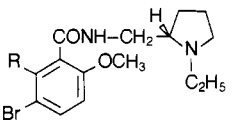
Potential Neuroleptic Agents. 3. Chemistry and Antidopaminergic Properties of Substituted 6-Methoxysalicylamides

Tomas de Paulis,† Yatendra Kumar, Lars Johansson, Sten Rämsby,* Lennart Florvall, Håkan Hall, Kristina Ängeby-Möller, and Sven-Ove Ögren

Research and Development Laboratories, Astra Läkemedel AB, S-15185 Södertälje, Sweden. *Received June 18, 1984*

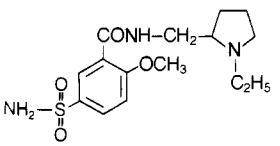
A series of substituted 6-methoxysalicylamides were synthesized from their corresponding 2,6-dimethoxybenzamides by demethylation of one methoxy group with boron tribromide. Substituted 6-methoxysalicylamides having a lipophilic aromatic substituent in the 3-position para with respect to the methoxy group, e.g. a bromo or an iodo atom or an ethyl or a propyl group, and having an (*S*)-*N*-(1-alkyl-2-pyrrolidinyl)methyl moiety as the side chain were found to be potent blockers of [^{3}H]spiperone binding in vitro and potent inhibitors of the apomorphine syndrome in the rat. Similar to remoxipride but in contrast to haloperidol, some of the substituted salicylamides show a 10–20-fold separation between the dose that inhibits hyperactivity and that which inhibits stereotypy. It was concluded that, besides the requirement of a lipophilic substituent in the position para to the methoxy group for antidopamine activity in vivo, the formation of a coplanar six-membered pseudoring involving the amide moiety and the methoxy group is a structural requirement for activity in vitro.

A variety of substituted benzamides has been shown to display properties associated with blockade of central dopamine (DA) receptors.[1] Recently, a series of substituted 2,6-dialkoxybenzamide derivatives have been characterized as potent and selective DA-blocking agents.[2-4] One of these compounds, remoxipride, was shown to be



remoxipride, R = OCH_3
compound *11*, R = OH

50 times more active than sulpiride as an inhibitor of the

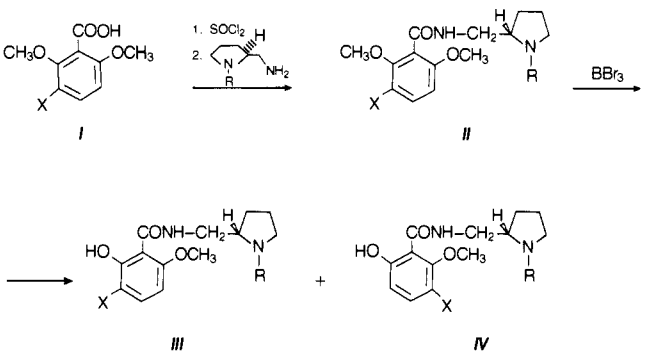


sulpiride

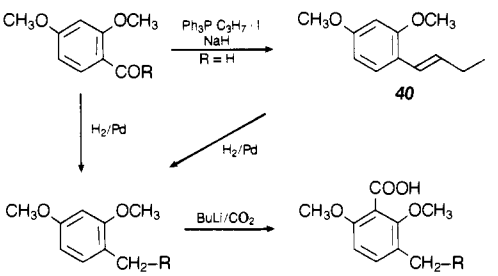
apomorphine syndrome in the rat and was predicted to have low propensity for induction of extrapyramidal side effects in man.[2] However, the potent antagonism of the apomorphine syndrome by remoxipride in vivo was not accompanied by a corresponding affinity for the [^{3}H]spiperone-labeled dopamine D-2 receptor binding site in vitro.[4] The discrepancy between the in vivo and in vitro activities in this series of substituted benzamides led us to initiate an investigation of the structural requirements of the affinity for the [^{3}H]spiperone binding site in rat striatal homogenates. Central DA blockade in vivo was evaluated by the ability to inhibit two behavioral components induced by the DA agonist apomorphine. Apomorphine-induced hyperlocomotion has been associated with activation of DA receptors in the limbic brain area, while stereotypic behavior is considered to reflect activation of DA receptor in the striatum.[4] Thus, this model might identify DA antagonists with reduced propensity for neurological side effects, e.g. drug-induced parkinsonism.

We now report a series of 3- or 5-substituted 2-hydroxy-6-methoxybenzamides (e.g., compound 11), their synthesis, structural characterization, and structure–activity relationships for DA-blocking activities.

Scheme I



Scheme II



27 R = H
28 R = CH_3
29 R = C_2H_5
30 R = C_3H_7

31 R = H
32 R = CH_3
33 R = C_2H_5
34 R = C_3H_7

Chemistry. The general synthetic route for N-substituted [(2-pyrrolidinyl)methyl]-2-hydroxy-6-methoxybenzamides (III and IV) is presented in Scheme I.

The 3-nitro- and 3-halogen-substituted 2,6-dimethoxybenzoic acids were prepared according to methods in the literature.[5-7] Catalytic hydrogenation of 2,4-dimethoxy-

† Present address: Chemistry Department, Vanderbilt University, Nashville, Tennessee 37235.

(1) Jenner, P.; Marsden, C. D. *Neuropharmacol.* **1981**, *20*, 1285.
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(5) Brian, E. G.; Doyle, F. P.; Metha, M. D.; Miller, D.; Nayler, J. H.; Stove, E. R. *J. Chem. Soc.* **1963**, 491.
(6) Durrani, A. A.; Tyman, J. H. P. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1658.
(7) Doyle, F. P.; Nayler, J. H. C.; Waddington, H. R. J.; Hanson, J. C.; Thomas, G. R. *J. Chem. Soc.* **1963**, 497.

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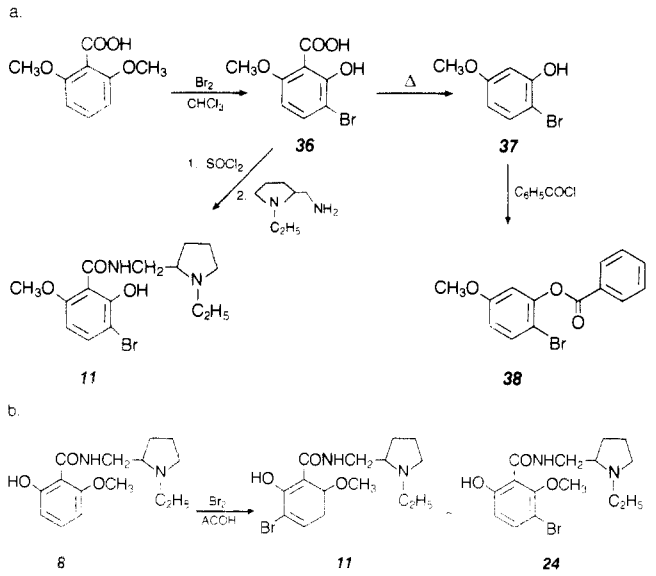
Scheme III



benzaldehyde, 2,4-dimethoxyacetophenone, and 2,4-dimethoxypropiophenone gave the methyl-, ethyl-, and propyl-substituted 2,4-dimethoxybenzene derivatives **27–29**, respectively (Scheme II). A Wittig reaction of 2,4-dimethoxybenzaldehyde gave 1-butenyl-2,4-dimethoxybenzene (**40**), which on hydrogenation gave the butyl-substituted homologue **30**. From these 1-alkyl-2,4-dimethoxybenzenes **27–30** the 3-alkyl-2,6-dimethoxybenzoic acids **31–34** were prepared by lithiation followed by carboxylation. Conversion of the substituted benzoic acids I (Scheme I) into the corresponding acid chlorides was conducted with thionyl chloride in the presence of a catalytic amount of dimethylformamide. The acid chlorides (not isolated) on treatment with the appropriate 1-alkyl-2-(aminomethyl)pyrrolidines[8] gave the (*S*)-(−)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamides (II, **1–7**) and the corresponding *N*-propyl derivative **19** (Table I). Selective monodealkylation with boron tribromide of the substituted benzamides **1–7**, **19**, **20**,[2] **35**,[2] and remoxipride[2] gave the 3-substituted 2-hydroxybenzamides (III, **8–16**, **21**, **26**) as the major products (Table I). The presence of varying amounts of the corresponding minor 5-substituted isomers IV (Scheme I) was detected by chromatographic and spectroscopic techniques. The minor isomers **22–25** were isolated by column chromatography from the mother liquors of their corresponding major isomers **21**, **11**, **10**, and **15**, respectively. Compound **17** was prepared by the same method from the acid **34** without isolating the corresponding 2,6-dimethoxybenzamide. Compound **18** was prepared by acetylation of compound **11** and also by direct amidation via the acid chloride of *O*-acetyl-3-bromo-6-methoxysalicylic acid (**39**). Attempted synthesis of **24** by bromination of the salicylamide **8** gave a mixture of both isomers **11** and **24** (Scheme IIIb). In this experiment the amount of the minor isomer **24** was 15% compared to about 5% in the demethylation reaction.

In the ^{1}H NMR spectra of the salicylamides, the aromatic proton in the 4-position was assigned to the signal appearing at 7.1–7.5 ppm (Table I). The other signal that appears at about 6.3 ppm in the spectra of the major isomers was assigned to the proton in the 5-position (ortho to the methoxy group). In all the minor isomers the corresponding signal of the proton in the 3-position (ortho to the hydroxy group) appears at about 6.7 ppm.

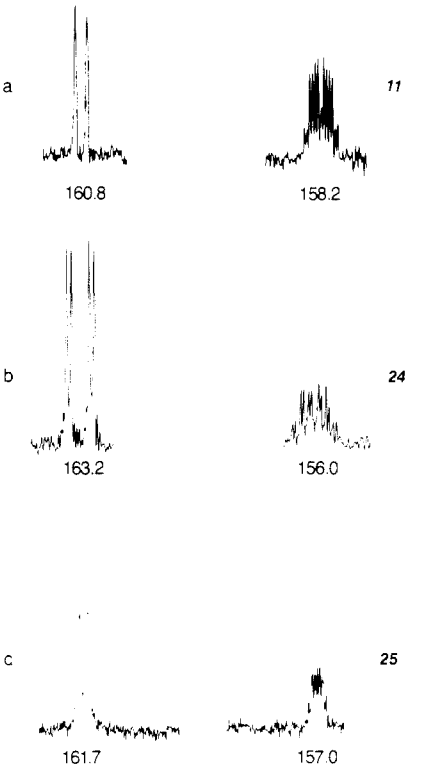


Figure 1. Carbon–proton coupling pattern of the 50-MHz ^{13}C NMR spectra in $CDCl_3$ of the carbons in the 2-position (hydroxy) and in the 6-position (methoxy) of compounds **11**, **24**, and **25**.

The correct assignments of these structures were established by examining their ^{13}C NMR long-range C–H coupling constants (gated decoupling technique). Thus, in the nondecoupled carbon spectrum of compound **11**, the hydroxy-substituted carbon signal appears at 160.8 ppm (assigned by its 15-Hz downfield shift upon the addition of D_2O) as a doublet due to coupling with the proton in the 4-position. The methoxy-substituted carbon signal appears as a multiplet due to its coupling both with the aromatic protons in the 4- and 5-positions and with the protons of the methoxy group (Figure 1a). In the ^{13}C NMR spectrum of the corresponding minor isomer **24**, the signal of the hydroxy-substituted carbon appears at 163.2 ppm as a double doublet due to its coupling with the aromatic protons in the 3- and 4-positions (Figure 1b). The structures of the other halogen-substituted salicylamides (**9**, **10**, **12**, **21–23**, **26**) were assigned by comparing their NMR spectra with those of **11** and **24**.

The assigned structure of compound **11** was confirmed by its synthesis in the following way: 2,6-Dimethoxybenzoic acid was treated with bromine in chloroform to give 3-bromo-2-hydroxy-6-methoxybenzoic acid (**36**) exclusively (Scheme IIIa). The acid chloride was prepared and coupled with 1-ethyl-2-(aminomethyl)pyrrolidine to give an amide that was identical with compound **11**. To establish the structure of the acid **36**, it was decarboxylated by heating and the resulting phenol **37** was reacted with benzoyl chloride (experimental data not shown), yielding a product identified as 2-(benzoyloxy)-4-methoxy-1-bromobenzene (**38**)[9] with a melting point of 65 °C. The isomeric 4-(benzoyloxy)-2-methoxy-1-bromobenzene has been reported to have a melting point of 140 °C.[9]

In the nondecoupled ^{13}C NMR spectrum of **15**, the hydroxy-substituted carbon signal (C-2), assigned by its shift

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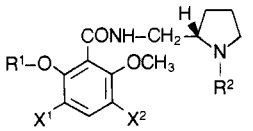
(9) Hodgson, H. H.; Dyson, R. J. H. *J. Chem. Soc.* **1935**, 946.

Table I. Structures and Physical Constants of 3-Substituted (*S*)-(−)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamides and (*S*)-(−)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamides



compd	struct X^1	X^2	R^1	R^2	yield, %	mp, °C	solv[a]	rotation[b] $[\alpha]_D^{25}$, deg
1	H	H	CH_3	C_2H_5	51	100–102	A	−59
2	F	H	CH_3	C_2H_5	25	63–66	A	−76
3	I	H	CH_3	C_2H_5	55	112–113	A	−67
4	NO_2	H	CH_3	C_2H_5	35	144–145	B	−67
5	CH_3	H	CH_3	C_2H_5	60	82–83	A	−80
6	C_2H_5	H	CH_3	C_2H_5	66	99–100	A	−72
7	n-C_3H_7	H	CH_3	C_2H_5	74	105–106	B	−64
8	H	H	H	C_2H_5	51	142–143	C	−56
9	F	H	H	C_2H_5	40	154–155	C	−59
10	Cl	H	H	C_2H_5	80	100 (dec)	C	−51
11	Br	H	H	C_2H_5	76	54–55	D	−46
12	I	H	H	C_2H_5	73	oil[d]		−68
13	NO_2	H	H	C_2H_5	29	156–158	E	−51
14	CH_3	H	H	C_2H_5	67	188–189	E	−58
15	C_2H_5	H	H	C_2H_5	35	101–102	C	−72
16	n-C_3H_7	H	H	C_2H_5	47	170–171	E	−73
17	n-C_4H_9	H	H	C_2H_5	25	164–167	C	−67
18	Br	H	CH_3CO	C_2H_5	83	156 (dec)	E	racemic
19	Br	H	CH_3	C_3H_7	50	103–104	D	−81
20[c]	Cl	H	CH_3	C_2H_5	76	164–165	E	
21	Br	H	H	C_3H_7	50	140–141	E	−78
22	H	Br	H	C_3H_7	20	137–138	E	−68
23	H	Cl	H	C_2H_5	90	oil[d]		−63
24	H	Br	H	C_2H_5	7	63–65	D	−71
25	H	C_2H_5	H	C_2H_5	7	57–59	D	−61
26[e]	Br	H	H	C_2H_5	56	83–84	F	−67
35[c,f]	Br	H	C_2H_5	C_2H_5	68	163–164	C	

formula	anal.	^{1}H NMR shift, ppm H-3	H-4	H-5	CH_3O
$C_{16}H_{24}N_2O_3$	C, H, N,	6.55	7.24	6.55	3.80
$C_{16}H_{23}FN_2O_3$	C, H, F, N				
$C_{16}H_{23}IN_2O_3$	C, H, I, N		7.66	6.21	3.85
$C_{16}H_{23}N_3O_5$·HCl	C, H, Cl, N		7.95	6.69	3.90
$C_{17}H_{26}N_2O_3$	C, H, N		7.11	6.60	3.80
$C_{18}H_{28}N_2O_3$	C, H, N, O		7.16	6.64	3.84
$C_{19}H_{30}N_2O_3$	C, H, N		7.14	6.60	3.82
$C_{15}H_{22}N_2O_3$·HCl·0.5H_2O	C, H, Cl, N	6.57	7.22	6.36	3.89
$C_{15}H_{21}FN_2O_3$·HCl·0.5H_2O	C, H, Cl, N		7.02	6.27	3.91
$C_{15}H_{21}ClN_2O_3$·HCl·0.5H_2O	C, H, Cl, N, O		7.34	6.27	3.87
$C_{15}H_{21}BrN_2O_3$	C, H, Br, N, O		7.48	6.34	3.94
$C_{15}H_{21}IN_2O_3$			7.68	6.27	4.08
$C_{15}H_{21}N_3O_5$·HCl·0.5H_2O	C, H, N		8.06	6.46	4.02
$C_{16}H_{24}N_2O_3$·HCl	C, H, Cl, N		7.12	6.30	3.88
$C_{17}H_{26}N_2O_3$·HCl	C, H, Cl, N		7.09	6.28	3.80
$C_{18}H_{28}N_2O_3$·HCl	C, H, Cl, N		7.08	6.31	3.87
$C_{19}H_{30}N_2O_3$·$C_4H_6O_6$	C, H, N, O		7.12	6.32	3.89
$C_{17}H_{23}BrN_2O_4$·HCl	C, H, N				
$C_{17}H_{25}BrN_2O_3$	C, H, Br, N, O				
$C_{16}H_{23}ClN_2O_3$·HCl					
$C_{16}H_{23}BrN_2O_3$·HCl	C, H, Br, Cl, N		7.41	6.25	3.88
$C_{16}H_{23}BrN_2O_3$·HCl		6.65	7.43		3.90
$C_{15}H_{21}ClN_2O_3$		6.72	7.31		3.91
$C_{15}H_{21}BrN_2O_3$	C, H, Br, N	6.70	7.48		3.89
$C_{17}H_{26}N_2O_3$	C, H, N	6.73	7.20		3.77
$C_{16}H_{23}BrN_2O_3$	C, H, Br, N, O				
$C_{17}H_{25}BrN_2O_3$·HCl					

[a] Key: A, diisopropyl ether; B, acetonitrile; C, ethanol–ether; D, hexane; E, acetone. [b] $[\alpha]_D^{25}$ (*c* 2, acetone) of base. [c] Prepared according to ref 2. [d] Characterized by NMR and mass spectroscopy. Purity was ascertained by GC and TLC. [e] 6-Ethoxy derivative. [f] (*S*)-(−)-3-Bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,6-diethoxybenzamide.

change upon addition of D_2O, appears as a multiplet (double triplet) at 162.1 ppm due to its coupling with methylene protons and a proton in the 4-position. The methoxy-substituted carbon signal (C-6) appears as a multiplet at 156.9 ppm due to its coupling with methoxy protons and protons in the 4- and 5-positions (Figure 2a). Irradiation of the methoxy protons gave a double doublet due to the coupling with protons in the 4- and 5-positions (Figure 2b). In the spectrum of the minor isomer **25**, the signal of the hydroxy-substituted carbon appears as a poorly resolved double doublet at 161.7 ppm due to coupling with protons in 3- and 4-positions (Figure 1c).

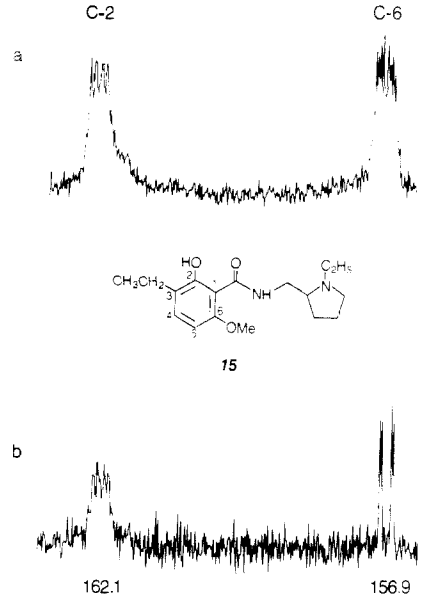


Figure 2. Carbon–proton coupling pattern of the 50-MHz ^{13}C NMR spectra of compound **15** in acetone-d_6, showing the signals of the carbons in the 2- (hydroxy) and 6- (methoxy) positions (a) without and (b) with selective decoupling of the methoxy protons.

In an analogous manner the structures of the alkyl-substituted salicylamides **14–17** were assigned as having the alkyl group in the 3-position by the similarity of their proton and carbon spectra with that of the major ethyl-substituted isomer **15**.

Results and Discussion

Displacement of [^{3}H]Spiperone Binding. The potencies of the compounds in displacing [^{3}H]spiperone from rat striatal membranes in vitro are presented in Table II.

In agreement with the findings with remoxipride, all the 2,6-dimethoxy-substituted compounds were found to have low affinity for [^{3}H]spiperone binding sites in vitro. Thus, compounds **3**, **6**, **19**, and remoxipride have IC_{50} values that are in the range of 1 μM, i.e. 4–5 times higher than the IC_{50} of sulpiride. In marked contrast, their corresponding salicylamides **12**, **15**, **21**, and **11** having an *o*-hydroxy group instead of a methoxy group were highly active. The potencies of the salicylamides were clearly dependent on the nature of the substituent in the 3-position. Thus, the compounds with a chloro atom or a bromo atom in the 3-position (**10**, **11**) have potencies in the same range as that of haloperidol. Interestingly, the potency of these salicylamides was retained or even increased by substituting with an alkyl group. The compounds with a methyl, an ethyl, or a propyl group in the 3-position (**14–16**) are highly active. In contrast, substitution of the 5-position with a halogen atom (**22–24**) or an alkyl group (**25**) produces less variation in the activity than the corresponding substitution in the 3-position, e.g. analogues **21**, **10**, **11**, and **15**, respectively.

The IC_{50} values of the 3-substituted salicylamides **8–16** were found to correlate with the lipophilicity of the substituents (Figure 3). Correlation between the DA-blocking activity and the lipophilicity contribution of the 3-substituent, using the Hansch π parameter, shows a linear relationship for the halogen series of substituents. The alkyl series of 3-substituents shows a parabolic relationship, with ethyl and propyl groups producing the most potent compounds **15** and **16**.

The introduction of a nitro group decreases the activity. The 3-nitro derivative **13** is one-tenth as active as the unsubstituted analogue **8** and 900 times less potent than the 3-iodosalicylamide **12**. The effect of the substituent on the pyrrolidine nitrogen atom was investigated by comparing the activities of the ethyl and *n*-propyl derivatives **11** and **21**, respectively, and by comparing the activity of the dimethoxybenzamide **19** with that of remoxipride. The *N*-propyl-substituted salicylamide derivative **21** was twice as potent as its *N*-ethyl analogue **11**, indicating a favorable effect with increasing chain length of the *N*-alkyl function.

Table II. Blockade of [^{3}H]Spiperone Binding and Antagonism of Apomorphine-Induced Hyperactivity and Stereotypy

compd	[^{3}H]spiperone binding (IC_{50}, μM)	apomorphine antagonism[a] (ED_{50}, μmol/kg ip) hyperactivity	stereotypy
1	15.3[b]	63.1 (61.7–64.6)	85 (85–85)
3	2.79	1.12 (1.07–1.15)	2.1 (2.0–2.0)
6	0.921	0.95 (0.78–1.07)	14.1 (13.8–14.1)
8	0.315	0.40 (0.36–0.43)	7.8 (7.6–8.3)
9	0.362		
10	0.039	0.20 (0.19–0.20)	0.87 (0.81–0.95)
11	0.012	0.055 (0.054–0.055)	0.32 (0.30–0.33)
12	0.0034	0.15 (0.11–0.16)	0.54 (0.52–0.55)
13	3.02		
14	0.019	1.23 (1.15–1.26)	0.95 (0.89–1.02)
15	0.0047	0.12 (0.11–0.13)	0.25 (0.24–0.25)
16	0.0048	0.16 (0.14–0.18)	0.79 (0.74–0.87)
17	0.026		
18	3.50[b]	0.30[b] (0.30–0.30)	0.46[b] (0.46–0.47)
19	0.987	3.4[b] (2.7–3.8)	15.5[b] (15.1–15.8)
20	3.19	6.3[c] (5.2–6.9)	11.7[c] (11.2–12.3)
21	0.0063	0.43 (0.42–0.43)	0.6 (0.65–0.66)
22	0.15		
23	0.064	0.78 (0.65–0.85)	2.5 (2.4–2.7)
24	0.056	6.2 (6.0–6.2)	6.6 (6.5–6.8)
25	0.123	0.47 (0.45–0.49)	0.79 (0.56–0.95)
26	0.021[b]	1.66[b] (1.58–1.78)	2.95[b] (2.82–3.02)
35	13.0	0.47[c] (0.46–0.49)	1.9[c] (1.8–2.2)
remoxipride	1.57	0.86 (0.81–0.98)	6.5 (6.2–6.7)
sulpiride[b]	0.233	65.6 (62.7–68.5)	212 (198–238)
haloperidol	0.012	0.29 (0.27–0.35)	0.27 (0.26–0.29)

[a] The compounds were injected ip 60 min prior to apomorphine (1 mg/kg sc). The apomorphine-induced hyperactivity and stereotypy were scored as described previously.[2] The ED_{50} values and the 90% confidence interval (calculated by Theil's method[18] from log dose–response curves) are based on five to six dose levels with six to eight animals per dose. [b] Racemic compound. [c] Value taken from Florvall and Ögren.[2]

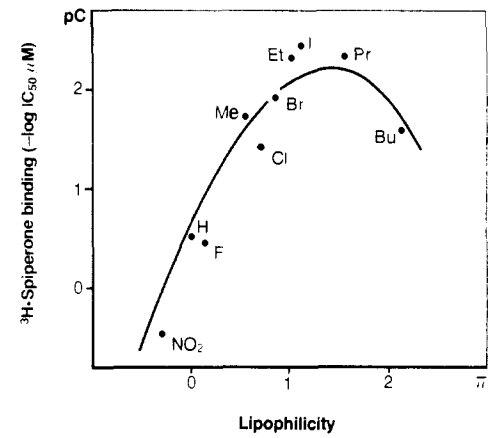


Figure 3. Relationship between blockade of [^{3}H]spiperone binding (pC) and the lipophilic contribution (π) of the aromatic 3-substituent in *S*-(−)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamides.

Inhibition of DA-Mediated Activity in Vivo. The abilities of some of the dimethoxybenzamides and the salicylamides to antagonize the behavioral syndrome caused by apomorphine in the rat are shown in Table II.

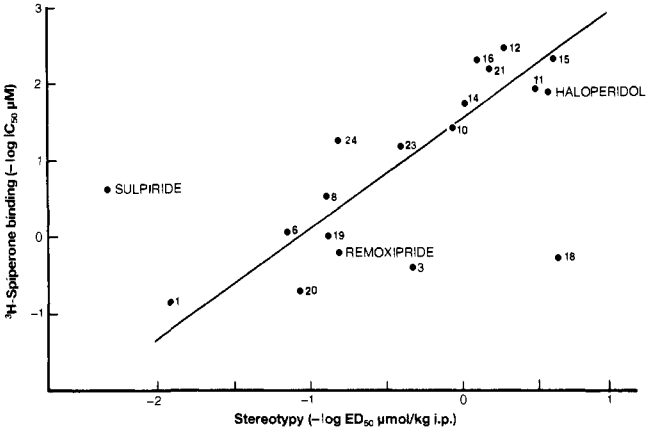


Figure 4. Correlation between blockade of [^{3}H]spiperone binding and apomorphine-induced stereotypy of six substituted 2,6-dimethoxybenzamides (remoxipride, 1, **3**, **6**, **19**, **20**) and 10 substituted 6-methoxysalicylamides (**8**, **10**–**12**, 14–**16**, **21**, **23**, **24**). For racemic compounds half the test value was used. Sulpiride and the acetate ester **18** were omitted in the regression analysis (r = 0.87, n = 16).

The in vivo inhibitory activity of these compounds was found to correlate positively with the blockade of [^{3}H]-spiperone binding (Figure 4) with the exclusion of the ethoxy derivatives **26** and **35** and the two outliers, sulpiride and the acetate ester **18** of the corresponding salicylamide **11**. A linear correlation (r = 0.87) exists between the logarithm of the inverse of the IC_{50} values of inhibition of the [^{3}H]spiperone binding and the inverse ED_{50} values of the stereotypy inhibition. Similar to sulpiride but unlike haloperidol, some salicylamides were more effective in blocking the hyperactivity induced by apomorphine than in blocking the stereotyped behavior such as chewing, licking, and sniffing. The compounds having a bromo atom (**11**) or a propyl group (**16**) are about 5–7 times more potent than haloperidol in inhibiting the hyperactivity paradigm (Table II). However, the highest separations between the ED_{50} values for inhibition of hyperactivity and inhibition of stereotypy were observed in the nonsubstituted salicylamide **8** and the ethyl-substituted analogue **6** of remoxipride. The separation ratio for **8** is 6 times that of sulpiride and more than 20 times that of haloperidol.

The present study addressed the question whether it was possible to enhance the in vitro potency of the dialkoxybenzamides related to remoxipride. The major finding of this study is that demethylation (deethylation) of one methoxy (ethoxy) group in the 2,6-dimethoxy- (diethoxy-) benzamides results in highly potent DA-blocking activity both in vitro and in vivo. Thus, the most active salicylamides (**11**, **12**, **15**, **21**) have potencies that are in the same range or higher than that of haloperidol. With regard to the stereotypy–hyperactivity separation paradigm, it is noteworthy that the compounds with the highest separation (**6** and **8**) are relatively weak blockers of [^{3}H]spiperone binding in vitro.

The importance of the hydroxy group is demonstrated by the lack of activity in vitro of the *O*-acetyl derivative **18**. When tested in vivo, **18** was equipotent with its corresponding hydroxy derivative **11**, most likely due to its rapid conversion into **11** by esterases present in the rat blood.

Most substituted benzamides contain either a halogen atom or a sulfonamide moiety in the 5-position (which corresponds to the 3-position in these salicylamides). The importance of the position para to the methoxy group was demonstrated by the relative low potency of the compounds **8** and **22**–**25**, which are all lacking a substituent

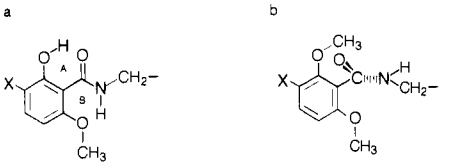


Figure 5. (a) Formation of two pseudorings, A and B, by hydrogen bonding in 6-methoxysalicylamides. (b) Prevention of the ring by out-of-plane crowding in 2,6-dimethoxybenzamides.

in the 3-position. Surprisingly, the incorporation of an electron-donating substituent, e.g. an ethyl or a propyl group, resulted in compounds with potencies superior to those of the corresponding compounds carrying an electron-attracting halogen substituent.[10]

The conclusion of these findings is that the structural requirement of the substituent in the 3-position is dependent on the lipophilic character of the substituent and not on its electronic nature. This is further substantiated by the poor activity of the nitro-substituted compound **13** (see Figure 1). On the other hand, the nature of the substituent in the 5-position of these salicylamides seems to have little influence on the activity.

The effect of replacing the *N*-ethyl group in the pyrrolidine ring with an *N*-propyl group was also investigated. The activity in vitro or in vivo is not much affected by these changes. These findings are consistent with the results obtained in the sulpiride series, e.g. sulmepride (TER 1546), sulpiride, and prosulpride (GRI 1665), where the *N*-methyl, *N*-ethyl, and *N*-propyl analogues of sulpiride showed similar antiapomorphine activities.[11]

In the in vitro–in vivo correlation (Figure 4) both remoxipride and sulpiride deviate from the linear relationship (sulpiride was excluded). The fact that sulpiride appears less active in vivo than predicted by its ability to displace [^{3}H]spiperone from its binding sites in striatum can be explained by the low bioavailability of sulpiride, which requires a large dose to achieve an adequate concentration inside the blood–brain barrier. In the case of remoxipride, the opposite deviation is found. The position of remoxipride in the correlation in Figure 4 is shifted toward being more active in vivo than predicted from the in vitro activity. This might be attributed to the presence of an active metabolite in the rat.[4]

It is of particular interest to note that compound **11** and its acetate ester **18** have similar activities in vivo in spite of a 300-fold difference in their potencies to inhibit [^{3}H]spiperone binding. Possibly, **18** behaves as a prodrug of **11**, thereby producing the same antiapomorphine effect in the rat as **11**. Similarly, remoxipride shows a considerable potency in blocking the behavioral syndrome of apormorphine without the corresponding effect on the [^{3}H]spiperone binding (Figure 4). This suggests that the formation of the active 5-bromo-6-methoxysalicylamide derivative **24** in the rat could be responsible for some of the antidopamine effects of remoxipride in vivo.

Examinations of the crystal structure of substituted benzamides have shown that there exists a strong hydrogen bonding between the oxygen atom of the *o*-methoxy group and the amide hydrogen atom, thus forming a rigid bicyclic ring system[12] (ring B, Figure 5a).

The presence of a hydroxy group adjacent to the amide carbonyl enhances this planar arrangement by forming another six-membered pseudoring with another hydrogen

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bond between the phenol hydrogen atom and the carbonyl oxygen atom (ring A, Figure 5a). The high in vitro potency of the salicylamides is probably due to the presence of this planar arrangement between the amide and the aromatic ring. In compounds **1–7**, **18–20**, and remoxipride, the bulky methoxy groups in both 2- and 6-positions prevent this ring system by introducing a steric hindrance that forces the amide group out of the plane and, thus, decreases the possibility of the hydrogen bond to form between the oxygen atom of the methoxy group and the amide hydrogen atom (Figure 5b). In crystals of remoxipride the dihedral angle between the amide carbonyl group and the benzene ring is 71°; that is, the amide is almost perpendicular to the aromatic plane.[13]

In conclusion, antidopamine activity in vivo of these substituted 6-methoxysalicylamides requires a lipophilic substituent in the para position with regard to the methoxy group. For dopamine receptor blockade in vitro, formation of a coplanar six-membered pseudoring, involving the amide moiety and the methoxy group, seems to be a structural requirement.

Experimental Section

Chemistry. Melting points are uncorrected. NMR spectra were recorded on a JEOL FT 200 spectrometer, and shifts are reported in parts per million from internal tetramethylsilane. Mass spectra were recorded at 70 eV on a LKB 9000 instrument. Optical rotations were obtained in acetone unless otherwise noted on a Perkin-Elmer 141 polarimeter. The elemental analyses were performed by Analytische Laboratories, Elbach, West Germany, and were within 0.4% of the theoretical values. Thin-layer chromatography was performed with Merck silica gel 60 F-254 plates (10 × 5 cm) in diisopropyl ether–methanol–concentrated ammonia (100:10:1). Gas chromatography was performed on a carlo Erba 4160 instrument using a 20-m fused silica capillary column (0.25 mm) with 0.22-μm cross-linked SE-30 as the stationary phase.

General Method for the Preparation of 3-Substituted 2,6-Dimethoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamides (II). The appropriate 3-substituted 2,6-dimethoxybenzoic acid I (0.10 mol) was dissolved in toluene (350 mL), and thionyl chloride (20 mL, 0.27 mol) was added. The mixture was heated to 65 °C, and a catalytic amount of dimethylformamide (0.5 mL) was added. After 1 h the solvent was removed in vacuo, and the residue was dissolved in chloroform (150 mL). A solution of (*S*)-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidine [prepared by chloroform extraction (2 × 75 mL) of a solution of 50 g of the pyrrolidine ditartrate[8] in 5 N NaOH (75 mL)] was added at 25 °C. After 30 min the solvent was removed, and the residue was dissolved in 1 N HCl (150 mL). The aqueous layer was washed with ether (50 mL) and neutralized with 5 N NaOH to give an oil, which was extracted with ether (3 × 300 mL). Crystallization from diisopropyl ether (500 mL) gave the pure benzamide. Physical constants are given in Table I.

General Method for the Preparation of 3-Substituted 2-Hydroxy-6-methoxy-*N*-[(1-substituted 2-pyrrolidinyl)-methyl]benzamides (III). To a solution of the appropriate 2,6-dimethoxybenzamide II (0.010 mol) in methylene chloride (75 mL) was added 3 N HCl–ether (3.3 mL) followed by dropwise addition of a solution of boron tribromide (2.5 g, 0.010 mol) in methylene chloride (25 mL) at 20 °C. After 30 min, 1 N ammonia (50 mL) was added. The aqueous layer was extracted with methylene chloride (2 × 50 mL), and the combined organic layer was washed with water and dried (Na_2SO_4), and the solvent was removed. The residue was dissolved in ether (60 mL) or hexane, and insoluble material was removed by filtration. Evaporation gave the corresponding 3-substituted 2-hydroxy-6-methoxybenzamide III as the major product. Column chromatography on silica using diisopropyl ether–methanol–ammonia (80:19:1) as eluent gave the minor 5-substituted isomer IV (**23–25**). The hydrochloride of the amine was prepared by adding 1 equiv of HCl–ether. Physical constants are given in Table I.

2-Acetoxy-3-bromo-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide (18). *O*-Acetyl-3-bromo-6-methoxysalicylic acid (**39**; 5.8 g, 0.02 mol) was dissolved in thionyl chloride (30 mL) and stirred for 16 h at 20 °C. The solvent was removed, and the residue was dissolved in toluene (100 mL). A solution of *N*-ethyl-2-(aminomethyl)pyrrolidine (2.6 g, 0.02 mol) in toluene (50 mL) was added, and the mixture was stirred for another 16 h at 20 °C. The precipitate was filtered and washed with toluene. Recrystallization from acetone gave 7.2 g (83%) of hydrochloride of **18**, mp 156 °C (dec). Anal. ($C_{17}H_{24}BrClN_2O_4$) C, H, Cl, N.

5-Bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-hydroxy-6-methoxybenzamide (24). Method A. The preparation and separation is given in the general synthesis of III.

Method B. To a solution of the salicylamide **8** (2.4 g, 0.0086 mol) in acetic acid (50 mL) was added bromine (0.46 mL, 0.0086 mol) in acetic acid (5 mL) dropwise at 20 °C. After 15 h the solvent was removed. GC analysis of the residue (3 g) showed a mixture of 85% of compound **11** and 15% of the minor isomer **24**. Separation on reverse-phase HPLC (methanol–0.5 N ammonium acetate–25% ammonia (60:40:1)) gave 0.6 g of **24** after crystallization from hexane: mp 63–65 °C; ^{13}C NMR of the aromatic region ($CDCl_3$) δ 169.08 (CONH), 163.22 (C-2, shift changes −14.9 Hz upon addition of D_2O), 156.03 (C-6, shift changes +1.5 Hz), 136.95 (C-4), 116.31 (C-3), 109.68 (C-1), 105.16 (C-5). Anal. ($C_{15}H_{21}BrN_2O_3$) C, H, Br, N.

3-Alkyl-2,6-dimethoxybenzoic Acids (31–33). 2,4-Dimethoxyphenyl alkyl ketone[14] (0.1 mol) was dissolved in ethanol (300 mL) and was hydrogenated at NTP in the presence of concentrated HCl (1 mL) and Pd/C (5%, 2.0 g). When the absorption of hydrogen ceased, the catalyst was filtered off. The solvent was removed to give 2,4-dimethoxyphenylalkyl benzene (**27–29**) as an oil.[14] The obtained oil was dissolved in tetrahydrofuran (250 mL), and butyllithium (75 mL, 1.6 M solution in hexane) was added dropwise under a nitrogen atomsphere at room temperature. After stirring for 2 h, the solution was poured onto dry ice. When the temperature reached 20 °C, 2 M HCl was added until pH 1 and the product was extracted with ether. The solution was dried (Na_2SO_4) and concentrated to give 3-alkyl-2,6-dimethoxybenzoic acid **31**: yield 70%; mp 114–115 °C (hexane–diisopropyl ether). Anal. ($C_{10}H_{12}O_4$) C, H, O. **32**: yield 75%; mp 94–96 °C (diisopropyl ether) lit.[15] mp 114–120 °C. Anal. ($C_{11}H_{14}O_4$) C, H. O. **33**: yield 60%, oil (used without further purification).

3-Butyl-2,6-dimethoxybenzoic Acid (34). Sodium hydride, 50% in oil (0.55 g, 0.011 mol), was dissolved in dimethyl sulfoxide (17 mL), and the mixture was heated to 80 °C for 1 h. After cooling to 25 °C, a solution of *n*-propyltriphenylphosphonium iodide (4.7 g, 0.011 mL) in dimethylsulfoxide (17 mL) was added. After 15 min, a deep red appeared. A solution of 2,4-dimethoxybenzaldehyde (1.66 g, 0.01 mol) in tetrahydrofuran (17 mL) was slowly added. The reaction mixture was stirred for 1 h at room temperature and poured into ice water (250 mL). Extraction with ether (3 × 50 mL) gave 4.0 g of crude product. Addition of diisopropyl ether (20 mL) precipitated crystalline triphenylphosphonium oxide (1.5 g). The filtrate was concentrated, and the residue was distilled to give a mixture of the cis and trans isomers of (2,4-dimethoxyphenyl)butene (**40**): yield 1.0 g (52%); bp 80–93 °C (0.3 mm). Hydrogenation of **40** in methanol (25 mL) with palladium (5% on carbon, 50 mg) gave 0.75 g of 2,4-dimethoxy-*n*-butylbenzene (**30**) as an oil, lit.[16] bp 264 °C (710 mm). Lithiation and carboxylation of **30**, performed as described for compounds **31–33**, gave **34** as an oil, yield 0.60 g (68%). The compound was used without further purification.

3-Bromo-6-methoxysalicylcic Acid (36). To a suspension of 2,6-dimethoxybenzoic acid (80.0 g, 0.44 mol) in chloroform (1500 mL) was added a solution of bromine (23 mL) in chloroform (200 mL) at 0 °C. The reaction mixture was stirred at room temperature for 30 h. The solvent was removed, and the residue was

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recrystallized from 95% methanol (350 mL) to give 82 g (76%) of **36**: mp 148–150 °C; ^{13}C NMR of the aromatic region ($CDCl_3$) δ 170.97 (CONH), 159.29 (C-2, shift changes −8.6 Hz upon addition of D_2O), 158.78 (C-6, no shift), 137.84 (C-4), 103.79 (C-5), 102.71 (C-1 and C-3). Anal. ($C_8H_7BrO_4$) C, H, Br, O.

***O*-Acetyl-3-bromo-6-methoxysalicyclic Acid (39).** 3-Bromo-2-hydroxy-6-methoxybenzoic acid (**36**; 24.7 g, 0.10 mol) was dissolved in acetic anhydride (50 mL). Concentrated H_2SO_4 (0.2 mL) was added, and the mixture was stirred at 60 °C for 20 h. After cooling, the reaction mixture was poured into an ice–water mixture. The solvent was removed in vacuo. The residue was crystallized from diisopropyl ether: yield 24.5 g (85%); mp 146–147 °C. Anal. ($C_{10}H_9BrO_5$) C, H, Br.

Pharmacology. [^{3}H]Spiperone Binding. The assays were performed essentially as described earlier.[4] Rats were killed by decapitation, and the striatum was rapidly dissected out on ice. After homogenisation in Tris-HCl buffer (0.05 M, pH 7.6) the homogenate was centrifuged twice for 10 min at 48000*g*, resuspended, and recentrifuged. The final pellet was resuspended in Tris-HCl buffer (0.05 M, pH 7.6) containing 0.1% ascorbic acid and various salts to a final concentration of 5 mg/mL. The incubations were performed at 37 °C for 10 min in plastic trays and were terminated by filtration and subsequent washing on glass fiber paper (Whatman GF/B). (+)-Butaclamol (1 μM) was used for the determination of unspecific binding. The radioactivity of the filters was determined by scintillation spectroscopy. The IC_{50} values were calculated by using log-logit regression analysis.

Apomorphine-Induced Behavior. Male Sprague-Dawley rats, (275–325 g), were used. The behavior was scored 5, 20, 40, and 60 min after injection of apomorphine (1 mg/kg), given subcutaneously into the neck. The scoring was performed as described previously.[2] The test compounds were dissolved in saline or acetic acid and distilled water and injected ip 60 min prior to apomorphine. The ED_{50}'s for stereotypies are the doses that reduce the strength of apomorphine-induced stereotypies by 50% over the total observation period of 60 min. The ED_{50}'s for hyperactivity are the doses that reduce the hyperactivity response by 50% over the observation period of 60 min. The ED_{50} values, based on at least six dose levels with six to eight animals per dose level, were calculated by Theil's method[18] and correlated for ties following Sen's procedure[17] based on Kendell's τ. A slightly modified version of Sen's procedure was used to determine the 90% confidence interval.

Registry No. 1, 96947-76-1; **2**, 96897-87-9; **3**, 96897-88-0; **4**, 96897-89-1; **4**·HCl, 96898-03-2; **5**, 96897-90-4; **6**, 84226-00-6; **7**, 96947-77-2; **8**, 84226-04-0; **8**·$^1/_2$HCl, 96947-81-8; **9**, 84226-05-1; **9**·$^1/_2$HCl, 96947-82-9; **10**, 96947-78-3; **10**·$^1/_2$HCl, 96947-83-0; **11**, 84226-14-2; **12**, 84226-06-2; **13**, 96897-91-5; **13**·$^1/_2$HCl, 96898-04-3; **14**, 96897-92-6; **14**·HCl, 96898-05-4; **15**, 84226-07-3; **15**·HCl, 96393-01-0; **16**, 84226-08-4; **16**·HCl, 96947-84-1; **17**, 96897-93-7; **17**·$C_4H_6O_6$, 96898-06-5; (±)-**18**, 96897-94-8; (±)-**18**·HCl, 84225-89-8; **19**, 84225-93-4; **20**, 82977-52-4; **20**·HCl, 82977-51-3; **21**, 96947-79-4; **21**·HCl, 84225-96-7; **22**, 96947-80-7; **22**·HCl, 84225-99-0; **23**, 84226-16-4; **24**, 96393-00-9; **25**, 96897-95-9; (±)-**26**, 84225-83-2; **27**, 38064-90-3; **28**, 19672-03-8; **29**, 36680-47-4; **30**, 54459-33-5; **31**, 96897-96-0; **32**, 96897-97-1; **33**, 96897-98-2; **34**, 96897-99-3; **35**, 82935-47-5; **35**·HCl, 82935-30-6; **36**, 84225-86-5; **37**, 63604-94-4; **38**, 96898-00-9; **39**, 84225-87-6; (*E*)-**40**, 96898-01-0; (*Z*)-**40**, 96898-02-1; I (X = H), 1466-76-8; I (X = F), 52189-67-0; I (X = I), 90347-70-9; I (X = NO_2), 55776-17-5; I (X = Br), 73219-89-3; $Ph_3P^+C_3H_7I^-$, 14350-50-6; 2,4-dimethoxybenzaldehyde, 613-45-6; 2′,4′-dimethoxyacetophenone, 829-20-9; 2′,4′-dimethoxypropiophenone, 831-00-5; (*S*)-*N*-ethyl-2-(aminomethyl)pyrrolidine, 22795-99-9; (*S*)-*N*-propyl-2-(aminomethyl)pyrrolidine, 84225-92-3; remoxipride, 80125-14-0.

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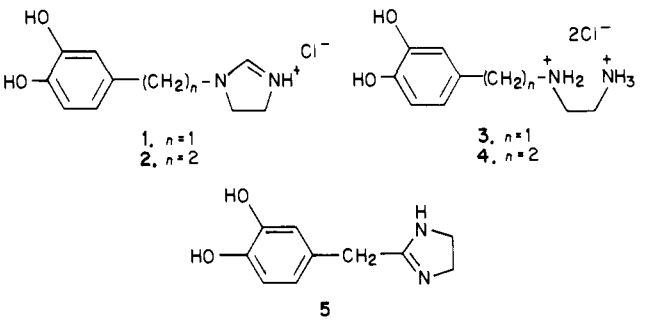
N-Substituted Imidazolines and Ethylenediamines and Their Action on α- and β-Adrenergic Receptors

Akihiko Hamada,† Emily L. Yaden,‡ J. S. Horng,‡ Robert R. Ruffolo, Jr.,‡§ Popat N. Patil,† and Duane D. Miller*†

Division of Medicinal Chemistry and Pharmacognosy and Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, and the Department of Cardiovascular Pharmacology, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285. *Received November 7, 1984*

A series of N-substituted imidazolines and ethylenediamines were synthesized and examined for their activity in α- and β-adrenergic systems. The length of the intermediate side chain between the catechol and imidazoline ring or the amine of the ethylenediamine segment was shown to affect the adrenergic activity. *N*-[2-(3,4-Dihydroxyphenyl)ethyl]imidazoline hydrochloride (**2**) and *N*-[2-(3,4-dihydroxyphenyl)ethyl]ethylenediamine dihydrochloride (**4**), both with two methylene groups between the catechol and amine segment, were found to be somewhat selective for α_2-adrenergic receptors while 1-(3,4-dihydroxybenzyl)imidazoline hydrochloride (**1**) and *N*-2-(3,4-dihydroxybenzyl)ethylenediamine dihydrochloride (**3**), both with one methylene group between the catechol and amine segment, were more selective for α_1-adrenergic receptors in a pithed rat model. Of the four compounds examined, only compound **2** showed significant direct activity on β_1- and β_2-adrenergic receptors.

We report the α-adrenergic activity of imidazolines **1** and **2** and their respective open-chain analogues **3** and **4**. The imidazoline **1** represents one of the positional isomers of the potent α-adrenergic agonist 2-(3,4-dihydroxybenzyl)-imidazoline (**5**).[1] Little work has been reported on N-substituted imidazolines,[1-5] and no investigation has appeared in adrenergic systems with the catechol segments substituted on the nitrogen atom of an imidazoline as shown with **1** and **2**. There have been extensive reports on the structure–activity relationships of 2-substituted imidazolines. Some of the results indicate the following:



† The Ohio State University.
‡ Eli Lilly and Co.
§ Present address: Director of Cardiovascular Pharmacology, Smith Kline and French Laboratories, Philadelphia, PA 19101.

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