Potential Neuroleptic Agents. 4. Chemistry, Behavioral Pharmacology, and Inhibition of [^{3}H]Spiperone Binding of 3,5-Disubstituted *N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamides

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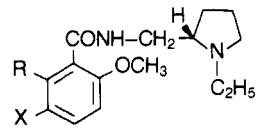
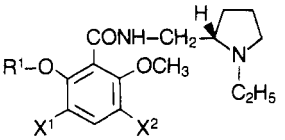
A series of 3,5-disubstituted *N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamides was synthesized, starting from the 2,6-dimethoxybenzoic acids, by boron tribromide demethylation of the corresponding 3,5-disubstituted 2,6-dimethoxybenzamides and separation of the two positional isomers. The correct structure assignments were based on selective decoupling studies on their ^{13}C NMR spectra. The salicylamide derivatives were tested for antidopamine activity in vivo by their ability to inhibit the apomorphine syndrome in the rat and in vitro by their ability to displace [^{3}H]spiperone from striatal preparations of the rat brain. The activity seems to reside exclusively in the *S* enantiomer. Several compounds were considerably more potent than haloperidol, particularly those having an ethyl group in the 3-position and a halogen atom in the 5-position of the aromatic ring. The corresponding 5-alkyl-3-halogen-substituted compounds were much less active. A low acute toxicity was found for the most potent compounds. Some of the salicylamides displayed a 10–20-fold separation between the dose which blocks apomorphine-induced hyperactivity and that which blocks apomorphine-induced stereotypy. One compound, *S*-(−)-3,5-dichloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamide (raclopride, FLA 870) (**13**) had a stereotypy–hyperactivity separation more than twice that of sulpiride while being 100 times more potent in blocking the apomorphine effects. On this basis, **13** was selected for clinical trials against schizophrenia.

In a previous report[1] we have described the development of a series of 3-substituted 6-methoxysalicylamides (I; R = OH) with potent antidopaminergic properties. A related



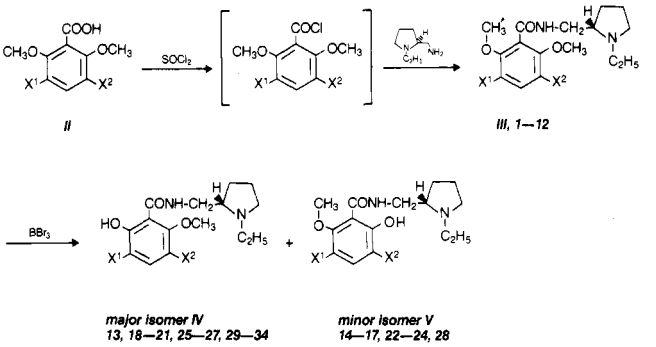
Ia (remoxipride): R = OCH_3; X = Br
Ib: R = OH, X = C_2H_5

substituted benzamide, remoxipride (Ia), is a potent inhibitor of the apomorphine syndrome in the rat without an accompanying ability to cause catalepsy.[2] Sulpiride [5-(aminosulfonyl)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide] is an effective antipsychotic agent with a low propensity to cause extrapyramidal side effects.[3] Remoxipride and sulpiride both inhibit apomorphine-induced motoric hyperactivity at doses lower than those that block apomorphine-induced stereotypy.[4] This separation was also found in the more potent 3-alkyl-substituted salicylamides, e.g. Ib.[1] We now report the synthesis and pharmacology of some *N*-[(1-ethyl-2-pyrrolidinyl)-methyl]-substituted 2-hydroxy-6-methoxybenzamides (IV) having alkyl or halogen substituents in both the 3- and 5-positions of the aromatic ring. The in vitro activities of these compounds were predicted with a QSAR method using the Hansch approach.[5]



III, $R^1 = CH_3$
IV, $R^1 = H$

Scheme I



Chemistry

The general synthetic route for the substituted 2,6-dimethoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamides **1–12** (Table I) and the substituted *N*-[(1-ethyl-2-pyrrolidinyl)methyl)]-6-methoxysalicylamides **13–34** (Table II) is presented in Scheme I. Conversion of the substituted 2,6-dimethoxybenzoic acids II into their corresponding acid chlorides with thionyl chloride in the presence of a catalytic amount of dimethylformamide gave the substituted 2,6-dimethoxybenzoic acid chlorides (not isolated). The acid chlorides were treated with the resolved 2-(aminomethyl)-1-ethylpyrrolidine to give the 3,5-disubstituted 2,6-dimethoxy-benzamides **1–12**. The *S*-(−) and *R*-(+) enantiomers of 2-(aminomethyl)-1-ethylpyrrolidine were obtained by resolution of their ditartrate salts of D-(-)- and L-(+)-tartaric acid, respectively.[4] (*S*)-(−)-3,5-Dibromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide (III; $X^1 = X^2 = Br$) was prepared as reported.[4] The dialkyl-substituted benzamide III ($X^1 = CH_3$, $X^2 = C_3H_7$), which was used for the synthesis of compounds **28**

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(1) de Paulis, T.; Kumar, Y.; Johansson, L.; Rämsby, S.; Florvall, L.; Hall, H.; Ängeby-Möller, K.; Ögren, S.-O. *J. Med. Chem.* **1985**, *28*, 1263.
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(3) O'Connor, S. E.; Brown R. A. *Gen. Pharmacol.* **1982**, *13*, 185.
(4) Florvall, L.; Ögren, S.-O. *J. Med. Chem.* **1982**, *25*, 1280.
(5) de Paulis, T.; Hall, H. In "QSAR in Design of Bioactive Compounds"; Kuchar, M, Ed.; Prous: Barcelona, 1984; p 199.

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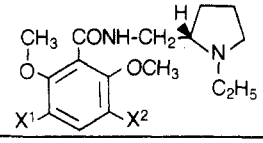
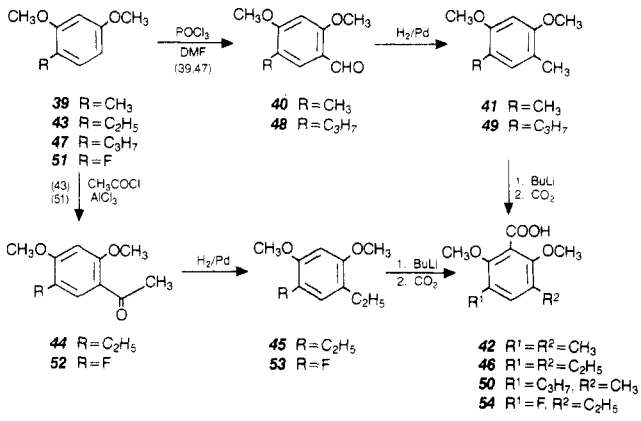
Table I. Structure and Physical Constants of the Substituted *N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamides (III)



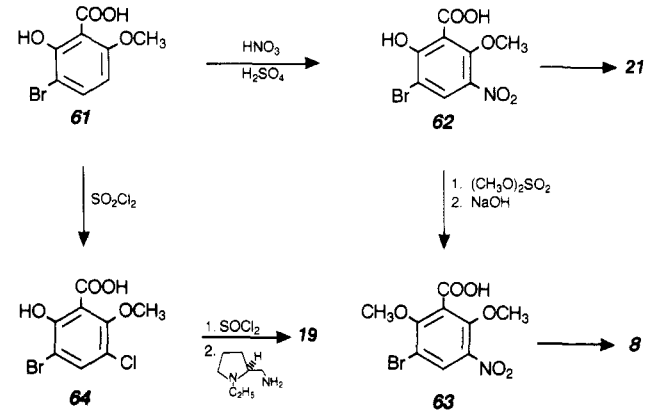
compd	X^1	X^2	yield,[a] %	mp,[a] °C	rotation,[b] deg	formula	anal.
1	Cl	Cl	82	144–145	−71	$C_{16}H_{22}Cl_2N_2O_3$	C, H, Cl, N
2	Cl	Br	55	152–153	−79	$C_{16}H_{22}BrClN_2O_3$	C, H, Br, Cl, N
3	Cl	CH_3	75	120–121	−71	$C_{17}H_{25}ClN_2O_3$	C, H, Cl, N
4	Cl	C_2H_5	62	103–104	−70	$C_{18}H_{27}ClN_2O_3$	C, H, Cl, N
5	Br	F	50	96–98	−72	$C_{16}H_{22}BrFN_2O_3$	C, H, Br, F, N
6	Br	CH_3	64	128–129	−76	$C_{17}H_{25}BrN_2O_3$	C, H, Br, N
7	Br	C_2H_5	27	115–116	−59	$C_{18}H_{27}BrN_2O_3$	C, H, Br, N
8	Br	NO_2	54	108–109	racemic	$C_{16}H_{22}BrN_3O_5$	C, H, Br, N
9	CH_3	CH_3	79	96–97	−58	$C_{18}H_{28}N_2O_3$	C, H, N
10	C_2H_5	F	60	oil[c]	nd[d]	$C_{18}H_{27}FN_2O_3$	
11	C_2H_5	C_2H_5	52	83–84	−70	$C_{20}H_{32}N_2O_3$	C, H, N
12	C_3H_7	Cl	43	oil[c]	−76	$C_{19}H_{29}ClN_2O_3$	

[a] Recrystallized from diisopropyl ether. [b] $[\alpha]^{20}_D$ (*c* 2% in acetone). [c] Characterized by NMR and mass spectrometry. Purity was checked by GC. [d] Not determined.

Scheme II



Scheme III



and **34**, was only characterized by NMR and mass spectrometry.

Demethylation of one of the methoxy groups in the 2,6-dimethoxybenzamides **III** by boron tribromide gave the substituted 6-methoxysalicylamides as a mixture of the two positional isomers **IV** and **V**. In concordance with the results obtained in the 3-substituted series,[1] the isomer having the larger substituent in the 3-position adjacent to the hydroxy group was invariably the major product. This is explained by the effect of a forced rotation of the methoxy group out of the plane of the aromatic ring, which results in a higher atomic charge on the oxygen atom and the subsequent facilitated complexation with the Lewis acid.[6]

The syntheses of the 3,5-dialkyl-substituted 2,6-dimethoxybenzoic acids are presented in Scheme II. Vielsmeyer–Haack formylation of 2,4-dimethoxymethylbenzene[21] (**39**) followed by reduction of the obtained 2,4-dimethoxy-5-methylbenzaldehyde (**40**) gave 2,4-dimethoxy-1,5-dimethylbenzene (**41**). Lithiation and carboxylation of **41** gave 2,6-dimethoxy-3,5-dimethylbenzoic acid (**42**). The same reaction sequence, starting from 2,4-dimethoxypropiophenone[7] produced 2,6-dimethoxy-3-methyl-5-propylbenzoic acid (**50**). An improved Friedel–Crafts acetylation of 2,4-dimethoxyethylbenzene (**43**) gave the substituted acetophenone[8] **44** in a quantitative yield.

Catalytic hydrogenation, subsequent lithiation, and carboxylation of the obtained 2,4-diethyl-1,5-dimethoxybenzene (**45**) gave 3,5-diethyl-2,6-dimethoxybenzoic acid (**46**). The same reaction sequence, starting from 2,4-dimethoxyfluorobenzene[9] gave 3-ethyl-5-fluorobenzoic acid (**54**). Chlorination of 3-methyl-2,6-dimethoxybenzoic acid,[1] 3-ethyl-2,6-dimethoxybenzoic acid,[1] and 3-propyl-2,6-dimethoxybenzoic acid[1] gave the corresponding 5-chloro-substituted benzoic acids **55–57**, respectively.

3-Bromo-2,6-dimethoxy-5-methylbenzoic acid (**58**) and 3-bromo-5-ethyl-2,6-dimethoxybenzoic acid (**59**) (not isolated) were obtained by bromination of 3-methyl-2,6-dimethoxybenzoic acid and 3-ethyl-2,6-dimethoxybenzoic acid, respectively. Bromination of 3-fluoro-2,6-dimethoxybenzoic acid[9] gave the corresponding 5-bromo-substituted benzoic acid **60**. The 3,5-dichloro-, 3,5-dibromo-, and 3-bromo-5-chloro-substituted 2,6-dimethoxybenzoic acids were prepared by halogenation as described by Florvall and Ögren.[4]

In order to confirm the structure assignments of those substituted salicylamides having different substituents in the 3-position and 5-position, some substituted 6-methoxysalicylic acids were prepared. Coupling of their corresponding acid chlorides with (*S*)-(−)-2-(aminomethyl)-1-ethylpyrrolidine gave the isomerically pure salicylamides **19**, **21**, **30**, and **31**. 3-Bromo-6-methoxysalicylic acid[1] (**61**)

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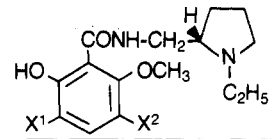
(7) Nakazawa, K. *J. Pharm. Soc. Jpn.* **1954**, *74*, 836; *Chem. Abstr.* **1955**, *49*, 9556*g*.
(8) Kachru, P. L.; Kachru, C. N. *J. Ind. Chem. Soc.* **1959**, *36*, 311; *Chem. Abstr.* **1969**, *76*, 70484*a*.
(9) Durrani, A. A.; Tyman, J. H. P. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1658.

Table II. Structure and Physical Constants of Halogen and Alkyl 3- and 5-Substituted *N*-[(1-Ethylpyrrolidinyl)methyl]-6-methoxysalicylamides



compd	X^1	X^2	yield, %	mp, °C	crystn solv[a]	rotation,[b] deg	formula[c]	anal.
13	Cl	Cl	95	142-143	A	−64	$C_{15}H_{20}Cl_2N_2O_3 \cdot C_4H_6O_6$	C, H, Cl, N, O
14	Cl	Br	35	249-250	B	−52	$C_{15}H_{20}BrClN_2O_3 \cdot HCl$	C, H, Br, Cl, N
15	Cl	CH_3	40	oil[d]		−68	$C_{16}H_{23}ClN_2O_3$	
16	Cl	C_2H_5	15	170-171	B	−65	$C_{17}H_{25}ClN_2O_3 \cdot HCl$	C, H, Cl, N
17	Cl	C_3H_7	10	oil[d]		−58	$C_{18}H_{27}ClN_2O_3$	
18	Br	F	60	136-137	A	−60	$C_{15}H_{20}BrFN_2O_3 \cdot HCl$	C, H, Br, Cl, F, N
19	Br	Cl	55	61-63	C	−55	$C_{15}H_{20}BrClN_2O_3$	C, H, Br, Cl, N
20	Br	Br	70	169-170	B	−50	$C_{15}H_{20}Br_2N_2O_3 \cdot CH_4O_3S$	C, H, Br, N, O, S
21	Br	NO_2	68	127-129	B	−67	$C_{15}H_{20}BrN_3O_5 \cdot HCl$	C, H, Br, N
22	Br	CH_3	42	oil[d]		nd	$C_{16}H_{23}BrN_2O_3$	
23	Br	C_2H_5	25	59-60	C	−47	$C_{17}H_{25}BrN_2O_3$	C, H, Br, N
24	NO_2	Br	10	108-110	C	racemic	$C_{15}H_{20}BrN_3O_5$	C, H, Br, N[e]
25	CH_3	Cl	40	114-116	B	nd	$C_{16}H_{23}ClN_2O_3 \cdot C_4H_6O_6$	C, H, Cl, N[f]
26	CH_3	Br	20	121-123	D	−55	$C_{16}H_{23}BrN_2O_3 \cdot C_4H_6O_6$	C, H, Br, N[g]
27	CH_3	CH_3	50	160-162	D	−73	$C_{17}H_{26}N_2O_3$	C, H, N, S
28	CH_3	C_3H_7	18	oil[d]		nd	$C_{19}H_{30}N_2O_3$	
29	C_2H_5	F	62	79-82	C	−54	$C_{17}H_{25}FN_2O_3 \cdot C_4H_6O_6$	C, H, F, N
30	C_2H_5	Cl	60	144-146	B	−69	$C_{17}H_{25}ClN_2O_3 \cdot HCl$	C, H, Cl, N
31	C_2H_5	Br	45	145-147	A	−46	$C_{17}H_{25}BrN_2O_3 \cdot C_4H_6O_6$[h]	C, H, Br, N, O
32	C_2H_5	C_2H_5	87	137-138	B	−65	$C_{19}H_{30}N_2O_3 \cdot CH_4O_3S$	C, H, N, O, S
33	C_3H_7	Cl	51	oil[d]		−48	$C_{18}H_{27}ClN_2O_3$	
34	C_3H_7	CH_3	20	oil[d]		nd	$C_{19}H_{30}N_2O_3$	
35	(*R*)-(+)-**13**		88	140-142	A	+63	$C_{15}H_{20}Cl_2N_2O_3 \cdot C_4H_6O_6$	C, H, Cl, N
36	deshydroxy-**20**		63	oil[d]		−47	$C_{15}H_{20}Br_2N_2O_2$	
37	desmethoxy-**20**		62	218-217	A	−70	$C_{14}H_{18}Br_2N_2O_2$	C, H, Br, N

[a] Solvents: A, ethanol; B, acetone; C, hexane, D, acetone-ether. [b] Rotation $[\alpha]^{20}_D$ of base (*c* 2% in acetone). [c] Base-salt. [d] Characterized by NMR and mass spectrometry. Purity ascertained by TLC and GC. [e] Br: calcd, 19.87; found, 18.64. N: calcd, 10.45; found, 9.71. [f] C: calcd, 50.37; found, 49.70. [g] C: calcd, 46.08; found, 45.55. [h] Semihydrate.

was chlorinated by sulfuryl chloride to give the 3-bromo-5-chloro-substituted acid (**60**) (Scheme III). Conversion of **60** to the corresponding salicylamide produced a compound identical with the major isomer **19** obtained by demethylation of (*S*)-(−)-3-bromo-5-chloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide (**2**). 3-Bromo-5-nitro-2,6-dimethoxybenzoic acid (**63**) could not be prepared by direct nitration. However, nitration of 3-bromo-6-methoxysalicylic acid gave 3-bromo-5-nitro-6-methoxysalicylic acid (**62**), which produced the corresponding salicylamide **21** upon amidation of its acid chloride. In order to obtain the 5-bromo-3-nitro isomer **24**, the nitro-substituted salicylic acid **62** was treated with dimethyl sulfate and hydrolyzed to give the dimethoxybenzoic acid **63**.

Coupling of the acid chloride of **63** with 2-(aminomethyl)-1-ethylpyrrolidine gave the 2,6-dimethoxybenzamide derivative **8**. Demethylation of **8** gave a mixture of compounds **21** and **24**, which were separated by column chromatography.

In some cases where the structure determination could not be based on the synthesis from a known salicylamide derivative, the correct assignments of the position of the substituents were deduced from their ^{13}C NMR spectra.

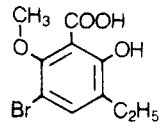
Demethylation of one of the methoxy groups of (*S*)-(−)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxy-3-methyl-5-propylbenzamide (III; $X^1 = CH_3$, $X^2 = C_3H_7$) produced the two substituted salicylamides **28** and **34** in a ratio of 1:1. They were separated by column chromatography and isolated as their noncrystalline free bases. In the nondecoupled ^{13}C NMR spectrum of compound **28**, the signals of the two oxygen-substituted carbon atoms appear as multiplets at 154.7 and 159.4 ppm. Irradiation of the protons of the aromatic methyl group changed one of the multiplets (the signal at 159.4 ppm) into a doublet. This signal was assigned to the hydroxy-substituted carbon (C-2) since this couples with the proton at the 4-position only. In the spectrum of the other isomer **34**, irradiation of the methyl protons did not produce any doublet of the corresponding hydroxy- (C-2) or methoxy- (C-6) substituted carbon signals because of additional coupling with the methylene protons of the propyl group or the methyl protons of the methoxy group, respectively. The structure of compound **34** is consequently the 3-propyl-5-methyl-substituted isomer.

The structures of the alkyl- and halogen-substituted positional isomers **15** and **25**, **22** and **26**, **16** and **30**, and **17** and **33** were assigned by comparing their ^{13}C NMR spectra with the spectra of the 5-bromo-3-ethyl-substituted salicylamide **23** and the 3-bromo-5-ethyl-substituted isomer **31**.

In the spectrum of the minor isomer **23**, the hydroxy-substituted carbon signal at 157.8 ppm appeared as a doublet due to coupling with the proton in the 4-position. In the spectrum of the other isomer **31**, the methoxy-substituted carbon signal at 153.7 ppm appeared as a multiplet due to coupling with the methoxy protons of the 4-position. Irradiation of the protons of the methoxy group changed this signal into a doublet due to coupling with the proton in the 4-position. In order to prove the assignment, compound **31** was synthesized by an alternative route shown in Scheme IV. 3-Ethyl-2,6-dimethoxybenzoic acid was demethylated with hydrogen bromide to give the corresponding 6-methoxysalicylic acid **65** as described previously.[10] Bromination of **56** with bromine in dioxane

(10) de Paulis, T.; Hall, H.; Ögren S.-O.; Wägner, A.; Stensland, B.; Csöregh, I. *Eur. J. Med. Chem.* **1985**, *20*, 273.

Table III. Assignment of the 50-MHz ^{13}C NMR Spectrum of 5-Bromo-3-ethyl-2-hydroxy-6-methoxybenzoic Acid (**66**)

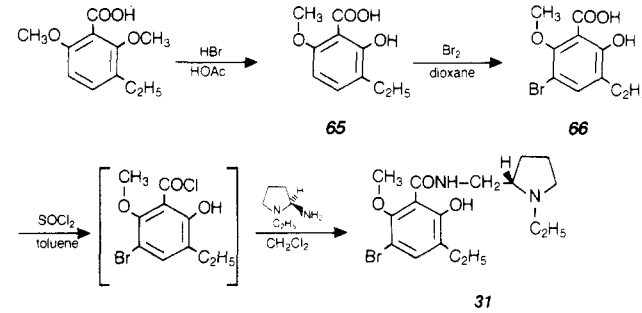


chem shift[a] (δ, Me_4Si)	mult[b]	J (proton–carbon), Hz	assignment posn	assignment subst
169.98	s	none	7	carbonyl
160.74	dt	11.0, 3.7	2	hydroxy
153.53	m	3.1	6	methoxy
138.14	dt	164, 5.5	4	proton
132.33	m	4.9	3	*C*-methylene
105.69	s	none	1	carboxamide
103.77	d	3.7	5	bromo
63.03	q	146.5	8	*O*-methyl
22.52	tq	129, 4.3	9	1-ethyl
13.28	dt	127, 4.9	10	2-ethyl

[a] Obtained from 5%, in $CDCl_3$ (δ 77.16) as internal reference. [b] Nondecoupled.

gave the 5-bromo-3-ethyl-substituted salicylic acid **66**. Assignment of the carbon signals of **66** was based on their multiplicities and coupling constants in the nondecoupled ^{13}C NMR spectrum (Table III). Compound **66** was converted into its acid chloride (not isolated) and treated with a solution of (*S*)-(−)-2-(aminomethyl)-1-ethylpyrrolidine to give the 5-bromo-3-ethyl-substituted salicylamide **31**, identical with one of the isomers obtained by the demethylation of compound **7**. Furthermore, the structure of compound **30**, the chloro analogue of **31**, was determined by X-ray crystallography after being synthesized from 3-ethyl-6-methoxysalicylic acid (**65**).[10]

Scheme IV



The structure of the fluoro-bromo- and fluoro-ethyl-substituted salicylamides **18** and **29** were determined by comparing the substituent-induced chemical shifts in their ^{13}C NMR spectra with those of the corresponding desfluorine derivatives.[1] The obtained isomers have shifts that are consistent with additive SCS values of the six aromatic carbons only if the fluorine atom is in the 5-position (unpublished observation).

In order to establish the relative importance of the 2-methoxy group vs. that of the 2-hydroxy group in stabilizing the planar benzamide conformation, the deshydroxy analogue **36** and the desmethoxy analogue **37** of the 3,5-dibromo-substituted salicylamide **20** were prepared by demethylation of **20** and by coupling with the corresponding benzoic acid chlorides, respectively.

Pharmacology

Pharmacological results are presented in Table IV.

Inhibition of [^{3}H]Spiperone Binding. The potencies (IC_{50}) of the substituted 6-methoxysalicylamides **13**–**34** in

Table IV. Inhibition of [^{3}H]Spiperone Binding, Antagonism of Apomorphine-Induced Hyperactivity, Stereotypy, and Acute Toxicity of 3- and 5-Disubstituted 6-Methoxysalicylamides

compd	[^{3}H]spiperone binding: IC_{50}, μM	apomorphine antag:[a] ED_{50}, μmol/kg ip hyperactivity	apomorphine antag:[a] ED_{50}, μmol/kg ip stereotypy	acute tox: LD_{50}, μmol/kg ip
13	0.032	0.13 (0.13–0.14)	1.70 (1.58–1.91)	660
14	0.058			
15	0.011			
16	0.012	2.5 (2.5–2.5)	1.51 (1.48–1.58)	
17	0.110			
18	0.0066	0.16 (0.16–0.17)	0.42 (0.38–0.48)	
19	0.017	0.95 (0.93–0.98)	1.35 (1.32–1.41)	
20	0.026	0.65 (0.62–0.68)	1.48 (1.45–1.55)	450
21	0.185			
22	0.011			
23	0.017	0.87 (0.87–0.89)	1.45 (1.38–1.55)	
24	6.28[b]			
25	0.0026	0.74 (0.74–0.76)	1.32 (1.12–1.82)	
26	0.0055			
27	0.0077	1.86 (1.86–1.86)	3.1 (2.6–5.2)	
28	0.15			
29	0.0015	0.065 (0.045–0.079)	0.31 (0.30–0.34)	
30	0.00092	0.032 (0.03–0.032)	0.19 (0.18–0.19)	330
31	0.0023	0.085 (0.083–0.087)	0.47 (0.47–0.47)	320
32	0.0018	0.076 (0.074–0.076)	0.46 (0.46–0.46)	530
33	0.003	0.10 (0.093–0.10)	0.35 (0.35–0.36)	
34	0.005			
35	2.92	1.07 (1.05–1.12)	32 (32–33)	
36	0.008	0.66 (0.52–0.76)	2.69 (2.63–2.75)	
37	12.9	28	>40	
38	3.39	0.44 (0.42–0.44)[c]	2.1 (2.0–2.2)[c]	160[c]
remoxipride (Ia)	1.57	0.86 (0.81–0.98)[c]	6.5 (6.2–6.7)[c]	820[c]
(*R*)-(+)-Ia	59.9	120 (112–148)[c]	296 (234–437)[c]	700[c]
Ib	0.0047[d]	0.12 (0.11–0.13)[d]	0.28 (0.26–0.28)[d]	370
(*S*)-(−)-sulpiride	0.21	28.2 (27.5–28.2)	162 (158–166)	
haloperidol	0.012	0.29 (0.27–0.35)	0.27 (0.26–0.29)	93

[a] The compounds were injected ip 60 min prior to apomorphine (1 mg/kg sc). The apomorphine-induced hyperactivity and stereotypy were scored and calculated as described previously.[1,4] The ED_{50} values and the 90% confidence intervals in parentheses, calculated by Thiel's method[19] 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.[4] [d] Value taken from de Paulis et al.[1]

displacing [^{3}H]spiperone from rat striatal membranes in vitro are in the same order of magnitude as that of haloperidol. The only exceptions are the nitro-substituted compounds **21** and **24** and the 5-propyl derivatives **17** and **28**, which are more than 10 times less active. In agreement with the results of the monosubstituted series,[1] the 3-methyl analogues **25–27**, the 3-ethyl analogues **29–32**, and the 3-propyl analogues **33** and **34** were the most potent salicylamide derivatives, clearly more active than the corresponding 3-halo analogues. Thus, the 3-ethyl-5-bromo-substituted compound **31** and the 3,5-diethyl-substituted compound **32** were found to be 17 and 14 times more potent than the corresponding 3,5-dibromo- and 3-bromo-5-ethyl-substituted compounds **20** and **23**, respectively. The nature of the substituent in the 5-position does not seem to influence the activity, providing it is smaller than a propyl or a nitro group. The different structural requirement of the 3- and 5-positions of the aromatic ring is clearly demonstrated by comparing the activities of the two propyl–methyl-substituted compounds **28** and **34** with that of the 3,5-diethyl-substituted compound **32**.

The 3-propyl-5-methyl analogue **34** has the same lipophilic property as compound **32**, the difference in structure being one carbon atom less in the 5-position and one more in the 3-position. The 3-propyl-substituted compound **34** showed the same high activity as compound **32**. The 3-methyl-5-propyl analogue **28**, on the other hand, which can be regarded as the opposite modification of **32**, was found to be more than 100 times less active than compound **32**. This was interpreted as a steric requirement of the methoxy group to adopt an in-plane conformation for activity, since otherwise the formation of the crucial hydrogen bonded six-membered ring with the amide group would be obstructed.[11]

The activity of the deshydroxy compound **36** was about 4 times that of the corresponding salicylamide **20**. In contrast, the desmethoxy derivative **37** was virtually inactive.

Having a nitro group in the 3-position drastically reduces activity, in agreement with the results of the 3-nitro analogue of the monosubstituted series.[1] The enantiomer of **13** with the opposite *R*-(+) configuration at the 2-position of the pyrrolidine ring, **35**, was 100 times less potent than the *S*-(−) enantiomer.

Inhibition of Apomorphine-Induced Behavioral Activity. In agreement with the findings of the receptor binding studies in vitro, the most potent compounds in vivo were those having an alkyl substituent in the 3-position of the aromatic ring. Thus, the 3-ethyl-substituted salicylamides **30–32** and the 3-propyl-substituted analogue **33** have antiapomorphine (stereotypy) activities equal to that of haloperidol. Similar to other substituted benzamides, but in contrast to haloperidol, the 3,5-disubstituted 6-methoxysalicylamides inhibited the apomorphine-induced motoric hyperactivity at much lower doses than those that inhibited the apomorphine-induced stereotypic behaviors such as sniffing and chewing/licking/biting. This separation might reflect a differential action on subpopulations of DA receptors[12] or it might reflect a specific action in different regions in the brain that govern these effects. Figure 1 shows a plot of the separation and the [^{3}H]spiperone receptor binding activities of the salicylamides. Several compounds, e.g. **30–33**, display stereotypy–hyperactivity separations in the same range (5–10 times) as that of sulpiride and remoxipride, while being considerably more potent on the receptor preparations in vitro. Compound **16**, which is the positional isomer of compound **30**, had less separation than haloperidol. This result is in sharp contrast to the result with the corresponding 5-chloro analogue of **16**. This compound, **13** (FLA 870, A 40664 proposed INN: raclopride) showed an ED_{50} value on the hyperactivity parameter that is 13 times lower than that of the stereotypy parameter, and **13** was the most outstanding salicylamide in this respect. On the basis of these results, together with pharmacokinetic investigations in the rat and the dog (data not shown), compound **13** was selected for clinical investigations as an antipsychotic agent.

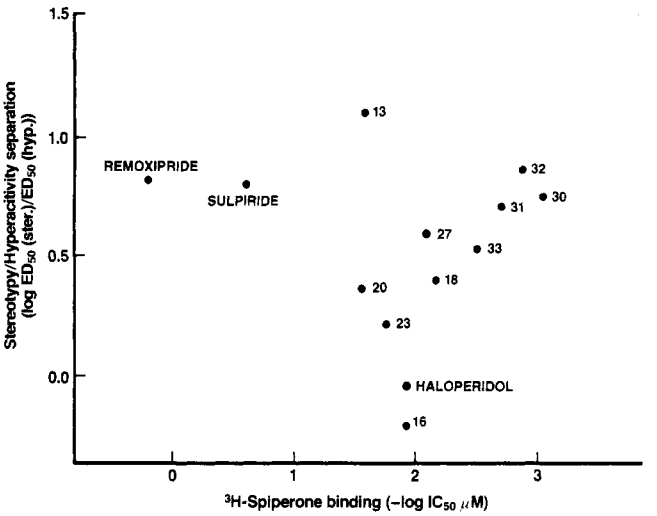


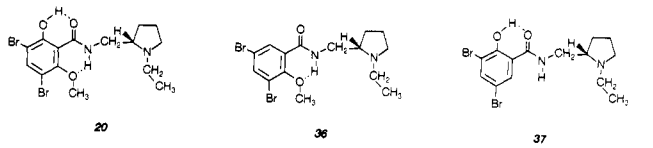
Figure 1. Plot of the separation between apomorphine-induced stereotypy and hyperactivity in vivo vs. blockade of [^{3}H]spiperone binding in vitro.

Acute Toxicity in the Male Rat. In the present study the acute toxicity of some of the substituted benzamides was determined and compared with that of the reference compounds.

The acute toxicity of the substituted salicylamides is generally low, 4–8 times less than that of haloperidol.

Discussion

The importance of having substituents in the aromatic 4- and 5-positions of the metoclopramide series was demonstrated by Testa et al.[13] We have recently shown the favorable influence of having a lipophilic substituent in the 5-position (which corresponds to the 3-position in the substituted salicylamides).[1] By comparing the activity of compound **20** with that of the corresponding analogues



20 36 37

without the methoxy and hydroxy groups, respectively, it was demonstrated that the hydroxy group does not contribute to the activity. In fact, the deshydroxy derivative **36** of compound **20** was 4 times more potent than **20** in blocking the [^{3}H]spiperone binding, and the racemic form of **36** is claimed to be a very potent antiemetic agent in the dog.[14]

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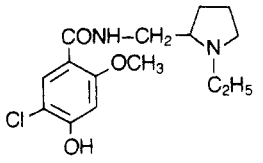
(13) Anker, L.; Testa, B.; van de Waterbeemd, H.; Bornan-Crausaz, A.; Theodorou, A.; Jenner, P.; Marsden, C. D. *Helv. Chim. Acta* **1983**, *66*, 542.

It seems that the presence of an electropositive hydrophilic substituent in the 6-position mainly reduces toxicity while retaining the antidopamine activity. The acute toxicity of **20** is one-third that of the corresponding dimethoxy analogue **38** upon intraperitoneal administration, despite its equipotent activity in vivo. A similar effect has been clearly demonstrated for the amino and hydroxy substituent in the 4-position of other substituted benzamides. Fournier used quantitative structure–toxicity relationships to establish the beneficial effects of a *p*-amino group in a series of thia isosteres of metoclopramide.[15] Thominet documented a 4-fold reduction in the acute toxicity of a 4-methoxy isomer of the chloro analogue of remoxipride upon demethylation to the corresponding 4-hydroxy derivative **67**.[16,17]



67

An increase in activity is found when comparing the symmetrically substituted dichlorosalicylamide **13** with the corresponding bromo–fluoro (**18**) and iodo–hydrogen[1] analogues. The same relationship is found in the dialkyl-substituted series; i.e., the 3-propyl-5-methylsalicylamide **34** is more potent than the 3-methyl-5-propyl isomer **28**. Since no strong correlation was found with the electronic parameter (Hammett's σ) of the substituents and the global lipophilicity is likely to be similar in the symmetrically and the unsymmetrically meta-substituted salicylamides, these effects might be explained by a negative correlation to the size of the substituent in the 5-position (3-position in sulpiride).

The structural requirement of having a small substituent in the position adjacent to the essential methoxy group means that this group must be able to adopt a conformation close to the aromatic plane in order to facilitate the formation of the amide hydrogen-bonded six-membered ring including the amide nitrogen atom and the methoxy oxygen atom.[17] Thus, our compounds seem to confirm Testa's modification[11] of Humber's model[18] of the receptor with regard to the aromatic pharmacophore. This implies that the interaction with the part of the receptor that binds the aromatic moiety of the dopamine antagonist is centered on the hydrogen-bonded six-membered ring and the amide carbonyl oxygen atom, rather than centered on the aromatic ring of the benzamide itself (Figure 2).[17] Since the deshydroxy compound **36** was equally potent the presence of the β-ring does not seem to be necessary. This would explain the lack of influence of the electronegative properties of the substituents, provided the formation of the α-ring is not affected. Furthermore, the interatomic distance in the crystal structure of compound **30** between the α-ring and the amine nitrogen atom is 4.03 Å,[10] close

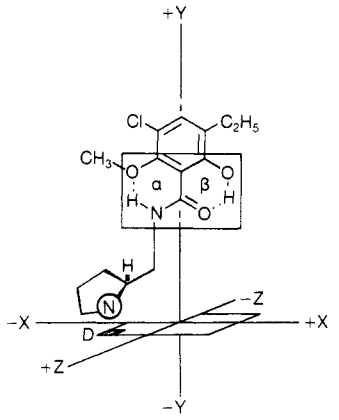


Figure 2. Application of compound **30** (pINN; eticlopride) to the dopamine antagonist receptor model of Philipp and Humber[18] as suggested by van de Waterbeemd and Testa.[11]

to the 5.1 Å to the point D (Figure 2) in the receptor model.[18]

It is concluded that the substituted 6-methoxysalicylamides, which carry an alkyl group in the 3-position and a halogen atom in the 5-position adjacent to the methoxy group, constitute a new type of very potent DA receptor blocking agent with an ability to show a differentiated inhibition of the apomorphine syndrome.

Experimental Section

Chemistry. Melting points are uncorrected. NMR spectra were recorded in deuteriochloroform 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 an LKB 9000 instrument. GC analyses were performed on a Carlo Erba 4200 instrument equipped with a 10 m × 0.35-mm fused silica capillary column and 3-μm cross-linked SE 54 as stationary phase. Hydrogen was used as carrier gas with an inlet pressure of 60 kPa. Optical rotations were obtained in acetone on a Perkin-Elmer 141 polarimeter. The elemental analyses were performed by Analytische Laboratorien, Elbach, West Germany, and were within 0.4% of the theoretical values.

General Method for the Preparation of 3,5-Disubstituted 2,6-Dimethoxybenzamides III. (*S*)-(–)-3,5-Dichloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide (1). In a 1-L flask 3,5-dichloro-2,6-dimethoxybenzoic acid[4] (150 g, 0.60 mol) was mixed with toluene (500 mL) and DMF (5 mL). The slurry was heated to 50 °C. Slow addition of thionyl chloride (150 mL, 2.0 mol) produced the corresponding acid chloride under evolution of gas. After 2 h, the solvent was removed at 50 °C. Toluene (500 mL) was again added and removed to give 160 g of pure *3,5-dichloro-2,6-dimethoxybenzoic acid chloride* as a light yellow oil. It was dissolved in 600 mL of CH_2Cl_2 and added under reflux to a solution of (2*S*)-2-(aminomethyl)-1-ethylpyrrolidine,[4] $[\alpha]^{20}_D$ –89° (*c* 2.8, DMF) (83 g, 0.65 mol), in 400 mL of CH_2Cl_2 and the resultant mixture stirred overnight at room temperature. The reaction mixture was shaken with 1 N NaOH (600 mL), and the aqueous layer was discarded. Drying (Na_2SO_4) and evaporation of the solvent gave compound **1**. At the beginning of crystallization of the residue, 2 L of diisopropyl ether was added: yield 192 g (89%); mp 145–146 °C.

General Method for the Preparation of 3,5-Disubstituted 6-Methoxysalicylamides IV and V. (*S*)-(–)-3,5-Dichloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamide L-(+)-Tartrate (13). Compound **1** (117 g, 0.32 mol) was dissolved in CH_2Cl_2 (1.0 L), and 3 N HCl–ether (110 mL, 033 mol) was added. A solution of BBr_3 (31 mL, 0.33 mol) in CH_2Cl_2 (300 mL)

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was slowly added at 10–15 °C (20 min). The reaction mixture was stirred at room temperature for 1.5 h. Ammonium hydroxide (2 N, 500 mL) was added. After stirring for 10 min, the organic layer was separated, washed with water (200 mL), and dried (Na_2SO_4) and the solvent was removed. The residue was dissolved in ether or hexane (400 mL), and insoluble material was removed by filtration. Evaporation of the solvent gave 108 g of **13**, mp 49–50 °C (isooctane). L-(+)-Tartaric acid (50 g, 0.33 mol) was dissolved in hot 2-PrOH (400 mL) and the resultant solution added to a solution of **13** in 2-PrOH (200 mL). Cooling overnight gave 154 g (95%) of the tartrate salt, mp 141–142 °C. Other suitable salts of **13** prepared in a similar manner are mesylate [mp 156–157 °C (acetone)] and phosphate [mp 148–149 °C (EtOH)]. The hydrochloride was hygroscopic.

(*S*)-(−)-3-Bromo-5-chloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamide (19). Compound **19** was prepared from the acid **64** as described for compound **31**: NMR δ 7.66 (s, 1 H), 3.92 (s, 3 H). It was identical with the major isomer obtained by demethylation of compound **2**.

(*S*)-(−)-3-Bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxy-5-nitrosalicylamide (21). Compound **21** was prepared from the acid **62** as described for compound **31**: NMR δ 8.35 (s, 1 H), 3.96 (s, 3 H). It was identical with the minor isomer obtained by demethylation of compound **8**.

(*S*)-(−)-5-Bromo-3-ethyl-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamide (31). To a solution of 5-bromo-3-ethyl-6-methoxysalicylic acid (**66**; 10.0 g, 0.036 mol) in toluene (100 mL) was added thionyl chloride (10 mL, 0.137 mol), followed by catalytic amounts (0.1 mL) of DMF. The mixture was heated to 70 °C for 30 min. The solvents were removed by evaporation. The residue, which consisted of 10.5 g of *5-bromo-3-ethyl-6-methoxysalicylic acid chloride*, was dissolved in CH_2Cl_2 (100 mL) and was added to a solution of (2*S*)-(−)-2-(aminomethyl)-1-ethylpyrrolidine in CH_2Cl_2 (150 mL). After 4 h at 23 °C the solvent was removed, ammonium hydroxide (1 N, 50 mL) was added, and the product was extracted with CH_2Cl_2 (3 × 100 mL). Drying (Na_2SO_4) and evaporation of the solvent gave 14 g of crude **31**: $[\alpha]^{20}_D$ −46° (*c* 1.0, acetone); NMR δ ~12 (br, OH), 8.84 (br, NH), 7.37 (s, 1 H), 3.86 (s, 3 H), 1.5–3.8 (m, 13 H), 1.20 (t, 3 H), 1.14 (t, 3 H). The tartrate semihydrate was prepared by mixing L-(+)-tartaric acid (5.5 g, 0.036 mol) in MeOH (100 mL) with the free base of **31** in 96% EtOH (100 mL). Recrystallization from 250 mL of EtOH gave 14.3 g (72%); mp 143–145 °C.

(*S*)-(−)-3,5-Dibromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide (36). A mixture of 3,5-dibromo-2-methoxybenzoic acid (4.5 g, 0.014 mol) and thionyl chloride (5.6 mL, 0.076 mol) in toluene (40 mL) was heated to 75 °C for 1.5 h. The solvent was removed, and the residue was dissolved in chloroform (50 mL). A solution of (2*S*)-2-(aminomethyl)-1-ethylpyrrolidine (2.0 g, 0.015 mol) in chloroform (25 mL) was added at 25 °C. After 1 h, water (100 mL) was added, the aqueous layer was separated, and the organic layer was shaken with 1 N HCl (50 mL). The combined aqueous layer was extracted with ether (3 × 75 mL), and the ether layers were dried (Na_2SO_4) and evaporated to yield 4.0 g (68%) of compound **36** as an oil: $[\alpha]^{20}_D$ −47° (*c* 0.53, acetone); NMR δ 16.5 (br, OH), 8.15 (d, 1 H, *J* = 2.4 Hz), 8.0 (br, NH), 7.79 (d, 1 H, *J* = 2.4 Hz), 3.88 (s, 3 H), 3.7 (dd, 1 H), 1.6–2.8 (m, 10 H), 1.13 (t, 3 H, *J* = 7.2 Hz).

(*S*)-(−)-3,5-Dibromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-salicylamide (37). One equivalent of HCl–ether was added to a solution of compound **36** (0.73 g, 0.0016 mol) in 15 mL of CH_2Cl_2. Boron tribromide (0.40 g, 0.0016 mol) in 10 mL of CH_2Cl_2 was added dropwise at 23 °C. After 1.5 h, 50 mL of 1 N NH_4OH was added and the product was extracted with CH_2Cl_2 (2 × 25 mL). Drying and evaporation gave 0.6 g (92%) of **37**. Recrystallization from 20 mL of ethanol gave 0.45 g: mp 217–218 °C; $[\alpha]^{20}_D$ −70° (*c* 0.20, acetone). Anal. ($C_{14}H_{18}Br_2N_2O_2$) C, H, Br, N.

2,4-Dimethoxy-5-methylbenzaldehyde (40). 2,4-Dimethoxy-1-methylbenzene[21] (**39**; 10 g, 0.066 mol) was mixed with phosphoroxychloride (7.5 mL, 0.079 mol) and dimethylformamide (5.8 g, 0.079 mol) at 5 °C. The temperature was raised to 80 °C for 1 h. The reaction mixture was poured into ice water (150 mL) and made alkaline with 2 N NaOH. Extraction with ether gave a solid residue. Recrystallization from EtOH (85 mL) gave 8.5 g (72%), mp 117–118 °C (lit.[22] mp 118–119 °C).

2,4-Dimethoxy-1,5-dimethylbenzene (41). Compound **41** was prepared from **40** as described for compound **45**: yield 7.5 g (100%); mp 74–76 °C (lit.[23] mp 75–76 °C).

2,6-Dimethoxy-3,5-dimethylbenzoic Acid (42). Compound **42** was prepared from **41** as described for compound **46**: yield 6.6 g (70%); mp 71–72 °C (lit.[24] mp 77–79 °C).

5-Ethyl-2,4-dimethoxyacetophenone (44). To a stirred mixture of 1,3-dimethoxy-4-ethylbenzene (125 g, 0.75 mol) and acetyl chloride (65 mL, 0.90 mol) in 1250 mL of CH_2Cl_2 was added $AlCl_3$ (4 × 30 g, 0.90 mol) at 2–8 °C (35 min). After stirring for 2 h at 0 °C, the reaction mixture was poured into a mixture of 2 kg of ice and 75 mL of concentrated HCl. The organic layer was separated. The aqueous layer was washed with CH_2Cl_2 (2 × 500 mL). The combined organic layer was washed with 200 mL of water and dried (Na_2SO_4). Evaporation of the solvent gave 159 g (100%) of crystalline residue. GC (column temperature 180 °C, retention time 1.25 min) and NMR show >95% purity. Data for an analytical sample recrystallized from hexane: mp 74–75 °C (lit.[8] mp 67 °C); NMR δ 7.7 (s, 1 H), 6.4 (s, 1 H), 3.9 (ds, 6 H), 2.5 (s + q, 5 H), 1.15 (t, 3 H).

2,4-Diethyl-1,5-dimethoxybenzene (45). Crude 5-ethyl-2,4-dimethoxyacetophenone (**44**; 145 g, 0.70 mol) was dissolved in EtOH (1500 mL). Ten milliliters of concentrated HCl and 7.5 g 10% palladium on charcoal were added. Hydrogenation at normal pressure and ambient temperature consumed 31 L (1.4 mol) of hydrogen (4.5 h). The catalyst was filtered through Hyflo, and the solvent was evaporated at 50 mm and 50 °C. The residue (112.5 g) was distilled to yield a colorless oil that solidified on standing: 100.7 g (75%); bp 118–120 °C (15 mm); mp 24–26 °C; NMR δ 6.9 (s, 1 H), 6.5 (s, 1 H), 3.8 (s, 6 H), 2.5 (q, 2 H), 1.1 (t, 3 H).

3,5-Diethyl-2,6-dimethoxybenzoic Acid (46). Butyllithium (430 mL of 1.65 M in hexane, 0.72 mol) was added to a solution of 2,4-diethyl-1,5-dimethoxybenzene (116 g, 0.60 mol) in anhydrous THF (1000 mL) under N_2 at 10 °C. The reaction mixture was allowed to reach room temperature (1.5 h) and poured into solid carbon dioxide (~200 g) in ether (1000 mL) with stirring. After 30 min water (800 mL) was added followed by 100 mL of concentrated HCl. The organic layer was separated, and the aqueous layer was shaken with ether (2 × 300 mL). The combined organic layer was extracted with 1 N NaOH (4 × 500 mL). Acidifying the aqueous solution with concentrated HCl (200 mL) and extraction with CH_2Cl_2 (3 × 500 mL) gave 148 g crude product. Recrystallization from 1.5 L of hexane gave 116 g (81%): mp 79–81 °C; NMR δ 7.0 (s, 1 H), 3.8 (s, 6 H), 2.5 (q, 4 H), 1.1 (t, 6 H). Anal. ($C_{13}H_{18}O_4$) C, H.

2,4-Dimethoxy-5-propylbenzaldehyde (48). Compound **48** was prepared from 1,3-dimethoxy-4-propylbenzene (**47**) as described for compound **40**: yield 13 g (76%); mp 80–81 °C (lit.[25] mp 82–83 °C); NMR δ 10.3 (s, 1 H), 7.6 (s, 1 H), 6.4 (s, 1 H), 3.9 (s, 6 H), 2.5 (dt, 2 H), 1.6 (m, 2 H), 0.95 (t, 3 H).

1,3-Dimethoxy-4-methyl-6-propylbenzene (49). Compound **49** was prepared from **48** as described for compound **45**: yield 12 g (93%); NMR δ 6.9 (s, 1 H), 6.5 (s, 1 H), 3.9 (s, 6 H), 2.5 (dt, 2 H), 2.2 (s, 3 H), 1.6 (m, 2 H), 0.95 (t, 3 H).

2,6-Dimethoxy-3-methyl-5-propylbenzoic Acid (50). Compound **50** was prepared from **49** as described for compound **46**: yield 0.7 g (70%); NMR δ 7.0 (s, 1 H), 3.8 (s, 6 H), 2.6 (dt, 2 H), 2.2 (s, 3 H), 1.6 (m, 2 H), 0.95 (t, 3 H).

5-Fluoro-2,4-dimethoxyacetophenone (52). Compound **52** was prepared from 2,4-dimethoxyfluorobenzene[9] (**51**) as described for compound **44**: yield 6.0 g (95%); mp 112–113 °C (EtOH). Anal. ($C_{10}H_{11}FO_3$) C, H, F.

1-Ethyl-3-fluoro-4,6-dimethoxybenzene (53). Compound **53** was prepared from **52** as described for compound **45**: yield 5.6 g (94%) as an oil; NMR δ 6.92 (d, 1 H, *J* = 12 Hz), 6.55 (d, 1 H, *J* = 7 Hz), 3.90 (d, 3 H, *J* = 2 Hz), 3.82 (s, 3 H), 2.53 (q, 2 H), 1.08 (t, 3 H).

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3-Ethyl-5-fluoro-2,6-dimethoxybenzoic Acid (54). Compound **54** was prepared from **53** as described for compound **46**: yield 6.1 g (89%) as an oil; NMR δ 6.90 (d, 1 H, J = 12.3).

5-Chloro-3-methyl-2,6-dimethoxybenzoic Acid (55). 2,6-Dimethoxy-3-methylbenzoic acid[1] (2.5 g, 0.013 mol) was dissolved in $CHCl_3$ (50 mL). SO_2Cl_2 (2.4 g, 0.018 mol) was added dropwise at 20 °C, and the reaction mixture was stirred overnight. The solvent was evaporated. The residue was treated with water and extracted with ether. The organic layer was dried (Na_2SO_4) and concentrated to give **55** as an oil, yield 2.7 g (92%). This acid was characterized as its amide. Into a solution in $CHCl_3$ of the acid chloride of **55** was passed gaseous NH_3. The solvent was removed, and the residue was treated with water. The solid thus obtained was filtered, dried, and crystallized from i-Pr_2O; mp 148–150 °C. Anal. ($C_{10}H_{12}ClNO_3$) C: calcd, 52.30; found, 53.35; H, Cl: calcd, 15.44; found, 14.80; N.

5-Chloro-3-ethyl-2,6-dimethoxybenzoic Acid (56). The acid **56** was prepared by chlorination of 2,6-dimethoxy-3-ethylbenzene[1] as described above: yield 17 g (96%) of compound **56** as an oil; GC (column temperature 200 °C, retention time 1.50 min) single peak; NMR δ 11.7 (b, 1 H), 7.27 (s, 1 H), 3.89 (s, 3 H), 3.81 (s, 3 H), 2.60 (q, 2 H), 1.19 (t, 3 H).

5-Chloro-3-propyl-2,6-dimethoxybenzoic Acid (57). The acid **57** was prepared by the chlorination of 2,6-dimethoxy-3-propylbenzoic acid[1] using the same procedure as described for the preparation of **55**. The acid **57** was obtained as an oil (7 g (87%)) characterized by ^1H NMR and was used in the next step without further purification: NMR δ 13.5 (b, 1 H), 7.3 (s, 1 H), 3.97 (s, 3 H), 3.92 (s, 3 H), 2.63 (t, 2 H), 1.6 (m, 2 H), 1.00 (t, 3 H).

5-Bromo-3-methyl-2,6-dimethoxybenzoic Acid (58). To a solution of 2,6-dimethoxy-3-methylbenzoic acid (1.57 g, 0.008 mol) in dioxane (40 mL) was added Br_2 (1.44 g, 0.009 mol) in dioxane (20 mL) at room temperature. The reaction mixture was stirred for 2 h. Water (200 mL) was added, and the product was extracted with ether. The ether layer was dried (Na_2SO_4) and concentrated to give **58** as an oil. To characterize the acid, it was converted into its amide. The acid was treated with $SOCl_2$ to prepare its acid chloride, and then NH_3 gas was passed to the solution of acid chloride in $CHCl_3$. The precipitated amide was collected and crystallized from $CHCl_3$–hexane; mp 152–154 °C. Anal. ($C_{10}H_{12}BrNO_3$) C, H, Br, N.

5-Bromo-3-ethyl-2,6-dimethoxybenzoic Acid (59). Compound **59** was prepared from 3-ethyl-2,6-dimethoxybenzoic acid[1] as described for compound **58**: yield 7.0 g (73%); mp 47–49 °C (hexane); NMR δ 7.48 (s, 1 H), 3.94 (s, 3 H), 3.87 (s, 3 H) 2.68 (q, 2 H), 1.23 (t, 3 H). Anal. ($C_{11}H_{13}BrO_4$) C, H, Br.

3-Bromo-5-fluoro-2,6-dimethoxybenzoic Acid (60). Compound **60** was prepared from 3-fluoro-2,6-dimethoxybenzoic acid[9] as described for compound **58**: yield 1.0 g (80%) as an oil; NMR δ 10.5 (b, 1 H), 7.42 (d, 1 H, J = 11.0 Hz), 4.00 (d, 3 H, J = 2.0 Hz), 3.96 (s, 3 H).

3-Bromo-5-nitro-6-methoxysalicylic Acid (62). A solution of 3-bromo-6-methoxysalicylic acid[1] (**61**; 8.4 g, 0.050 mol) in $CHCl_3$ (50 mL) was treated with 65% HNO_3 (6.9 mL, 0.10 mol) at 30 °C for 1 h. Crystals of the product precipitated. Filtration and washing with $CHCl_3$ (50 mL) and water (50 mL) gave **62**. Recrystallization from $CHCl_3$ (500 mL) gave 5.45 g (43%): mp 168–169 °C; ^1H NMR δ 8.33 (s, 1 H), 4.10 (s, 3 H); ^{13}C NMR (Me_2SO) δ 170.6 (CONH), 163.5 (C-2), 155.9 (C-6), 136.8 (C-5), 133.7 (C-4), 109.6 (C-1), 106.1 (C-3), 64.2 (OCH_3). Anal. ($C_8H_6BrNO_6$) C, H, Br, N, O.

3-Bromo-5-nitro-2,6-dimethoxybenzoic Acid (63). 3-Bromo-5-nitro-6-methoxysalicylic acid (**62**; 14.0 g, 0.048 mol) was dissolved in acetone (300 mL); K_2CO_3 (35 g, 0.254 mol) was added followed by dimethyl sulfate (14 mL, 0.147 mol). The mixture was heated to reflux for 4 h. After cooling, the product was poured into ice water (600 mL) and extracted with ether (3 × 400 mL). Drying (Na_2SO_4) and evaporation of the solvent gave *methyl 3-bromo-2,6-dimethoxy-5-nitrobenzoate* as an oil. A one-third portion (4.5 g, 0.014 mol) was mixed with KOH (2.0 g, 0.035 mol), water (45 mL), and ethanol (25 mL) and heated to reflux for 2.5 h. The clear solution was diluted with ice water (500 mL) and washed with ether (150 mL). The aqueous layer was made acidic with 2 N HCl. Extraction with ether (3 × 150 mL), drying, and evaporation of the solvent gave 4.0 g of residue. Crystallization from cyclohexane (60 mL) gave 2.0 g (47%) of **63**: mp 129–130 °C; NMR δ 11.0 (b, 1 H), 8.2 (s, 1 H), 4.04 (s, 3 H), 4.01 (s, 3 H). Anal. ($C_9H_8BrNO_6$) C, H, Br, N.

3-Bromo-5-chloro-6-methoxysalicylic Acid (64). Compound **64** was prepared from 3-bromo-6-methoxysalicylic acid (**61**) as described for compounds **55**: yield 24.2 g (86%); mp 195–196 °C (MeOH); ^1H NMR δ 7.76 (s, 1 H), 3.95 (s, 3 H); ^{13}C NMR δ 170.5 (CONH), 158.3 (C-2), 155.8 (C-6), 138.3 (C-4), 118.8 (C-5), 108.9 (C-1), 106.9 (C-3), 62.2 (OCH_3). Anal. ($C_8H_6BrClO_4$) C, H, Br, Cl.

5-Bromo-3-ethyl-6-methoxysalicylic Acid (66). 3-Ethyl-6-methoxysalicylic acid[10] (**65**; 12.8 g, 0.065 mol) was dissolved in acetic acid (60 mL). Anhydrous NaOAc (6.4 g, 0.078 mol) was added. A solution of bromine (10.5 g, 0.065 mol) in acetic acid (40 mL) was added dropwise. The temperature rose to 38 °C. After 15 min, ice water (200 mL) was added. Extraction with $CHCl_3$ (2 × 100 mL), drying, and evaporation of the solvent gave a residue of crystalline **66**. Recrystallization from 80% MeOH (50 mL) gave 12.3 g plus 4.2 g in a second crop (93%): mp 109–111 °C; ^1H NMR δ 12.1 (br, 2 H), 7.49 (s, 1 H), 4.07 (s, 3 H), 2.64 (q, 2 H), 1.21 (t, 3 H); ^{13}C NMR δ 170.0 (CONH), 160.7 (C-2), 153.6 (C-6), 138.1 (C-4), 132.3 (C-3), 105.7 (C-1), 103.8 (C-5), 63.0 (OCH_3), 22.5 (CH_2), 13.3 (CH_3). Anal. ($C_{10}H_{11}BrO_4$) C, H, Br.

Pharmacology. [^{3}H]Spiperone Binding. The assays were performed essentially as described earlier.[2] Rats were killed by decapitation, and the striatum was rapidly dissected out on ice. After homogenization in Tris-HCl buffer (0.05 M, pH 7.6) the homogenate was centrifuged for 10 min at 48000g, 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 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 were 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[19] and correlated for ties following Sen's procedure[20] based on Kendell's τ. A slightly modified version of Sen's procedure was used to determine the 90% confidence interval.

Acute Toxicity. Male Sprague–Dawley rats (ALAB, Sollentuna, Sweden) weighing 150–200 g were used. The rats were housed five per cage and had free access to food and water. The rats were kept in a temperature- (21–22 °C), humidity- (40–50%), and light-controlled room (12/12 h dark/light cycle). The number of surviving animals was recorded 2, 24, and 48 h after the injection. The LD_{50}'s were calculated by probit analysis and based on three to five dose levels with five rats per dose (Table IV).

Registry No. 1, 84225-94-5; **2**, 98575-95-2; **3**, 98526-84-2; **4**, 98575-96-3; **5**, 98526-85-3; **6**, 98526-86-4; **7**, 98526-87-5; **8**, 98526-88-6; **9**, 98526-89-7; **10**, 98526-90-0; **11**, 98575-97-4; **12**, 98526-91-1; **13** (free base), 84225-95-6; **13** (tartrate), 98185-20-7; **13** (mesylate), 98527-30-1; **13** (phosphate), 98527-31-2; **13**·HCl, 98527-32-3; **14**·HCl, 98632-69-0; **15**, 98526-96-6; **16**·HCl, 97612-24-3; **17**, 98526-95-5; **18**·HCl, 98526-92-2; **19**, 88936-01-0; **20** (mesylate), 98526-94-4; **21** (free base), 98526-99-9; **21**·HCl, 98526-93-3; **22**, 98526-97-7; **23**, 98526-98-8; **24**, 98526-99-9; **25** (tartrate), 98527-00-5; **26** (tartrate), 98527-01-6; **27**, 98527-02-7; **28**, 98527-03-8; **29** (tartrate), 98527-05-0; **30**·HCl, 97612-24-3; **31** (free base), 98526-98-8; **31** (tartrate), 98527-06-1; **32** (mesylate), 89020-43-9; **33**, 98526-95-5; **34**, 98527-03-8; **35** (tartrate), 98527-08-3; **36**, 98527-09-4; **37**, 98527-10-7; **39**, 38064-90-3; **40**, 7149-91-9; **41**,

24953-82-0; **42**, 91971-25-4; **43**, 19672-03-8; **44**, 98527-11-8; **45**, 52959-34-9; **46**, 98527-12-9; **47**, 36680-47-4; **48**, 36674-07-4; **49**, 98527-13-0; **50**, 98527-14-1; **51**, 17715-70-7; **52**, 98527-15-2; **53**, 98527-16-3; **54**, 98527-17-4; **55**, 98527-18-5; **55** (acid chloride), 98527-33-4; **55** (amide), 98527-34-5; **56**, 98527-19-6; **57**, 98527-20-9; **58**, 98527-21-0; **58** (amide), 98527-35-6; **59**, 98527-22-1; **60**, 98527-23-2; **61**, 84225-86-5; **62**, 98527-24-3; **63**, 98527-25-4; **64**, 98527-26-5; **65**, 98527-27-6; **66**, 98527-28-7; II (X^1 = X^2 = Cl), 73219-91-7; II (X^1 = Cl, X^2 = Br), 73219-92-8; II (X^1 = H, X^2 = Br), 96897-96-0; II (X^1 = H, X^2 = Pr), 96897-98-2; II (X^1 = H, X^2 = F), 52189-67-0; (2*S*)-2-(aminomethyl)-1-ethylpyrolidine, 22795-99-9; 2,5-dibromo-2-methoxybenzoic acid, 13130-23-9; 2,6-dimethoxy-3-ethylbenzene, 19672-03-8; methyl 3-bromo-2,6-dimethoxy-5-nitrobenzoate, 98527-36-7; **66**, 98527-28-7; II (x^1 = x^2 = Cl), 73219-91-7; II (x^1 = Cl, x^2 = Br), 73219-92-8; II (x^1 = H, x^2 = Br), 96897-96-0; II (x^1 = H, x^2 = Pr), 96897-98-2; II (x^1 = H, x^2 = F), 52189-67-0; (2*S*)-2-(aminomethyl)-1-ethylpyrolidine, 22795-99-9; 2,5-dibromo-2-methoxybenzoic acid, 13130-23-9; 2,6-dimethoxy-3-ethylbenzoic acid, 96897-97-1; methyl 3-bromo-2,6-dimethoxy-5-nitrobenzoate, 98527-36-7.

Potential Antitumor Agents. 45. Synthesis, DNA-Binding Interaction, and Biological Activity of Triacridine Derivatives

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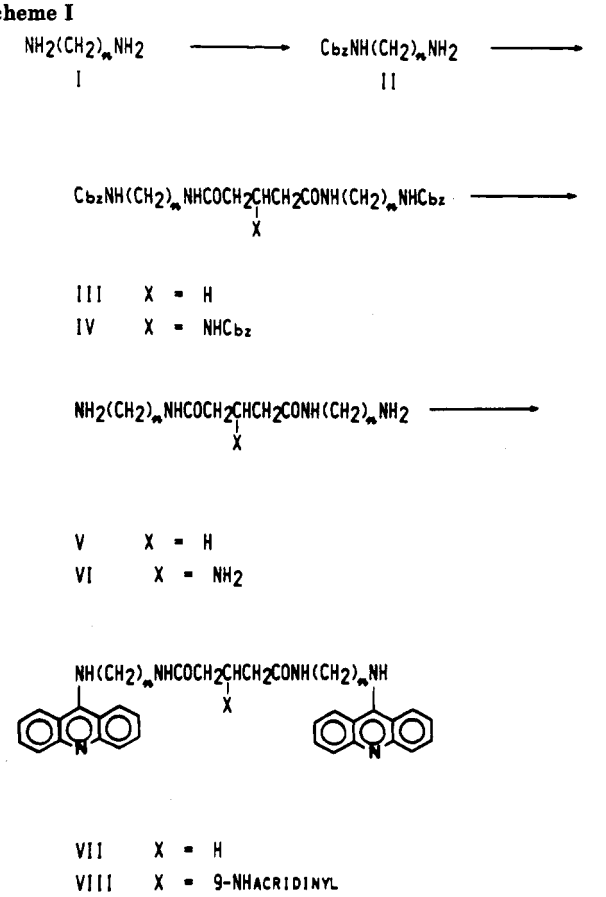
A series of amide-linked triacridines of varying interchromophore separation were synthesized as potential DNA trisintercalating agents. The corresponding diamide diacridines (lacking the central chromophore) were also prepared, and the DNA-binding and biological activities of both series of compounds were evaluated. Although one of the triacridines shows evidence of a trisintercalative binding mode, most of the triacridines (and all the diacridines) bound by bisintercalation. The diacridines showed greater cytotoxicity and higher DNA association constants than the corresponding 9-[[3-(dimethylamino)propyl]amino]acridine monomer, but addition of a third chromophore did not provide corresponding increases in either DNA affinity, inhibition of RNA synthesis, or cytotoxicity. Some members of both series show minimal in vivo antileukemic activity. The results suggest that further development of trimeric molecules as potential antitumor agents should focus on smaller chromophores with lower capacity for nonspecific binding and/or the employment of rigid linker chains to provide true molecular "staples" for DNA.

The acridine-derived DNA-intercalating agents comprise an important class of antitumor drugs.[1] The mode of action of such compounds is surmised to be inhibition of nucleic acid synthesis[2] and/or the induction of irreparable DNA strand breaks.[3,4] The drug physicochemical properties that contribute most to the cytotoxic processes include strong equilibrium binding to DNA,[5,6] long drug residence times at a particular DNA site,[7] and the ability to deliver radical species to the DNA.[8,9] While it is conceptually clear how the latter property might facilitate DNA breakage, the biochemical reasons for the favorable effect of the binding requirements have not been elucidated. Suggestions include the blocking of polymerase progression along the DNA[2,10] and selective reaction of the drug–DNA complex with proteins involved in DNA replication, such as the topoisomerases.[11,12] In efforts to obtain compounds with higher equilibrium binding constants than achievable by the monomers, much work has gone into the synthesis and evaluation of dimeric molecules, where the two chromophores are joined by a linker chain of sufficient length and flexibility to allow both chromophores to intercalate when bound to DNA. Most of this work has involved derivatives of 9-aminoacridine, and compounds of significantly higher binding constants and lower dissociation rates[13,14] than those of the monomer 9-aminoacridine have been obtained. In a logical extension of this argument, several groups[15–18] have now prepared and evaluated the DNA-binding properties of triacridine derivatives that appear to act as DNA trisintercalating agents. Some preliminary reports on the biological activity of these compounds have appeared.[17,19]

Scheme I



In this paper we report the synthesis, physicochemical properties, and biological activity of a series of triacridine

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