unsubstituted patterns give potent compounds in the three series, but there are different optimal requirements, depending on the nature of the amide N-substituent.

The 3-ethyl-5-chlorosalicylamide which is ideal in the (*S*)-*N*-ethyl series (eticlopride, **3**) is not remarkable in the (*R*)-*N*-benzyl series, i.e. **18** is only equipotent with **15** and **17**, or in the 4-piperidinylbenzamides. The 3-bromo-5-methoxy substitution of the salicylamides is apparently a good choice in all the investigated side chain cases (**4**, **9**, **21**, and **22**). The corresponding non-salicylamides in the pyrrolidine series (**5**, **24**, and **26**) are as potent as the salicylamides (**4**, **21**, and **22**) but 1 order of magnitude less active in the piperidine type (**10** vs **9**). The lipophilicity of the 3-substituent is of major importance for the activity of the monomethoxysalicylamides of the *N*-ethyl-2-pyrrolidinyl type.[5,13] A certain influence of the corresponding 5-bromo group in the 2,3-dimethoxybenzamides is evident when comparing **24**/**26** and **25**. A similar loss in activity is also found in the *N*-ethyl-2-pyrrolidinyl series when the bromo group in **5** is removed (**30**).[6] On the other hand, in the 4-piperidinyl series the bromo (**10**) and desbromo (**31**) compounds are equipotent (Table III). In a large set of 4-piperidinylbenzamides, QSAR studies also showed that electronic properties primarily determine the activity.[14]

In Vivo Studies. Some of the compounds were also evaluated for their in vivo activity (Table II). The lack of potency in vivo of **14** relative to **12** is in agreement with the stereoselective receptor affinity noted earlier. Com-

pounds **12** and **15** were less potent in blocking the apomorphine-induced stereotypies than **22**, **24**, and **26**, in line with the 10-fold difference in [^{3}H]spiperone displacing affinity. This difference in potency was moderate or absent in the blockade of the apomorphine-induced hyperactivity consistent with the affinity for the [^{3}H]raclopride binding site. Higher dose levels were necessary to induce catalepsy than to block the apomorphine-induced responses, suggesting a lower liability to produce EPS at antiphsychotically effective doses. However, no significant differences in the inhibition of the apomorphine-induced hyperactivity and stereotypies were noted among the most potent compounds in this class of *N*-benzylated benzamides, which makes them less interesting candidates as atypical antipsychotics. Benzamides **12** and **15** with a lower ability to displace [^{3}H]spiperone show, on the other hand, a certain functional separation in the behavioral tests. Whether this observation relates to the different relative affinities for the [^{3}H]spiperone and [^{3}H]raclopride binding site remains to be determined. The compounds investigated blocked the apomorphine-induced hypothermia and hyperactivity in similar doses (Table II). (*S*)-*N*-Ethyl benzamide **5** and salicylamides **1**, **2**, and **4** were more potent in vivo than the corresponding (*R*)-*N*-benzyl derivatives despite their comparable in vitro potencies (Table II and III), which might be due to metabolic and pharmacokinetic reasons.

Conclusions

The substituted [(*N*-benzyl-2-pyrrolidinyl)methyl]-benzamides display a high and stereoselective affinity for the dopamine D-2 receptor. In contrast to the corresponding *N*-ethyl derivatives, the affinity is confined to the *R* enantiomer. The 4-piperidinyl and the *R* isomer, but not the *S* isomer, of (2-pyrrolidinylmethyl)benzamides display the same N-substitution requirements for antidopaminergic activity. The aromatic substituents have slightly varying influence on activity in the three side chain series. The 3-bromo-5,6-dimethoxysalicylamides (**4**, **9**, and **22**) are highly potent in all series investigated.

The substituted (*R*)-*N*-[[1-(4-fluorobenzyl)-2-pyrrolidinyl]methyl]benzamides (**26**) and -salicylamides (**22**) are suitable for development into ^{18}F radioligands for use in PET without altering the parent structure. Thus, [^{18}F]-**26** ([^{18}F]NCQ 115) given intravenously to a Cynomolgus monkey has been found to accumulate to a greater extent in striatum than in cerebellum.[37] Likewise, [^{3}H]-**26** ([^{3}H]NCQ 115) is a potent and selective dopamine D-2 ligand in vitro.[38]

Experimental Section

Chemistry. Melting points were determined in open capillary tubes on a Mettler FP61 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL FX 200 or a Varian Gemini-300 spectrometer with Me_4Si as internal standard. Mass spectra were obtained on a Finnigan-MAT TSQ-70 instrument in the thermospray mode with loop injection. GLCs were run on an SE 30 capillary column, and the amounts determined by a Hewlett-Packard 3390A integrator. Optical rotations were measured on an Optical Activity AA-100 polarimeter. Preparative, centrifugally accelerated TLCs were conducted on a Chromatotron from Harrison Research. Elemental analyses were performed by Analytische Laboratorium, Elbach, West Germany, and are within ±0.4% of the theoretical values.

(+)-(*R*)-3,5-Dichloro-*N*-[[1-(4-fluorobenzyl)-2-pyrrolidinyl]methyl]-2,6-dimethoxybenzamide (16; Method A). A mixture of 3,5-dichloro-2,6-dimethoxybenzoic acid[19] (1.5 g, 6 mmol), thionyl chloride (2.0 mL, 28 mmol), and 3 drops of dimethylformamide as catalyst was stirred in 30 mL of toluene at 60 °C under N_2 for 3 h. After cooling, the solvent was evaporated in vacuo and the residue was dissolved in CH_2Cl_2 and evaporated again. The residue consisting of 3,5-dichloro-2,6-dimethoxybenzoyl chloride was dissolved in 10 mL of CH_2Cl_2. A solution of (*R*)-2-(aminomethyl)-1-(4-fluorobenzyl)pyrrolidine[29,30] (5.0 mmol obtained from 2.54 g of ditartrate by partitioning between 2 M NaOH and CH_2Cl_2) in 15 mL of CH_2Cl_2 was added and the mixture was stirred overnight at room temperature. The solvent was evaporated and the residue was partitioned between 2 M NaOH and EtOAc/Et_2O. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated to give 2.2 g (100%) of amide **16** as an oil which solidified. Recrystallization from *i*-Pr_2O/Et_2O gave 1.72 g (78%) of pure **16**: mp 126–128 °C; ^{1}H NMR ($CDCl_3$) δ 7.44 (s, 1), 7.25 (m, 2), 6.97 (m, 2), 6.43 (br, 1), 3.88 (s, 6); MS (TSP) *m/z* (rel int) 445/443/441 (M + 1, 13/67/100).

The substituted benzamides and salicylamides **11**, **12**, **15**, **16**, **18–20**, **24–26** were prepared analogously from the corresponding amine and benzoyl chloride. See Table I for details.

(+)-(*R*)-3,5-Dichloro-*N*-[[1-(4-fluorobenzyl)-2-pyrrolidinyl]methyl]-6-methoxysalicylamide (17; Method B). The 3,5-dichloro-2,6-dimethoxybenzamide **16** (1.7 g, 3.9 mmol) was dissolved in 25 mL of CH_2Cl_2 and cooled with ice. Solutions of HCl in Et_2O (1.7 M, 4 mmol) followed by boron tribromide in CH_2Cl_2 (3.7 mL of 1.08 M) were added dropwise at a temperature of <5 °C. The temperature was allowed to reach room temperature and the stirring was continued for 2 h. The reaction mixture was poured into 300 mL of ice-cooled 1 M NH_3 and 50 mL of CH_2Cl_2. The mixture was separated and the aqueous phase was extracted with 50 mL of CH_2Cl_2. The combined organic phases were washed with 100 mL of water, dried (Na_2SO_4), and evaporated to give 1.66 g (100%) of pure salicylamide **17**. A hydrochloride salt was prepared and recrystallized from EtOH/*i*-Pr_2O/H_2O 15:5:2 to furnish 1.24 g (69%): mp 165–167 °C; ^{1}H NMR ($CDCl_3$) δ 8.98 (br, 1), 7.52 (s, 1), 7.3 (m, 2), 7.0 (m, 2), 3.87 (s, 3); MS (TSP) *m/z* (rel int) 431/429/427 (M + 1, 13/67/100).

Salicylamides **14**, **17**, **21–23** were prepared analogously (Table I).

(−)-(*S*)-*N*-[[1-Benzyl-2-pyrrolidinyl]methyl]-3-bromo-2,6-dimethoxybenzamide (13; Method C). Benzyl bromide (94 mg, 0.55 mmol) was added to a mixture of (*S*)-*N*-(2-pyrrolidinylmethyl)-2,6-dimethoxy-3-bromobenzamide hydrochloride[19] (210 mg, 0.55 mmol), potassium carbonate (182 mg, 1.32 mmol), and 7 mL of dimethylformamide at ice-cooling. The mixture was allowed to reach room temperature and the stirring was continued overnight. The reaction mixture was poured into 20 mL of water and 3 mL of 2 M NaOH and extracted with Et_2O twice. The organic layer was washed with water and brine. Drying (Na_2SO_4) and evaporation gave a residue which was purified by chromatography on SiO_2 in a pipet with *i*-Pr_2O/MeOH/NH_3 100:10:1 as eluent to afford 235 mg (99%) pure amide **13**, which solidified upon standing: mp 76–78 °C; ^{1}H NMR ($CDCl_3$) δ 7.44 (d, 1), 7.23 (m, 5), 6.56 (d, 1), 6.44 (br d, 1), 3.84 (s, 3), 3.73 (s, 3); ^{13}C NMR ($CDCl_3$) δ 165.8, 157.3, 155.3, 139.8, 134.4, 129.4, 128.9, 127.6, 123.8, 108.9, 108.7, 62.7, 62.3, 58.7, 56.4, 54.6, 40.9, 28.1, 22.9; MS (TSP) *m/z* (rel int) 435/433 (M + 1, 98/100).

Compound **27** was prepared by an analogous method (Table I).

Single-Crystal X-ray Analysis. A colorless crystal of dimensions 0.26 × 0.24 × 0.20 mm, recrystallized from ethanol, was mounted along the crystallographic *b* axis for data collection on a Phillips PW1100 automatic four-circle diffractometer equipped with graphite-monochromated CuKα radiation. The unit-cell parameters were determined from a Guinier powder photograph taken with strictly monochromated CuKα$_1$ radiation and with Si (*a* = 5.4309 Å) as internal standard; space group $P2_12_12_1$ was based on the systematic absences and structure analysis. A total of 2057 unique reflections were collected using the ω–2θ scan technique to $2\theta_{max}$ = 136°. The scan width was 1.6° and the scan speed 0.035° s^{-1}. Total time of background counts, recorded on each side of the reflection, was equal to the scan time of the measured reflection. Three reference reflections, monitored periodically every second hour, showed no significant intensity

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variation. 1699 reflections were considered observed with $I \geq 3\sigma(I)$ and corrected for Lorenz, polarization, and absorption effects.

The structure was solved by direct methods using MULTAN80[39] in combination with different Fourier recycling. The full-matrix least-squares refinement was carried out using the SHELX76[40] system with all non-H atoms treated anisotropically. All H atoms, except H(2), H(8), and H(14) participating in hydrogen bonding, were introduced at calculated positions after each cycle with the restricted bond length 1.08 Å. The methyl group was treated as a rigid group with free rotation around the O–C bond. Two different isotropic thermal factors were applied: *general*-H and *methyl*-H taking the values 6.1 (4) and 6.7 (9) Å^2, respectively. The H(2), H(8), and H(14) atoms, located from the last difference Fourier, were held at fixed positions but permitted to take individual isotropic thermal factors 7 (2), 2 (1), and 6 (1) Å^2, respectively, in the final cycles. The final refinement converged to $R = 0.0349$ and $R_w = 0.0383$ for 261 parameters and 1699 reflections. The function minimized was $\sum w(|F_o| - |F_c|)^2$ with $w^{-1} = \sigma^2(F) + 0.0004F^2$; $(\Delta/\sigma)_{max} = 0.036$, average value $(\Delta/\sigma) = 0.007$; highest and lowest residual peaks in the final difference map, 0.21 and −0.22 e Å^{-3}. Atomic scattering factors for non-H atoms were taken from the SHELX76 system.

The absolute configuration of **15**·HCl, unambiguously establishing the *R* enantiomer, was determined by measuring Bijvoet ratios.[31] The intensities of 15 Friedel paired reflections, exhibiting the greatest influence of anomalous scattering effects in CuKα radiation, were selected for intensity measurements. The values of the anomalous scattering factors were taken from Cromer and Liberman.[41] Lists of calculated and observed Bijvoet differences are included in the supplementary material.

Pharmacology. [^{3}H]Spiperone and [^{3}H]Raclopride Binding.[35] Male Sprague–Dawley rats were killed by decapitation. The striata were rapidly dissected out on ice and homogenized in Tris-HCl buffer (0.05 M, pH 7.6). The homogenate was centrifuged for 10 min at 48000*g*, resuspended, and recentrifuged. The final pellet was suspended in Tris-HCl buffer (0.05 M, pH 7.6) containing 0.1% ascorbic acid and various salts to a final concentration corresponding to 5 mg of original wet weight/mL. The incubations were performed at 37 °C for 10 min with [^{3}H]spiperone or 25 °C for 60 min with [^{3}H]raclopride in plastic trays. The incubations were terminated and bound radioligand was separated from free by filtration and subsequent washing on glass-fiber filters (Whatman GF/B). (+)-Butaclamol (1 μM) was used for the determination of nonspecific binding. The radioactivity was determined by a liquid scintillation counter. The IC_{50} values were calculated using log–logit regression analysis ([^{3}H]spiperone). The K_i values were calculated by using nonlinear-regression analysis (LIGAND)[42] according to Cheng and Prusoff ([^{3}H]raclopride).[43]

Apomorphine-Induced Behavior. Male Sprague–Dawley rats (270–325 g) were used. The behavior was scored 5, 20, 40, and 60 min after injection of apomorphine hydrochloride (1 mg/kg, 0.02%), given subcutaneously into the neck. The scoring was performed as described previously.[3,4] The test compounds were dissolved in saline (0.9% NaCl) or acetic acid and distilled water and injected (5 ml/kg) ip 60 min prior to apomorphine. The ED_{50}'s for stereotypies refer to the calculated doses that reduce the scores of apomorphine-induced stereotypies over the total observation period of 60 min by 50%. The ED_{50}'s for hyperactivity refer to the calculated doses that reduce the scores of hyperactivity over the observation period of 60 min by 50% of that of the apomorphine control. The ED_{50} values (based on at least six dose levels with six to eight animals per dose level) for stereotypies and hyperactivity have been calculated by regression analysis using Fieller's theorem for calculation of the 95% confidence limit.[44] The ED_{50} value of the response has been defined as the midpoint between the mean of the apomorphine control group and the mean of saline control group.

Apomorphine-Induced Hypothermia. The method has recently been described.[36] Male Sprague–Dawley rats (200–250 g) were used and were housed at 21 ± 0.5 °C. Groups of rats (n = 6) were pretreated ip with saline or test compounds 30 min prior to the injection of apomorphine hydrochloride (1 mg/kg sc). Core temperature was measured by a rectal probe.

The temperature was recorded before administration of the test compounds as well as before apomorphine and 15 min after apomorphine. The means of the temperature variations were calculated and the changes were expressed as percent of respective control mean value. The ED_{50} value, calculated by regression analysis using Fieller's theorem for calculation of the 95% confidence limit,[44] refers to the dose level which blocks the hypothermic effect of apomorphine by 50%.

Measurement of Catalepsy. Catalepsy was measured in the horizontal bar test 20, 40, 60, 90, 120, and 240 min after ip injection of the test compound. In addition, a control group receiving saline was tested in the same manner as drug-treated groups. A test cage (Macrolon type III) fitted with a horizontal bar (diameter of 1.5 cm) placed 7 cm above the cage floor was used. The rat was placed with both front limbs extended over the bar, and the catalepsy interval was defined as the time (descent latency) required for both front limbs to be removed from the bar. The test was limited to a cutoff time if the rat had not moved after 60 s. A rat was considered to be cataleptic if it showed a mean time of more than 15 s from the three test occasions showing the longest descent latencies. The proportion of animals that were cataleptic according to this criterion (≥15 s) was calculated for each dose level of the drugs, and presented in dose–response curves. The ED_{50}, determined by probit analysis, is defined as the dose at which 50% of the animals are cataleptic.

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Supplementary Material Available: Tables of fractional atomic coordinates, anisotropic thermal parameters, hydrogen-bonding scheme, Bijvoet ratios, bond distances, bond angles, and torsion angles (7 pages); a table listing observed and calculated structure factors (8 pages). Ordering information is given on any current masthead page.

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