22. Quantitative Structure-Affinity Relationships of Dopamine D_2 Receptor Antagonists: A Comparison between Orthopramides and 6-Methoxysalicylamides

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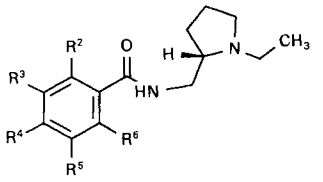
A large series of orthopramides (= 2-methoxybenzamides), 6-methoxysalicylamides, and 2,6-dimethoxybenzamides were examined for their affinity to the dopamine D_2 receptor. The binding data were correlated with physicochemical parameters and ^{13}C-NMR chemical shifts using the cross-validated partial least-squares method and multiple linear regression analysis. The results quantitate the influence of electronic factors and lipophilicity to D_2 receptor binding. They also show that the *N*-[(1-ethylpyrrolidin-2-yl)methyl] and *N*-(1-benzylpiperidin-4-yl) side-chains affect the mode of binding of these compounds.

Introduction. – Neuroleptic agents belonging to the chemical class of substituted benzamides are classified as atypical antipsychotic agents, since they do not induce extrapyramidal side effects. These agents exert their action by blocking dopamine D_2 receptors in the mesolimbic areas of the brain [1] [2]. Besides the classical orthopramides (*ortho*-methoxybenzamides) [3–6], 6-methoxysalicylamides, and 2,6-dimethoxybenzamides have recently been reported [7] [8], a number of which are undergoing clinical trials. Highly potent compounds are also found in the 5,6-dimethoxysalicylamide series [9] [10].

Several quantitative structure-affinity relationship (QSAR) studies have demonstrated that antidopaminergic properties of these compounds strongly depend on the substitution pattern of the aromatic ring [7–14]. For congeneric orthopramides, a good correlation was found between D_2 receptor affinity and electronic properties of ring substituents [11]. *de Paulis et al.* [7] have also shown how lipophilic substituents at C(3) of 6-methoxysalicylamide derivatives increase affinity. By comparing the affinity of some 6-methoxysalicylamides with corresponding analogues devoid of either the 6-MeO or the 2-OH group, it was demonstrated that the latter does not contribute to the affinity of



	R^2	R^3	R^4	R^5	R^6
Raclopride	OH	Cl	H	Cl	MeO
Eticlopride	OH	Et	H	Cl	MeO
Remoxipride	MeO	Br	H	H	MeO

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these compounds [8] [10]. In fact, the influence of the 2-OH group on dopamine D_2 receptor affinity is not yet clear.

Variations in the side chain are also interesting from a SAR viewpoint. Many potent compounds contain the *N*-[(1-ethylpyrrolidin-2-yl)methyl] group, which can be replaced by a piperidine or a nortropane ring, *e.g.* in tropapride [12]. Lipophilic N substituents may give additional binding to an auxiliary binding site, thus possibly compensating suboptimal substitutions in the aromatic ring [12].

Here, we report a study of 107 compounds belonging to the orthopramide, 2,6-dimethoxybenzamide, and 6-methoxysalicylamide classes, and having either a *N*-[(1-ethylpyrrolidin-2-yl)methyl] or a *N*-(1-benzylpiperidin-4-yl) side chain. The aromatic part had different combinations of substituents in all positions, except C(4) which was unsubstituted. The activity of the compounds was measured *in vitro* as D_2 receptor affinity (pK_i). The electronic influence of substituents was assessed by ^{13}C-NMR chemical shifts. In addition, a number of classical substituent parameters such as π (hydrophobic substituent constant), σ (electronic substituent constant), and MR (molar refractivity of substituent) were also considered [15] [16].

The objectives of this study were to assess the structural factors governing D_2 receptor affinity, and to verify whether the same structural factors govern the affinity of orthopramides, 2,6-dimethoxybenzamides, and 6-methoxysalicylamides having either an *N*-[(1-ethylpyrrolidin-2-yl)methyl] or an *N*-(1-benzylpiperidin-4-yl) side chain. In addition, the intriguing behavior of the 5,6-dimethoxy derivatives was also investigated. To find meaningful quantitative structure-affinity relationships (QSAR), the statistical analyses were performed using principal component analysis (PCA), multiple linear regression (MLR) analysis, and the cross-validated partial least squares (PLS) method [17–19].

Results and Discussion. – The [^{3}H]spiperone binding affinity of all compounds, as expressed by pK_i values, is given in *Table 1*. Note that all derivatives with an *N*-[(1-ethylpyrrolidin-2-yl)methyl] side chain were the eutomers of (*S*)-configuration. The ^{13}C-NMR chemical shifts of the C-atoms in the aromatic ring and in the amide bridge are

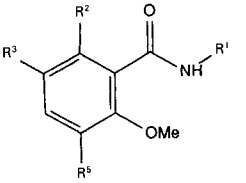

Table 1. *Binding of Substituted Benzamides to the D_2 Receptor* (displacement of [^{3}H]spiperone)

No.	R^2	R^3	R^5	I_1[a])	pK_i[b])	No.	R^2	R^3	R^5	I_1[a])	pK_i[b])
Orthopramides						**9**	H	NH_2SO_2	H	0	1.16
1	H	H	H	0	0.41	**10**	H	I	MeO	0	3.28
2	H	H	MeO	0	1.76	**11**	H	I	H	0	2.47
3	H	Cl	Cl	0	2.18	**12**	H	Me	MeO	0	2.76
4	H	Cl	MeO	0	3.88	**13**	H	MeS	MeO	0	2.43
5	H	Br	H	0	1.96	**14**	H	Et	H	0	1.86
6	H	Br	Br	0	2.57	**15**	H	Et	Cl	0	2.85
7	H	Br	MeO	0	3.40	**16**	H	Et	Br	0	2.44
8	H	Br	OH	0	2.48	**17**	H	Et	MeO	0	3.36

Table 1 (cont.)

No.	R^2	R^3	R^5	I_1[a])	pK_i[b])
18	H	Pr	H	0	1.65
19	H	H	H	1	0.55
20	H	H	MeO	1	2.68
21	H	Br	H	1	1.12
22	H	Br	Br	1	2.18
23	H	Br	MeO	1	2.78
24	H	Et	H	1	0.77
25	H	Pr	H	1	0.63
2,6-Dimethoxybenzamides					
26	MeO	H	H	0	–0.41
27	MeO	Cl	H	0	–0.03
28	MeO	Br	H	0	0.28
29	MeO	Br	Cl	0	–0.40
30	MeO	Br	Br	0	–0.05
31	MeO	Cl	Cl	0	–0.04
32	MeO	Br	NH_2	0	–0.01
33	MeO	Br	OH	0	0.48
34	MeO	Br	MeO	0	0.23
35	MeO	I	H	0	0.03
36	MeO	I	MeO	0	–[c])
37	MeO	MeO	H	0	–[c])
38	MeO	Et	H	0	0.51
39	MeO	H	MeO	0	–[c])
40	MeO	NO_2	Br	0	–[c])
41	MeO	Et	MeO	0	–[c])
42	MeO	H	OH	0	–[c])
43	MeO	Cl	Cl	0	–0.06
44	MeO	Br	H	0	0.39
45	MeO	Br	Br	0	–0.09
6-Methoxysalicylamides					
46	OH	H	H	0	0.98
47	OH	H	Cl	0	1.67
48	OH	H	Br	0	1.73
49	OH	H	MeO	0	2.53
50	OH	H	Et	0	1.39
51	OH	F	H	0	0.92
52	OH	Cl	H	0	1.89
53	OH	Cl	Cl	0	2.06
54	OH	Cl	Br	0	1.71
55	OH	Cl	MeO	0	4.00
56	OH	Cl	Me	0	2.44
57	OH	Cl	Et	0	2.40
58	OH	Cl	Pr	0	1.44
59	OH	Br	H	0	2.41
60	OH	Br	F	0	2.66
61	OH	Br	Cl	0	2.25
62	OH	Br	Br	0	2.05
63	OH	Br	MeO	0	3.48
64	OH	Br	OH	0	3.50
65	OH	Br	NH_2	0	1.96
66	OH	Br	Me	0	2.44
67	OH	Br	Et	0	2.25
68	OH	Br	NO_2	0	1.21
69	OH	I	H	0	2.92
70	OH	I	Cl	0	2.52
71	OH	I	MeO	0	3.30
72	OH	MeO	H	0	1.17
73	OH	MeO	Cl	0	1.63
74	OH	MeO	Br	0	1.65
75	OH	MeO	Et	0	–[c])
76	OH	MeO	Pr	0	–[c])
77	OH	Me	H	0	2.20
78	OH	Me	Cl	0	3.06
79	OH	Me	Br	0	2.74
80	OH	Me	Me	0	2.59
81	OH	Me	Pr	0	1.33
82	OH	Et	H	0	2.81
83	OH	Et	F	0	3.30
84	OH	Et	Cl	0	3.51
85	OH	Et	Br	0	3.22
86	OH	Et	MeO	0	3.63
87	OH	Et	Et	0	3.40
88	OH	Pr	H	0	2.80
89	OH	Pr	Cl	0	2.97
90	OH	Pr	MeO	0	3.10
91	OH	Pr	Me	0	2.78
92	OH	Bu	H	0	2.06
93	OH	NO_2	H	0	–0.01
94	OH	NO_2	Br	0	–0.01
95	OH	H	H	1	1.56
96	OH	H	Et	1	2.36
97	OH	Cl	H	1	1.58
98	OH	Cl	Cl	1	2.76
99	OH	Cl	Et	1	2.36
100	OH	Br	H	1	1.86
101	OH	Br	Br	1	2.60
102	OH	Br	Et	1	1.80
103	OH	Et	H	1	1.38
104	OH	Et	Cl	1	2.50
105	OH	Et	Br	1	1.93
106	OH	Et	Et	1	2.07
107	OH	Et	I	0	3.30

[a]) $I_1 = 0$ when $R^1 = N$[(1-ethylpyrrolidin-2-yl)methyl], absolute configuration (*S*); $I_1 = 1$ when $R^1 = N$-(1-benzylpiperidin-4-yl).

[b]) Affinity constant in μM; SD = ±2%.

[c]) Not measured.

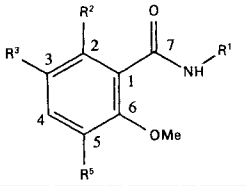

Table 2. *^{13}C-NMR Chemical Shifts in ppm* ($\delta(CDCl_3) = 77.15$ ppm)

No.	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)
Orthopramides							
1	121.8	132.3	120.9	131.9	111.2	157.4	165.3
2	126.6	122.4	123.9	114.8	152.3	147.4	165.0
3	129.6	130.4	130.1	139.9	130.2	152.9	163.5
4	127.6	129.3	122.2	115.3	146.3	153.1	163.9
5	123.5	134.5	113.3	134.7	113.1	156.4	163.8
6	129.9	133.8	117.9	138.3	118.6	154.3	163.2
7	128.3	125.6	116.9	118.3	153.4	146.9	164.0
8	127.4	123.5[a])	116.8	123.3[a])	152.2	146.2	165.5
9	119.7	128.3	138.8	129.5	111.7	156.9	165.0
10	128.2	131.9	87.1	124.0	147.9	153.3	164.1
11	124.4	140.5	83.4	140.8	114.0	157.4	163.7
12	126.3	122.8	133.9	116.1	152.2	145.5	165.5
13	126.8	119.8[a])	134.1[a])	113.8[a])	152.5	145.3	164.8
14	121.5	131.2	136.9	131.3	111.3	155.7	165.6
15	126.5	129.7	141.5	132.6	127.9	152.0	164.9
16	127.1	130.6	142.5	135.6	116.7	153.3	165.1
17	126.4	121.7	140.2	114.9	152.3	145.6	165.5
18	121.5	132.3	135.4	132.0	111.3	155.8	165.7
19	122.0	132.3	121.2	131.9	111.2	157.0	164.0
20	126.8	122.4	124.2	114.8	152.3	147.0	163.0
21	126.8	134.5	113.6	134.7	113.2	156.8	162.0
22	130.1	133.8	118.2	138.3	118.6	153.9	162.1
23	128.5	125.6	117.2	118.3	153.5	146.5	163.1
24	121.7	131.2	137.2	131.3	111.3	155.3	164.5
25	121.7	132.3	135.7	132.0	111.2	155.4	164.6
2,6-Dimethoxybenzamides							
26	116.9	157.5	104.2	130.3	104.2	157.5	166.0
27	123.2	153.4	119.4	130.4	107.6	155.6	164.5
28	122.4	155.9[a])	107.3[a])	133.6	108.3[a])	153.9[a])	165.1
29	124.1	153.4	112.2	133.8	129.4[a])	152.9[a])	163.8
30	129.5	154.1	112.8	136.7	112.8	154.1	163.9
31	[b])	–	–	–	–	–	–
32	128.8	146.2	112.9	119.6	137.4	143.2	164.6
33	127.1	146.7	111.3	120.9	144.3	147.1	164.5
34	128.6	147.6	111.1	117.0	149.9	145.9	164.6
35	122.9	157.9	80.8	139.7	109.3	157.2	165.0
36	128.5	149.9	84.2	122.6	150.4	149.5	164.7
37	122.8	147.0	146.9	114.3	106.3	150.7	165.6
38	[b])	–	–	–	–	–	–
39	122.8	150.7	106.3	114.3	146.9	147.0	165.6
40	136.7	151.7	140.0	130.1	111.7	158.7	164.8
41	127.2	149.1	133.0	113.8	144.3	148.2	166.0
42	121.1	150.6	107.7	116.1	143.3	145.1	165.8
43	[b])	–	–	–	–	–	–
44	[b])	–	–	–	–	–	–
45	[b])	–	–	–	–	–	–

Table 2 (cont.)

No.	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)
6-Methoxysalicylamides							
46	104.2	164.5	111.6	133.0	100.9	158.9	170.3
47	109.2	162.4	115.4	134.1	116.9	157.4	169.0
48	109.7	163.2	116.3	137.0	105.2	156.0	169.1
49	108.3	156.9	113.1	118.9	144.4	148.3	169.8
50	107.8	161.7	114.7	134.1	126.8	157.0	169.2
51	105.6	152.8	156.8	118.0	99.0	154.4	169.7
52	105.2	160.0	115.6	132.8	101.2	157.5	169.8
53	109.7	158.2	119.0	133.5	116.5	153.7	168.6
54	110.0	158.8	119.7	136.3	104.7	154.8	168.6
55	108.8	153.1	115.7	115.9	146.6	148.0	169.2
56	108.9	157.2	118.1	135.0	121.0	156.0	169.4
57	109.0	157.0	118.5	133.5	127.3	155.6	169.4
58	[b])	–	–	–	–	–	–
59	105.2	160.8	104.2	135.8	102.0	158.2	169.8
60	108.1	156.2	105.0	124.2	147.1	145.7	168.9
61	109.7	159.1	107.8	136.5	117.3	154.4	168.6
62	110.1	159.6	108.4	139.1	105.2	155.4	168.5
63	109.0	153.5	105.5	121.9	144.6	147.9	169.2
64	107.5	152.6	105.7	125.8	139.0	149.1	169.1
65	109.2	152.1	107.3	124.5	132.1	145.2	169.2
66	108.9	158.0	107.1	138.0	125.7	156.7	169.3
67	108.9	157.8	107.4	136.4	128.0	156.3	169.3
68	118.8	169.0	109.7	130.6	127.2	153.9	169.1
69	103.5	162.3	77.8	142.6	103.5	159.6	170.3
70	109.0	161.3	81.4	142.3	117.9	155.4	168.6
71	107.7	155.6	79.1	127.4	144.8	148.7	168.7
72	104.9	155.0	144.0	115.1	99.2	152.6	170.5
73	109.1	152.6	147.2	119.0	116.8	144.1	169.3
74	109.1	153.6	146.9	118.5	103.9	149.0	169.3
75	108.0	151.9	146.1	115.8	125.2	149.8	170.2
76	107.9	151.8	145.8	115.8	123.9	149.9	170.1
77	102.8	162.5	120.2	133.4	100.2	157.0	170.6
78	108.1	160.5	124.8	134.3	115.8	152.6	169.4
79	108.5	161.1	125.5	137.1	104.3	153.7	169.4
80	107.3	159.5	123.9	135.8	120.3	154.9	170.2
81	107.3	159.4	122.9	135.8	124.2	154.7	170.2
82	102.8	162.1	125.9	131.8	100.2	156.9	170.6
83	106.7	157.2	127.7	120.4	145.6	143.8	169.7
84	108.1	160.1	130.5	132.7	115.8	152.5	169.4
85	108.7	161.0	131.5	135.7	104.6	153.8	169.6
86	107.5	154.7	128.2	119.0	143.8	146.3	170.2
87	107.1	159.4	129.3	133.1	125.8	154.8	170.5
88	102.8	162.8	124.4	132.5	100.2	157.0	170.7
89	108.1	160.4	129.3	133.5	115.9	152.7	169.4
90	107.5	154.9	126.9	119.9	143.6	146.3	170.2
91	107.3	159.3	127.3	135.0	119.3	155.0	170.2
92	102.8	163.1	124.0	132.7	100.3	157.0	170.7
93	106.7	159.1	132.1	130.6	101.1	162.9	168.6
94	114.3	155.4	131.1	132.4	109.6	167.9	169.1
95	104.4	164.5	111.9	133.0	100.9	158.5	169.1
96	108.0	161.6	114.9	134.0	126.4	156.5	168.9
97	104.8	159.8	115.6	132.8	101.1	157.3	168.0

Table 2 (cont.)

No.	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)
98	109.9	158.1	119.3	133.5	116.5	153.3	167.5
99	109.0	157.0	118.8	133.6	127.4	155.1	168.3
100	104.8	160.6	104.2	135.8	102.0	157.8	168.7
101	110.3	159.5	108.7	139.1	105.2	155.0	167.4
102	108.8	157.7	107.7	136.5	128.0	155.8	168.1
103	103.6	162.3	127.4	132.0	100.3	156.8	169.6
104	108.1	160.0	130.9	132.8	116.0	152.2	168.3
105	108.5	160.7	131.5	135.6	104.6	153.1	168.2
106	[b])	–	–	–	–	–	–
107	108.6	162.0	132.3	141.5	77.2	156.5	169.5

[a]) Tentative assignments.
[b]) Not measured.

given in *Table 2*. The classical substituent parameters used in this study are reported in *Table 3*.

Inspection of *Table 1* reveals that 6-methoxysalicylamides are more potent than corresponding orthopramide analogues in blocking the dopamine D_2 receptor. The introduction of a MeO group at C(5) significantly increases the binding affinity of both orthopramides and 6-methoxysalicylamides to dopamine D_2 receptors. In contrast, 2,6-dimethoxybenzamides are 100 times less potent than the corresponding orthopramides and salicylamides. Presumably, steric hindrance of the 2,6-dimethoxy groups forces the

Table 3. *Substituent Parameter Values*[a])

Aromatic substituents	π[b])	MR[c])	V[d])	σ_m[e])	σ_p[e])	κ[f])
H	0.00	1.03	4.62	0.00	0.00	0.00
Me	0.56	5.65	18.08	–0.07	–0.17	0.11
Et	1.02	10.30	31.54	–0.07	–0.15	0.13
Pr	1.55	14.96	45.00	–0.07	–0.13	0.04
Bu	2.13	19.61	58.46	–0.08	–0.16	0.07
MeO	–0.02	7.87	23.14	0.12	–0.27	0.44
MeS	0.61	13.82	33.08	0.07	–0.22	0.40
OH	–0.67	2.85	10.90	0.12	–0.37	0.40
NH_2	–1.23	5.42	14.91	–0.16	–0.66	0.66
NO_2	–0.28	7.36	20.46	0.71	0.78	0.26
NH_2SO_2	–1.82	12.28	39.12	0.46	0.57	1.24
F	0.14	0.92	6.79	0.34	0.06	–0.16
Cl	0.71	6.03	15.70	0.37	0.23	–0.01
Br	0.86	8.88	20.55	0.39	0.23	0.00
I	1.12	13.94	26.18	0.35	0.18	0.01

[a]) Data taken from [16].
[b]) Hydrophobic substituent constant.
[c]) Molar refractivity.
[d]) Molar volume in $cm^3 \cdot mol^{-1}$.
[e]) *Hammet*'s electronic parameter (σ_m and σ_p are defined with respect to the amido group).
[f]) Electron donor-acceptor parameter.

amide moiety in an almost perpendicular conformation, thereby preventing formation of the intramolecular CONH···OCH_3 H-bond, a critical requirement for D_2 receptor binding [7].

Principal Component Analysis. Principal component analysis (PCA) was performed to extract the structural information content of the ^{13}C-NMR chemical shifts of C-atoms in the aromatic ring and the C-atom in the amide group (*Table 2*). This analysis yielded three significant principal components (PCs) accounting for 91% of the data variance (first component 50%, second component 23.5%, and the third component 16.5%). The correlation matrix (*Table 4*) of ^{13}C-NMR chemical shifts reveals that the chemical shifts of the C-atom in the amide group (C(7)) and of the C-atom in the aromatic ring (C(1)) are highly correlated. A plot (*Fig. 1*) of PC_1 scores (*i.e.*, the coordinate of a given compound along the axis PC_1) *vs.* PC_2 scores illustrates the distribution of compounds into two groups according to the electronic character of their aromatic moiety, orthopramides and 2,6-dimethoxybenzamides on one side, 6-methoxysalicylamides on the other. Interestingly, compounds **68**, **93**, and **94**, although being salicylamide derivatives, cluster with orthopramides. This effect may be due to the presence of a NO_2 group at C(3) or C(5)

Table 4. *Correlation Matrix of ^{13}C-NMR Chemical Shifts*

	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)
C(1)		–0.77	–0.07	–0.26	0.42	–0.27	–0.94
C(2)			–0.15	0.39	–0.46	0.35	0.78
C(3)				–0.19	–0.24	0.01	0.15
C(4)					–0.64	0.74	0.14
C(5)						–0.75	–0.32
C(6)							0.17

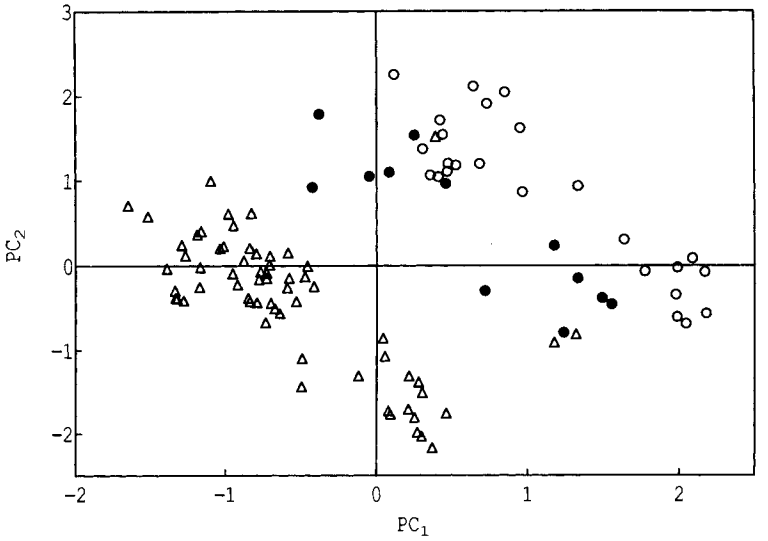

Fig. 1. *Scores plot of orthopramides* (○), *2,6-dimethoxybenzamides* (●), *and 6-methoxysalicylamides* (△) *using the first two principal components* (PC_1 *vs.* PC_2) *in a PCA of ^{13}C-NMR chemical shifts* (*Table 2*)

which strongly influences electronic distribution. *Fig. 1* clearly indicates that the electronic character of both orthopramides and 2,6-dimethoxybenzamides is very similar and markedly differs from that of 6-methoxysalicylamides.

To assess the influence of structural factors on dopamine D_2 receptor binding, we have analyzed the affinity of orthopramides, 2,6-dimethoxybenzamides, and 6-methoxysalicylamides having an *N*-[(1-ethylpyrrolidin-2-yl)methyl] side chain. In addition, *N*-(1-benzylpiperidin-4-yl) derivatives were analyzed separately in order to verify, whether the same structural factors govern the affinity of compounds having either an *N*-[(1-ethylpyrrolidin-2-yl)methyl] or an *N*-(1-benzylpiperidin-4-yl) side chain. The data set to be analyzed consisted of one biological dependent variable and 30 structural variables. Beside multiple linear regression (MLR) analysis, the cross-validated partial least-squares (PLS) statistical method was also used to analyze this data set. It has been shown that PLS has some advantages compared to the MLR method, in particular the ability to handle more variables than compounds and to overcome the problem of collinearity [18] [19]. Furthermore, it provides strong assurance that a QSAR equation will have a predictive utility, and is not obtained by chance [20]. In all equations, only those compounds were included for which all data points were available; no outlier was excluded from analysis (*Tables 1–3*).

PLS and MLR of Orthopramides with an N-*[(1-Ethylpyrrolidin-2-yl)methyl] Side Chain.* The final MLR equations (*Eqns. 1* and *2*) for orthopramides were obtained as follows. First, a PLS analysis (see *Exper. Part*), using all the explanatory variables and a number of cross-validated groups equal to the number of compounds ($n = 18$), provided a cross-validated correlation coefficient (r^2_{CV}) equal to 0.57 and two significant principal components. Using these two significant components, the second run of PLS without cross-validation (*i.e.*, the number of cross-validated groups is equal zero) gave the following statistical parameters:

$$n = 18 \qquad r^2 = 0.782 \qquad s = 0.422 \qquad F = 26.86$$

where n is the number of compounds, r^2 the squared correlation coefficient, s the standard deviation of the equation, and F the *Fischer*'s test of significance. One should keep in mind that the squared correlation coefficient (r^2) is a measure of the goodness of the fit, while the squared cross-validated correlation coefficient (r^2_{CV}) is a real measure of the predictive power of the equation. A closer inspection of the modeling power (*i.e.*, the relative contribution) of each explanatory variable to the overall variance explained by the two significant principal components reveals that the chemical shifts of the C-atoms C(2), C(3), and C(5) and the steric effect (expressed by the sum of molar refractivity of substituent ΣMR or by the sum of molar volumes of substituents ΣV) are the most relevant variables. Excluding irrelevant explanatory variables is known to significantly improve the predictive power of the model [18]. Therefore, PLS with cross-validation was repeated using only these relevant parameters. A highly significant cross-validated correlation coefficient ($r^2_{CV} = 0.818$) is obtained, and the final PLS without cross-validation yielded the following statistical parameters:

$$n = 18 \qquad r^2 = 0.899 \qquad s = 0.308 \qquad F = 29.00$$

In a stepwise procedure, MLR analysis was applied to the same data set. Taking into account the problem of collinearity, MLR showed the same trends as PLS analysis, *i.e.*,

the chemical shifts of the C-atoms C(2), C(3), and C(5), and the steric effect are the most important structural factors. The statistically best equation (*Fig. 2*) obtained is as follow:

$$pK_i = 0.254\ (\pm 0.043)\ \delta(C(2)) + 0.030\ (\pm 0.009)\ \delta(C(3)) + 0.083\ (\pm 0.011)\ \delta(C(5)) + 0.026\ (\pm 0.007)\ \Sigma MR - 46.1\ (\pm 8.0) \quad (1)$$

$$n = 18 \qquad r^2 = 0.899 \qquad s = 0.308 \qquad F = 29.00$$

In parentheses, the standard error of the coefficients are given. The statistical parameters of this equation are the same as those obtained above by PLS. In this particular case (no collinearity), there is no differences between MLR and PLS methods. To emphasize the relative contributions of each explanatory variable, normalized coefficients [21] were also calculated as shown in *Eqn. 2*.

$$pk_i = 1.58\ \delta(C(2)) + 0.71\ \delta(C(3)) + 1.80\ \delta(C(5)) + 0.35\ \Sigma MR \quad (2)$$

Eqn. 2 clearly demonstrates that the chemical shifts of C(5) and C(2) in the aromatic ring are the predominant structural properties related to the affinity of orthopramides for the dopamine D_2 receptor. The chemical shifts, in particular that of C(5), are mainly influenced by the substituent at position 5; substituents such as MeO or OH increase the chemical shift and enhance affinity, while substituents such as H, Cl, and Br decrease the chemical shift and diminish affinity. In a previous study [12], the high potency of nortropane-substituted benzamides with a 5-MeO group was explained in terms of stability of the intramolecular benzamidic H-bond, a linear relationship having been obtained between anti-dopaminergic activity and the amidic hydrogen chemical shifts of a series of 5-substituted derivatives. In full agreement with our results, substituents such as MeO or EtO increased the chemical shift and enhanced the potency, while substituents such as H, Br, and NO_2 decreased the chemical shift and diminished the potency.

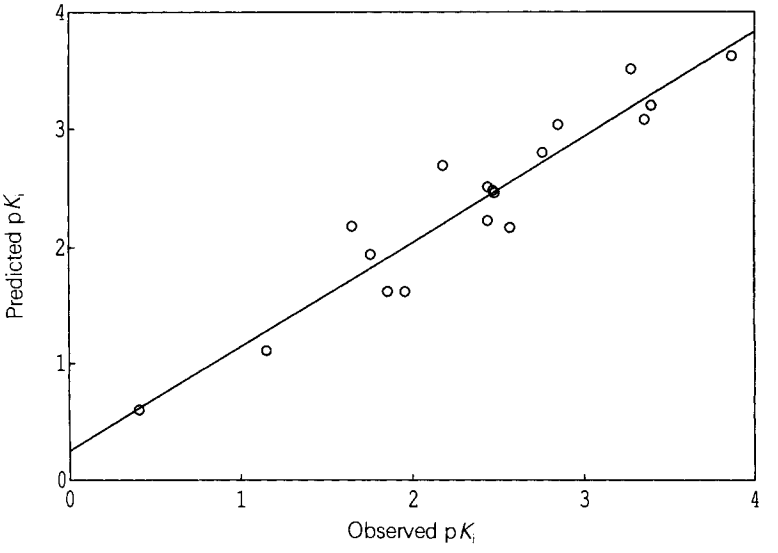

Fig. 2. *Observed* vs. *predicted D_2 receptor affinity of orthopramides* (*Eqn. 1*)

PLS and MLR of 6-Methoxysalicylamides with an N-[(1-Ethylpyrrolidin-2-yl)-methyl] Side Chain. The same PLS procedure was applied to the data set of 6-methoxysalicylamides using all explanatory variables and a number of cross-validated groups equal to the number of compounds ($n = 48$). The exploratory PLS analysis provided a cross-validated correlation coefficient (r^2_{CV}) equal to 0.54 and three significant principal components. Using these significant components, the second run of PLS without cross-validation gave the following statistical parameters:

$$n = 48 \qquad r^2 = 0.701 \qquad s = 0.515 \qquad F = 32.80$$

Unlike orthopramides, an inspection of the modeling power of variables reveals that the lipophilic contribution of substituents, in particular at C(3) (π_3), and δ(C(6)) are the most relevant variables. MLR confirmed that the lipophilicity of substituents at C(3) (π_3) and δ(C(6)) are the most relevant variables, but a quadratic term for π_3 must be included providing *Eqn. 3*

$$\begin{aligned} \mathrm{p}K_i = &-0.754\ (\pm 0.217)\ \pi_3^2 + 2.10\ (\pm 0.36)\ \pi_3 - 0.060\ (\pm 0.017)\ \delta(\mathrm{C}(6)) \\ &+ 10.6\ (\pm 2.3) \end{aligned} \tag{3}$$

$$n = 48 \qquad r^2 = 0.668 \qquad s = 0.543 \qquad F = 28.81$$

Normalized coefficients are as follow:

$$\mathrm{p}K_i = -0.703\ \pi_3^2 + 1.20\ \pi_3 - 0.321\delta(\mathrm{C}(6)) \tag{4}$$

Using *Eqn. 3*, the relationship between observed and predicted affinity was depicted in *Fig. 3* which illustrates the relatively poor prediction of some compounds.

These results confirm and extend those of *Norinder* and *Högberg* [13], who also applied the PLS method to analyze the D_2 receptor affinity of a series of salicylamides and showed the lipophilic character of the substituent at C(3) to be the most predominant

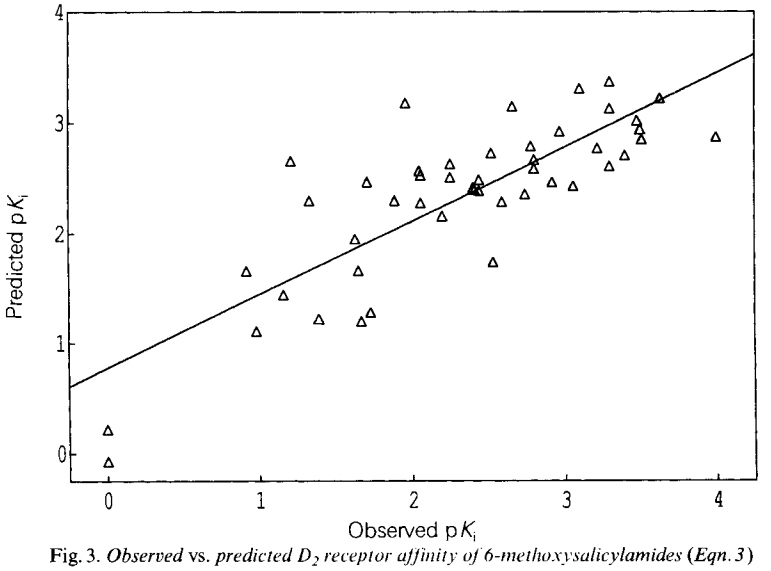

Fig. 3. *Observed* vs. *predicted D_2 receptor affinity of 6-methoxysalicylamides (Eqn. 3)*

factor. In a previous study, *de Paulis et al.* [7] found a parabolic relationship between blockade of [^{3}H]spiperone binding and the lipophilic constant of the substituent at C(3) of a congeneric series of 6-methoxysalicylamides.

A comparison between *Eqns. 1*, *2*, and *3*, *4* indicates that there are marked differences in the structural properties responsible for D_2 receptor affinity of orthopramides and 6-methoxysalicylamides, strongly suggesting different modes of binding to the dopamine D_2 receptor. Support for this hypothesis comes from a recent study of the ionization and conformational behavior of raclopride, a 6-methoxysalicylamide analogue, where ionization and conformational behavior differ significantly from that of orthopramide analogues [22].

PLS and MLR of 2,6-Dimethoxybenzamides. As discussed above, both orthopramides and 2,6-dimethoxybenzamides possess similar electronic characters (*Fig. 1*), suggesting that both classes could be merged into a single QSAR analysis. However, the poor affinity of 2,6-dimethoxybenzamides results in weak structural effects of substituents, and their variation in affinity (*Table 1*) is too small to allow a substituent-affinity relationship to be analyzed.

PLS and MLR of N-*(1-Benzylpiperidin-4-yl) Derivatives.* It should be noted that an attempt to treat both orthopramides and 6-methoxysalicylamides in the same analysis proved unsuccessful (results not shown). However, by considering separately all *N*-(1-benzylpiperidin-4-yl) derivatives ($I_1 = 1$), correlations were obtained which include both orthopramides and 6-methoxysalicylamides. Using all explanatory variables and a number of cross validated group equal to 18, PLS analysis yielded a cross-validated correlation coefficient (r^2_{CV}) equal to 0.802 and three significant principal components. Using these three significant components, the second run of PLS without cross-validation gave the following statistical parameters:

$$n = 18 \qquad r^2 = 0.922 \qquad s = 0.226 \qquad F = 55.27$$

The chemical shifts of the C-atoms C(2), C(3), and C(6), and the steric effect (expressed by the sum of molar refractivity of substituents ΣMR or by the sum of molar volumes of substituents ΣV) are the most relevant variables. The statistically best equation and its normalized form obtained by the stepwise procedure of MLR are as follow:

$$\mathrm{p}K_i = 0.030\,(\pm 0.006)\,\delta(\mathrm{C}(2)) - 0.029\,(\pm 0.008)\,\delta(\mathrm{C}(3)) - 0.226\,(\pm 0.025)\,\delta(\mathrm{C}(6)) + 35.8\,(\pm 3.8) \quad (5)$$

$$n = 18 \qquad r^2 = 0.871 \qquad s = 0.291 \qquad F = 31.50$$

$$\mathrm{p}K_i = 0.629\,\delta(\mathrm{C}(2)) - 0.381\,\delta(\mathrm{C}(3)) - 1.020\,\delta(\mathrm{C}(6)) \quad (6)$$

The inclusion of steric parameters such as molar refractivity and molar volumes did not significantly improve the correlation (results not shown).

In the *Eqn. 6*, some structural factors governing the binding of *N*-(1-benzylpiperidin-4-yl) derivatives, with or without a 2-OH group, are similar to those obtained in *Eqns. 2* and *4*. It has been suggested that orthopramides and 6-methoxysalicylamides having an *N*-[(1-ethylpyrrolidin-2-yl)methyl] side chain share the ability to adopt a low-energy folded conformation, whereas compounds belonging to the rigid piperidinyl series, (*e.g.* tropapride and emonapride) cannot behave similarly. Interestingly, the distance between

the basic N-atom and the centre of the six-membered pseudo-ring in *N*-(1-benzylpiperidin-4-yl) derivatives is the same (6 Å) as that existing in *N*-[(1-ethylpyrrolidin-2-yl)methyl] derivatives between the basic N-atom and the centre of the aromatic ring (*Fig. 4*). This topographical analogy may explain the different QSAR of *N*-(1-benzylpiperidin-4-yl) and *N*-[(1-ethylpyrrolidin-2-yl)methyl] derivatives.

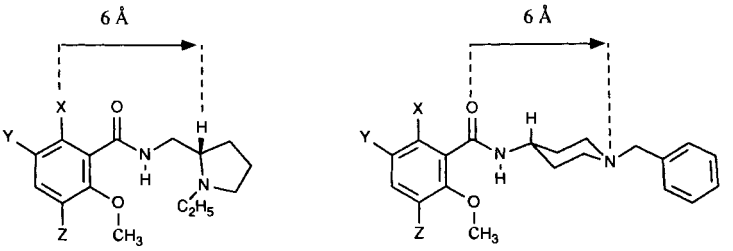

Fig. 4. *Corresponding intramolecular distances in folded* N-*[(1-ethylpyrrolidin-2-yl)methyl]derivatives* (left) *and* N-*(1-benzylpiperidin-4-yl) derivatives* (right)

Conclusion. – The present study shows that ^{13}C-NMR chemical shifts, used alone or in combination with physicochemical parameters, can be useful electronic descriptors in QSAR equations. While the nature of the electronic effect(s) they quantitate may not be easy to unravel, these chemical shifts will at least prove that electrostatic forces do play a role in the drug-receptor interactions being analyzed. Furthermore, a comparison between distinct chemical classes of ligands may indicate comparable or distinct modes of binding to a single receptor. This type of comparison can be performed in the present study, showing the importance of the basic side chain in influencing the mode of binding of orthopramides and 6-methoxysalicylamides. Thus, a piperidinyl side chain results in orthopramides and 6-methoxysalicylamides binding by the same mode to the D_2 receptor. In contrast, a pyrrolidinyl side chain renders the presence or absence of a *ortho* OH group critical for the mode of binding, as evidenced by the large differences between *Eqns. 2* and *4*. The reason for this unexpected difference is being investigated.

Experimental Part

Chemistry. Most compounds were synthesized as already described [7] [8] by reacting the appropriate substituted PhCOCl with either (*S*)-2-(aminomethyl)-1-ethylpyrrolidine or 4-amino-1-benzylpiperidine, resp. The phenolic benzamides (methoxysalicylamides) were obtained by BBr_3 demethylation of the corresponding 2,6-dimethoxybenzamide derivatives.

NMR Chemical Shift. Noise-decoupled ^{13}C-NMR spectra were recorded on a *Fourier*-transform *Bruker NB 200* instrument operating at 250 MHz and 37°. Samples of the free base were *ca.* 20% (*w*/*v*) in $CDCl_3$. Structural assignments of the signals were based on their correlation with tabulated chemical shifts of monosubstituted benzenes [23] [24].

Dopamine Receptor Binding. The reported IC_{50} values for displacing [^{3}H]spiperone from its binding to striatal homogenates of the rat brain were converted to pK_i values by *Cheng* and *Prusoff* formula [25]. Experimental procedures were previously described [26] [27]. The binding assays from different laboratories gave the similar values for raclopride and eticlopride.

Substituent Descriptors and Statistics. Substituent parameter values were taken from [15] [16]. Multiple linear regression analysis was performed using SAS program running on a *VAX 8550* computer of the university of Lausanne. PCA and PLS statistical methods were performed using the QSAR module of SYBYL [28] running on a *SUN Sparc 1* workstation. The SIMCA package [29] including also the PLS algorithms was run on an IBM PC/AT3.

Conceptually, principal component analysis is a bilinear projection method which represents the compounds as points in a multidimensional space, and then projects these points down onto a two- or three-dimensional subspace. This provides an efficient way to convert a data table to a few pictures showing the relations between compounds as illustrated in *Fig. 1.* The mathematical and geometric description of this method is reported in [17] [30]. Unlike PCA, partial least-squares analysis converts a data table to a linear equation relating the target property (*e.g.* biological activity) and the explanatory properties (*e.g.* π, MR). PLS analysis rotates the data matrix to maximize the overlap between the explanatory variables and the target property. For more detail, the reader is refered to the original study of *Wold* and coworkers [18]. In cross-validation of PLS, the analysis is repeated with a omitting random subset of compounds or one compound at a time (when the number of cross-validated group is equal to the number of compounds), and the resulting equation is used to predict the binding affinity of the omitted compounds. The standard errors of the cross-validated predictions are calculated as the sum of squares of deviations of observed *vs.* predicted values. This procedure is repeated, till every compound has been predicted exactly once. A cross-validated r^2 is defined as:

$$r^2_{CV} = (SD - PRESS)/SD \tag{7}$$

where SD is the sum of squares of deviations of the observed values from their mean and PRESS is the prediction error sum of squares [19].

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