125174-41-6; 3 (X = O, R = 2-$NO_2$), 125174-42-7; 3 (X = O, R = 4-NHCONHMe), 125174-43-8; 3 (X = S, R = 4-$NO_2$), 115256-40-1; 3 (X = O, R = 2-$NO_2$-5-Me), 125174-44-9; 3 (X = O, R = 2-Cl-4-$NO_2$), 125174-45-0; 3 (R = 4-$NO_2$), 115256-47-8; 3 (R = 4-$NO_2$, X = $-CH_2-$), 125174-46-1; 3 (X = -$CH_2O$-, R = 4-$NO_2$), 125174-47-2; 3 (X = $(CH_2)_2O$, R = 4-$NO_2$), 125174-48-3; 3 (X = $(CH_2)_2$, R = 4-$NO_2$), 125174-49-4; 3 (X = OCH(Ph), R = 4-$NO_2$), 125174-50-7; 4 (X = O, R = 4-$CO_2$Me), 125174-51-8; 4 (X = O, R = 4-$CONH_2$), 115256-19-4; 4 (X = O, R = 4-CONHMe), 115256-27-4; 4 (X = O, R = $CONEt_2$), 125174-52-9; 4 (X = O, R = 4-CO-c-N$(CH_2CH_2)_2$O), 125174-53-0; 4 (X = O, R = 3-$CONH_2$), 125174-54-1; 4 (X = O, R = 4-$CF_3$), 125174-55-2; 4 (X = O, R = 2-$CONH_2$-4-Me), 125174-56-3; 4 (X = O, R = 4-$CH_2CONH_2$), 125174-57-4; 4 (X = O, R = 4-$SO_2NH_2$), 125174-58-5; 4 (X = O, R = 4-$NH_2$), 115256-13-8; 4 (X = O, R = 3-$NH_2$), 125174-59-6; 4 (X = O, R = 2-$NH_2$), 125174-60-9; 4 (X = O, R = 4-NHCONHMe), 125174-61-0; 4 (X = S, R = 4-$NH_2$), 115256-41-2; 4 (X = O, R = 2-$NH_2$-5-Me), 125174-62-1; 4 (X = O, R = 2-Cl-4-$NH_2$), 125174-63-2; 4 (R = 4-$NH_2$), 115256-48-9; 4 (X = $-CH_2-$, R = 4-$NH_2$), 125174-64-3; 4 (X = $-CH_2O-$, R = 4-$NH_2$), 125174-65-4; 4 (X = $(CH_2)_2O$, R = 4-$NH_2$), 125174-66-5; 4 (X = $(CH_2)_2$, R = 4-$NH_2$), 125174-67-6; 4 (X = OCH(Ph), R = 4-$NH_2$), 125174-68-7; 6, 125174-69-8; 7, 115256-20-7; 8, 115256-29-6; 8·HCl, 115256-28-5; 9, 115256-21-8; 10, 125174-70-1; 10·HCl, 115256-22-9; 11, 115256-23-0; 12, 125174-71-2; 12·HCl, 125174-72-3; 13, 125174-73-4; 13·HCl, 115256-24-1; 14, 125174-74-5; 14·$MeSO_3H$, 125174-75-6; 15, 125197-04-8; 16, 115256-11-6; 17, 115256-30-9; 18, 125174-76-7; 18·HCl, 115256-31-0; 19, 125174-77-8; 19·HCl, 125174-78-9; 20, 115256-42-3; 21, 125174-79-0; 21·HCl, 115256-32-1; 22, 115256-43-4; 23, 115256-50-3; 24, 125174-80-3; 25, 125174-81-4; 25·HCl, 115256-33-2; 26, 125174-82-5; 26·HCl, 115256-34-3; 27, 125174-83-6; 28, 125174-84-7.

# Potential Antipsychotic Agents. 5.[†] Synthesis and Antidopaminergic Properties of Substituted 5,6-Dimethoxysalicylamides and Related Compounds[‡]

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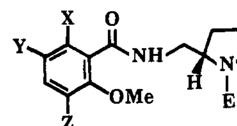
A series of 3-substituted 5,6-dimethoxysalicylamides III (9–13 and 15) has been synthesized from the corresponding 2,5,6-trimethoxybenzoic acids. Relaxation times $T_1$ and carbon chemical shifts of the methoxy groups in III showed that the 6-methoxy group adopts a nearly perpendicular orientation and the 5-methoxy group takes on a more coplanar orientation with respect to the ring plane in solution. The salicylamides III display a very high and stereoselective affinity for the [$^3$H]spiperone and [$^3$H]raclopride binding sites in vitro. Regioisomeric salicylamides IV also exhibit pronounced, but lower than III, affinity for the [$^3$H]spiperone binding site. The structural requirements were further assessed by studies of the related amino analogues 23 and 24 and hydroxy analogue 27. The 3-bromo compound 11 (FLB 463) was studied in various in vivo models and compared with the dopamine-$D_2$ antagonists sulpiride, raclopride, eticlopride, and haloperidol. The high potency of 11 to selectively block dopamine-$D_2$ receptors in vitro and in vivo combined with indications on a low potential for motor side effects makes it a very interesting new member of the class of substituted salicylamides.

The clinical efficacy of most currently used antipsychotic agents, e.g. the phenothiazines and butyrophenones, is attributed to their ability to block dopamine-$D_2$ receptors.[1,2] The introduction of more selective compounds, e.g. the substituted benzamides sulpiride,[3,4] and remoxipride,[5,6] indicated that the antidopaminergic effect associated with the clinical efficacy could be separated from the induction of extrapyramidal side effects (EPS).[1,7] A recently developed series of salicylamides, e.g. 28 (FLA 797),[8] eticlopride,[9] and raclopride (Chart I),[9,10] are highly potent and selective dopamine-$D_2$ antagonists.[7] Importantly, these compounds combine a very high and stereoselective affinity for dopamine-$D_2$ receptors with a low liability to produce EPS in behavioral studies in the rat.[1,7-10] Accordingly, raclopride has been selected for clinical investigations and found to be well tolerated.[11] The suitable binding characteristics also led to the development of tritiated and carbon-11 labeled raclopride as radioligands.[12-14] As further support for the abovementioned mode of action of antipsychotics, positron

**Chart I**




| | X | Y | Z |
|---|---|---|---|
| (S)-sulpiride | H | $NH_2SO_2$ | H |
| remoxipride | $OCH_3$ | Br | H |
| FLA 797 (28) | OH | Br | H |
| raclopride (29) | OH | Cl | Cl |
| eticlopride (30) | OH | $C_2H_5$ | Cl |

[†] Part 6: de Paulis, T.; Hall, H.; Kumar, Y.; Rämsby, S.; Ögren, S. O.; Högberg, T. *Eur. J. Med. Chem.* In press.

[‡] Presented in part at the 194th National Meeting at the American Chemical Society, New Orleans, 1987, MEDI 43 and the Xth International Symposium on Medicinal Chemistry, Budapest, 1988, p 66.

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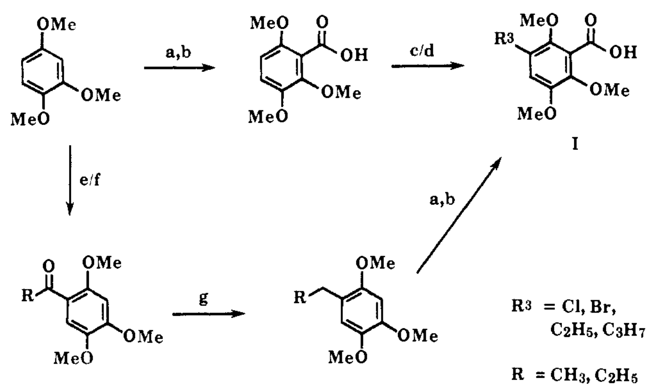
Scheme I[a]

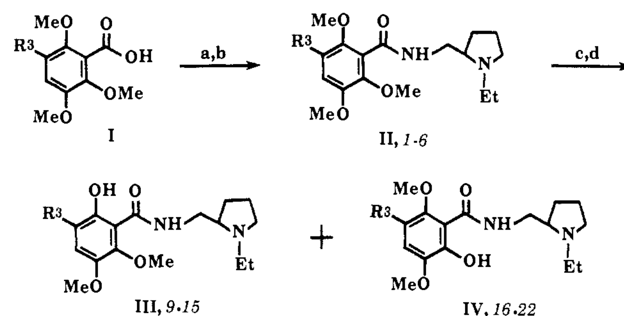



[a] (a) n-BuLi, THF, 20 °C; (b) $CO_2$(s), THF/$Et_2O$; (c) $SO_2Cl_2$, $CHCl_3$, 0 °C; (d) $Br_2$, dioxane, 20 °C; (e) $AlCl_3$, $CH_3COCl$, $CH_2Cl_2$, 5-10 °C; (f) $AlCl_3$, $C_2H_5COCl$, $CH_2Cl_2$, 10 °C; (g) $H_2$, Pd/C 5%, EtOH.

emission tomography studies with [$^{11}$C]raclopride indicate that the therapeutic action of a number of classical and atypical antipsychotic drugs is related to a substantial occupancy of dopamine-$D_2$ receptors.[15]

The quantitative structure–activity relationships (QSAR) of the substituted salicylamides showed that the lipophilicity and steric bulk of the 3-substituent mainly determine the affinity for the [$^3$H]spiperone binding site.[7,16] This contrasts with studies of other types of substituted benzamides which require electron-attracting groups in the corresponding position.[17] This diverging picture is also evident from molecular electrostatic potential (MEP) calculations on salicylamides[7,18] and other orthopramides.[19,20] Thus, the MEPs of salicylamides suggest that the aromatic region tolerates a greater variation of electronic features.

Scheme II[a]




[a] (a) $SOCl_2$, DMF, toluene, 50 °C; (b) (S)- or (R)-2-(aminomethyl)-1-ethylpyrrolidine; (c) 1 equiv of HCl in $Et_2O$; (d) $BBr_3$, $CH_2Cl_2$, room temperature.

The conformational properties of substituted salicylamides have been investigated by X-ray crystallography and force-field calculations.[7,21-23] On the basis of molecular modeling studies, the compounds are suggested to have a planar internally hydrogen bonded salicylamide moiety and a folded or half-folded side-chain conformation during the receptor interaction.[7,23]

The present paper describes the development of a series of highly potent 5,6-dimethoxysalicylamides (**9–13**, **15**). This substitution pattern is clearly ideal for high and selective affinity for the dopamine-$D_2$ receptor, which prompted the synthesis of a corresponding anthranilamide **23** in order to see if a primary amino group would mimic the phenol group in **11**. The electronic, lipophilic, and steric requirements in the 5-position were further explored by the introduction of amino (**24**) and hydroxyl (**27**) groups instead of the 5-methoxy substituent in **11**. The stereoselective dopamine receptor blockade was ascertained by investigations of the corresponding *R* isomer **14**.

## Chemistry

The syntheses of the required 3-halo- and 3-alkyl-2,5,6-trimethoxybenzoic acids are outlined in Scheme I. Friedel–Crafts acylations of 1,2,4-trimethoxybenzene followed by catalytic hydrogenation gave 3-ethyl- and 3-propyl-2,4,5-trimethoxybenzene in high overall yields. Regiospecific ortho lithiation followed by quenching with carbon dioxide furnished the benzoic acids in high yields. Standard halogenations of 2,3,6-trimethoxybenzoic acid gave the corresponding 3-chloro and 3-bromo acids. The acids were usually used without additional purification after the extractive workup. The 3-bromo-5,6-dimethoxyanthranilic acid was obtained by hydrolysis of the corresponding Boc-protected aniline.[24] 3,6-Bis(benzyl-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oxy)-2-methoxybenzoic acid was prepared via ortho lithiation of 2,5-bis(1-ethoxyethoxy)anisole followed by quenching with carbon dioxide and benzylation.[25]

Acids I were converted to the acid chlorides and reacted with (*S*)- or (*R*)-2-(aminomethyl)-1-ethylpyrrolidine, obtained by resolution[12b] or by an efficient stereoconservative synthesis from L- or D-proline,[26] to give trimethoxybenzamides II (**1**–**6**) according to Scheme II. Compound **7** was obtained by reaction of the acid chloride with (*S*)-1-trityl-2-(aminomethyl)pyrrolidine[5] followed by hydrolysis of the trityl group with ethanolic hydrochloric acid in 79% yield. Subsequent reaction of **7** with an excess of allyl bromide and potassium carbonate in dimethylformamide provided benzamide **8** in excellent yield. Demethylation of trimethoxybenzamides II to a mixture of salicylamides III and IV was effected with treatment of the hydrochloride salt with boron tribromide in dichloromethane (Scheme II). Isomers III were formed in increasing amounts with increasing steric hindrance from the adjacent substituent, i.e. 20%, 65%, 75% and 85% in the case of $R^3$ being hydrogen (**9**), chloro (**10**), bromo (**11**), and ethyl (**12**) or propyl (**13**), respectively. Isomers III and IV were separated by column chromatography. Propyl derivatives **13** and **20** were separated by partitioning the phenols between diethyl ether and aqueous sodium hydroxide, which allowed for an efficient recovery of the minor isomer from the aqueous layer.

A more efficient way to prepare salicylamides **9** and **16**, than by demethylation of **1**, was based on the availability of the pure bromo derivatives **11** and **18**. Hydrogenolysis over palladium on charcoal resulted in a quantitative conversion to the corresponding desbromo compounds. Furthermore, this reaction was part of the structure elucidation of the salicylamides (see below). Compound **9** has also been made via ortho lithiation of (*S*)-2,3-dimethoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide followed by oxidation, which allows for a regiospecific synthesis of the 5,6-dimethoxysalicylamide system.[24] Anthranilamide **23** was made from 2-amino-3-bromo-5,6-dimethoxybenzoic acid,[24] which was reacted with ethyl chloroformate to generate the isatoic anhydride. Subsequent reaction with (*S*)-2-(aminomethyl)-1-ethylpyrrolidine furnished **23** in a modest yield after isolation by reversed-phase chromatography. Two improved synthesis methods of **23** have recently been described.[24] 5-Aminosalicylamide **24** was prepared by reduction of (*S*)-3-bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-nitro-6-methoxysalicylamide[9] with iron(II) sulfate in methanolic ammonia. The corresponding hydroquinone **27** was obtained by amination of 3,6-bis(benzyloxy)-2-methoxybenzoyl chloride to give **25**. The benzyl groups were readily removed by hydrogenolysis, giving **26**, which was brominated with bromine–dioxane in the presence of acetic acid to yield compound **27** in a good overall yield.

The determination of the regiochemistry of the benzamides resides mainly on $^1H$ and $^{13}C$ NMR data. The hydroxy-bearing carbons were distinguished from the methoxy-substituted carbons by the upfield shift induced by addition of $D_2O$ or by gated decoupling. The assignments of the aromatic $^{13}C$ chemical shifts of substituted 6-methoxysalicylamides have previously been based on long-range C–H coupling constants.[8] Analogously, the salicylamides **9** and **16** obtained by hydrogenolysis of **11** and **18**, respectively, were subjected to gated-decoupling experiments. Thus, the aromatic hydroxy-substituted carbon at 156.9 ppm in one of the isomers displayed a double doublet through $^3J$ and $^2J$ couplings with the aromatic protons ($^3J$ = 9.8 Hz and $^2J$ = 2.4 Hz) in accordance with structure **9**. In the other isomer (**16**) the hydroxylated carbon at 155.0 ppm showed only one doublet ($^3J$ = 8.6 Hz). The anticipated $^4J$ coupling was not resolved. The other oxygenated carbons in both isomers displayed complex coupling patterns due to additional coupling with the protons of the methoxy groups. This experiment also indirectly confirms the structures of **3**, **11** and **18**. Likewise, the ethyl-substituted compounds **12** and **19** were assigned on basis of long-range C–H coupling constants of the phenolic carbons. The sole doublet ($^3J$ = 8.2 Hz) at 151.9 ppm confirmed the structure of **19** due to the lack of $^3J$ couplings to the methylene protons of the ethyl group. By applying substituent parameters for $^{13}C$ chemical shifts to the assigned compounds the structures of the remaining derivatives were verified.

In the analysis one should note the conformational differences between the 2,6-dimethoxy (II) and the 2-hydroxy-6-methoxy (III and IV) and 2-amino-6-methoxy (**23**) compounds. The former derivatives display a twisted benzamide conformation.[7,22,27] This fact is reflected in the different chemical shifts of the C-1 carbons, e.g. 128.6 (**3**) ppm versus 109.0 (**11**), 109.1 (**18**), 109.2 (**24**), 108.6 (**27**), and 113.0 (**23**) ppm in the series of bromo-substituted compounds. Likewise, C-1 has a chemical shift of 123.4 ppm in remoxipride and 105.2 ppm in **28**.

Additional support for the validity of the structures rests on the fact that aromatic methoxy groups flanked by two ortho substituents have longer relaxation times $T_1$ and higher downfield carbon shifts, suggesting a location out of the plane of the ring (Table II).[28,29] The difference in conformations between the two series (**1**, **2**, **3**, and remoxipride vs **10**, **11**, **17**, and **18**) is of importance (vide supra). The longer relaxation times and larger chemical shifts for the 5-methoxy and 6-methoxy groups in the 5,6-dimethoxysalicylamides (**10** and **11**) in comparison with the corresponding groups in the 2,5,6-trimethoxybenzamides (**1**–**3**) is indicative of a more coplanar arrangement of the salicylamide moiety forcing the methoxy groups further out of planarity with the benzene ring. The 6-methoxy group is likely to adopt a nearly perpendicular orientation in **10** and **11**, whereas the 5-methoxy group exhibits a more restricted rotation closer to the ring plane. These conformational aspects in relation to the biological activity will be discussed later.

## Pharmacology

The compounds were primarily investigated for their ability to inhibit the binding of [$^3H$]spiperone to rat striatal membranes in vitro (Table I).[30] The incubations were performed at 37 °C and (+)-butaclamol (1 $\mu$M) was used for the determination of nonspecific binding. The $IC_{50}$ values were calculated by log–logit regression analysis.

Consistent with previous studies[8] 2,6-dimethoxy derivatives II (**2**–**5**) exhibit a modest affinity for the [$^3H$]spiperone binding site. On the other hand, 5,6-dimethoxysalicylamides III (**9**–**13** and **15**) were found to be extremely

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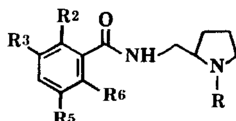
**Table I.** Structure, Physical Data, and Inhibition of [$^3$H]Spiperone Binding ($IC_{50}$, nM) and [$^3$H]Raclopride Binding ($K_i$, nM) of Substituted 2-Methoxybenzamides




| compd | R² | R³ | R⁵ | R⁶ | R | stereo-chem | % yield | mp, °C | recrystn solvent | formula | anal. | spiperone[a] $IC_{50}$, nM | raclopride $K_i$, nM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $OCH_3$ | H | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *S* | 43 | oil[b] | | $C_{17}H_{26}N_2O_4$ | | – | |
| 2 | $OCH_3$ | Cl | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *S* | 54 | 118–120 | *i*-$Pr_2O$ | $C_{17}H_{25}ClN_2O_4$ | C,H,Cl,N | 420 | |
| 3 | $OCH_3$ | Br | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *S* | 79 | 106–107 | *i*-$Pr_2O$ | $C_{17}H_{25}BrN_2O_4$ | C,H,N | 1750[c] | |
| 4 | $OCH_3$ | $C_2H_5$ | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *S* | 71 | 85–87 | *i*-$Pr_2O$ | $C_{19}H_{30}N_2O_4$ | C[d],H,N,O | 820 | |
| 5 | $OCH_3$ | $C_3H_7$ | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *S* | 32 | 68–70 | *i*-$Pr_2O$ | $C_{20}H_{32}N_2O_4$ | C,H,N,O | 2960 | |
| 6 | $OCH_3$ | Br | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *R* | 73 | 106–108 | *i*-$Pr_2O$ | $C_{17}H_{25}BrN_2O_4$ | C,H,N | – | |
| 7 | $OCH_3$ | Br | $OCH_3$ | $OCH_3$ | H | *S* | 79 | 181–182 | acetone/ $Et_2O$ | $C_{15}H_{21}BrN_2O_4$·HCl | C,H,N | – | |
| 8 | $OCH_3$ | Br | $OCH_3$ | $OCH_3$ | $CH_2$=CH-$CH_2$ | *S* | 97 | 118–122 | *i*-$Pr_2O$ | $C_{18}H_{25}BrN_2O_4$ | C,H,N | – | |
| 9 | OH | H | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *S* | 100 | oil[b] | | $C_{16}H_{24}N_2O_4$ | | 8.8 | 0.75 |
| 10 | OH | Cl | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *S* | 37 | 165–167 | acetone | $C_{16}H_{23}ClN_2O_4 \cdot CH_4O_3S$ | C,H,Cl,N,O,S | 1.7 | 0.087 |
| 11 | OH | Br | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *S* | 48 | 179–180 | acetone | $C_{16}H_{23}BrN_2O_4 \cdot CH_4O_3S$ | C,H,Br,N,O,S | 1.4 | 0.064 |
| 12 | OH | $C_2H_5$ | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *S* | 54 | 157–158 | acetone | $C_{18}H_{28}N_2O_4 \cdot CH_4O_3S$ | C,H,N,O,S | 0.77 | 0.033 |
| 13 | OH | $C_3H_7$ | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *S* | 63 | 84–85 | aq acetone | $C_{19}H_{30}N_2O_4 \cdot C_4H_6O_6 \cdot H_2O$ | C,H,N | 2.4 | 0.023 |
| 14 | OH | Br | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *R* | 53 | 180–181 | acetone | $C_{16}H_{23}BrN_2O_4 \cdot CH_4O_3S$ | C,H,N | 79 | 6.54 |
| 15 | OH | Br | $OCH_3$ | $OCH_3$ | $CH_2$=CH-$CH_2$ | *S* | 48 | 146–148 | acetone | $C_{17}H_{23}BrN_2O_4 \cdot CH_4O_3S$ | C,H,Br,N,S | 2.3 | |
| 16 | $OCH_3$ | H | $OCH_3$ | OH | $C_2H_5$ | *S* | 100 | oil[b] | | $C_{16}H_{24}N_2O_4$ | | 205 | |
| 17 | $OCH_3$ | Cl | $OCH_3$ | OH | $C_2H_5$ | *S* | 11 | oil | | $C_{16}H_{23}ClN_2O_4$ | C,H,N | 71 | |
| 18 | $OCH_3$ | Br | $OCH_3$ | OH | $C_2H_5$ | *S* | 13 | 97–99 | hexane/ EtOH | $C_{16}H_{23}BrN_2O_4$ | C,H,N | 67 | 8.38 |
| 19 | $OCH_3$ | $C_2H_5$ | $OCH_3$ | OH | $C_2H_5$ | *S* | 5 | 137–138 | acetone | $C_{18}H_{28}N_2O_4 \cdot CH_4O_3S$ | C,H,N | 19[e] | |
| 20 | $OCH_3$ | $C_3H_7$ | $OCH_3$ | OH | $C_2H_5$ | *S* | 14 | oil[b] | | $C_{19}H_{30}N_2O_4$ | | – | |
| 21 | $OCH_3$ | Br | $OCH_3$ | OH | $C_2H_5$ | *R* | 14 | 97–99 | hexane/ EtOH | $C_{16}H_{23}BrN_2O_4$ | C,H,N | 6170[f] | |
| 22 | $OCH_3$ | Br | $OCH_3$ | OH | $CH_2$=CH-$CH_2$ | *S* | 10 | oil[b] | | $C_{17}H_{23}BrN_2O_4$ | | 44 | |
| 23 | $NH_2$ | Br | $OCH_3$ | $OCH_3$ | $C_2H_5$ | *S* | 25 | oil | | $C_{16}H_{24}BrN_3O_3$ | C,H,N | 21 | |
| 24 | OH | Br | $NH_2$ | $OCH_3$ | $C_2H_5$ | *S* | 53 | 105 dec | EtOH | $C_{15}H_{22}BrN_3O_3 \cdot 2HCl \cdot H_2O$ | C,H,Br,Cl,N | 33 | |
| 25 | OBzl | H | OBzl | $OCH_3$ | $C_2H_5$ | *S* | 90 | 124–125 | *i*-$Pr_2O$ | $C_{29}H_{34}N_2O_4$ | C,H,N | – | |
| 26 | OH | H | OH | $OCH_3$ | $C_2H_5$ | *S* | 92 | 107–108 | $Et_2O$ | $C_{15}H_{22}N_2O_4$ | C,H,N | 56 | |
| 27 | OH | Br | OH | $OCH_3$ | $C_2H_5$ | *S* | 47 | 189–190 | acetone/ MeOH | $C_{15}H_{21}BrN_2O_4$·HCl | C,H,Br,Cl,N | 1.9 | |
| (*S*)-sulpiride | H | $SO_2NH_2$ | H | $OCH_3$ | $C_2H_5$ | *S* | | | | | | 210 | 6.9 |
| FLA 797 (28) | OH | Br | H | $OCH_3$ | $C_2H_5$ | *S* | | | | | | 12 | 0.55 |
| raclopride (29) | OH | Cl | Cl | $OCH_3$ | $C_2H_5$ | *S* | | | | | | 32 | 1.30 |
| eticlopride (30) | OH | $C_2H_5$ | Cl | $OCH_3$ | $C_2H_5$ | *S* | | | | | | 0.92 | 0.12 |
| haloperidol | | | | | | | | | | | | 12 | 0.33 |

[a] Correlation coefficients r > 0.95 unless otherwise indicated. [b] Characterized by NMR and mass spectrometry. Purity ascertained by TLC, GC, and/or HPLC. [c] *r* = 0.90. [d] C: calcd, 65.15; found, 64.14. [e] *r* = 0.91. [f] *r* = 0.89.

**Table II.** Spin–Lattice Relaxation Times ($T_1$) and $^{13}C$ Chemical Shifts ($\delta$) of Methoxy Substituents of Selected *N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamides

| compd | substituent | | | | chemical shift, ppm | | | relaxation time, s | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $R^2$ | $R^3$ | $R^5$ | $R^6$ | $R^2$ | $R^5$ | $R^6$ | $R^2$ | $R^5$ | $R^6$ |
| 1 | $OCH_3$ | H | $OCH_3$ | $OCH_3$ | 55.8 | 56.0 | 60.9 | 3.0 | 3.3 | 5.7 |
| 2 | $OCH_3$ | Cl | $OCH_3$ | $OCH_3$ | 61.7 | 56.2 | 61.0 | 4.7 | 2.5 | 5.9 |
| 3 | $OCH_3$ | Br | $OCH_3$ | $OCH_3$ | 61.8 | 56.2 | 61.0 | 4.6 | 2.6 | 5.6 |
| remoxipride | $OCH_3$ | Br | H | $OCH_3$ | 61.8 | | 55.9 | 5.7 | | 2.8 |
| 10 | OH | Cl | $OCH_3$ | $OCH_3$ | | 56.8 | 61.3 | | 4.2 | 7.3 |
| 11 | OH | Br | $OCH_3$ | $OCH_3$ | | 56.9 | 61.3 | | 3.8 | 6.5 |
| 17 | $OCH_3$ | Cl | $OCH_3$ | OH | 61.6 | 56.2 | | 6.2 | 3.2 | |
| 18 | $OCH_3$ | Br | $OCH_3$ | OH | 61.8 | 56.2 | | 5.0 | 2.6 | |

potent inhibitors of [$^3$H]spiperone binding. The high potency of unsubstituted parent compound **9** is remarkable in light of the structure–activity relationships for 3-substituted 6-methoxysalicylamides.[8] Modest differences in in vitro activity for 3-halo- (**10** and **11**) and 3-alkyl- (**12** and **13**) 5,6-dimethoxysalicylamides were found. The introduction of a *N*-allyl (**15**) instead of a *N*-ethyl (**11**) group in the pyrrolidine ring results in a negligible loss in affinity. The binding of this series of compounds to the [$^3$H]spiperone binding site is stereoselective by analogy with other salicylamides[9] and substituted benzamides.[31] Thus, *R* isomer **14** is considerably less active than **11**.

Isomeric series IV also display pronounced affinity for the [$^3$H]spiperone binding site—albeit markedly lower than that of III. In analogy with 5,6-dimethoxysalicylamides III, unsubstituted 3,6-dimethoxysalicylamide **16** is the least active and 5-substituted compounds **17**–**19** are equipotent in this series (IV). Notably, compounds **16**–**19** have virtually the same activity as the corresponding 5-substituted 6-methoxysalicylamides described previously.[8] The stereoselective binding is apparent also for the compounds IV (cf. **18** and **21**).

Salicylamides III were also assessed for their ability to block the binding of the selective dopamine-$D_2$ ligand [$^3$H]raclopride (Table I).[13,30] In accordance with the studies using [$^3$H]spiperone, the 3-halo (**10** and **11**), and 3-alkyl (**12** and **13**) derivatives were about equipotent. They displayed a 10-fold higher activity in comparison to unsubstituted compound **9**. The stereoselective binding was confirmed in the comparison with **14**. Likewise, the regioisomer **18** was markedly less active than **11**. Thus, the same relative affinities of the investigated compounds were observed with the two radioligands.

Interestingly, anthranilamide **23** is only $^1/_{10}$ as active as the corresponding salicylamide **11**, but still the activity is considerable in relation to other classes of dopamine-$D_2$ antagonists (cf. Table I). The other amino isostere **24**, having the 5-methoxy group of potent 5,6-dimethoxysalicylamide **11** replaced with an amino group, is about equipotent with **23** and accordingly less active than **11**. The 5-aminosalicylamide **24** is also somewhat less potent than the corresponding 6-methoxysalicylamide **28** (cf. Table I). On the other hand, the 5-hydroxy analogue **27** is almost equipotent with 5-methoxy compound **11**.

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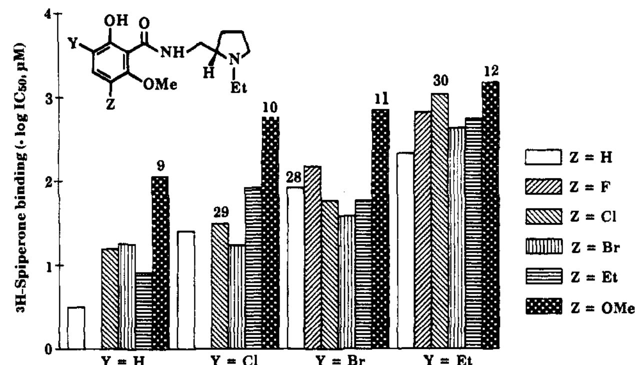


**Figure 1.** Relationship between blockade of [$^3$H]spiperone binding and the substitution pattern of 3,5-substituted 6-methoxysalicylamides.

The potent bromo compound **11** was studied in greater detail in various in vivo models in relation to potent dopamine-$D_2$ antagonists (Table III). Pretreatment of the rats with the test compounds produced a dose-related blockade of behavioral effects induced by the dopamine agonist apomorphine. A preferential inhibition of the apomorphine induced increase in locomotor activity (hyperactivity) over the oral stereotypies (e.g. licking, chewing, and biting) and a weak tendency to induce catalepsy have been regarded as indicators for a lowered EPS potential.[1,7,10] Haloperidol displays no separation in the blockade of the two apomorphine-induced behaviors in contrast to sulpiride, raclopride, and eticlopride.[9,10] Furthermore, the benzamides have a considerably lower ability to induce catalepsy. The high in vitro potency of **11** corresponds to a remarkably good in vivo activity (Table III). Importantly, the apomorphine-induced hyperactivity was inhibited at a significantly lower dose than the blockade of oral stereotypies. The blockade of hypothermia induced by apomorphine confirmed that the functional blockade of dopamine-$D_2$-mediated responses[32] occurs at a very low dose. The *R* isomer **14** showed the expected low tendency to inhibit the apomorphine-induced responses, confirming the stereoselective dopamine receptor blockade in vivo. Likewise, regioisomer **18** was markedly less active than **11**. The moderate tendency for **11** to produce catalepsy in the rat combined with a functional blockade of dopamine-mediated behaviors in very low doses is noteworthy.

## Discussion

The previously investigated 5-alkyl- and 5-halo-substituted 6-methoxysalicylamides display QSAR features[7,16] differing from those of other types of orthopramides.[17] As can be seen in Figure 1, the 3-substituent (Y) adjacent to the phenol group has the major influence on the affinity for the [$^3$H]spiperone binding site. The 5-substituent (Z) flanking the 6-methoxy group merely modulates the activity within each cluster of different Y groups. Notably, 5,6-dimethoxysalicylamides III (Z = OMe) are anomalous in this respect; i.e. the activity is not as markedly affected by the nature of the 3-substituent (Y). The cluster in Figure 1 with compounds lacking 3-substituents (Y = H) reveals the superior properties of the 5-methoxy over the 5-alkyl or 5-halo substituted 6-methoxysalicylamides. Also the clusters of 3-chloro (Y = Cl) and 3-bromo (Y = Br) derivatives show this effect. 5,6-Dimethoxysalicylamides III is the most active class of benzamides, lacking lipophilic nitrogen substituents, we have investigated. However, other types of 2,3-dimethoxybenzamides, such as tropa-

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**Table III.** In vivo Activities of 11 (FLB 463), 14, and 18 in Relation to Known Antipsychotics in the Rat ($ED_{50}$, μmol/kg ip)

| compd | block of apomorphine-induced responses: hyperactivity[a] | stereotypies[a] | hypothermia[b] | catalepsy, bar test[c] |
|---|---|---|---|---|
| 11 | 0.009 (0.004–0.017) | 0.049 (0.042–0.057) | 0.005 (0.003–0.007) | 0.14 (0.07) |
| 14 | 12.6 (8.3–19.0) | 5.3 (4.1–6.8) | | |
| 18 | 1.3 (0.2–8.6) | 12.0 (10.3–13.9) | | |
| sulpiride | 55 (34–87) | 290 (252–334) | 8.0 (3.5–14.4) | 280 (34.4) |
| raclopride | 0.13 (0.05–0.23) | 1.80 (1.57–2.13) | 0.20[d] | 11 (4.1) |
| eticlopride | 0.015 (0.003–0.083) | 0.19 (0.16–0.21) | 0.09 (0.06–0.14) | 0.74 (0.32) |
| haloperidol | 0.37 (0.23–0.60) | 0.27 (0.23–0.33) | 0.35 (0.18–1.1) | 0.9 (0.13) |

[a] The compounds were injected ip 60 min prior to apomorphine (1 mg/kg sc). The hyperactivity and stereotypies were scored and calculated as described previously.[10] The $ED_{50}$ values were calculated by regression analysis using Fieller's theorem for estimates of the 95% confidence limits.[43] [b] The compounds were injected ip 60 min prior to apomorphine (1 mg/kg sc). The body temperature was measured by a rectal probe 15 min following apomorphine as described previously.[32] The $ED_{50}$ values were calculated by regression analysis using Fieller's theorem for estimates of the 95% confidence limits.[43] [c] Catalepsy was measured by the bar test 20, 40, 60, 90, 120, and 240 min after injection of the test compound. The rat was placed with both fore paws on a 7 cm high horizontal bar, and the catalepsy interval was defined as the time required for both fore limbs to be removed from the bar. A rat was considered to reach catalepsy if it showed a mean time of at least 15 s from the three test occasions showing the largest descent latencies. The $ED_{50}$ (SEM) was calculated by probit analysis. [d] Not possible to determine.

pride, are known as potent dopamine receptor antagonists.[31,33] It has been pointed out earlier that the lipophilic *N*-substituents required in this type of compounds, e.g. benzyl, might bind to an accessory binding site, thereby compensating for a nonoptimal aromatic substitution pattern.[7,23]

Molecular modeling studies of the salicylamides with piquindone[23] and a 2-phenylpyrrole derivative,[7] a conformationally restricted benzamide isostere,[34] support the view[35] of the importance of a planar hydrogen-bonded benzamide moiety. It has been suggested that the 5-substituent flanking the methoxy group must be small enough to permit a coplanar orientation of the 6-methoxy group in order to facilitate formation of this intramolecular hydrogen bond with the amide.[9] However, force-field calculations have shown that several of the more potent salicylamides do have a preferred perpendicular orientation of the 6-methoxy group.[7] This methoxy orientation is also found in several of the solid-state conformations, e.g. eticlopride,[21] raclopride,[23] and 3-bromo-5,6-dimethoxysalicylamide 11.[27] The $^{13}C$ NMR investigations on **10** and **11** provide valuable information about the conformation of the methoxy groups in solution (Table II). Thus, the longer relaxation times $T_1$ and higher chemical shifts of the 6-methoxy groups are strongly indicative of a perpendicular orientation.[28] A perpendicular methoxy orientation has been calculated to be accompanied with a higher electron density,[29] which in fact would facilitate the hydrogen bonding. However, the energy difference between a perpendicular and coplanar 6-methoxy conformation is within 1 kcal $mol^{-1}$ in compounds having adjacent 5-chloro[7] or 5-methoxy[37] groups. Thus, in the series III (**9–13**) a coplanar orientation cannot be ruled out during the receptor interaction.

In the tropapride series, an interesting correlation between the chemical shift of the amide hydrogen and the antidopaminergic effect has been found.[36] This effect was suggested to reflect reinforcement of the intramolecular hydrogen bond by electron-donating groups flanking the *o*-methoxy group being in an out of plane orientation. We have found no such indications in the salicylamide series; e.g. the chemical shift of the amide hydrogen is identical for **11** (5-methoxy) and the 100-fold less potent 5-nitro analogue[9] (unpublished observations). The stereoelectronic requirements on the 2-methoxy group will be treated more thoroughly in a series of related non-salicylamides with pyrrolidinylmethyl side chains.[38]

The 5-methoxy group in **11** could be altered to a 5-hydroxy group (**27**) without markedly affecting the affinity for the [$^3$H]spiperone binding site, which shows that the methyl part of the 5-methoxy group is not crucial for activity. It is therefore of interest to note that the more hydrophilic 5-amino analogue **24** is 1 order of magnitude less active than **27** ($\Delta\pi$-values 0.56). Clearly, the electronic features provided by the 5-oxy-6-methoxysalicylamide moiety are essential.

It has been proposed that the salicylamides exist in planar intramolecular hydrogen bonded form in aqueous media to a larger extent than common orthopramides lacking the additional phenol group.[7] The latter type of compounds have been shown to be internally hydrogen bonded in aprotic solvent but not in water.[35b] The 10-fold drop in activity of the more hydrophilic anthranilamide **23** in relation to **11** could be due to an enhanced competition to form external hydrogen bonds with water leading to a larger population of nonplanar benzamide conformations.

5,6-Dimethoxysalicylamide **11** was also found to be extremely potent in vivo (Table III). The substituted benzamides sulpiride, raclopride, and eticlopride display dopamine receptor blockade in vivo with the same relative order as predicted by the affinity for the [$^3$H]spiperone binding site (Table I). However, compound **11** is more potent than the heretofore most active salicylamide eticlopride in vivo. Moreover, salicylamide **11** in analogy with sulpiride, raclopride, and eticlopride showed inhibition of apomorphine-induced hyperactivity in lower doses than that needed for blockade of stereotypies. The indication of a lowered tendency to induce EPS is also reflected in the moderate ability to cause catalepsy. The high potency to selectively and stereoselectively block dopamine-$D_2$ receptors in vitro and in vivo combined with the indications on a low EPS potential makes **11** a very interesting

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new member of the substituted-salicylamide class. This class of salicylamides should also be suitable as radioligands due to the possibilities of introducing 3-substituents of different types.[39]

## Experimental Section

**Chemistry.** Melting points were determined in open capillary tubes on a Mettler FP 61 apparatus and are uncorrected. NMR spectra were recorded on a Varian EM 360 A or a JEOL FX 200 spectrometer using $Me_4Si$ as internal standard. Mass spectra were recorded on an LKB 9000 (EI/70 eV) or an LKB 2091 (EI/70 eV or CI) instrument. Optical rotations were measured on an Optical Activity AA-100 polarimeter. UV spectra were obtained on a HP 8450A VIS spectrometer. GLCs were run on an SE 30 capillary column and the amounts were determined by a Hewlett-Packard 3390A integrator. Preparative HPLC was conducted on a Waters LC 500 apparatus. Preparative, centrifugally accelerated, radial TLC was conducted on a Chromatotron from Harrison Research. Elemental analyses, performed by Analytische Laboratorium, Elbach, West Germany, were within ±0.4% of the theoretical values unless otherwise noted.

**2,3,6-Trimethoxybenzoic acid** was synthesized according to the method of Gilman and Thirtle by lithiation of 1,2,4-trimethoxybenzene and subsequent reaction with carbon dioxide in 86% yield: mp 149–150 °C (lit.[40] 47%, mp 148–149 °C); $^1$H NMR ($CDCl_3$) δ 7.1 and 6.8 (AB q, 2).

**3-Bromo-2,5,6-trimethoxybenzoic Acid.** A solution of bromine (8.0 g, 0.05 mol) in 50 mL of dioxane was added to a stirred solution of 2,3,6-trimethoxybenzoic acid (10.6 g, 0.05 mol) in 100 mL of dioxane at ambient temperature. The reaction mixture was stirred for 1 h and partitioned between water and $Et_2O$. The organic layer was dried ($Na_2SO_4$) and evaporated to give 12.2 g (84%) of crude product, which crystallized upon standing: mp 93–95 °C; $^1$H NMR ($CHCl_3$) δ 7.1 (s, 1). Anal. ($C_{10}H_{11}BrO_5$) C: calcd, 41.26; found 42.38; H; Br: calcd, 27.45; found, 26.61.

**3-Chloro-2,5,6-trimethoxybenzoic acid** was obtained by chlorination of 2,3,6-trimethoxybenzoic acid (5.0 g, 0.024 mol) with $SO_2Cl_2$ (3.2 g, 0.024 mol) in 75 mL of $CHCl_3$ at 0 °C. After 2 h the reaction mixture was partitioned between $CHCl_3$ and water. The organic layer was dried ($Na_2SO_4$) and evaporated to give 6.0 g (100%) title compound as an oil: $^1$H NMR ($CHCl_3$) δ 7.1 (s, 1).

**2,4,5-Trimethoxyacetophenone.** Aluminum chloride (66 g, 0.50 mol) was added portionwise to a solution of 1,2,4-trimethoxybenzene (75 g, 0.45 mol) and acetyl chloride (40 g, 0.50 mol) in 1200 mL of $CH_2Cl_2$ at 5–8 °C. After stirring for 1 h at 10 °C, 1500 mL of 0.5 M HCl was added. The organic layer was separated, washed with water, dried ($Na_2SO_4$), and evaporated to give a crystalline crude product. Recrystallization from MeOH gave 84 g (89%) of pure product, mp 98–100 °C. Anal. ($C_{11}H_{14}O_4$) C, H, O.

**1-Ethyl-2,4,5-trimethoxybenzene.** 2,4,5-Trimethoxyacetophenone (60 g, 0.3 mol) was hydrogenated at normal pressure in 1200 mL of EtOH with palladium on charcoal (5%, 8 g) as catalyst. After 1.5 h, 13 L (0.6 mol) of hydrogen was consumed. Filtration and evaporation gave 55 g (94%) of an oil, which was used without further purification.

**3-Ethyl-2,5,6-trimethoxybenzoic Acid.** Butyllithium (250 mL of 1.3 M in hexane, 0.32 mol) was added to a solution of 1-ethyl-2,4,5-trimethoxybenzene (55 g, 0.3 mol) in 750 mL of anhydrous THF under $N_2$ at 20–30 °C. After stirring for 1.5 h the reaction mixture was poured into solid carbon dioxide in $Et_2O$. Water (1000 mL) was added and the phases were acidified with concentrated HCl and extracted with $CH_2Cl_2$. Evaporation of the solvent furnished 60 g (83%) of a crystalline product, which was recrystallized from *i*-$Pr_2O$/hexane to give 50 g (69%) pure acid, mp 77–78 °C. Anal. ($C_{12}H_{16}O_5$) C, H, O.

**2,4,5-Trimethoxypropiophenone.** Aluminum chloride (18.6 g, 0.14 mol) was added to a solution of 1,2,4-trimethoxybenzene (23.4 g, 0.14 mol) and propionyl chloride (11.4 g, 0.14 mol) in 250 mL of $CH_2Cl_2$ at 10 °C. After stirring for 1 h, standard workup and recrystallization from MeOH gave 25.0 g (80%) of pure product, mp 108–110 °C. Anal. ($C_{12}H_{16}O_4$) C, H, O.

**1-Propyl-2,4,5-trimethoxybenzene** was prepared by hydrogenation (4.5 L $H_2$, 0.2 mol) of the corresponding propiophenone (22.4 g, 0.1 mol) in 350 mL of MeOH at normal pressure with palladium on charcoal (5%, 2.3 g) as catalyst. Filtration and evaporation gave 20 g (0.1 mol) of an oil which was lithiated directly.

**3-Propyl-2,5,6-trimethoxybenzoic acid** was prepared in analogy with the ethyl derivative to give a crude acid contaminated with some pentanoic acid: $^1$H NMR ($CDCl_3$) δ 6.8 (s, 1), 3.8–3.9 (3 s, 9), 2.4 (t, 2), 1.6 (m, 2), 0.9 (t, 3). Characterized as primary amide, mp 130–131 °C (*i*-$Pr_2O$).

**3-Bromo-5,6-dimethoxyanthranilic Acid.** To a solution of 3-bromo-5,6-dimethoxy-*N*-*tert*-butoxyanthranilic acid[24] (1.50 g, 4.0 mmol) in 20 mL of THF and 20 mL of $H_2O$ was added 20 mL of concentrated HCl dropwise with cooling. After stirring for 5 h at room temperature, the cooled mixture was adjusted to pH 7 with 45% NaOH and washed with $Et_2O$. The aqueous layer was concentrated and triturated with three 25-mL portions of EtOH. Evaporation of the EtOH gave 1.10 g (100%) of the hygroscopic deprotected aniline, which was used directly: $^1$H NMR (EtOH-$d_6$) δ 7.03 (s, 1), 3.83 (s, 3), 3.75 (s, 3).

**3,6-Bis(benzyloxy)-2-methoxybenzoic acid** was prepared according to the method of Högberg et al. from 3,6-dihydroxy-2-methoxybenzoic acid, mp 119–120 °C (*i*-$Pr_2O/Et_2O$).[25]

**(*S*)-3-Bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,5,6-trimethoxybenzamide (3).** A solution of 3-bromo-2,5,6-trimethoxybenzoic acid (4.5 g, 0.015 mol), thionyl chloride (4.5 g, 0.038 mol), and a few drops of dimethylformamide (DMF) in 60 mL of toluene was stirred at 60 °C for 1 h. The solvent was evaporated and the residue was dissolved in 150 mL of $CHCl_3$ and evaporated again. The residual acid chloride was dissolved in 20 mL of $CHCl_3$ and a solution of (*S*)-2-(aminomethyl)-1-ethylpyrrolidine[26] (2.6 g, 0.02 mol) in 40 mL of $CHCl_3$ was added. The temperature rose to 50 °C during the addition. After stirring for 30 min, the solvent was evaporated and the residue was partitioned between 100 mL of 1 M NaOH and 100 mL of $Et_2O$. After additional extraction of the aqueous phase with $Et_2O$, the combined ethereal extracts were dried ($Na_2SO_4$) and evaporated to give 5.8 g of **3**. Recrystallization from *i*-$Pr_2O$ gave 4.8 g (79%) of pure amide **3**: mp 106–107 °C; $^{13}$C NMR ($CDCl_3$) δ 164.6, 149.9, 147.6, 145.9, 128.6, 117.0, 111.1; $^1$H NMR ($CDCl_3$) δ 7.07 (s, 1), 3.86 (s, 3), 3.85 (s, 3), 3.84 (s, 3).

**2,5,6-Trimethoxybenzamides 1, 2, and 4–6** and 3,6-bis(benzyloxy)-2-methoxybenzamide **25** were prepared in an analogous way (Table I).

**(*S*)-3-Bromo-*N*-(2-pyrrolidinylmethyl)-2,5,6-trimethoxybenzamide (7).** 3-Bromo-2,5,6-trimethoxybenzoyl chloride (see previous example, 1.48 g, 4.8 mmol) was reacted with (*S*)-1-trityl-2-(aminomethyl)pyrrolidine[5] (1.51 g, 4.4 mmol) in 10 mL of $CH_2Cl_2$ at room temperature for 1 h. The solvent was evaporated and the residue was treated with 10 mL of EtOH and 0.1 mL of concentrated HCl during 1 h. After evaporation of the solvent, the residue was partitioned between 0.5 M HCl and $Et_2O$. The aqueous phase was made alkaline, extracted with $CH_2Cl_2$, dried ($Na_2SO_4$), and evaporated to give 1.30 g (79%) of **7**. An analytical sample of hydrochloride was prepared and recrystallized from acetone/$Et_2O$ and a small amount of EtOH, mp 181–182 °C.

**(*S*)-*N*-[(1-Allyl-2-pyrrolidinyl)methyl]-3-bromo-2,5,6-trimethoxybenzamide (8).** A solution of allyl bromide (0.40 g, 3.3 mmol) in 4 mL of DMF was added dropwise to a stirred mixture of **7** (base, 0.82 g, 2.2 mmol) and $K_2CO_3$ (0.40 g, 3 mmol) in 10 mL of DMF at room temperature. After 1 h, 150 mL of water was added and the product was extracted with 1 M HCl three times. The aqueous phase was made alkaline and extracted with $CH_2Cl_2$. Drying ($Na_2SO_4$) and evaporation gave 0.8 g (97%) of solid **8**. Recrystallization from *i*-$Pr_2O$ gave 0.33 g (40%): mp 118–122 °C; $[\alpha]^{20}_D$-85° (*c* 0.44, acetone); $^1$H NMR ($CDCl_3$) δ 8.9 (br), 7.07 (s, 1), 6.34 (br, 1), 5.85 (m, 1), 5.12 (dd, 2), 3.86 (s, 3, OMe), 3.84 (s, 6, $(OMe)_2$), 3.76 (m, 1), 3.35 (m, 2), 3.04 (m, 1), 2.88 (m, 1), 2.67 (br, 1), 2.22 (m, 1), 1.60–1.98 (m, 3).

**(*S*)-3-Bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5,6-dimethoxysalicylamide (11) and Isomer 18.** The trimethoxy

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compound 3 (8.1 g, 0.020 mol) was dissolved in 100 mL of $CH_2Cl_2$ and 7.3 mL of 3 M HCl in $Et_2O$ (0.022 mol) was added. A solution of $BBr_3$ (5.5 g, 0.022 mol) in 40 mL of $CH_2Cl_2$ was added dropwise at 10–15 °C. After stirring at ambient temperature for 1 h, 50 mL of 2 M $NH_3$ was added. The organic layer was separated, dried ($Na_2SO_4$), and evaporated to give 6.1 g of a mixture of 11 and 18 in a 75:25 ratio according to capillary GLC. The phenols were separated by preparative HPLC chromatography on $SiO_2$ with $i$-$Pr_2O$/MeOH/$NH_3$ (100:3:0.3) as eluent to give 3.7 g (48%) of 11 and 1.0 g (13%) of 18 in isomerically pure forms.

11: hydrochloride salt, mp 135–137 °C (acetone/$Et_2O$); mesylate, mp 179–180 °C (acetone); $^{13}C$ NMR (base, $CDCl_3$) $\delta$ 169.2, 153.5, 147.9, 144.6, 121.9, 109.0, 105.5; $^1H$ NMR (base, $CDCl_3$) $\delta$ 9.1 (br, NH), 7.28 (s, 1), 3.93 (s, 3), 3.84 (s, 3), 3.70 (dd, 1), 3.30 (m, 2), 2.84 (dq, 1), 2.6 (m, 1), 2.20 (m, 2), 1.4–1.8 (m, 4), 1.13 (t, 3). Anal. ($C_{16}H_{23}BrN_2O_4$·HCl) C, H, N, Br, Cl. 18: base, mp 97–99 °C (hexane/EtOH 20:1); $^{13}C$ NMR ($CDCl_3$) $\delta$ 169.3, 153.6, 149.0, 146.9, 118.5, 109.1, 103.9; $^1H$ NMR ($CDCl_3$) $\delta$ 8.9 (br, NH), 7.06 (s, 1), 3.86 (s, 3), 3.84 (s, 3), 3.9–1.6 (multiplets, 12), 1.13 (s, 3); $[\alpha]^{20}_D$-53° ($c$ 1.52, acetone).

The remaining salicylamides 9, 10, 12, 14–17, 19, 21, and 22 were prepared in an analogous way (Table I). Alternatively, derivatives 9 and 16 were obtained by the following procedure.

**(*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-5,6-dimethoxysalicylamide (9).** A solution of 11·HCl (0.20 g, 0.47 mmol) in 10 mL of EtOH was hydrogenated for 2.5 h at ambient temperature and pressure with Pd/C (10 mg, 5%) as catalyst. Filtration and evaporation of the solvent gave 0.18 g of an oily residue, which was partitioned between $CH_2Cl_2$ and aqueous $NH_3$ to give the free base identical with the minor isomer obtained by demethylation of 1: $^{13}C$ NMR ($CDCl_3$) $\delta$ 169.8, 156.9, 148.3, 144.4, 118.9, 113.1, 108.3; $^1H$ NMR ($CDCl_3$) $\delta$ 8.4 (br), 7.02 and 6.70 (AB, 2), 3.93 (s, 3), 3.83 (s, 3), 3.8–1.7 (multiplets, 11), 1.13 (t, 3).

16: $^{13}C$ NMR ($CDCl_3$) $\delta$ 170.5, 155.0, 152.6, 144.0, 115.1, 104.9, 99.2; $^1H$ NMR ($CDCl_3$) $\delta$ 8.9 (br), 6.87 and 6.29 (AB, 2), 3.88 (s, 3), 3.85 (s, 3), 3.8–1.7 (multiplets, 11), 1.13 (t, 3).

**(*S*)-5,6-Dimethoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-3-propylsalicylamide (13) and Isomer 20.** A solution of 5 (10.0 g, 0.027 mol) and HCl (0.027 mol in $Et_2O$) in 250 mL of $CH_2Cl_2$ was treated with $BBr_3$ (6.8 g, 0.027 mol) in 50 mL of $CH_2Cl_2$ at 10 °C. The reaction mixture was stirred for 2 h at 20 °C and quenched with 100 mL of 2 M $NH_3$. Extraction with $CH_2Cl_2$, drying ($Na_2SO_4$), and evaporation of the solvent gave 9.2 g of 13 and 20 in a ratio of 85:15. The residue was dissolved in 300 mL of $Et_2O$ and extracted with 50 mL of 1 M NaOH twice, which removed the minor compound 20 from the ethereal layer. Drying and evaporation of the solvent gave 6.0 g (63%) of 13, which was converted to 4.5 g (32%) of the L-tartrate: mp 84–85 °C (acetone/water 98:2); $^{13}C$ NMR (base, $CDCl_3$) $\delta$ 170.2, 154.9, 146.3, 143.6, 126.7, 119.9, 107.5.

The combined aqueous layer was washed with 50 mL of $Et_2O$ twice. The pH was adjusted to 8.5 and extraction with $Et_2O$ gave 1.3 g (14%) of pure 20 as an oil: $^{13}C$ NMR ($CDCl_3$) $\delta$ 170.1, 151.8, 149.9, 145.8, 123.9, 115.8, 107.9.

**(*S*)-2-Amino-3-bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5,6-dimethoxybenzamide (23).** Ethyl chloroformate (0.32 mL, 3.4 mmol) was added to a solution of 3-bromo-5,6-dimethoxyanthranilic acid (0.96 g, 3 mmol) and triethylamine (0.58 mL, 4.2 mmol) in 15 mL of THF/$CH_2Cl_2$ (1:1) at −20 °C. After stirring for 45 min at −20 °C, a solution of (*S*)-2-(aminomethyl)-1-ethylpyrrolidine (0.50 g, 3.8 mmol) in 10 mL of $CH_2Cl_2$ was added at −20 °C. After stirring for 3 h at room temperature the mixture was washed with water and extracted with 0.5 M HCl. The aqueous phase was made alkaline and extracted twice with $CH_2Cl_2$. Drying ($Na_2SO_4$) and evaporation gave 0.45 g crude material, which was purified by chromatography on a $C_{18}$ reversed-phase column with $H_2O$/MeOH/$NH_3$ (40:60:0.3) as eluent to give 0.28 g (25%) pure 23 as an oil: $^{13}C$ NMR ($CDCl_3$) $\delta$ 167.0, 148.1, 143.7, 140.9, 120.3, 113.0, 104.9; $^1H$ NMR ($CDCl_3$) $\delta$ 7.9 (br), 7.14 (s, 1), 5.80 (br), 3.82 (s, 3), 3.80 (s, 3), 3.9-1.7 (multiplets, 11), 1.11 (t, 3).

**(*S*)-5-Amino-3-bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamide (24).** Iron(II) sulfate heptahydrate (11 g, 0.04 mol) was dissolved in 25 mL of water and added to a solution of (*S*)-3-bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxy-5-nitrosalicylamide[9] (2.0 g, 0.005 mol) in 50 mL of MeOH. Concentrated ammonia (30 mL) was added and the mixture was stirred for 1 h at 60 °C. After filtration and washing of the filter cake with MeOH/$H_2O$ (1:1), the filtrate was acidified and washed with $Et_2O$. The pH of the aqueous layer was adjusted to 8 with $NH_3$. Extraction four times with $Et_2O$ and drying ($Na_2SO_4$) and evaporation of the solvent gave 1.0 g (53%) of a solid residue of 24. The dihydrochloride monohydrate was prepared and recrystallized from EtOH: mp 105 °C dec; $^{13}C$ NMR (base, $CDCl_3$) $\delta$ 169.2, 152.1, 145.2, 132.1, 124.5, 109.2, 107.3.

**(*S*)-*N*-[(Ethyl-2-pyrrolidinyl)methyl]-5-hydroxy-6-methoxysalicylamide (26).** Dibenzyl ether 25 (4.7 g, 0.010 mol) was dissolved in 100 mL of EtOH and hydrogenated for 1 h (0.45 L, 0.020 mol, $H_2$) at normal pressure with Pd on charcoal (5%, 0.7 g) as catalyst. After filtration and evaporation, the residue solidified upon treatment with $Et_2O$ to give 2.7 g (92%) of 26: mp 107–108 °C; $^1H$ NMR ($CDCl_3$/MeOD) $\delta$ 7.13 and 6.67 (AB, 2), 3.99 (s, 3), 3.9–1.7 (multiplets, 11), 1.13 (t, 3).

**(*S*)-3-Bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-hydroxy-6-methoxysalicylamide (27).** To a stirred solution of compound 26 (1.48 g, 5.0 mmol) in 10 mL of dioxane and 5 mL of acetic acid a solution of bromine (0.29 mL, 5.5 mmol) in 10 mL of dioxane was added dropwise at 0 °C. The reaction mixture was stirred for 0.5 h at room temperature and then partitioned between 400 mL of water and 75 mL of $Et_2O$. The aqueous layer was made alkaline with $NH_3$ to pH 9.5 and extracted with four 100-mL portions of $Et_2O$. The combined organic phases were dried ($Na_2SO_4$), concentrated to 200 mL, and treated with HCl/$Et_2O$ to give 1.22 g crude hydrochloride. Recrystallization from acetone/MeOH afforded 0.96 g (47%): mp 189–190 °C; $^{13}C$ NMR (base, $CDCl_3$) $\delta$ 169.3, 152.9, 145.9, 141.4, 125.4, 108.6, 106.5; $^1H$ NMR (base, $CDCl_3$) $\delta$ 10.2 (br), 9.2 (br), 7.40 (s, 1), 3.94 (s, 3), 3.9–1.4 (multiplets, 11), 1.15 (t, 3).

**Pharmacology. [$^3$H]Spiperone Binding.** The assays were performed essentially as described earlier.[6,30] Rats were killed by decapitation, and their striata were 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 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 $\mu$M) was used for the determination of nonspecific binding. The radioactivity of the filters was determined by scintillation spectroscopy. The $IC_{50}$ values were calculated by using log-logit regression analysis.

**[$^3$H]Raclopride Binding.** The assays were done in analogy with the above protocol for [$^3$H]spiperone as detailed previously.[30] The $K_i$ values were calculated by using nonlinear-regression analysis (Ligand[41]) according to Cheng and Prusoff.[42]

**Apomorphine-Induced Behavior.** Male Sprague–Dawley rats (270–325 g) were used. The behavior was scored 5, 20, 40, and 60 min after injection of apomorphine hydrochloride (1 mg/kg), given subcutaneously into the neck. The scoring was performed as described previously.[6] 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 refer to the calculated doses that reduce the scores of apomorphine-induced stereotypies by 50% over the total observation period of 60 min. The $ED_{50}$'s for hyperactivity refer to the calculated doses that reduce the scores of hyperactivity by 50% over the observation period of 60 min of that of the apomorphine control. The $ED_{50}$ values (based on at least six dose levels with six to eight animals per dose level) for stereotypies and hyperactivity have been calculated by regression analysis using Fieller's theorem for calculation of the 95% confidence limit.[43] The $ED_{50}$ value of the response has been defined as the midpoint between the mean of the apomorphine control group and the mean of the saline control group.

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**Apomorphine-Induced Hypothermia.** The method has recently been described.[32] Male Sprague–Dawley rats (200–250 g) were used and were housed at 21 ± 0.5 °C. Groups of rats ($n$ = 6) were pretreated ip with saline or test compounds 60 min prior to the injection of apomorphine hydrochloride (1 mg/kg sc). Core temperature was measured by a rectal probe. The temperature was recorded before administration of the test compounds as well as before apomorphine and 15 min after apomorphine. The means of the temperature variations were calculated and the changes were expressed in percent of respective control mean value. The $ED_{50}$ value, calculated by regression analysis using Fieller's theorem for calculation of the 95% confidence limit,[43] refers to the dose level which blocks the hypothermic effect of apomorphine by 50%.

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

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**Registry No.** 1, 101460-26-8; 2, 101460-23-5; 3, 101460-21-3; 4, 101460-27-9; 5, 101460-30-4; 6, 125198-20-1; 7, 125198-32-5; 7·HCl, 101460-63-3; 8, 101460-53-1; 9, 101460-64-4; 10, 101460-41-7; 10·$MeSO_3H$, 101460-43-9; 11, 101460-66-6; 11·$MeSO_3H$, 125198-22-3; 12, 101460-44-0; 12·$MeSO_3H$, 101460-46-2; 13, 101460-48-4; 13-tartrate, 125198-23-4; 14, 125198-24-5; 14·$MeSO_3H$, 125198-25-6; 15, 101460-51-9; 15·$MeSO_3H$, 125198-26-7; 16, 98601-22-0; 17, 101460-42-8; 18, 102727-69-5; 19, 101460-45-1; 19·$MeSO_3H$, 101460-47-3; 20, 101460-50-8; 21, 125198-27-8; 22, 101460-52-0; 23, 101460-34-8; 24, 101460-57-5; 24·2HCl, 101460-59-7; 25, 101460-68-8; 26, 125198-28-9; 27, 101460-71-3; 27·HCl, 125198-29-0; 2,3,6-trimethoxybenzoic acid, 60241-74-9; 3-bromo-2,5,6-trimethoxybenzoic acid, 101460-22-4; 3-chloro-2,5,6-trimethoxybenzoic acid, 101460-24-6; 1,2,4-trimethoxybenzene, 135-77-3; 2,4,5-trimethoxyacetophenone, 1818-28-6; 1-ethyl-2,4,5-trimethoxybenzene, 125198-30-3; 3-ethyl-2,5,6-trimethoxybenzoic acid, 101460-28-0; 2,4,5-trimethoxypropiophenone, 3904-18-5; 1-propyl-2,4,5-trimethoxybenzene, 6906-65-6; 3-propyl-2,5,6-trimethoxybenzoic acid, 101460-31-5; 3-bromo-5,6-dimethoxy-*N*-*tert*-butoxyanthranilic acid, 125198-31-4; 3-bromo-5,6-dimethoxyanthranilic acid, 101460-35-9; 3,6-bis(benzyloxy)-2-methoxybenzoic acid, 101460-69-9; (*S*)-2-(aminomethyl)-1-ethylpyrrolidine, 22795-99-9; 3-bromo-2,5,6-trimethoxybenzoyl chloride, 101460-33-7; (*S*)-1-trityl-2-(aminomethyl)pyrrolidine, 98598-84-6; (*S*)-3-bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxy-5-nitrosalicylamide, 101460-58-6.

# Design, Synthesis, and 5-Lipoxygenase-Inhibiting Properties of 1-Thio-Substituted Butadienes

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The synthesis of novel 1-thio-substituted butadienes, designed as mechanism-based 5-lipoxygenase inhibitors, is described. The structure of these compounds closely resembles a proposed high-energy intermediate during the lipoxygenation of arachidonic acid. They demonstrate 5-lipoxygenase inhibition in vitro and in vivo. The most potent compound is **15a** with an $IC_{50}$ of 1.8 $\mu$M in vitro. $LTC_4$ release was inhibited by 80% after intraperitoneal administration of **15c** at a dose of 2 mg/kg.

There is strong evidence for the role of leukotrienes as mediators of asthma and inflammation.[1,2] As a key role in their synthesis is played by 5-lipoxygenase,[3] much effort has been directed toward the identification and development of inhibitors of this enzyme. Numerous compounds of diverse chemical structure have been described, many of which have been found to possess inadequate potency or unacceptable toxicity.[4] This latter effect could be explained by a lack of specificity, perhaps resulting from their antioxidant activity[5] or structural features leading to blood cell toxicity, specifically methemoglobinemia.[6] Second generation inhibitors of 5-lipoxygenase will therefore require both better selectivity and enhanced potency. As a rational approach we considered the possibility of designing transition-state analogue[7] inhibitors of 5-lipoxygenase.

Central to the transition-state analogue theory is the hypothesis that, during the course of an enzyme-catalyzed reaction, intermediates and transition states are formed that are bound more tightly by the enzyme than either substrate or product.[7] Chemical structures mimicking the transition state or a high-energy intermediate might reasonably be expected to act as selective and potent inhibitors of the enzyme.

Since the enzymatic conversion of arachidonic acid by 5-lipoxygenase proceeds through a cascade of complex reaction steps, it raises the question whether the transition-state analogue concept, originally formulated by

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