Inhibitors of Neuronal Monoamine Uptake. 2. Selective Inhibition of 5-Hydroxytryptamine Uptake by α-Amino Acid Esters of Phenethyl Alcohols

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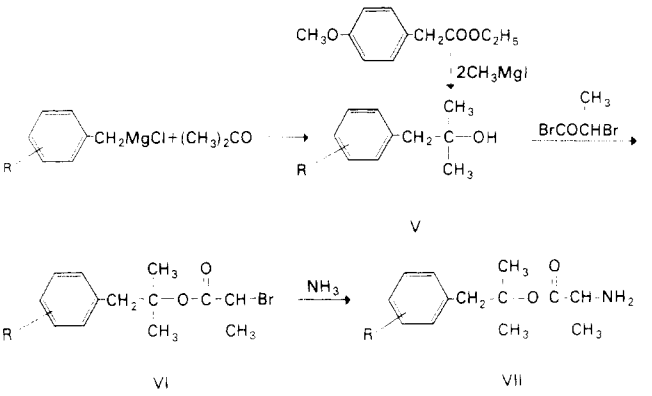
A series of α-amino acid esters of substituted phenethyl alcohols was prepared and tested as inhibitors of the neuronal reuptake of noradrenaline and 5-hydroxytryptamine. Some of the compounds are potent and very selective in blocking the 5-hydroxytryptamine uptake, as evidenced by biochemical data and behavioral tests. The most promising agent, alaproclate [2-(4-chlorophenyl)-1,1-dimethylethyl 2-aminopropanoate hydrochloride (1, IV)], was selected for further studies as a potential antidepressant agent. A discussion on structure–activity relationships (SAR) is given. In an attempt to explain the selective action on the mechanism of 5-hydroxytryptamine uptake by the new inhibitors, their structures are compared with those of the two neurotransmitters. From the tentative pharmacophore and conformations of transmitter (5-HT) and inhibitor (alaproclate) derived from SAR, a hypothetic carrier site for 5-HT uptake is deduced in terms of geometry and electronic properties.

The introduction of imipramine (I)[1] as an agent for the treatment of depressed patients about 2 decades ago marked the start of modern pharmacotherapy of depression. Since then imipramine has become a model for numerous agents, and it is the parent compound of the class of tricyclic antidepressant drugs. These drugs are known to inhibit the presynaptic uptake of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in the central nervous system (CNS).[2a] These effects are believed to account for the clinical efficacy of the tricyclic antidepressants.[2a] However, these agents also show undesirable pharmacological properties such as anticholinergic and cardiotoxic effects.[2b] In our laboratory we have previously modified the tricyclic system in several ways in an attempt to separate the antidepressant properties from the side effects.[3,4]

It has been suggested[5-7] that serotonin (5-HT) is involved in the regulation of mood, whereas noradrenaline is responsible for the psychic and motoric activity. Most tricyclics are much stronger inhibitors of the uptake of NA than of the uptake of 5-HT. The potential relationship between inhibition of the neuronal uptake of 5-HT and antidepressant effect has initiated a search for selective inhibitors of the 5-HT uptake in several laboratories.[8-14]

In the first paper[14] in this series we reported a new class of selective inhibitors of the neuronal uptake of 5-HT consisting of α-amino acid amides of phenethylamines (e.g., compound II). The way in which the effect of compound II was related to the time after administration in mice showed, however, a dual effect which probably was due to formation of chlorphentermine (III). The latter compound is about equally active on the 5-HT and NA uptake.[15] In order to avoid the formation of chlorphentermine under in vivo conditions, which abolishes the selectivity for the 5-HT uptake, we have now synthesized the corresponding α-amino acid esters of phenethyl alcohols (e.g., compound IV). In the present report we describe the synthesis and inhibitory activities on the accumulation of 5-HT and NA in mouse brain slices of this series of compounds. We have also studied the interaction of these agents with the behavioral effects of 5-hydroxytryptophan (5-HTP). For some of the compounds, anticholinergic (mydriatic) effects and acute toxicities have been determined.

Scheme I



Chemistry. Compounds 1 and 4–13 reported in Table I were prepared by the reaction sequence outlined in Scheme I. Tertiary alcohols of type V were previously prepared by Ferrari[16] by reacting the Grignard reagents of benzyl halides with acetone. This method was convenient for most tertiary alcohols in our program, but the yields varied somewhat (Table II). In those cases where a low yield of alcohol was obtained, the main products were dimers (VIII) formed from the magnesium halides. In analogy with a method of Vollmar and Dunn[17] for the preparation of tertiary alkyl esters of alanine, we successfully prepared the 2-bromopropanoates of the aryl-substituted tertiary alcohols VI. In this method dimethylaniline was used as a catalytic base. Experiments

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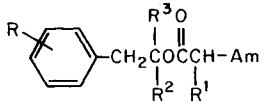
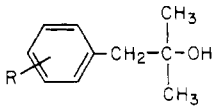
Table I. Substituted α-Amino Acid Esters of Phenethyl Alcohols



Compd	R	R¹	R²	R³	Am	Formula	Analyses	Mp, °C	Recrystn solvent	Mol wt
1	4-Cl	CH_3	CH_3	CH_3	NH_2	$C_{13}H_{18}ClNO_2 \cdot HCl$	C, H, Cl, N	123–125	$CHCl_3$–Et_2O	292.2
2	4-Cl	CH_3	CH_3	CH_3	NH_2	$C_{13}H_{18}ClNO_2 \cdot HCl$[a]	C, H, Cl, N	153–154	Me_2CO	292.2
3	4-Cl	CH_3	CH_3	CH_3	NH_2	$C_{13}H_{18}ClNO_2 \cdot HCl$[b]	C, H, Cl, N	153–154	Me_2CO	292.2
4	3-Cl	CH_3	CH_3	CH_3	NH_2	$C_{13}H_{18}ClNO_2 \cdot HCl$	C, H, Cl, N	117–118	Me_2CO	292.2
5	2-Cl	CH_3	CH_3	CH_3	NH_2	$C_{13}H_{18}ClNO_2 \cdot HCl \cdot 0.5H_2O$	H, Cl, N; C[c]	104–106	Me_2CO–EtOH	310.2
6	4-F	CH_3	CH_3	CH_3	NH_2	$C_{13}H_{18}FNO_2 \cdot HCl \cdot H_2O$	C, H, Cl, N	112–113	Me_2CO	293.7
7	4-Br	CH_3	CH_3	CH_3	NH_2	$C_{13}H_{18}BrNO_2 \cdot HBr$	C, H, Br, N	147–148	Me_2CO–*i*-PrOH	381.1
8	3,4-Cl_2	CH_3	CH_3	CH_3	NH_2	$C_{13}H_{17}Cl_2NO_2 \cdot HBr$	C, H, Br, Cl, N	135–136	$CHCl_3$–Et_2O	371.1
9	H	CH_3	CH_3	CH_3	NH_2	$C_{13}H_{19}NO_2 \cdot HCl$	C, H, Cl, N	132–133	Me_2CO	257.8
10	4-CH_3	CH_3	CH_3	CH_3	NH_2	$C_{14}H_{21}NO_2 \cdot HBr$	C, H, Br, N, O	154–155	Me_2CO	316.2
11	4-C_2H_5	CH_3	CH_3	CH_3	NH_2	$C_{15}H_{23}NO_2 \cdot HCl$	C, H, Cl	139–141	Me_2CO	285.8
12	4-OCH_3	CH_3	CH_3	CH_3	NH_2	$C_{14}H_{21}NO_3 \cdot HCl$	C, H, Cl, N, O	133–135	Me_2CO	287.8
13	3-CF_3	CH_3	CH_3	CH_3	NH_2	$C_{14}H_{18}F_3NO_2 \cdot HCl$	C, H, Cl, N	140–141	Me_2CO	325.8
14	4-Cl	H	CH_3	CH_3	NH_2	$C_{12}H_{16}ClNO_2 \cdot HCl$	C, H, Cl, N, O	147–148	$CHCl_3$–Et_2O	278.2
15	4-Cl	CH_3	H	CH_3	NH_2	$C_{12}H_{16}ClNO_2 \cdot HCl$[d]		132–133	Me_2CO	278.2
16	4-Cl	CH_3	H	CH_3	NH_2	$C_{12}H_{16}ClNO_2 \cdot HCl$[d]		167–170	Me_2CO	278.2
17	4-Cl	CH_3	H	H	NH_2	$C_{11}H_{14}ClNO_2 \cdot HCl$[e]	C, H, Cl, N	145–146	EtOH–Et_2O	264.1
18	4-Cl	CH_3	H	H	NH_2	$C_{11}H_{14}ClNO_2 \cdot HCl$[f]	C, H, Cl, N	145–146	Me_2CO–H_2O	264.1
19	H	CH_3	H	H	NH_2	$C_{11}H_{15}NO_2 \cdot HCl$	C, H	138–139	Me_2CO	229.7
20	4-OCH_3	CH_3	H	H	NH_2	$C_{12}H_{17}NO_3 \cdot HCl$[g]	C, H, N	132–133	Me_2CO	259.7
21	4-Cl	$-CH_2C_6H_5$	CH_3	CH_3	NH_2	$C_{19}H_{22}ClNO_2 \cdot HCl$	C, H, Cl, N	163–164	Me_2CO	368.3
22	4-Cl	$-CH(CH_3)_2$	CH_3	CH_3	NH_2	$C_{15}H_{22}ClNO_2 \cdot 0.6(COOH)_2$	H, N; C, Cl[h]	128–131	*i*-PrOH	337.8
23	H	$-CH(CH_3)_2$	CH_3	CH_3	NH_2	$C_{15}H_{23}NO_2 \cdot 0.6(COOH)_2$	C, H, N	134–135	*i*-PrOH	303.4
24	4-Cl	CH_3	C_6H_5	CH_3	NH_2	$C_{18}H_{20}ClNO_2 \cdot HCl \cdot H_2O$	C, H, Cl, N, O	129–130	EtOAc	372.3
25	4-Cl	CH_3	CH_3	CH_3	$N(CH_3)_2$	$C_{15}H_{22}ClNO_2 \cdot HCl$	C, H, Cl, N, O	109–110	EtOAc	320.3
26	4-Cl	CH_3	CH_3	CH_3	c-$C_5H_{10}N$	$C_{18}H_{26}ClNO_2 \cdot HCl$	H, Cl, N, O; C[i]	155–156	*i*-PrOH–MeOH	360.3
27	4-Cl	CH_3	CH_3	CH_3	c-C_5H_9N-4-CH_3	$C_{19}H_{28}ClNO_2 \cdot HCl$	C, H, Cl, N, O	141–142	EtOAc	374.4

[a] $[\alpha]^{20}_D$ +2.52° (1% base in EtOH). [b] $[\alpha]^{20}_D$ −2.53° (1% base in EtOH). [c] C: calcd, 51.8; found, 51.4. [d] $[\alpha]^{20}_D$ ±0° (1% base in EtOH). The mass spectrum was in agreement with the assigned structure. [e] $[\alpha]^{20}_D$ −13.7° (1% in H_2O). [f] $[\alpha]^{20}_D$ +13.4° (1% in H_2O). [g] $[\alpha]^{20}_D$ −11.0° (1% in H_2O). [h] C: calcd, 57.6; found, 58.1. Cl: calcd, 10.5; found, 10.1. [i] C: calcd, 61.0; found, 60.5.

Table II. Substituted 2-Methyl-1-phenyl-2-propanols



R	Yield,[a] %	R	Yield,[a] %
4-Cl	70	H	31
3-Cl	80	4-CH_3	13
2-Cl	74	4-C_2H_5	50
4-F	64	3-CF_3	73
4-Br	37	4-OMe	76[b]
3,4-Cl_2	57		

[a] Yields refer to the Grignard reactions from the substituted benzyl halides if not otherwise stated (Scheme I). [b] Yield refers to the Grignard reaction from the 4-methoxyphenylacetic ester (Scheme I).

with other bases in this reaction gave either a poor yield of the ester or none at all, a fact which may indicate that dimethylaniline takes part in the reaction in another way and not only as a hydrogen chloride acceptor. The bromo esters VI were finally transformed into the desired alanine esters VII by reaction with saturated ammonia in alcohol. Compound **14** was prepared analogously via the intermediate 2-(4-chlorophenyl)-1,1-dimethylethyl 2-bromoacetate. Compounds **2** and **3** are enantiomers, which were obtained by resolution of the racemic compound **1** by means of (+)- and (−)-tartaric acids.

The advantage of finding a direct process for esterification of the described alcohols V with amino acids is obvious. With such a method, enantiomeric compounds would be easily accessible by using commercially available enantiomers of alanine, for example. Such a method would also permit the preparation of labeled esters of amino acids directly from radioactive amino acids, e.g., [^{14}C]alanine. We tried to solve this problem mainly by applying methods used in peptide chemistry. A base-catalyzed esterification without a protective group on the primary amino function is excluded because of polypeptide formation. Consequently, an acidic process is preferable. As remarked in the literature[18] the disadvantage of tertiary butyl esters is that they decompose under relatively mild acidic treatment to the corresponding acid and alcohol. However, Roeske[19] succeeded in preparing the *tert*-butyl ester from isobutylene and alanine in the presence of concentrated sulfuric acid. Taschner[20] prepared the same compound by transesterification of *tert*-butyl acetate with alanine in the presence of a 60% perchloric acid solution. Rudinger and Pravda[21] have described a stereoselective esterification in which they reacted an *N*-carboxyanhydride of alanine with benzyl alcohol in an ether solution saturated with hydrogen chloride gas. Compounds **17**, **18**, and **20** in Table I were prepared in analogy with the *N*-carboxyanhydride method from primary alcohols IX and an optically pure alanine derivative. Likewise, compound **16** was prepared by the same method from a racemic secondary alcohol X. In the latter case one could expect some optical activity of the product in contrast to compound **15**, which was prepared via amination of the corresponding bromo ester. However, compound **16**, like **15**, was found not to rotate the plane of polarized light to any measurable extent, but the melting points of the two compounds differed appreciably.

When we used the corresponding tertiary alcohol V, the esterification failed and the only isolated products were crystalline alanyl chloride hydrochloride and the dehydrated product XI from the tertiary alcohol. This negative result was general for all applications in which we used acid-catalyzed methods on our tertiary alcohols or their corresponding dimethylstyrene derivatives XI and alanine with or without amino group protection. Under neutral or basic conditions no reaction at all was observed. Attempts to use *N*-carbobenzoxyalanine in the activated form, e.g., as the acid chloride, nitrobenzyl ester, or dicyclohexylcarbodiimide complex, also failed. Likewise, experiments with the tertiary halide XII and the silver or tetrabutylammonium salt of protected alanine were unsuccessful. In a previous publication[14] we described the stereoselective synthesis of the corresponding amides. The fact that the amides are easily prepared in contrast to the esters, although the sterical interactions are probably quite similar, indicates that the competing dehydration reaction of the tertiary alcohols dominates in acidic media. Under neutral or alkaline conditions, the high activation energy needed apparently obstructs the esterification. This interpretation is based on the fact that the tertiary esters are very stable toward alkaline hydrolysis. In addition, no aminolysis was observed even when ammonia was used under pressure and heating during the amination of the α-bromo esters.

A small number of analogous esters of other amino acids, compounds **21**–**23**, were prepared from the corresponding α-bromo esters via reduction of the azide derivatives. The remaining compounds in Table I (**19** and **24**–**27**) were prepared by minor variations of the methods described above.

Pharmacological Results. Structure–Activity Relationships. Inhibition of the Accumulation of 5-HT and NA. In Vitro Activity. Most of the compounds examined were much more potent in inhibiting the accumulation of 5-HT than that of NA (Table III). It is obvious from the results obtained that the design of the side chain is very important for the selectivity and also for the activity. Thus, the 1,1-dimethyl groups of the phenethyl radical ($R^2 = R^3 = CH_3$) probably impart a favorable conformation on the side chain, since the compounds without methyl groups ($R^2 = R^3 = H$, **17**–**20**) had much less activity in vitro than the corresponding compounds with the dimethyl groups (**1**–**3**, **9**, and **12**). One methyl group in that position ($R^3 = CH_3$) is, however, sufficient to give a compound with high and selective activity (**15**). In compound **16**, the presence of both methyl groups (R^1 and R^3) on asymmetric carbons seems to give an unfavorable configuration for activity on comparison with the racemic compound **15**. Substituting one methyl group (**1**) with a phenyl group (**24**) does not increase the potency or selectivity. The terminal methyl group (R^1) is also important for activity, since **1** was much more potent than **14**. Increasing the size of this substituent to isopropyl (**22**, **23**) or benzyl (**21**) strongly decreased the activity. In similarity with the corresponding amide derivatives studied previously,[14] the tertiary amine derivatives **25**–**27** were inactive in vitro. The primary amine is a necessary requirement for activity in the amide series,[14] and this apparently holds true also in the ester series.

The aromatic substituents are also of importance for the potency and to some extent for the selectivity. Thus, compounds with a chlorine atom at the 4 or 3 position (**1**, **4**) were more selective for the 5-HT accumulation than the compound with a 2-chlorine (**5**) or without any aromatic substituent (**9**). A 4-bromo (**7**) or 4-methyl (**10**) substituent was equivalent to a chlorine atom, whereas a 4-methoxy group (**12**) decreased the activity. The 3-trifluoromethyl- (**13**) and 3,4-dichloro- (**8**) substituted derivatives had activities similar to the other halogen-substituted compounds.

Table III. Inhibition of the Accumulation of [^{3}H]-(−)-Noradrenaline (NA) and [^{14}C]-5-Hydroxytryptamine (5-HT) in Mouse Brain Slices and Potentiation of the 5-Hydroxytryptophan (5-HTP) Syndrome in Mice

	Inhibn of amine accumulation[a]						
	In vitro, IC_{50}, μM		In vivo, ED_{50}, μmol/kg ip		Potentiation of 5-HTP,[b] ED_{50}, μmol/kg ip		
Compd	NA	5-HT	NA	5-HT	Head twitches	Tremor	Abduction
1	>34	0.7	>137	51	4 (2-7)	18 (14-22)	>34
2	>34	1.0	>137	68	16 (10-25)	15 (10-23)	13 (8-22)
3	>34	1.5	>137	>137	80 (62-101)	49 (36-67)	48 (33-72)
4	>34	1.0	>137	17	11 (2-16)	35 (27-44)	66 (37-117)
5	8.6	1.4	>137	>137	53 (35-80)	76 (43-136)	>86
6	>34	2.7	>136	102	29	32 (19-58)	37 (26-60)
7	>26	2.6	>105	66	14 (10-21)	>66	>66
8	>27	1.3	>106	40	22 (16-31)	>77	>77
9	23	2.7	>155	58	30 (19-48)	>98	>98
10	>32	1.3	>127	32	37 (16-85)	>79	>79
11	>34	1.0	>136	136			
12	>35	6.9	>139	>139	39 (26-57)	40 (28-54)	80 (41-156)
13	>31	1.5	>123	92	52 (31-85)	72 (44-116)	97 (46-203)
14	>36	9.0	>144	>144	>90	>90	>90
15	>36	1.8	>144	>144	>90	>90	>90
16	>36	>36	>144	>144			
17	>38	>38	>151	>151	>95	>95	>95
18	>38	23	>151	>151	>95	>95	>95
19	>44	8.7	>174	>174	>109	>109	>109
20	>39	>39	>154	>154	>96	>96	>96
21	>27	>27	>109	>109	>68	>68	>68
22	>30	>30	>118	>118	>74	>74	>74
23	>33	>33	>132	>132	>83	>83	>83
24	>27	1.6	>27	>27			
25	>31	>31	>125	>125	>78	>78	>78
26	>28	>28	>111	>111	>55	>55	>55
27	>27	27	>107	>107	>53	>53	>53
Chlorimipramine	0.9	0.09	142	20	8 (5-11)	24 (19-30)	43 (36-51)
Desipramine	1.3	10	17	>198	>170	>170	170
Zimelidine	25	1.7	>98	49	9 (5-13)	12 (8-16)	15 (10-26)

[a] Slices from the middle brain regions of the mice were incubated with 1×10^{-7} M [^{3}H]-(−)-NA and 1×10^{-7} M [^{14}C]-5-HT for 5 min in the presence of the test compounds or 0.5 h after their injection. The inhibition of the neuronal accumulation was determined in percent, and the IC_{50} and ED_{50} values were determined from log dose-response curves. [b] The test compounds were injected 1 h prior to 5-HTP, 90 mg/kg iv. The ED_{50} values are based on dose-response curves. 95% confidence limits in parentheses.

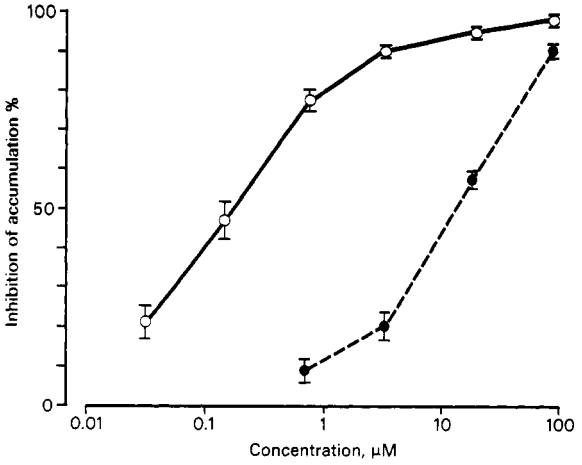


Figure 1. Inhibition by alaproclate (1) of the simultaneous accumulation of [^{14}C]-5-hydroxytryptamine (○) and [^{3}H]-(−)-noradrenaline (●) in the cell-free homogenate of rat hypothalamus. The concentration of the labeled amines was 5×10^{-8} M. The incubation time was 5 min.

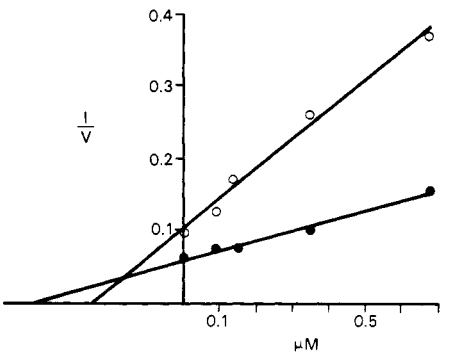


Figure 2. Dixon plot of the inhibition by alaproclate (1) of [^{14}C]-5-HT accumulation in the cell-free homogenate of rat hypothalamus. [^{14}C]-5-HT concentrations: 5×10^{-8} M (○), 2×10^{-7} M (●). The incubation time was 4 min; v = pmol of [^{14}C]-5-HT accumulated per 10 mg of original tissue per 4 min of incubation.

Compound 1, alaproclate (pINN) hydrochloride, exhibited the most potent and selective overall activity in the primary evaluation (Table III), and it was therefore chosen for further studies. The selectivity of the 5-HT uptake was verified by recording the inhibition of the accumulation of 5-HT and NA in synaptosomes of a cell-free homogenate of the rat hypothalamus (Figure 1). The IC_{50} for inhibition of the 5-HT accumulation was 1.4 $\times 10^{-7}$ M and that of the NA accumulation was 1.4×10^{-5} M, i.e., a selectivity 100 times in favor of the 5-HT accumulation. Experiments determining the release of [^{3}H]-NA and [^{3}H]-5-HT from mouse brain slices showed that alaproclate released NA and 5-HT at high concentrations (EC_{50} values 260 and 120 μM for NA and 5-HT release, respectively). It is possible that release of NA is involved in the apparent inhibition of the NA accumulation. Alaproclate is, on the other hand, a true inhibitor of the 5-HT accumulation. The compound inhibited competitively the 5-HT accumulation in a rat hypotha-

Table IV. Mydriatic Action and Acute Toxicity of Some of the New Inhibitors of the 5-HT Uptake (Mice)

Compd	Mydriasis,[a] PD_{200}, μmol/kg iv	Toxicity,[b] LD_{50} μmol/kg iv	μmol/kg ip	μmol/kg po
1	>137	185	684	3082
2	>205	205-342	514	
3	>205	240	548-684	
4	89	205	415	
8	108	148	674	
9	194	256	581-775	
10	111	253-316	601	
12	>139	209	1045	
Chlorimipramine	6	142	477	1790
Desipramine	63	116	330	1650

[a] PD_{200} refers to the dose which increases the pupil width by 200%. [b] LD_{50} based on four doses refers to the dose which kills 50% of the animals within 24 h.

lamus homogenate (Figure 2). The K_i value obtained from this experiment was 1.3×10^{-7} M.

In Vivo Activity. The inhibition of the amine accumulation in mouse brain slices after administration of the test compounds in vivo gave on the whole the same results as in the in vitro tests. The correlation between the in vitro and in vivo inhibition of the 5-HT accumulation was highly significant ($r_s = 0.80$, $n = 18$, $p < 0.01$) according to the Spearman rank test. The low potencies in vivo of the (−) enantiomer of alaproclate (3) and of compound 14 may be explained by a stereospecific enzymatic hydrolysis of the ester linkage. The poor effect of the 2-chloro derivative 5 in vivo may be due to a rapid aromatic 4-hydroxylation of this compound.

Alaproclate hydrochloride was tested for its ability to inhibit the accumulation of dopamine (DA) in mouse striatal slices after ip administration. It had no significant effect at 40 mg/kg.

Potentiation of the 5-Hydroxytryptophan Syndrome in Mice. Compounds inhibiting the membrane 5-HT uptake potentiate the syndrome (head twitches, tremor, and abduction of hind legs) produced by 5-hydroxytryptophan (5-HTP) in mice. As shown in Table III compounds 1, 4, and 7 were very active in potentiating the head twitches. The correlation between inhibition of the 5-HT accumulation after administration in vivo and potentiation of the head twitches was significant ($r_s = 0.70$, $n = 11$, $p < 0.05$; Spearman rank test). Thus it seems likely that potentiation of 5-HTP is mainly due to 5-HT uptake inhibition. Tremor and abduction of hind legs were much less potentiated and most of the compounds had slight or no effect at the highest dose tested. This indicates that some of these compounds including alaproclate (1) may have a differential action on the 5-HT systems of the brain.

Mydriatic Effect. The mydriatic effect of the new compounds (Table IV) was generally weak compared with chlorimipramine and desipramine which indicates that the new compounds have weak peripheral anticholinergic effects.

Toxicity. The acute toxicity of the most potent uptake inhibitors was generally lower than that of the reference compounds (Table IV). The ratio between the effective dose (ED_{50}, ip) for uptake inhibition of 5-HT (Table III) and the lethal dose (LD_{50}, ip) was of the order of 10 for some of the most potent compounds (1, 2, 4, and 8–10).

Discussion

Structural Comparison of Alaproclate (1) with 5-HT and NA. It can be supposed that the biogenic amines are attached to active sites for their membrane transport by electrostatic and dispersion forces involving the cationic, protonated amino groups and the substituted benzene rings, respectively. In addition, it is possible that other forces contribute to stabilize a transmitter–carrier complex on the outside of the membrane, e.g., by hydrogen bonding between the hydroxyl groups of the biogenic amines and corresponding functions in the carrier structure. In the case of 5-HT a localized charge transfer[22-24] from the nucleophilic 2 position in the indole nucleus to an electron acceptor in a membrane biopolymer may be involved in the formation of the complex. It is usually assumed that the competitive inhibitors of the bioamine uptake are attracted partly by the same type of forces to the same carrier site as the respective neurotransmitter. On this basis hypotheses have been presented to explain the inhibition of NA uptake by superimposition of proposed conformations of NA and potent inhibitors of the tricyclic type.[25,26] However, since some potent inhibitors with rigid structures are not able to adapt to the same receptor,[26,27] other explanations have been proposed. The possibility that the NA carrier exists in two different conformational states has been considered to explain these observations.[27] Alternatively, polycyclic inhibitors might be attracted to biopolymer structures adjacent to those which accommodate NA and phenethylamine inhibitors at the carrier site.[28]

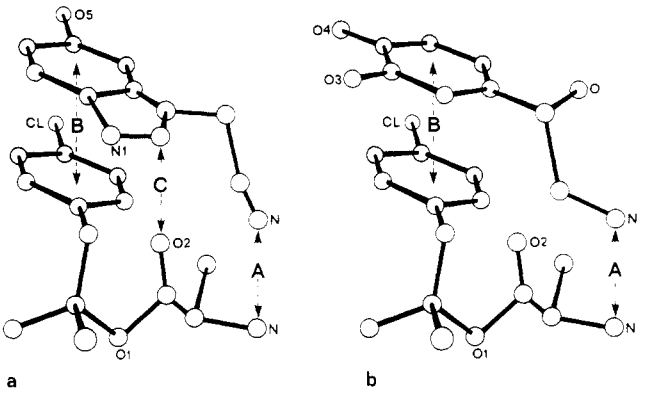


Figure 3. A perspective view of superimposed molecular models of (*R*)-alaproclate (2), 5-HT (a) and NA (b), respectively. These conformations of neurotransmitters and alaproclate (1) are postulated to be involved in the interaction with the uptake sites. The high affinity of alaproclate (1) to the active site for 5-HT uptake is explained by the presence of a pharmacophore (A–C) of similar electronic properties and geometry to those exhibited by 5-HT. The lack of affinity of alaproclate (1) to the NA site, in spite of the possibility of a two-point overlap (A, B), is explained by the presence of bulky substituents (e.g., Cl).

The competitive nature of the potent inhibition of 5-HT accumulation indicates that alaproclate has a rather high affinity to the 5-HT carrier in the neuron membrane. The poor effect on the NA accumulation shows, on the other hand, that this compound has a low affinity to the NA carrier. As alaproclate is a monocyclic compound, like the phenethylamines, and unrelated to the tricyclic drugs, we assume that it competes for the same uptake sites as 5-HT and NA, respectively. Therefore, a comparison of a Dreiding molecular model of alaproclate with those of 5-HT and NA may give some information on the reason for the selectivity (Figure 3).

Correlation of chemical structure and pharmacological effect in the present series of α-amino acid esters, the previously described series of α-amino acid amides,[14] and a few analogous compounds (to be published) with and without a polarizable group in the position of the carbonyl function of alaproclate reveals the importance of three

molecular features in these agents to attain a potent inhibition of 5-HT uptake. In the transmitter molecule, 5-HT, three corresponding molecular elements are also present. Thus, alaproclate and 5-HT have the following structural elements in common: A, a terminal primary amino group, positively charged at physiological pH; B, an aromatic benzene nucleus influenced by substituents; and C, an electron-rich or nucleophilic position capable of a localized charge transfer (ester carbonyl oxygen[29] in alaproclate and an indole 2-carbon in 5-HT,[22] respectively). These elements (A–C) define a pharmacophore postulated to account for affinity to an active site on the carrier effecting the transport of 5-HT through the neuronal membrane.

Because of steric interactions between the 4-chlorophenyl and the two geminal methyl groups, this part of the molecule of alaproclate ought to be rather rigid. By mutual adjustment of the side chains of the inhibitor and transmitter in molecular models, it is then possible to superimpose the pharmacophore parts of alaproclate and 5-HT as shown in Figure 3. These conformations are probably close to those engaging the carrier. A recent conformational study of the *R* enantiomer of alaproclate, compound **2**, by a single-crystal x-ray determination and energy calculations gives support for this supposition.[30] It is interesting to note that this conformation of 5-HT is close to a gauche form reported for the crystal structure of serotonin picrate, where stacking interactions of a charge-transfer type between the indole and picric acid nuclei were indicated.[31] It appears therefore possible that the favored conformation of 5-HT for binding to the uptake site is the gauche form, in which the side chain is approximately perpendicular to the indole plane and the terminal nitrogen is bent toward the 2 position of the indole in a staggered conformation. This hypothesis is supported by other classes of compounds which also inhibit selectively the uptake of 5-HT, e.g., 3-(4-bromophenyl)-*N*,*N*-dimethyl-3-(3-pyridyl)allylamine, zimelidine (H 102/09),[11] norzimelidine (A 24356),[11] and *dl*-8-chloro-11-*anti*-aminobenzo[*b*]bicyclo[3.3.1]nona-3,6a(10a)-diene hydrochloride (Org 6582).[12] They all structurally agree better with a gauche than with an anti form of 5-HT. Since the interaction with the postsynaptic 5-HT receptors seems to be poor for these compounds, the favored conformation of 5-HT for interaction with these receptors might be different, presumably an anti form.

In view of the preceding discussion, it would be tempting to explain the lack of inhibition of the NA uptake with alaproclate by comparison of the favored conformation of the latter and a gauche form of NA. In this case, no superimposition of aromatic nuclei and amino groups is possible due to too short a distance between these parts in the molecule of NA. However, as the unsubstituted compound **9** and the 2-substituted analogue **5** are moderate to good inhibitors of the NA uptake, it seems more likely that it is the extended anti conformation of NA that engages its carrier site. This conformation of NA was also inferred by Horn and Snyder from studies on the inhibition of uptake achieved by rigid amphetamine analogues.[26,32] The conformation of NA, shown in Figure 3, with a fully extended side chain and the amino group above the plane of the ring, is supported by x-ray crystallography[33] and molecular orbital calculations.[34] A fairly good overlap is possible when comparing this conformation of NA with the favored conformation of alaproclate as shown in Figure 3. It is suggested that the 3- and 4-substituted compounds, like **4** and alaproclate, but in contrast to the unsubstituted **9**, become too bulky to be accepted by the carrier for the NA uptake. As a result these agents do not inhibit NA uptake. It is also evident from Figure 3 that a 2-substituent, as in **5**, can be accommodated on the carrier site at the same place as that where the 3-hydroxyl group or the hydroxy-substituted side-chain carbon of NA engages this carrier, thereby explaining the inhibiting potency of compounds with this substituent pattern on the NA uptake.

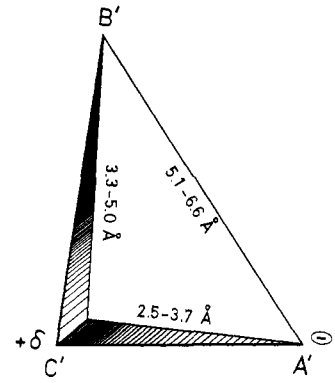


Figure 4. Hypothetic geometry and electronic nature of the carrier site for neuronal 5-HT uptake: A', center of negative charge capable of ionic binding; B', center of flat area (aromatic nucleus) capable of van der Waals interactions; and C', center of partial positive charge capable of localized charge transfer.

Table V. Distances between Postulated Pharmacophore Elements (A–C) in 5-HT and Alaproclate (1) in Two Different Conformations of Each Molecule

Conformation	Distance, Å		
	A–B	A–C	B–C
5-HT[a]	5.4	3.1	3.4
5-HT[b]	5.1	3.7	3.3
Alaproclate[a]	5.7	3.0	3.5
Alaproclate[c]	6.6	2.7	5.0

[a] Conformation shown in Figure 3a (distances measured on superimposed Dreiding molecular models of 5-HT and alaproclate). [b] Conformation in the crystal structure of serotonin picrate[31] (distances calculated from the x-ray data). [c] Conformation in the crystal structure of (*R*)-alaproclate hydrochloride[30] (distances calculated from the x-ray data).

A Hypothetic Carrier Site Model for Neuronal Uptake of 5-HT. From the postulated conformations of agonist (5-HT) and antagonist [alaproclate (1)], in which the pharmacophore parts A–C can be superimposed, the geometry and electronic properties of the complementary carrier site A'–C' can be deduced. This hypothetical model for the active site on the carrier effecting the transmembrane passage of 5-HT is depicted in Figure 4.

The distances between the pharmacophore groups A–C in the matching models of 5-HT and alaproclate shown in Figure 3a vary not more than 0.3 Å, as evident from the data presented in Table V. However, the wider intervals (≤1.7 Å) of the distances in the carrier site model (Figure 4) are chosen so as to allow for the accommodation of conformations known to exist for 5-HT[31] and (*R*)-alaproclate[30] in the crystal state (Table V).

The conformation in the crystal state of another selective inhibitor of 5-HT uptake, zimelidine, was recently reported.[35] In this molecule a pharmacophore is discernible in which A is a terminal amino group, B is a substituted benzene ring, and C is an electron-rich allylic double bond. In this conformation of zimelidine the pharmacophore has the electronic properties and triangular dimensions satisfying the carrier site model presented in Figure 4. A similar pharmacophore can be identified also in other

selective inhibitors of 5-HT uptake, e.g., Org 6582[12] and fluoxetine (Lilly 110140).[8] In the rigid compound Org 6582 the distances between the pharmacophore elements fall within the ranges shown in Figure 4. In a molecular model of the flexible fluoxetine molecule it is possible to accommodate the amino group (A), the unsubstituted benzene ring (B), and the ether oxygen atom (C) on the same carrier site model of Figure 4.

Experimental Section

Chemistry. Melting points were determined on a capillary apparatus (Tottoli-Büchi) and are uncorrected. Refractory indices were measured on a Zeiss (Jena) refractometer. Mass spectra were recorded at 70 eV on a LKB 2091 mass spectrometer. The structures of all compounds were confirmed by their NMR spectra which were run on a Varian T-60 instrument. Microanalyses were performed by A. Bernhardt Laboratorium, Elbach, Germany, and are within ±0.4% of the calculated values unless otherwise stated. Thin-layer chromatography was performed on precoated Merck silica gel F_{254} plates.

2-(4-Chlorophenyl)-1,1-dimethylethyl 2-Aminopropanoate Hydrochloride (1). A solution of 4-chlorobenzyl chloride (165 g, 1.02 mol) in ether (500 mL) was added dropwise during 1 h to magnesium (24.3 g, 1.00 mol) in a three-necked flask equipped with a stirrer and reflux condenser. The spontaneous reflux was allowed to continue for another hour and the solution was cooled. Acetone (60.0 g, 1.30 mol) was added dropwise and the solution heated to reflux for 3 h. After cooling, the reaction mixture was poured onto a mixture of ice (500 mL) and concentrated hydrochloric acid (92 mL). The phases were separated. The organic phase was washed with water, dried (Na_2SO_4), and evaporated in vacuo. The residual oil was fractionated affording 1-(4-chlorophenyl)-2-methyl-2-propanol[16] [bp 123–124 °C [1.6 kPa (12 mmHg)]; n^{25}_D 1.5300; 128.5 g, 70% yield].

The substituted propanol (10.0 g, 54.2 mmol) was mixed with dimethylaniline (6.7 g, 55.3 mmol) and ether (25 mL). The solution was cooled and 2-bromopropionyl bromide (11.6 g, 53.7 mmol) was added dropwise during 40 min. Stirring at room temperature for 4 h gave a crystalline precipitate of dimethylaniline hydrochloride. Water (25 mL) was added to the reaction mixture. The phases were separated. The organic phase was washed with 5% sulfuric acid, saturated sodium hydrogen carbonate solution, and water. The ether solution was dried (Na_2SO_4) and evaporated in vacuo. The residual oil was fractionated affording 2-(4-chlorophenyl)-1,1-dimethylethyl 2-bromopropanoate [bp 105–115 °C [0.02 kPa (0.15 mmHg)]; 11.6 g, 60% yield].

The bromo ester (7.5 g, 23.5 mmol) was dissolved in ethanol (200 mL) and the solution was cooled to 0 °C, whereupon it was saturated with ammonia (4 h). Stirring was continued at room temperature for 24 h. The solvent was removed under vacuum. The residual oil was dissolved in ether (250 mL) and the solution was extracted with 0.5 N hydrochloric acid. The acidic phase was made alkaline by the addition of concentrated ammonia. The alkaline phase was extracted twice with ether. The organic layer was dried (Na_2SO_4) and the solvent evaporated. From the basic oily residue the hydrochloride was prepared. Recrystallization from chloroform–ether afforded 2-(4-chlorophenyl)-1,1-dimethylethyl 2-aminopropanoate hydrochloride (mp 123–124 °C; 5.1 g, 70% yield). Recrystallization from acetone–water yielded the stable monohydrate, mp 140–144 °C. Anal. ($C_{13}H_{18}ClNO_2 \cdot HCl \cdot H_2O$) C, H, Cl, N, O.

(+)-2-(4-Chlorophenyl)-1,1-dimethylethyl 2-Aminopropanoate Hydrochloride (2). The racemic compound 1 (as base) (20.0 g, 78.4 mmol) was dissolved in ethanol (200 mL) and heated to 60 °C. A solution of D(−)-tartaric acid (9.40 g, 62.7 mmol) in ethanol (200 mL) was added slowly until crystals began to form. Stirring was continued at room temperature for 30 min whereupon the rest of the solution was added (30 min). The precipitate was filtered off and recrystallized from boiling ethanol containing a few drops of water until a constant melting point and optical rotation were obtained (four times). By insulating the vessel the hot solution attained room temperature slowly to favor the crystallization process. The tartaric acid salt (5.50 g; mp 173.5–174.5 °C) was transformed to the base, $[\alpha]^{20}_D$ +2.52° (1% in absolute ethanol). From the base the hydrochloride was prepared and recrystallized from acetone: yield, 2.85 g.

(−)-2-(4-Chlorophenyl)-1,1-dimethylethyl 2-Aminopropanoate Hydrochloride (3). Mother liquors from the preparation of the enantiomeric compound 2 were pooled and made alkaline, and a crude base (13.0 g, 51.9 mmol) was obtained by extraction. The resolution then proceeded in analogy with the method for compound 2 using L(+)-tartaric acid (6.60 g, 44.0 mmol). After five recrystallizations from absolute ethanol the tartaric acid salt (5.32 g) melted at 173.5–174.5 °C. The base had a specific rotation of $[\alpha]^{20}_D$ −2.53° (1% in absolute ethanol). The hydrochloride was prepared and recrystallized from acetone: yield, 2.53 g.

Compounds 4–13 (Table I) were prepared in analogy with the method described for compound 1 via the appropriately substituted 2-methyl-1-phenyl-2-propanol (Table II).

Compound 14 was prepared in analogy with the method described for compound 1 using bromoacetyl bromide instead of bromopropionyl bromide.

2-(4-Chlorophenyl)-1-methylethyl 2-Aminopropanoate Hydrochloride (15). This compound was prepared via 4-chlorophenyl-2-propanone, obtained according to the method of Counsell et al.[36] for the 3-chloro analogue, as follows. 4-Chlorobenzyl cyanide (15.2 g, 100 mmol) and ethyl acetate (13.2 g, 150 mmol) were mixed in a dropping funnel and added dropwise to a boiling solution of sodium (3.1 g, 130 mmol) in ethanol (45 mL). The reaction mixture was refluxed for 8 h, cooled, and poured into ice water (400 mL). The aqueous phase was washed twice with ether and acidified with concentrated acetic acid to pH 4.8. The product was isolated by extraction with ether. After drying with anhydrous sodium sulfate and removal of the solvent in vacuo, yellow needles were obtained. Two recrystallizations from ethanol gave a yield of 14.3 g (74%) of α-acetyl-4-chlorobenzyl cyanide. A mixture of this compound (7.8 g, 40 mmol), hydrochloric acid (14 mL), and acetic acid (30 mL) was refluxed for 24 h. The mixture was cooled. Water (100 mL) was added and the mixture was extracted with benzene. The benzene solution was washed with 10% sodium hydroxide solution and water. After drying with anhydrous sodium sulfate the solvent was removed. The residual oil (6.1 g, 91% yield) consisted of 4-chlorophenyl-2-propanone as shown by the NMR spectra. A solution of this compound (6.0 g, 36 mmol) in ethanol (60 mL) was cooled in an ice bath. Sodium borohydride (2.39 g, 72 mmol) was added in portions and the mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo. The residue was treated twice with 50 mL of ether and filtered. A brown oily product was obtained when the filtrate was evaporated. The oil was purified by distillation affording 1-(4-chlorophenyl)-2-propanol: bp 90–91 °C [0.03 kPa (0.20 mmHg)]; yield 3.8 g (63%). From the alcohol, compound 15 was prepared in the usual way (cf. compound 1).

(+)-2-(4-Chlorophenyl)-1-methylethyl (−)-2-Aminopropanoate Hydrochloride (16). A stream of phosgene was passed into a suspension of L-alanine (8.9 g, 10.0 mmol) in dry tetrahydrofuran (200 mL). After 3 h a clear solution was obtained. A stream of dry air was led through the cooled solution for 1 h. The solvent was removed in vacuo on a water bath at 30 °C. The white crystalline product was mixed with ether (150 mL) and stirred at room temperature for 30 min. The ether was distilled off under reduced pressure. The residue was dissolved in boiling ether (500 mL), ligroine (200 mL) was added, and, after standing at 0 °C overnight, a white precipitate was obtained. The crystals of L-4-methyl-2,5-oxazolidinedione[20] were filtered off, washed with ether, and dried: yield, 8.6 g (75%); mp 84–89 °C dec.

A suspension of L-4-methyl-2,5-oxazolidinedione (0.30 g, 2.00 mmol) in a 5 N ethereal hydrogen chloride solution (5 mL) was cooled on an ice bath. 1-(4-Chlorophenyl)-2-propanol (0.8 g, 4.69 mmol; see compound 15) was diluted with 3 mL of ether and added dropwise for 5 min. A clear solution was obtained and carbon dioxide was evolved. After slow stirring for 16 h the reaction mixture was evaporated under reduced pressure. The residual oil was treated with ether until the desired white crystalline hydrochloride was obtained. The compound was filtered off, washed on the filter with ether, dried, and recrystallized from acetone: yield, 250 mg (33%).

(−)-2-(4-Chlorophenyl)ethyl 2-Aminopropanoate Hydrochloride (17). This compound was prepared from 2-(4-

chlorophenyl)ethanol and L-4-methyl-2,5-oxazolidinedione in analogy with the method described for compound **16**.

(+)-2-(4-Chlorophenyl)ethyl 2-Aminopropanoate Hydrochloride (18). This compound was prepared analogously to compound **17** using D-4-methyl-2,5-oxazolidinedione (prepared as described for the L isomer but using D-alanine; see compound **16**).

2-Phenylethyl 2-Aminopropanoate Hydrochloride (19). This compound was prepared from 2-phenylethanol in the usual way (cf. compound **1**).

(−)-2-(4-Methoxyphenyl)ethyl 2-Aminopropanoate Hydrochloride (20). This compound was prepared from 2-(4-methoxyphenyl)ethanol and L-4-methyl-2,5-oxazolidinedione in analogy with the method described for compound **16**.

2-(4-Chlorophenyl)-1,1-dimethylethyl 2-Amino-3-phenylpropanoate Hydrochloride (21). 1-(4-Chlorophenyl)-2-methyl-2-propanol (5.54 g, 30.0 mmol; see compound **1**) was mixed with dimethylaniline (3.64 g, 30.0 mmol) and dichloromethane (15 mL). The solution was cooled and 2-bromo-3-phenylpropanoic acid bromide (prepared in analogy with a method of Cason et al.[37]) (13.1 g, 45.0 mmol) was added dropwise during 25 min. After stirring at room temperature for 22 h, dilute sodium hydroxide solution was added. The phases were separated and the organic layer was washed with water, 5% sulfuric acid to remove dimethylaniline (ion-pair formation keeps the desired amine in the dichloromethane phase), water, and 0.5 N sodium hydrogen carbonate solution. Drying over anhydrous sodium sulfate and evaporation of the solvent afforded a crude oil which was chromatographed on a silica gel column with chloroform as eluent. After working up the desired fractions, the 2-(4-chlorophenyl)-1,1-dimethylethyl 2-bromo-3-phenylpropanoate was obtained as a colorless oil (5.14 g, 44%).

The bromo ester (5.14 g, 13.0 mmol) was dissolved in methanol (135 mL) and a solution of sodium azide (1.27 g, 19.5 mmol) in water (8.5 mL) was added. The solution was transferred to a steel autoclave and heated at 90 °C for 16 h. After cooling, the reaction mixture was evaporated. The residue was distributed between water and ether. After separation the aqueous phase was extracted with ether. The combined ether solutions were dried and evaporated, yielding 2-(4-chlorophenyl)-1,1-dimethylethyl 2-azido-3-phenylpropanoate as a colorless oil (4.10 g, 88%).

The azido ester (4.10 g, 11.4 mmol) was dissolved in methanol (15 mL), palladium on charcoal (5%, 35 mg) was added, and a slow stream of hydrogen gas was led through the stirred mixture during 60 h. The reaction mixture was filtered and evaporated, and the crude oil was chromatographed on silica gel [*n*-hexane–2-propanol (3:2), containing 0.14 mol/L of ammonia]. The yield of the desired amino ester was 2.8 g (74%), as the base from which the hydrochloride was prepared.

2-(4-Chlorophenyl)-1,1-dimethylethyl 2-Amino-3-methylbutanoate Oxalate (22). This compound was prepared in analogy with the method described for compound **21** using 1-(4-chlorophenyl)-2-methyl-2-propanol and 2-bromo-3-methylbutyryl bromide.

When reducing catalytically the intermediate azido ester, some dechlorinated amine was obtained in admixture with the desired compound (cf. compound **23**). Purification was achieved by recrystallization of the oxalate salts from 2-propanol: mp 128–131 °C (mixture of mono- and dibasic salts).

2-Phenyl-1,1-dimethylethyl 2-Amino-3-methylbutanoate Oxalate (23). This compound was isolated from the mixture obtained in the preparation of compound **22** (cf. above): mp 134–135 °C (mixture of mono- and dibasic salts).

2-(4-Chlorophenyl)-1-methyl-1-phenylethyl 2-Aminopropanoate Hydrochloride Monohydrate (24). This compound was prepared from 4-chlorobenzyl chloride and acetophenone in analogy with the method described for compound **1**.

2-(4-Chlorophenyl)-1,1-dimethylethyl 2-Dimethylaminopropanoate Hydrochloride (25). 2-(4-Chlorophenyl)-1,1-dimethylethyl 2-bromopropanoate (10.0 g, 31.3 mol, prepared as described under compound **1**), dimethylamine (5.62 g, 125 mmol), and benzene (30 mL) were mixed in a steel autoclave and heated at 90 °C for 16 h. After cooling the reaction mixture was evaporated, the residue was dissolved in 1 N hydrochloric acid, and the solution was washed with ether. The aqueous phase was made alkaline and extracted with ether. The ether phase was washed with water, dried over sodium sulfate, and evaporated in vacuo. From the residual oily base (6.7 g) the hydrochloride was prepared.

2-(4-Chlorophenyl)-1,1-dimethylethyl 2-Piperidinopropanoate Hydrochloride (26). This compound was obtained by heating 2-(4-chlorophenyl)-1,1-dimethylethyl 2-bromopropanoate and piperidine (2 equiv) in toluene in a steel autoclave during 90 h in the presence of a few crystals of sodium iodide. The reaction mixture was worked up in the same way as for compound **25**: yield, 60%.

2-(4-Chlorophenyl)-1,1-dimethylethyl 2-(4-Methylpiperidino)propanoate Hydrochloride (27). This compound was obtained as described for compound **26** using 4-methylpiperidine.

Pharmacology. White male mice (NMRI) weighing 18–22 g were used. The injections were given intraperitoneally if otherwise not stated. The compounds were dissolved in water and given in a volume of 10 mL/kg.

Accumulation of [^{3}H]-(−)-Noradrenaline and [^{14}C]-5-Hydroxytryptamine. The simultaneous accumulation of [^{3}H]-NA and [^{14}C]-5-HT in slices of the mouse or rat brain was determined as described previously.[14] The in vitro values were obtained by adding the test compounds to the incubation medium. In the in vivo tests the compounds were injected 0.5 h before the sacrifice of the mice, and the decrease in the accumulation of 5-HT and NA in brain slices was measured in vitro. The active accumulation was defined as that inhibited by 3×10^{-4} M cocaine. The inhibition was determined in percent. IC_{50} and ED_{50} values are determined from dose–response curves based on at least three different doses with four animals in each dose group.

Potentiation of the 5-Hydroxytryptophan (5-HTP) Syndrome. Potentiation of the 5-HTP response in mice was tested as described previously.[10] The doses producing head twitches, tremor, and abduction of the hind legs in 50% of the animals were determined by probit analyses of the dose–response curves.

Mydriatic Effect and Acute Toxicity. The mydriatic effect and acute toxicity in mice were determined as described previously.[14] The PD_{200} and LD_{50} values were determined from log dose–response curves and are based on four to six dose levels with six animals per dose level. PD_{200} refers to the dose which increases the pupil width by 200%.

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Inactivation of Trypsin-like Proteases by Sulfonylation. Variation of Positively Charged Group and Inhibitor Length

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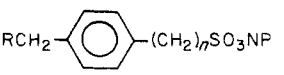
Attempts to achieve selective inactivation of serine proteases of closely related specificity (trypsin-like) by aryl sulfonylation have been extended. Nitrophenyl esters of benzenesulfonic acid and phenylmethanesulfonic acid containing various positively charged groups have been synthesized and examined as inactivators of trypsin, thrombin, plasmin, plasma kallikrein, and urokinase. Examples of selective inactivation by isothiouronium derivatives were found and attributed to differences among these enzymes in geometry and flexibility of the primary specificity sites.

Physiologically important proteases whose action involves a limited proteolysis of trypsin-like specificity often at unique sites in their normal substrate are found in coagulation, fibrinolysis, fertilization, and other processes[1]. Synthetic reagents capable of inactivating one of these enzymes without affecting the others may be of medicinal value. Affinity labeling offers a rational approach to enzyme inactivation based on the specificity of the target enzyme. However, proteases of similar specificity such as the trypsin-like enzymes cited which hydrolyze proteins at certain arginine or lysine residues have extended active centers which may encompass five or more amino acid residues[2,3] in their normal macromolecular substrate and may therefore achieve specificity by utilizing a large region of complementary interaction to position the substrate and promote productive cleavage. In view of this, the possibility of achieving selective inactivation with a small reagent occupying only the primary specificity site does not seem likely. Nevertheless, in earlier work we have shown that benzamidine and phenylguanidine are competitive inhibitors of trypsin[4] acting as structural analogues of the basic side chains of arginine and lysine and that carboxylate[5,6] and sulfonate[7,8] derivatives having these or other positively charged substituents may act as substrate analogues. Furthermore, among carboxylate derivatives the formation of acyl enzymes (intermediates of the normal hydrolytic pathway) of considerable stability in the case of plasmin[5,6] in contrast to thrombin indicated that selective inhibition was possible, involving a very limited region of the active centers.

Meanwhile the possibility has been explored[7,8] of using aryl sulfonate ester analogues instead of carboxylate in the expectation that selectivity of inhibition might be achieved in the acylation (sulfonylation) step rather than in the subsequent hydrolytic step since it was expected that desulfonylation would in all cases be negligible. In fact, *p*-nitrophenyl *p'*-amidinophenylmethanesulfonate was prepared and found to inactivate thrombin[8] but not trypsin, plasmin, or plasma kallikrein, although it complexed at the active center of all four proteases as shown by competition of the esterase action.

In the present work the effect of positively charged groups other than amidino and guanidino was examined on the sulfonylation of selected trypsin-like enzymes by *p*-nitrophenyl esters of the general structure



in which R is a positively charged group, $n = 0$ or 1, and NP is *p*- or *m*-nitrophenyl.

Chemistry. The *p*-nitrophenyl esters of *p*-bromomethylbenzenesulfonic acid and its next higher analogue, *p*-bromomethylphenylmethanesulfonic acid, were synthesized and reacted with dimethyl sulfide, thiourea, and its *N*-methyl derivatives, trimethylamine and pyridine, to

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