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Original article

Prodrugs of neuron-selective monoamine oxidase inhibitors: amino acid derivatives of 1-(4-aminophenyl)-2-aminopropanes

Lennart Florvall[a], Ingrid Fagervall[b], Lars-Gunnar Larsson[b], Svante B. Ross[b]*

[a]*Department of Medicinal Chemistry, Preclinical R&D, Astra Arcus AB, 15185 Södertälje, Sweden*
[b]*Department of Biochemical Pharmacology, Preclinical R&D, Astra Arcus AB, 15185 Södertälje, Sweden*

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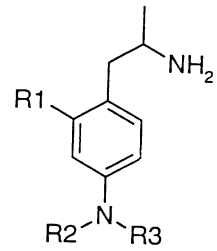
Abstract – Six amino acid derivatives of 1-(4-aminophenyl)-2-aminopropanes and their parent amines were synthezised and tested for their potency and selectivity in inhibiting monoamine oxidase (MAO) in vitro and in vivo. The amino acid derivatives were 300–1000 times less potent than the parent amines in inhibiting the MAO-A activity in a rat brain mitochondrial preparation in vitro. All compounds, except the (*R*)-valinamide derivative (**22**), were potent inhibitors of MAO in the rat brain in vivo and were, like the parent amines markedly more potent within the monoaminergic neurons than in other neurons. The glycinamide derivative **7** showed the largest difference between intra- and extra-neuronal inhibition in serotonergic neurons. The time course of the inhibitory effect of **7** in vivo showed that it is a reversible inhibitor with a long duration. © Elsevier, Paris

monoamine oxidase inhibitors / amiflamine / prodrug / serotonin / noradrenaline / dopamine

1. Introduction

The enzyme monoamine oxidase (MAO; amine: oxygen oxidoreductase (deaminating, flavin-containing); EC 1.4.3.4) exists in two forms, A and B, with different substrate specifications. 5-Hydroxytryptamine (5-HT) is a prefered substrate for the A form and β-phenylethylamine (PEA) for the B form. Since there are two forms of the enzyme, it is possible to develop compounds that selectively inhibit the A form and leave the B form active to deaminate amines, like tyramine, ingested in the food. Although the B form is abundant in the brain and the A form in peripheral tissues, the A form is functionally responsible for the main part of the deamination of 5-HT, noradrenaline (NA) and dopamine (DA) in the aminergic neurons in the brain [1, 2]. During the last two decades a new generation of selective and reversible MAO-A inhibitors has been developed among which moclobemide is clinically used as an antidepressant drug (for a review see [3]).



(*S*)-**1a**: $R_1 = R_2 = R_3 = CH_3$
(*R,S*)-**1b**: $R_1 = F$; $R_2 = R_3 = H$
(*R,S*)-**1c**: $R_1 = F$; $R_2 = R_3 = CH_3$

Amiflamine (**1a**) and related p-amino substituted amphetamine derivatives have been shown to preferably inhibit the MAO activity within the monoaminergic neurons by being accumulated within these neurons via the membranal amine transporters [4–7]. Because of their high concentration within these neurons, these compounds inhibit MAO therein at considerably lower doses than those inhibiting MAO in cells lacking these transporters [7]. The purpose of the present study was to

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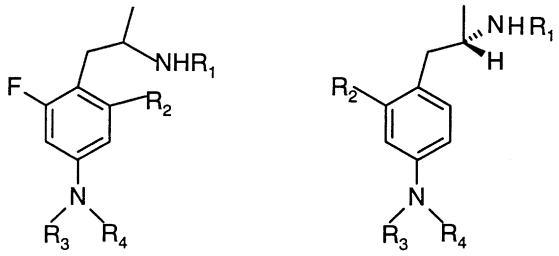
Table I. 1-(4-Amino-2-fluorophenyl)-2-aminopropane and amino acid derivatives.



Compound	R_1	R_2	R_3	R_4	m.p., °C	Formula
2	H	F	H	Me	208–209	$C_{10}H_{14}F_2N_2 \times 0.5(COOH)_2$
3	H	F	H	H	> 180 (D)	$C_9H_{12}F_2N_2 \times 1.5(COOH)_2 \times H_2O$
4	H	H	H	Mé	148–149	$C_{10}H_{15}FN_2 \times HAc$
5	Glycyl	F	H	Me	106–107	$C_{12}H_{17}F_2\ N_3\ O \times HAc$
6	Glycyl	H	H	H	102–103	$C_{11}H_{16}FN_3\ O \times HAc$
7	Glycyl	H	H	Me	112–113	$C_{12}H_{15}FN_3O \times HAc$
8	Glycyl	H	Me	Me	183–184	$C_{13}H_{20}FN_3O \times (COOH)_2$
21	(*S*)-Valyl	Me	Me	Me	246–248	$C_{17}H_{29}N_3O \times 2HCl$
22	(*R*)-Valyl	Me	Me	Me	225–226	$C_{17}H_{29}N_3O \times 2HBr$

[a] Configuration (*R*,*S*); [b] configuration (*S*).

synthesize low-potency prodrugs to neuronselective MAO-A inhibitors structurally related to amiflamine. The advantage of this approach would be that the interaction between ingested tyramine and MAO inhibitor in the intestines should be minimized. It is likely that the MAO-A activity in the intestinal mucosa is responsible for the main part of the deamination of tyramine ingested. Consequently, a useful pro-drug should not be deassociated in the intestines but be taken up into blood circulation, forming the active compound in the liver or the brain. In previous works with amiflamine analogues, we found that 2-fluoro substitution, e.g. **1b** and **1c**, gave very high MAO-A inhibitory potency in vitro and in vivo [4, 7, 8]. Hence these structures were chosen as the basis for the synthesis of a series of glycinamides as potential pro-drugs. In this report we describe the synthesis and pharmacological testing of these compounds as MAO-A inhibitors with neuron selective action in vivo. In addition, the *S*- and *R*-valine amides of amiflamine ((*S*)-**1a**) were prepared and evaluated as MAO-inhibitors.

2. Chemistry

The target fluoro compounds prepared in this study are listed in *table I* and their synthesis are outlined in figures 1–6.

Compound **2** was prepared as illustrated in *figure 1*. 3,5-Difluorobenzoic acid was converted to 3,5-difluorobenzoyl chloride by treatment with thionyl chloride and then reacting with concentrated aqueous ammonia to give the amide. Degradation of the obtained amide via the Hofmann reaction gave the corresponding aniline. The desired intermediate **9** was then obtained by the reaction of the aniline with formic acid. Compound **9** was

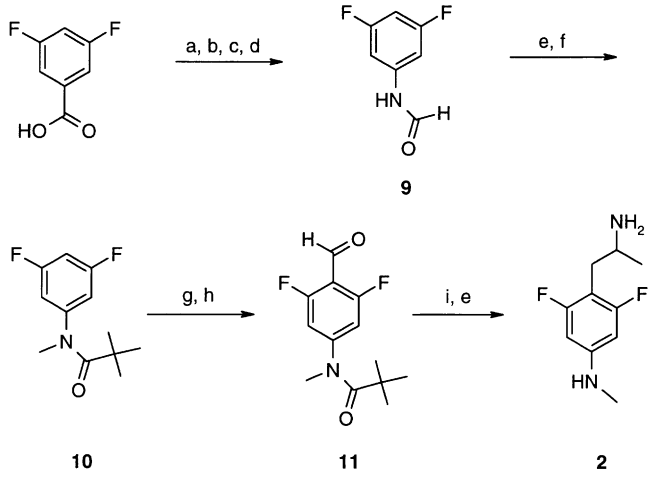


Figure 1. Reagents: (a) $SOCl_2$; (b) NH_3; (c) Br_2/NaOH; (d) HCOOH; (e) LAH; (f) *t*-BuCOCl; (g) *n*-BuLi; (h) DMF; (i) $MeCH_2NO_2$.

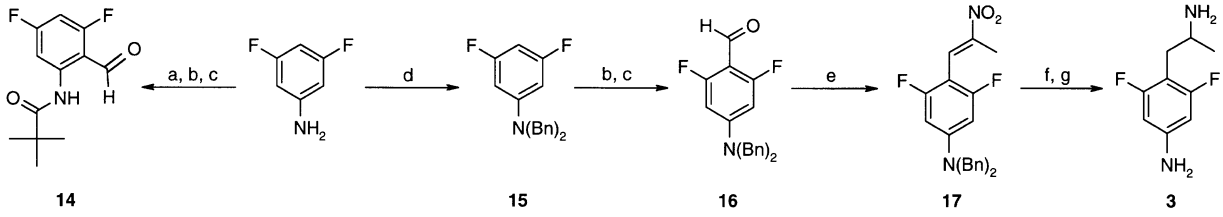


Figure 2. Reagents: (a) *t*-BuCOCl; (b) *n*-BuLi; (c) DMF; (d) $BnCl/K_2CO_3$; (e) $MeCH_2NO_2$; (f) LAH; (g) H_2, Pd/C.

also prepared from commercially available 3,5-difluoroaniline and formic acid. The formanilide **9** was reduced with lithium aluminium hydride and the obtained *N*-methylaniline was used directly in the next step to yield the *N*-pivaloylamide **10**. The pivaloyl protecting group was considered to be ideal since the absence of acidic α-hydrogens excludes undesired lithiations of the amide alkyl residue [9]. Furthermore the pivaloyl group proved sufficiently labile for removal in the final lithium aluminum hydride reduction step.

Lithiation of compound **10** with butyllithium and then reaction with N,N-dimethylformamide gave the desired aldehyde **11**. In the next step the 2-nitropropene intermediate was prepared by the condensation of compound **11** with nitroethane in the presence of ammonium acetate. Preparation of the target amine **2** was then achieved by the treatment with lithium aluminum hydride, which in one step effected both reduction and deprotection of the 2-nitropropene intermediate. Keeping the pivaloyl amide in excess and performing the reaction at low temperature was found to be important for the outcome of the deprotection.

In an attempt to synthesise compound **3**, lithiation and DMF treatment of 3,5-difluoro-*N*-pivaloylaniline was found to give rise to the benzaldehyde **14** in *figure 2*. Using a different strategy 3,5-difluoroaniline was *N*,*N*-dibenzylated with benzyl chloride in the presence DMF and anhydrous potassium carbonate. The *N*,*N*-dibenzylaniline **15** was reacted with butyllithium and DMF to give the desired aldehyde **16**. The 2-nitropropene derivative **17** was then prepared by the condensation of compound **16** and nitroethane in the presence of ammonium acetate. Selective reduction of the 2-nitropropene group with lithium aluminum hydride and subsequent debenzylation in situ by catalytic hydrogenation in the presence of palladium, gave the desired target amine **3**.

The synthesis of compound **4** in *figure 3* started from 3-fluoroaniline, which was reacted with formic acid. The formed formanilide was reduced with lithium aluminum hydride and the obtained 3-fluoro-*N*-methylaniline was benzylated directly to the corresponding *N*-benzyl-*N*-methyl derivative. The intermediate aniline was not isolated at this stage, but formylated by the Vilsmeier–Haack procedure to the desired intermediate 4-(*N*-benzyl-*N*-methylamino)-2-fluorobenzaldehyde. The condensation of this compound and nitroethane in the presence of ammonium acetate gave the 2-nitropropene derivative **18**.

Reduction of **18** with lithium aluminium hydride and debenzylation by catalytic hydrogenation of the obtained amine, afforded compound **4**. Reduction of **18** with lithium aluminum hydride and chloracetylation of the obtained amine with chloroacetyl chloride, gave amide **19**. Amination of **19** by means of dibenzylamine yielded

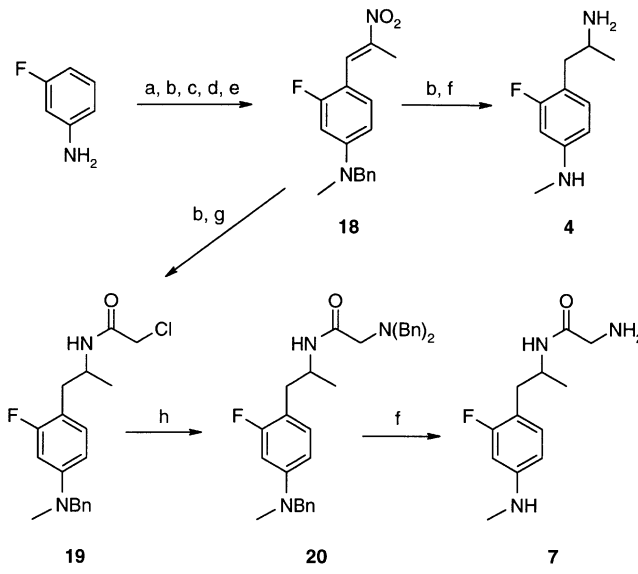


Figure 3. Reagents: (a) HCOOH; (b) LAH; (c) BnCl; (d) $POCl_3$/DMF; (e) $MeCH_2NO_2$; (f) H_2, Pd/C; (g) $ClCH_2COCl$; (h) $HNBn_2$.

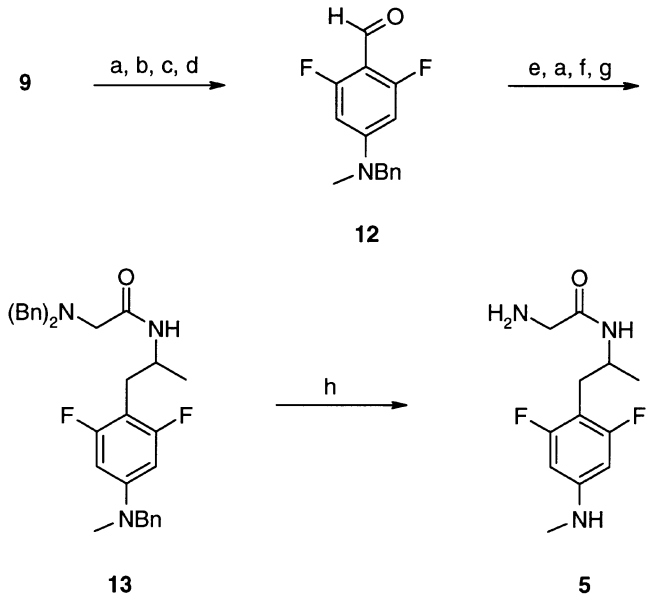


Figure 4. Reagents: (a) LAH; (b) BnCl; (c) *n*-BuLi; (d) DMF; (e) $MeCH_2NO_2$; (f) $ClCH_2COCl$; (g) $HNBn_2$; (h) H_2, Pd/C.

amide **20**, which was debenzylated by catalytic hydrogenation to give the target glycinamide derivative **7**.

The preparation of compound **5** in *figure 4* started from the formanilide **9** which was reduced with lithium aluminum hydride, benzylated and treated with butyllithium and *N,N*-dimethylformamide to give the desired aldehyde **12**. The conversion of compound **12** into the glycinamide **5** was then effected by the same route used for the preparation of compound **7**. It may be noted that an attempt to transform the intermediate *N*-benzyl-*N*-methyl-3,5-difluoroaniline into aldehyde **12** by means of the Vilsmeier–Haack formylation process, was unsuccessful.

The preparation of compound **8** started from 1-(4-dimethylamino-2-fluorophenyl)-2-aminopropane [4] which was treated with chloroacetyl chloride *(figure 5)*. Amination of the obtained amide with ammonium hydroxide at room temperature gave the target glycinamide. Chloroacetylation of 1-(4-dibenzylamino-2-fluorophenyl)-2-aminopropane [4], amination of the yielded chloroacetamide with dibenzylamine and subsequent catalytic hydrogenation, gave the desired compound **6** *(figure 5)*.

The preparation of compound **21** was effected by the acylation of (*S*)-(+)-1-(4-dimethylamino-2-methylphenyl)-2-aminopropane [10] with (*S*)-*N*-carbobenzyloxyvaline [11] in the presence of CDI. The deprotection of the obtained carbobenzyloxy intermediate to the target

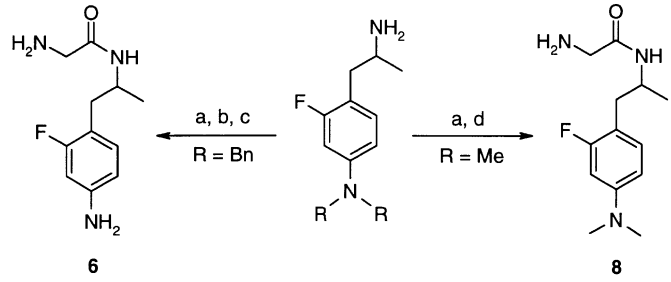


Figure 5. Reagents: (a) $ClCH_2COCl$; (b) $HNBn_2$; (c) H_2, Pd/C; (d) NH_3.

compound **21** was obtained by catalytic hydrogenation. Starting from (*R*)-*N*-carbobenzyloxyvaline [12] compound **22** was prepared in a similar procedure. However, in this case the deprotection was effected by using hydrobromic acid *(figure 6)*.

3. Pharmacology

3.1. MAO inhibition in vitro

The results are given in *table II*. They show that the fluorinated aminopropanes (**1b**, **1c**, **2**, **3**, **4**) were selective and potent inhibitors of the A form of MAO in vitro, since they inhibited the deamination of 5-HT at much lower concentrations than those required to inhibit the deamination of PEA. The di-fluoro derivative **3** was 10 times more potent than the corresponding mono-fluoro com-

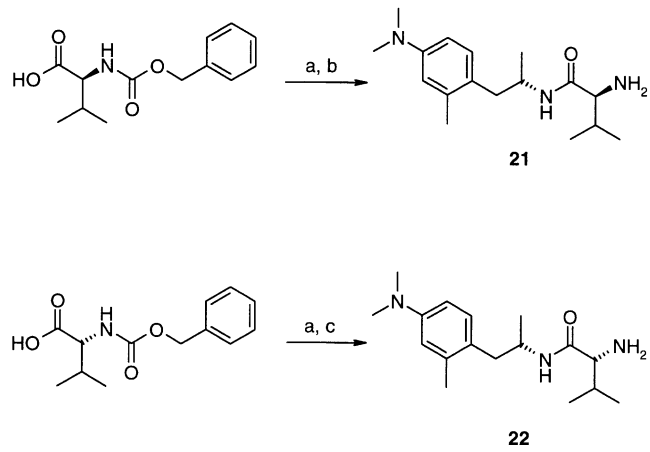


Figure 6. Reagents: (a) CDI, (*S*)-(+)-1-(4-dimethylamino-2-methylphenyl)-2-aminopropane; (b) H_2, Pd/C; (c) HBr.

Table II. Inhibition of MAO in vitro and inside (IN) and outside (EN) the monoaminergic neurons ex vivo.

Compound	In vitro inhibition IC_{50} (μM)		Ex vivo inhibition [a] ED_{50} (μmol/kg p.o.)					
	PEA	5-HT	5-HT		NA		DA	
NA			IN	EN	IN	EN	IN	EN
1b [b]	800	0.75	5.2*	48	0.8*	57	0.8*	4.4
6	>100	>100	14*	>70	1.8*	>70	nt	nt
4	>100	0.08	1.1*	5.8	1.2*	5.4	1.0*	6.2
7	>100	27	0.7*	12	1.2*	5.0	1.0*	6.3
1c [c]	120	1.2	0.45*	3.0	0.45*	3.0	1.1*	2.2
8	>100	>100	0.6*	2.3	0.7*	3.5	nt	nt
3	>100	0.07	0.5*	3.8	0.24*	6.2	nt	nt
2	40	0.01	0.5*	2.0	0.2*	2.9	nt	nt
5	>100	4.5	1.2*	4.4	0.5*	2.7	nt	nt
21	>100	>100	1.7*	17	nt	nt	nt	nt
22	>100	>100	>>17 (2 %)	>>17 (2 %)	nt	nt	nt	nt
Amiflamine (**1a**)[b]	>1000	0.8	1.1*	5.2	2.0*	8.0	3.3*	4.4

[a] Test compounds administered 1 h before the subcutaneous injection of phenelzine and the rats were sacrificed 2 days later. [b] Data from [7]. [c] Data from [4] and [5]. *: denotes significant ($p < 0.05$) larger inhibition inside than outside the neurons (Mann–Whitney U-test); nt = not tested. Between brackets: % inhibition at the dose noted.

pound **1b**. The 4-methylamino derivative **4** had 10–15 times higher potency than **1b**. The 4-methylamino-2,6-difluoro derivative **2** had the highest potency to inhibit both forms of the enzyme. The glycinamide derivatives (**5**–**8**) were 300–1000 times weaker than the corresponding amines *(table II)*. The inhibition curves of **4** and **7** are shown in *figure 7*. The (*S*)- and (*R*)-valine derivatives of amiflamine (**21**, **22**) also had weak MAO inhibitory potencies in vitro compared with amiflamine (**1a**) itself *(table II)*.

3.2. MAO inhibition in vivo

All compounds except the (*R*)-valine derivative of amiflamine **22** were potent inhibitors of MAO in vivo *(table II)*. They were all more potent in the monoaminergic neurons than in other neurons or cells, i.e. had neuron-selective action. With the exception of **5** and the primary 4-amino derivatives **Ib** the compounds were about equal active in the three types of aminergic neuron systems examined. These primary 4-amino derivatives had considerably lower potency in the 5-HT neurons than in NA and DA neurons but had quite high intraneuronal selectivity in the two former systems. The glycinamide derivative **7**, related to the 4-methylamino-2-fluoro compound **4**, had the highest selectivity with regards to the inhibition of neuronal 5-HT deamination *(table II, figure* 8). This compound was therefore chosen for further studies. The time courses of the MAO-A inhibition in serotonergic and noradrenergic neurons in hypothalamus after 1 mg/kg (3.3 μmol/kg) p.o. are shown in *figure 9*. The inhibition in serotonergic neurons was significant 8 h but not 20 h after the administration. In noradrenergic neurons the inhibition was still significant (about 40%)

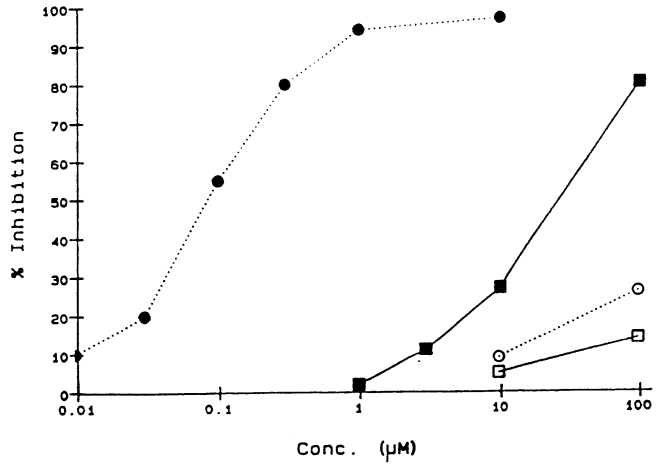


Figure 7. Inhibition by **7** (squares) and **4** (circles) of the deamination of ^{14}C-5-HT (MAO-A) (solid symbols) and ^{14}C-phenethylamine (MAO-B) (open symbols) in vitro. A mitochondrial preparation from rat brain was used as enzyme. The concentration of ^{14}C-5-HT was 50 μM and that of ^{14}C-PEA was 2.5 μM.

Table III. The effect of compound **7** on the monoamine levels in hypothalamus, hippocampus and striatum 1 h after oral administration.

Dose (μmol/kg)	Amine concentration, per cent of controls, ± S.E.M.						
	Hypothalamus			Hippocampus		Striatum	
	5-HT	NA	DA	5-HT	NA	5-HT	DA
3.3	127 ± 5 [c]	117 ± 3 [a]	127 ± 7	130 ± 8 [b]	159 ± 22 [a]	129 ± 7 [b]	105 ± 4
16	149 ± 11 [b]	116 ± 3 [a]	122 ± 12	146 ± 14 [a]	154 ± 12 [b]	153 ± 11 [b]	122 ± 5 [b]

Control values (nmol/g tissue): hypothalamus: 5-HT: 3.16 ± 0.05; NA: 9.21 ± 0.45; DA: 2.14 ± 0.29; hippocampus: 5-HT: 1.99 ± 0.06; NA: 1.71 ± 0.06; striatum: 5-HT: 1.57 ± 0.07; DA: 45.3 ± 1.8. [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$ (Dunnett's *t*-test following ANOVA) compared with saline controls.

after 20 h. Maximal effect was obtained 2 h after the administration.

3.3. Effect on monoamines and their metabolites

In the first experiment the rats were killed 1 h after the oral administration of **7** (3.3 and 16 μmol/kg). The 5-HT level in the three regions analyzed (hypothalamus, hippocampus and striatum) increased with 25–30% of the control values at the lower dose and with 45–50% at the higher dose *(table III)*. The NA level increased much more in hippocampus (58%) than in hypothalamus (17%) and both doses produced similar elevation. The DA level in striatum was significantly increased only after the high dose. The concentration of 5-HIAA hippocampus and hypothalamus decreased with 20–25% and 45–55%, respectively *(table IV)*. DOPAC was strongly decreased in hypothalamus (35% at 3.3 μmol/kg and 70% at 16 μmol/kg) and striatum (70 and 85%, respectively) *(table IV)*. The HVA levels were also markedly decreased in these regions.

The time courses of the changes in the concentrations of the monoamines and their metabolites after 3.3 mmol/kg of **7** were determined in a second experiment. The maximal increase in the amine levels were obtained 2 h after the administration whereas the concentrations of the metabolites had the lowest values already 1 h after administration (data not shown). The duration of the elevation of the NA concentration in hypothalamus and hippocampus was somewhat longer compared with that of the 5-HT concentration.

4. Discussion

In previous studies it has been shown that it is possible to develop neuron-selective MAO-A inhibitors by utilizing the specific transport mechanisms in the the cell membranes of the monoaminergic neurons [5–7]. By being transported into the neurons, compounds like α-ethyltryptamine, p-methoxyamphetamine, amiflamine and related p-aminoamphetamine derivatives are accumulated in the amine neurons and thereby are inhibiting MAO-A therein at lower doses than those inhibiting MAO-A in cells or neurons lacking these transporters [7]. Since the transporters are not identical in the various amine neurons, the structural requirements for uptake is different which makes it possible to obtain MAO-A selectivity in a certain monoamine system, e.g. noradrenaline neurons [7].

The aim of the present study was to develop prodrugs to amiflamine-like compounds with low in vitro affinity for MAO-A. This was achieved by making amino acid derivatives which had a hundred-fold lower in vitro

Table IV. Effect of compound **7** on monoamine metabolites in the rat hypothalamus, hippocampus and striatum.

Dose (μmol/kg)	Concentration of monoamine metabolites in per cent of controls, ± S.E.M.						
	Hypothalamus			Hippocampus		Striatum	
	5-HIAA	DOPAC	HVA	5-HIAA	5-HIAA	DOPAC	HVA
3.3	77 ± 3 [c]	66 ± 3 [a]	69 ± 5	80 ± 5 [a]	78 ± 4 [b]	41 ± 4 [c]	57 ± 4 [c]
16	45 ± 5 [c]	31 ± 2 [c]	35 ± 6 [c]	49 ± 5 [c]	55 ± 6 [b]	15 ± 1 [c]	28 ± 6 [c]

The concentration of 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in brain 1 h after the oral administration of **6** were analysed as described in the experimental part. Control values (nmol/g tissue): hypothalamus: 5-HIAA: 2.54 ± 0.08; DOPAC: 0.44 ± 0.04; HVA: 0.25 ± 0.04; hippocampus: 5-HIAA: 1.98 ± 0.09; striatum: 5-HIAA: 2.31 ± 0.08; DOPAC: 6.15 ± 0.34; HVA: 4.67 ± 0.29. [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$ (Dunnett's *t*-test following ANOVA) compared with saline controls.

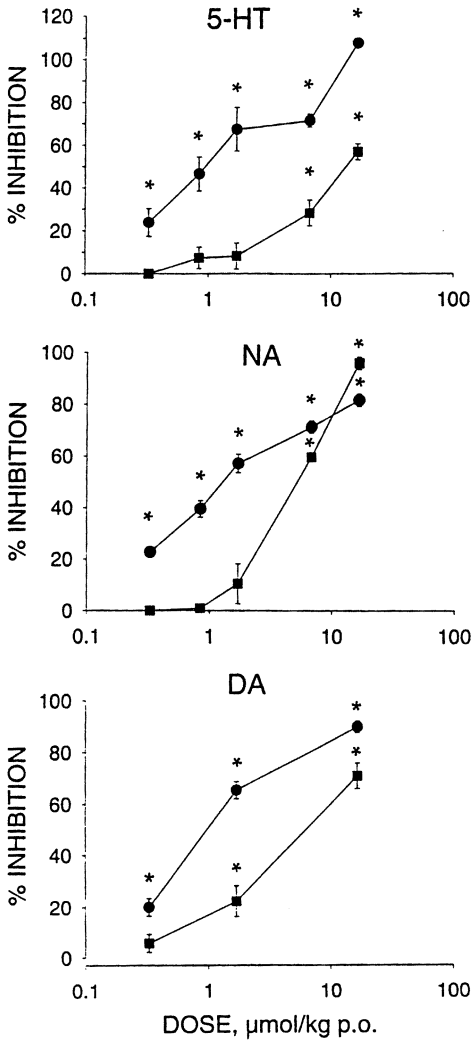


Figure 8. Dose–response curves of the in vivo inhibition by **7** of MAO-A inside (circles) and outside (squares) 5-HT neurons (top) and NA neurons (middle) in rat hypothalamus and DA-neurons (bottom) in rat striatum. Different doses of **7** were given orally 1 h before the subcutaneous injection of phenelzine sulfate (4 mg/kg in the experiment with 5-HT and NA and 2.5 mg/kg in the experiment with DA) and the rats were killed 48 h later. Hypothalami and striata were dissected out and homogenized. The homogenate was incubated with 0.1 µM ^{14}C-5-HT, 0.25 µM ^{3}H-NA or 0.25 µM ^{14}C-DA in the absence and the presence of selective uptake inhibitors. The protection against the irreversible inhibition caused by phenelzine was taken as a measure of the MAO inhibition that was calculated as described in the experimental section. Each value is the mean from 4 rats. *: $p < 0.05$ vs. phenelzine controls (Mann–Whitney U-test).

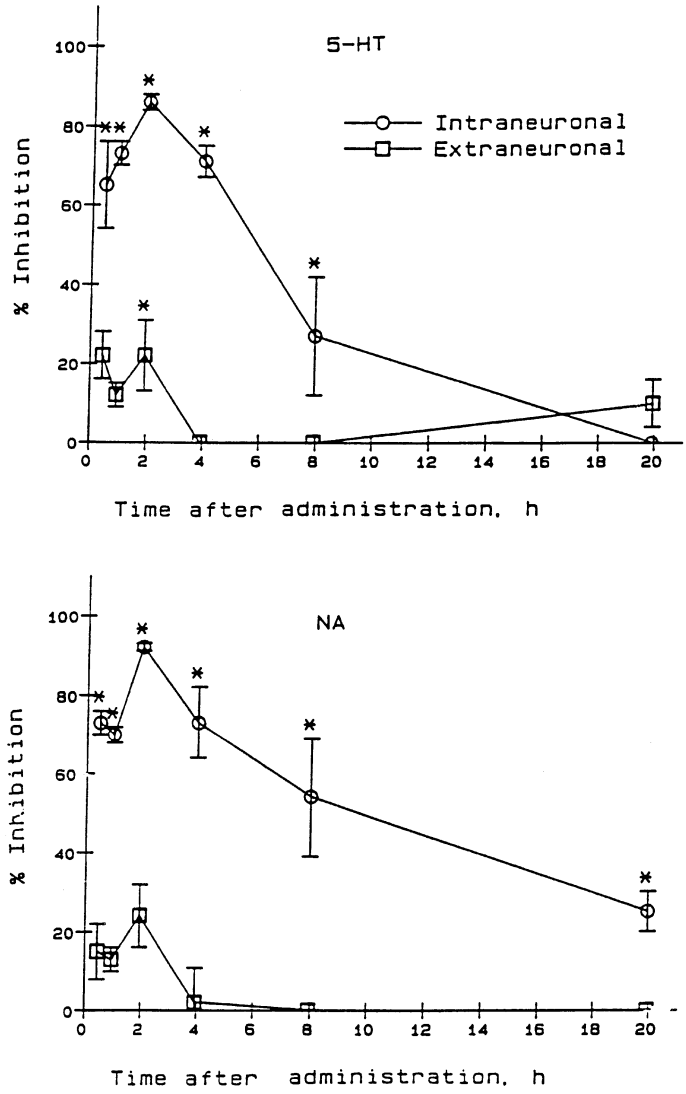


Figure 9. Time course of the inhibition of MAO-A by compound **7** within (circles) and outside (squares) 5-HT (top) and NA (bottom) neurons in the rat hypothalamus. **7** (3.3 µmol/kg) was administered orally at different times before the subcutaneous injection of phenelzine sulfate, 4 mg/kg, and the rats were killed 48 h after the latter injection. The MAO activity was determined and the inhibition at the times for the phenelzine injection was calculated as described in the experimental section. Each value is the mean ± S.E.M. (bars) from 4 rats. *: significant protection against phenelzine ($p < 0.05$; Mann–Whitney U-test).

potency to inhibit MAO-A than the parent drug. Since these amides can be enzymatically hydrolyzed in vivo forming the active inhibitors, it might be possible to overcome the main obstacle with MAO-A inhibitors: potentiation of tyramine-induced hypertension. This is thought to involve MAO-A activity in the intestine mucosa which is the first metabolic barrier for ingested tyramine. Thus, a pro-drug of MAO-A inhibitor which is able to pass into the blood circulation intact and thereafter form the active compound in the liver may be a therapeutically useful antidepressant.

The MAO inhibition in vivo by the compounds was established with two methods. The ex vivo deamination of the labelled monoamines in synaptosomal preparations from rats treated 2 days earlier with the test compound before the injection of phenelzine measures the protection by the test compound against the irreversible inhibition of MAO by phenelzine. This protection is consequently a measure of the inhibitory potency of the test compound competing with phenelzine for the enzyme [13]. If the enzyme activity is measured with low concentrations of the labelled transmitter amine in the absence and the presence of selective upptake inhibitors, it is possible to measure the activity within a particular aminergic neuron system [7]. This technique showed that the compounds inhibited the MAO activity in monoamine neurons at considerably lower doses than in other neurons or cells. Thus, compound **7** was 17 times more potent in inhibiting MAO-A in 5-HT neurons in hypothalamus than within other hypothalamic cells. This preference for inhibition of the aminergic MAO is obviously due to uptake of the inhibitor by the amine transporters, resulting in high concentration inside these neurons [6]. The increase in the concentrations of the transmitter amines and the decrease in their metabolites in various brain regions confirm the MAO inhibitory action of **7** in vivo. The time courses of the elevation of the amine levels followed those of the inhibition within the neurons whereas the decrease in the metabolites decayed more rapidly, probably due to extraneuronal deamination of the transmitter amines.

It must be noted that the glycine derivatives studied may not be the optimal compounds, since they were synthesized from the racemic amines. The enantiomers may differ in the rate of being formed by the enzymatic hydrolysis of the prodrugs and also in the capability of being actively transported into the monoaminergic neurons. In addition, it has been shown that the (*S*)-enantiomer of **1a** (amiflamine) is about 4 times more potent than the corresponding (*R*)-enantiomer as an inhibitor of MAO-A in vitro [5].

In accordance with previous observations [5–8], it was found that the primary aminophenyl derivative **1b** was more potent in inhibiting MAO in noradrenergic than in serotonergic neurons which indicates that the 1-(4-aminophenyl)-2-aminopropanes are better solutes for the noradrenaline transporter than for the serotonin transporter whereas the mono- and dimethylaminophenyl derivatives, e.g. **4** and **1c** appear to be similar good substrates for both transport systems. This structure–activity relationship seems, however, to be revoked by substituting the phenyl with two fluorine atoms at *ortho* positions, e.g. compound **3** which had similar potency in 5-HT and NA neurons and the very potent compound **2** which tended to be most active in NA neurons.

The results obtained in this study show that the amino acid derivatives of 1-(4-aminophenyl)-2-aminopropanes were very weak MAO inhibitors in vitro but quite strong and neuron-selective inhibitors in vivo. The lack of in vivo activity of the *R*-valine derivative of amiflamine (**22**) shows that enzymatic hydrolysis of the amide is necessary for MAO inhibitory effect. The hydrolysis of the amides may occur in the intestines by peptidases, in the liver by amidases or at both sites. Although the present study does not give a definite answer to this, the time course of the in vivo inhibitory action of **7**, the compound chosen for extended study, with optimal effect 2 h after oral administration indicated that the glycinamide was mainly hydrolyzed in the liver. In accordance with this view, it has been reported the midodrine (the glycinamide of 2,5-dimethoxy-β-phenylethanolamine) is almost completely absorbed (90% bioavailability) after oral administration in man and is rapidly hydrolyzed in the liver forming the parent amine [14]. Further studies have to be performed in order to show if this pro-drug principle might result in MAO inhibitors with potential clinical value.

5. Experimental protocols

5.1. Chemistry

Melting points were determined with a Mettler FP62 automatic melting point recorder. The elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Most of the intermediates were analyzed by means of GC/MS and used directly in the subsequent reaction step without any purification. Gas chromatography was carried out on a HP 5890 instrument with a 10 m × 0.1 mm CP-Sil-5 capillary column. ^{1}H NMR spectra were recorded with a Varian Gemini-300

NMR spectrometer. Mass spectra were obtained at 70 eV on an ITD800 (C I mode, CH_4) or a LKB291 (EI mode) mass spectrometer.

*5.1.1. 3,5-Difluoroformanilide **9***

Method I: A solution of 5.0 g (0.039 mol) of 3,5-difluoroaniline in 15 mL of 98% formic acid was refluxed for 2 h and then poured into 250 mL of crushed ice. After stirring for 1 h the obtained precipitate was filtered, washed with water and dried: Yield 5.0 g (82%); m.p. 88–89 °C. GC = 99%. MS (CI): *m/z* (M^+ + 1) 158.

Method II: To a stirred mixture of 31.6 g (0.2 mol) of 3,5-difluorobenzoic acid, 200 mL of CH_2Cl_2 and a catalytical amount of DMF $SOCl_2$ (80 mL, 1 mol) was added portionwise. The mixture was refluxed for 4 h, the solvent was evaporated and the residual oil dissolved in 80 mL of dry THF. The solution was added dropwise under stirring and cooling in ice to 400 mL of concentrated NH_4OH. The obtained amide was collected by filtration and dried. Yield 27.7 g (88%); m.p. 156–157 °C. The product (27.7 g, 0.17 mol) was added in portions, whith stirring, to an ice-cold solution of 10.6 mL Br_2 and 33.3 g of NaOH in 300 mL of water. The mixture was heated at reflux temperature for 2 h and then extracted with ether. The extract was dried with Na_2SO_4 and the ether was evaporated. To the residual oil was added 70 mL of 98% formic acid and the solution was refluxed for 2 h and then worked up as described in Method I: yield 18.9 g (71%); m.p. 88–89 °C; MS (CI): *m/z* (M^+ + 1) 158. Anal. ($C_7H_5F_2NO$) H, N; C: calc. 53.51; found 53.0, 52.9.

*5.1.2. 3,5-Difluoro-N-methyl-N-pivaloylaniline **10***

A solution of 5.0 g (0.03 mol) of compound **9** in 50 mL of ether was added dropwise under nitrogen to a stirred mixture of 3.0 g LAH in 50 mL of ether. The mixture was stirred and heated under reflux for 3.5 h. After dropwise addition of 15 mL of a saturated Na_2SO_4 solution while stirring and cooling in ice-water, the mixture was filtered. The filtrate was dried over anhydrous Na_2SO_4 and evaporated. The residual oil was dissolved in 20 mL of dry pyridine and 4.5 mL (0.04 mol) of pivaloyl chloride was added in one portion while stirring. After 12 h in room temperature the mixture was poured in 500 mL of ice-water. The obtained precipitate was filtered, washed with water and dried to give **9** (6.5 g, 96%); m.p. 69–70 °C. MS (CI): *m/z* (M^+ + 1) 228.. Anal. ($C_{12}H_{15}F_2NO$) H, N; C: calc. 63.42; found 62.9, 62.6.

*5.1.3. 2,6-Difluoro-4-(N-methylpivalolylamino)benzaldehyde **11***

To a stirred solution of 4.54 g (0.02 mol) of compound **10** in 40 mL of dry THF was added under nitrogen, while cooling in a solid CO_2–acetone bath, 12.5 mL of a 1.6 M solution of n-BuLi in hexane. After 0.5 h at –80 °C 4.5 mL (0.05 mol) of dry DMF in 10 mL of THF was added dropwise at the same temperature and the mixture was stirred for 1 h. The mixture was poured into dilute H_2SO_4 and ice and the aldehyde was extracted with ether. The extract was washed with water and the ether was evaporated. The obtained crude aldehyde was purified by means of the hydrogen sulfite addition compound as follows. To the residue was added 5.0 g of $NaHSO_3$ and 75 mL, of water. The mixture was stirred overnight at room temperature and extracted with ether. The water layer was separated and 10 g of K_2CO_3 was added. After stirring for 0.5 h the mixture was extracted with ether. The extract was washed with water, dried and the ether evaporated to yield 2.7 g (51%) of an oil, containing 96% (GC) of the wanted aldehyde. The oil solified when stirred with cool petroleum ether. The obtained solid aldehyde melted at 32–33 °C. MS (CI): *m/z* (M^+ + 1) 256; ^{1}H NMR ($CDCl_3$) δ 10.3 (s, 1H, CHO), 6.9 (d, 2H, $J_{H,F}$ = 9.3 Hz, Ar), 3.3 (s, 3H, CH_3), 1.2 (s, 9H, $3CH_3$).

*5.1.4. 1-(2,6-Difluoro-4-methylaminophenyl)-2-aminopropane semioxalate **2***

A mixture of 10.5 g (0.04 mol) of compound **11**, 5.3 mL (0.07 mol) of nitroethane and 5 g of ammonium acetate in 100 mL of acetic acid was heated under reflux for 1 h. The acetic acid was evaporated and after the addition of water the mixture was extracted with ether. The extract was washed with a $NaHCO_3$ solution and with water and dried over Na_2SO_4. The ether extract gave when evaporated, 12.3 g of an oil. Examination of the above residue by GC/MS indicated that the product contained 70% of the wanted β-nitrostyrene. MS (CI): *m/z* (M^+ + 1) 313.

To a stirred solution of 12.3 g of the obtained crude intermediate in 125 mL of ether was added by portions and cooling in ice 4.3 g (0.11 mol) of LAH. The mixture was stirred for 2 h. After dropwise addition of 20 mL of saturated Na_2SO_4 solution the mixture was stirred for 2 h and filtered. The filtrate was extracted with dilute aqueous HCl and the acid layer was alkalized with NaOH and extracted with ether. The extract was dried over anhydrous Na_2SO_4 and the ether was evaporated. The residual oil was dissolved in 20 mL of ethanol and added to a solution of 7.5 g (0.06 mol) of oxalic acid dihydrate in 20 mL of ethanol. Ether was added and the solution was cooled over night. The yielded oxalate salt was filtered off and dried; yield 3.0 g (30%); GC purity 98%, MS (CI): *m/e* (M^+ + 1) 201. Calc. for $C_{10}H_{14}F_2N_2$ 200.24. Recrystallization from ethanol–ether gave an analytical pure sample, m.p. 208–209 °C; ^{1}H NMR (D_2O) δ 6.3 (d, 2H, $J_{F,H}$ = 9 Hz, Ar), 3.4–3.6 (m, 1H, CH), 2.8 (d, 2H, $J_{H,H}$ =

7 Hz, CH_2), 2.6 (s, 3H, CH_3), 1.2 (d, 3H, $J_{H,H}$ = 7 Hz, α-CH_3). Anal. ($C_{10}H_{14}F_2N_2$•0.5$(COOH)_2$) C, H, N.

5.1.5. 3,5-Difluoro-N-pivaloylaniline

To a stirred solution of 5.0 g (0.039 mol) 3,5-difluoroaniline in 30 mL of dry pyridine was added 5.0 mL (0.04 mol) of pivaloyl chloride in one portion. After 2 h at room temperature the mixture was poured into 500 mL of water. The precipitate was filtered off and dried: yield 8.2 g (98%); m.p. 110–111 °C (m.p. 113.5 °C [15]) MS (CI): *m/e* (M^+ + 1) 214.

*5.1.6. 2,4-Difluoro-6-(pivaloylamino)benzaldehyde **14***

To a stirred solution of 4.26 g (0.2 mol) of 3,5-difluoro-N-pivaloylaniline in 40 mL of dry THF was added under nitrogen while cooling in a solid CO_2–acetone bath, 25 mL of a 1.6 M solution of n-BuLi in hexane. After 1 h at –80 °C, 4.0 mL of dry DMF (0.05 mol) in 10 mL of dry THF was added at the same temperature and the mixture was stirred for 1 h. The mixture was poured into dilute hydrochloric acid and ice and extracted with ether and the solvent was evaporated. To the residue was added 5.0 g of $NaHSO_4$ and 75 mL of water. The mixture was stirred overnight at room temperature and washed with ether. The water layer was separated and 10 g of K_2CO_3 was added. The mixture was stirred and cooled in an ice-bath. The formed precipitate was filtered off and yielded, after recrystallization three times from dilute ethanol, 1.0 g (21%) of the pure aldehyde, m.p. 67–68 °C. MS (CI): *m/z* (M^+ + 1) 242; ^{1}H NMR ($CDCl_3$) δ 11.8 (s, 1H, NH), 10.3 (s, 1H, CHO), 8.4 (dt, 1H, Ar), 6.6 (m, 1H, Ar), 1.2 (s, 9H, $3CH_3$); Anal. ($C_{12}H_{13}F_2NO_2$) C, H, N.

*5.1.7. N,N-Dibenzyl-3,5-difluoroaniline **15***

To a mixture of 10.0 g (0.078 mol) of 3.5-difluoroaniline, 22.0 g (0.16 mol) of K_2CO_3 and a catalytical amount of KI in 30 mL of DMF was added dropwise while stirring and heating at 100 °C, 20.7 mL (0.18 mol) of benzyl chloride. The mixture was heated overnight at 100 °C and 5 g K_2CO_3 and 5.0 mL of benzyl chloride were added. The mixture was stirred and heated for additional 6 h at 100 °C and then poured into 500 mL of an ice-water mixture. The obtained solid was filtered and recrystallized from dilute ethanol: yield 19.3 g (80%), m.p. 74–75 °C. MS (CI) *m/z* (M^+ + 1) 310; ^{1}H NMR ($CDCl_3$) δ 7.4–7.2 (m, 10H, $2C_6H_5$), 6.3–6.1 (m, 3H, Ar), 4.6 (s, 4H, $2CH_2$); Anal. ($C_{20}H_{17}F_2N$) C, H, N.

*5.1.8. 2,6-Difluoro-4-(N,N-dibenzylamino) benzaldehyde **16***

To a stirred solution of 6.2 g (0.02 mol) of compound **15** in 40 mL of dry THF under N_2 was added while cooling in a solid CO_2–acetone bath 18.0 mL of a 1.6 M n-BuLi hexane solution. After 1.5 h 4.0 mL (0.05 mol) of dry DMF was added. The mixture was stirred under cooling for 1.5 h and then poured into dilute H_2SO_4 and extracted with ether. The extract was washed with water, dried and the solvent was removed under reduced pressure. The residual semi-solid product was recrystallized from ethanol to yield 5.5 g (82%) of the desired aldehyde, m.p. 105–106 °C; ^{1}H NMR ($CDCl_3$) δ 10.2 (s, 1H, CHO), 7.4–7.1 (m, 10H, $2C_6H_5$), 6.2 (d, 2H, $J_{F,H}$ = 12.9 Hz, Ar), 4.7 (s, 4H, $2CH_2$); Anal. ($C_{21}H_{17}F_2NO$) C, H, N.

*5.1.9. 3,5-Difluoro-4-(2-nitropropen-l-yl)-N,N-dibenzylaniline **17***

A mixture of 5.3 g (0.016 mol) of compound **16**, 2.0 mL (0.028 mol) of nitroethane and 1.5 g of NH_4OAc was heated under reflux while stirring for 5 h. After cooling the obtained red precipitate was filtered off and recrystallized from ethanol to yield 4.2 g (67%) of **17**, m.p. 96–97 °C. MS (CI); *m/z* (M^+ + 1) 395; ^{1}H NMR ($CDCl_3$) δ 7.9 (s, 1H, CH), 7.4–7.2 (m, 10H, $2C_6H_5$), 6.3 (d, 2H, $J_{H,F}$ = 12.0 Hz, Ar), 4.7 (s, 4H, $2CH_2$), 2.2 (s, 3H, CH_3). Anal. ($C_{23}H_{20}F_2N_2O_2$) H, N; C: calc. 70.04; found 69.5 69.4.

*5.1.10. 1-(2,6-Difluoro-4-aminophenyl)-2-aminopropane sesquioxalate monohydrate **3***

A solution of 13.5 g (0.034 mol) of compound **17** in 200 mL of dry ether was added dropwise under N_2 to a stirred mixture of 4.5 g (0.12 mol) of LAH in 100 mL of dry ether. The mixture was stirred and heated under reflux for 4 h. After dropwise addition of 20 mL of saturated Na_2SO_4 solution while stirring and cooling in ice, the mixture was filtered. The filtrate was collected, and the solvent was evaporated under reduced pressure. The residual oil was dissolved in mixture of 25 mL of 12 M HCl and 140 mL of 50% AcOH and was hydrogenated with H_2/Pd-C (10%) at normal pressure and at approximately 50 °C. When the uptake of H_2 had stopped, the catalyst was filtered off and the filtrate was evaporated to about 50 mL and alkalized with NaOH. The mixture was extracted with ether and the extract was dried over Na_2SO_4 and the solvent was removed. The residual oil was dissolved in 100 mL of ethanol and added to a solution of 8.5 g (0.068 mol) of oxalic acid dihydrate in 100 mL of ethanol. The formed precipitation was dissolved by dropwise addition of water while stirring at reflux temperature. After cooling the yielded oxalate was filtered off and again recrystallized from dilute ethanol: yield 4.3 g (37%); m.p. > 180 °C (dec.). MS (CI): *m/z* (M^+ + 1) 187, ^{1}H NMR ($CDCl_3$) δ 6.2 (d, 2H, $J_{H,F}$ = 10 Hz, Ar), 3.1–3.2 (m, H, CH), 2.6 (dd, 1H, J_{gem} = 13 Hz,

$J_{H,H}$ = 6 Hz, CH_2), 2.5 (dd, 1H, J_{gem} = 13 Hz, $J_{H,H}$ = 8 Hz, CH_2), 1.1 (d, 3H, $J_{H,H}$ (6 Hz, CH_3). Anal. ($C_9H_{12}F_2N_2$•1.5$(COOH)_2$ H_2O) C, H, N. Anal. after drying at 140 °C ($C_{19}H_{12}F_2N_2$•1.5$(COOH)_2$) C, H, N, F.

*5.1.11. 3-Fluoro-4-(2-nitropropen-l-yl)-N-methyl-N-benzylaniline **18***

A solution of 26.0 g (0.23 mol) of 3-fluoro-aniline in 70 mL of 98% formic acid was refluxed for 2h and then poured into 500 mL of water. The obtained 3-fluoroformanilide was filtered off, washed with water and dried: yield 23.0 g (72%); m.p. 61–62 °C (m.p. 63–64 °C) [16]. The product (23.0 g, 0.16 mol) was dissolved in 100 mL of dry THF and the solution was added dropwise under N_2 while stirring to 15.2 g of LAH (0.4 mol) in 200 mL of ether. The mixture was stirred and heated under reflux for 4 h. After dropwise addition of 70 mL of saturated Na_2SO_4 solution while stirring and cooling in ice, the mixture was filtered. The filtrate was dried over Na_2SO_4 and the ether was removed. The residual, crude 3-fluoro-N-methylaniline was dissolved in 50 mL of DMF and 44.0 g of K_2CO_3 (0.32 mol) and a catalytical amount of KI was added. While stirring and heating 20.7 mL (0.18 mol) of benzyl chloride was added. The mixture was stirred overnight at 100 °C and then poured into 500 mL of water and extracted with ether. The extract was washed with water, dried with Na_2SO_4 and the ether was evaporated. The residual oil (32.0 g) was analyzed by means of GC/MS and was shown to contain 94% of 3-fluoro-N-methyl-N-benzylaniline. The crude product (32.0 g, 0.14 mol) was dissolved in 52.0 mL of DMF and 15 mL of $POCl_3$ was added dropwise while stirring and cooling with tap water. The mixture was heated for 2 h at 100 °C and poured into 500 mL of an ice-water mixture. The mixture was made basic with 10 M NaOH and extracted with ether. The extract was washed with water, dried and the ether was evaporated. GC/MS analysis of the residue (36.0 g of an oil) showed the presence of 81% of the intermediate, 4-(N-benzyl-N-methylamino)-2-fluorobenzaldehyde. The crude product (36.0 g, 0.12 mol) was dissolved in 200 mL of ethanol and 7.0 g of ammonium actate and 10.0 mL of nitroethane were added. The mixture was heated under reflux for 5 h. After cooling overnight, the obtained precipitate was filtered off and washed with ethanol yielding 27.8 g (77%) of a red compound, containing 96% (GC/MS) of the wanted nitro compound. Recrystallization from ethanol gave an analytical pure sample, m.p. 91–92 °C; MS (EI): *m/z* (M^+) 300. Anal. ($C_{17}H_{17}FN_2O_2$) C, H, N.

*5.1.12. 2-Chloro-N-[1-(4-N-methyl-N-benzylamino-2-fluorophenyl)-2-propyl]acetamide **19***

A solution of 27.6 g (0.092 mol) of compound **18** in 200 mL of dry THF was added dropwise while stirring under N_2 to 12.5 g of LAH (0.33 mol) in 200 mL of dry ether. The mixture was stirred and heated under reflux for 2 h. After dropwise addition of 60 mL of saturated Na_2SO_4 solution while stirring and cooling in ice, the mixture was filtered. The filtrate was dried over Na_2SO_4 and the ether was evaporated. The residual oil (24.8 g) was dissolved in 200 mL of toluene and a solution of 16.0 g NaOH (0.4 mol) in 100 mL of water was added. To the mixture was added dropwise while stirring and cooling in ice-water, 20 mL (0.24 mol) of chloroacetyl chloride. The mixture was stirred at 0 °C for 1 h and the toluene layer was separated, dried and the solvent was evaporated. The residual oil solified gradually upon standing. GC/MS analysis of the obtained product (24.1 g, 66%) showed the presence of 88% of the target compound **19**. Recrystallization from ethanol–isopropyl ether yielded 16.6 g (52%) of the pure amide, m.p. 85–86 °C; MS (EI): *m/z* 348. Anal. ($C_{19}H_{22}FClN_2O$) C, H, N.

*5.1.13. N'-[1-(4-N-benzyl-N-methylamino-2-fluorophenyl)-2-propyl]-N,N-dibenzylglycinamide **20***

A mixture of 16.6 g (0.047 mol) of compound **19**, a catalytic amount of KI, 9.6 mL (0.05 mol) of dibenzylamine and 6.9 g (0.05 mol) of K_2CO_3 in 25 mL of DMF was stirred for 4 h at 100 °C. To the mixture was added 1 L of water and the separated oil was extracted with ether. The ether extract was dried with Na_2SO_4 and the solvent was evaporated. The obtained oil solified gradually and showed, when analyzed, the presence of 95% of compound **20**: yield 22.9 g (96%), m.p. 85–87 °C. Recrystallization from isopropyl ether–petroleum ether gave an analytical pure sample, m.p. 87–88 °C; MS (CI: *m/z* (M^+ + 1) 510. Anal. ($C_{33}H_{36}FN_3O$) C, H, N.

*5.1.14. N'-[1-(4-Methylamino-2-fluorophenyl)-2-propyl]glycinamide acetate **7***

Pd/C (10%) was added to a solution of 10.2 g (0.02 mol) of compound **20** in 100 mL of acetic acid and hydrogenated at 50 °C and normal pressure until no more hydrogen was absorbed (3 h). The catalyst was filtered off and the solvent was evaporated under reduced pressure. The residue was dissolved in 20 mL of hot ethanol and ether was added until a weak turbidity was observed. The mixture was cooled overnight and the yielded precipitation was filtered off and washed with ether: yield 3.3 g

(55%), m.p. 112–113 °C. ^{1}H NMR (D_2O) δ 7.0–7.1 (m, 1H, Ar), 6.5–6.6 (m, 2H, Ar), 4.1–4.2 (m, 1H, CH), 3.7 (d, 1H, J_{gem} = 16 Hz, $COCH_2$), 3.6 (d, 1H, J_{gem} = 16 Hz, $COCH_2$), 2.8 (dd, 1H, J_{gem} = 14 Hz, $J_{H,H}$ = 5.5 Hz, CH_2), 2.6 (dd, 1H, J_{gem} = 14 Hz, $J_{H,H}$ = 8.2 Hz, CH_2), 2.7 (s, 3H, N–CH_3), 1.9 (s, 3H, HAc), 1.15 (d, 3H, $J_{H,H}$ = 6.6 Hz, α-CH_3); MS (EI): *m/z* (M^+) 239. Anal. ($C_{12}H_{18}FN_3O$•CH_3COOH) H; C: calc. 56.17; found 55.5, 55.2; N: calc. 14.04, found 13.5, 13.5.

5.1.15. 4-(N-Benzyl-N-methylamino)-2,6-difluorobenzaldehyde ***12***

A solution of 18.7 g (0.12 mol) of compound **9** in 100 mL of THF was added dropwise under N_2 while stirring to 11.4 g of LAH (0.3 mol) in 150 mL of ether. The mixture was stirred under reflux for 4 h. After dropwise addition of 60 mL of saturated Na_2SO_4 solution the mixture was filtered, dried with Na_2SO_4 and the ether was evaporated. The residual oil (17.0 g) was analysed by means of GC/MS and was shown to contain 98% of 2,6-difluoro-*N*-methylaniline. The product (17.0 g, 0.12 mol) was dissolved in 50 mL of DMF and 33.2 g (0.24 mol) K_2CO_3 and a catalytical amount of KI was added. While stirring and heating at 100 °C, 32.2 mL (0.28 mol) of benzyl chloride was added dropwise. The mixture was stirred overnight at 100 °C and 200 mL of $CHCl_3$ was added. After filtration the solvent was evaporated. The residual oil was dissolved in n-hexane and the solution was acidified with hydrochloric acid in ether. The precipitated hydrochloric salt was filtered off, washed with ether, suspended in 500 mL of water which was made alkaline with NaOH. The obtained oil was extracted with ether, dried with Na_2SO_4 and the ether was evaporated. The residue (23.6 g) was shown by GC/MS to contain 95% of 2,6-difluoro-*N*-benzyl-*N*-methylaniline. The oil solified gradually, m.p. 46–48 °C. To a stirred solution of 23.2 g (0.1 mol) of the obtained product in 200 mL of dry THF was added under nitrogen, while cooling in a solid CO_2–acetone bath, 90 mL of a 1.6 M solution of n-BuLi in hexane. After 1.5 h at –80 °C, 20 mL (0.15 mol) of dry DMF was added at the same temperature and the mixture was stirred for 1.5 h. The solution was poured into dilute H_2SO_4 and ice and the obtained aldehyde was filtered off and washed with water. The product, yielded after recrystallization from dilute ethanol, 22.9 g (88%) of the pure aldehyde, m.p. 83–84 °C: MS (EI): *m/z* (M^+) 261; ^{1}H NMR ($CDCl_3$) δ 10.1 (s, 1H, CHO), 7.1–7.4 (m, 5H, Ar), 6.2 (d, 2H, $J_{H,F}$ = 13 Hz, Ar), 4.6 (s, 2H, CH_2), 3.1 (s, 3H, CH_3). Anal. ($C_{15}H_{13}F_2NO$) C, H, N.

5.1.16. N'-[1-(4-N-Benzyl-N-methylamino-2,6-difluorophenyl)-2-propyl]-N,N-dibenzylglycinamide ***13***

To a solution of 22.7 g (0.087 mol) of compound **12** in 300 mL of ethanol was added 6.0 g of ammonium acetate and 8.6 mL of nitroethane. The mixture was heated under reflux for 5 h and the ethanol was removed at reduced pressure. After the addition of 500 mL of water the mixture was extracted with ether. The extract was washed with water, dried with Na_2SO_4 and the ether was evaporated. The GC/MS analysis of the residual red oil (27.1 g) showed that the product contained 92% of the intermediate nitrostyrene.

A solution of 27.1 g (0.08 mol) of the yielded oil in 150 mL of dry THF was added dropwise under N_2 while stirring to 12.0 g (0.3 mol) LAH in 200 mL of dry ether. The mixture was stirred and heated under reflux for 2 h. After dropwise addition of 60 mL of saturated Na_2SO_4 solution while stirring and cooling in ice, the mixture was filtered. The residual oil (24.2 g) was shown (GC/MS) to contain 89% of the desired amine. The product (24.1 g, 0.07 mol) was dissolved in 200 mL of toluene and a solution of 14.0 g (0.36 mol) of NaOH in 100 mL of water was added. To the mixture was added by portions while stirring and cooling in ice, 17.7 mL (0.22 mol) of chloroacetyl chloride. The mixture was stirred for 1 h and the toluene layer was separated, dried and the solvent was evaporated. The residual oil was stirred with isopropyl ether and the obtained solid was filtered. GC/MS analysis of the yielded product (19.6 g) showed the presence of 92% the intermediate amide. The compound (19.6 g, 0.05 mole) was dissolved in 25.0 mL of DMF and a catalytical amount of KI, 10.0 mL (0.053 mol) of dibenzylamine and 7.0 g of K_2CO_3 were added. The mixture was stirred for 5 h at 100 °C and then added to 1 L of water and extracted with ether. The extract was washed with water, dried and the solvent was evaporated to yield 28.0 g of an oil containing 92% (GC) of the intermediate dibenzylamine. An analytical sample, obtained by recrystallization of the crude substance from isopropyl ether–petroleum ether melted at 87–88 °C: MS (EI): *m/z* (M^+) 528; Anal. ($C_{33}H_{35}F_2N_3O$) C, H, N.

5.1.17. N'-[1-(4-Methylamino-2,6-difluorophenyl)-2-propyl]glycinamide acetate ***5***

This compound was prepared analogously to compound **7** from 27.5 g (0.048 mol) of the crude amide **13**: yield 6.6 g (43%); m.p. 106–107 °C: MS (EI): *m/z* (M^+) 257. ^{1}H NMR (D_2O) δ 6.35 (d, 2H, $J_{H,F}$ = 10.2 Hz, Ar), 4.1–4.2 (m, 1H, CH), 3.7 (d, 1H, J_{gem} = 16 Hz, $COCH_2$), 3.6 (d, 1H, J_{gem} = 16 Hz, $COCH_2$), 2.8 (dd, 1H, *J* = unres., CH_2), 2.65 (dd, 1H, *J* = unres., CH_2), 2.7 (s, 3H,

NCH_3), 1.8 (s, 3H, AcOH), 1.15 (d, 3H, $J_{H,H}$ = 6.7 Hz, α-CH_3). Anal. ($C_{12}H_{17}F_2N_3O$•CH_3COOH) C, H, N.

5.1.18. N'-[1-(4-Dimethylamino-2-fluorophenyl)-2-propyl]glycinamide oxalate ***8***

To a mixture of 1.2 g (0.004 mol) of 1-(4-dimethylamino-2-fluorophenyl)-2-aminopropane dihydrochloride, 25.0 mL of water, 50.0 mL of ether and 2.0 g NaOH (0.05 mol) was added dropwise while stirring and cooling in ice-water, 3.5 mL (0.04 mol) of chloroacetyl chloride. The mixture was stirred at 0 °C for 0.5 h and the ether layer was separated, dried and the ether was evaporated. The residual oil was dissolved in 50.0 mL of ethanol, 50.0 mL of a 25% aqueous NH_3 and left for 5 days at room temperature. The ethanol was evaporated and the residue was extracted with ether. The ether layer was dried and the solvent was evaporated. The residual oil was dissolved in 50.0 mL of hot ethanol and a solution of 1.26 g (0.01 mol) of oxalic acid dihydrate was added. The mixture was cooled overnight and the precipitate was filtered off and recrystallized from ethanol to give **8** (0.5 g, 37%): m.p. 183–184 °C. ^{1}H NMR (D_2O) δ 7.1–7.2 (m, 1H, Ar), 6.8–7.0 (m, 2H, Ar), 4.0–4.1 (m, 1H, CH), 3.6 (d, 1H, J_{gem} = 16.1 Hz, $COCH_2$), 3.5 (d, 1H, J_{gem} = 16.1 Hz, $COCH_2$), 2.9 (s, 6H, $(NCCH_3)_2$), 2.8 (d, 1H, *J* = unres., CH_2), 2.6 (d, 1H, *J* = unres., CH_2), 1.0 (d, 3H, $J_{H,H}$ = 6.8 Hz, α-CH_3); MS (EI): *m/z* (M^+) 253. Anal. ($C_{13}H_{20}FN_3O$•$(COOH)_2$) C, H, N.

5.1.19. N'-[1-(4-Amino-2-fluorophenyl)-2-propyl] glycinamide acetate ***6***

To a solution of 30.0 g (0.08 mol) of 1-(4-dibenzylamino-2-fluorophenyl)-2-aminopropane in 100.0 mL of toluene was added a solution of 13.2 g (0.33 mol) of NaOH in 100 mL of water. To the mixture was added dropwise while stirring and cooling in ice-water, 17.0 mL (0.2 mol.) of chloroacetyl chloride. The mixture was stirred at 0 °C for 1 h and the obtained precipitate was filtered off, washed with water and dried. The obtained product (21.3 g) was dissolved in 25 mL of DMF and 10.0 mL (0.053 mol) of dibenzylamine, a catalytical amount of KI and 7.0 g (0.05 mol) of K_2CO_3 were added. The mixture was stirred for 4 h at 100 °C and was then diltuted to 500 mL with water and extracted with ether. The extract was washed with water, dried with Na_2SO_4 and the solvent was evaporated. The residual oil (29.8 g) was dissolved in 100 mL of AcOH and was hydrogenated with H_2/Pd-C 10% at normal pressure and approximately 50 °C. When the uptake of hydrogen had stopped, the catalyst was filtered off and the acetic acid was evaporated at reduced pressure. The residue was dissolved in 20 mL of hot ethanol and ether was added until a weak turbidity was observed. The mixture was cooled overnight and the yielded precipitate was filtered off and washed with ether. The crude acetate salt (6.7 g), m.p. 99–102 °C, was recrystallized from THF to yield 5.0 g (35%) of the pure compound, m.p. 102–103 °C: MS (EI): *m/z* (M^+) 225. ^{1}H NMR (D_2O) δ 6.9–6.8 (m, 1H, Ar), 6.4–6.3 (m, 2H, Ar), 4.0–3.9 (m, 1H, CH), 3.5 (d, 1H, J_{gem} = 15.8 Hz, $COCH_2$), 3.4 (d, 1H, J_{gem} = 15.8 Hz, $COCH_2$), 2.6 (dd, 1H, J_{gem} = 14 Hz, $J_{H,H}$ = 5.3 Hz, CH_2), 2.4 (dd, 1H, J_{gem} = 14 Hz, $J_{H,H}$ = 8 Hz, CH_2), 1.2 (d, 3H, $J_{H,H}$ = 6.7 Hz, CH_3). Anal. ($C_{11}H_{16}FN_3O$•CH_3COOH) C, H, N.

5.1.20. 1-(2-Fluoro-4-methylaminophenyl)-2-aminopropane acetate ***4***

Compound **18** (45.9 g, 0.15 mol) was reduced with 21.0 g LAH as described for compound **17** 13.6 g (0.05 mol) of the obtained crude oil (42.6 g, purity 88%) was dissolved in 100 mL AcOH and hydrogenated similar to compound **17**. The yielded oil was dissolved in 50 mL of ethanol and ether was added until a weak turbidity was observed. After cooling the acetate salt (8.5 g, 70%) was filtered off, m.p. 148–149 °C: MS (EI): *m/z* (M^+) 182. ^{1}H NMR (D_2O) δ 7.05–7.15 (m, H, Ar), 6.5–6.6 (m, 2H, Ar), 3.5–3.6 (m, H, CH), 2.85 (d, 2H, $J_{H,H}$ = 7.1 Hz, CH_2), 2.7 (s, 3H, NCH_3), 1.9 (s, 3H, AcOH) 1.3 (d, 3H, $J_{H,H}$ = 6.7 Hz, α-CH_3). Anal. ($C_{10}H_{15}FN_2$•CH_3COOH) C, H, N.

5.1.21. N'-(S)-[1-(4-Dimethylamino-2-methylphenyl)-2-propyl]-(S)-valinamide dihydrochloride ***21***

To a stirred solution of 5.0 g (0.02 mol) of *N*-carbobenzyloxy-(*S*)-valine in 25.0 mL of dry THF was added 3.2 g (0.02 mol) of CDI. The reaction mixture was heated at reflux temperature until evolution of CO_2 ceased and 3.8 g (0.02 mol) of (*S*)-**1a** was added. The mixture was stirred at reflux temperature over night. The THF was evaporated and 200 mL of water was added. The obtained precipitate was filtered off and washed with water. Recrystallization from aqueous ethanol yielded 5.3 g of pure *N*-carbobenzyloxy-*N'*-(*S*)-[1-(4-dimethylamino-2-methylphenyl)-2-propyl]-(*S*)-valinamide; MS (EI): *m/z* (M^+) 425.

The obtained product (5.3 g) was dissolved in 100 mL of acetic acid and hydrogenated with H_2/Pd-C 10% at normal pressure and approximately 50 °C. The catalyst was filtered off and the acetic acid was evaporated at reduced pressure. The residue was dissolved in hot ethanol and acidified by the addition of hydrogen chloride in ether. To the solution was added petroleum ether (b.p. 40–60 °C) and the mixture was cooled over night. The yielded precipitation was filtered off and washed with ether: yield 2.8 g (62%), m.p. 246–248 °C: ^{1}H NMR

(D_2O) δ 7.46 (d, 1H, *J* = unres., Ar), 7.45 (d, 1H, $J_{H,H}$ = 8.1 Hz, Ar), 7.40 (d, 1H, $J_{H,H}$ = 8.1 Hz, Ar), 4.43 (m, 1H, CH), 3.67 (d, 1H, $J_{H,H}$ = 5.4 Hz, CH), 3.38 (s, 3H, CH_3), 3.28 (s, 3H, CH_3), 3.06 (dd, 1H, J_{gem} = 14.4 Hz, $J_{H,H}$ = 4.7 Hz, CH_2), 2.85 (dd, 1H, J_{gem} = 14.4 Hz, $J_{H,H}$ = 10.7 Hz, CH_2), 2.47 (s, 3H, CH_3), 1.96 (m, 1H, CH), 1.33 (d, 3H, $J_{H,H}$ = 6.6 Hz, CH_3), 0.73 (d, 3H, $J_{H,H}$ = 6.9 Hz, CH_3), 0.64 (d, 3H, $J_{H,H}$ = 6.9 Hz, CH_3); $[\alpha]^{20}$ = + 23.2 (*c* = 1, H_2O). Anal. ($C_{17}H_{29}N_3O$•2HCl) C, H, Cl, N; N: calc. 11.53; found 10.9, 10.8, 10.9.

5.1.22. N'-(S)-[1-(4-Dimethylamino-2-methylphenyl)-2-propyl]-(R)-valinamide dihydrobromide ***22***

The intermediate compound *N*-carbobenzyloxy-*N*'-(*S*)-[1-(4-dimethylamino-2-methylphenyl)-2-propyl]-(*R*)-valinamide was prepared as described for compound **21**. From 3.8 g (0.02 mol) of amiflamine and 5.0 g (0.02 mol) of carbobenzyloxy protected (*R*)-valine was thus obtained 6.2 g amide melting at 175–77 °C. The yielded compound was added by portions to 20 mL of 30% HBr in acetic acid. The mixture was stirred at room temperature for 0.5 h. Ether was added and the obtained hydrobromic salt was filtered off and recrystallized twice from ethanol–petroleum ether: yield 4.6 g (68%), m.p. 225–226 °C: MS (EI): *m/z* (M^+) 291. ^{1}H NMR (D_2O) δ 7.49 (s, 1H, Ar), 7.44 (br.m., 2H, Ar), 4.26 (m, 1H, CH), 3.76 (d, 1H, $J_{H,H}$ = 5.8 Hz, CH), 3.32 (s, 6H, $2CH_3$), 2.98 (dd, 1H, J_{gem} = 14.1 Hz, $J_{H,H}$ = 7.8 Hz, CH_2), 2.94 (dd, 1H, J_{gem} = 14.1 Hz, $J_{H,H}$ = 6.9 Hz, CH_2), 2.48 (s, 3H, CH_3), 2.25 (m, 1H, CH), 1.26 (d, 3H, $J_{H,H}$ = 6.6 Hz, CH_3), 1.06 (d, 6H, $J_{H,H}$ = 6.9 Hz, $2CH_3$); $[\alpha]^{20}$ = –4.2 (*c* = 1, H_2O). Anal. ($C_{17}H_{29}N_3O$•2HBr) C, H, Br, N.

5.2. Pharmacology

5.2.1. Animals

Male Sprague–Dawley rats (Alab-SD) weighing 160–200 g were obtained from B& K International (former Alab Laboratorietjänst AB), Sollentuna, Sweden. They were housed in plastic cages with sawdust in groups of 5 under constant temperature and lighting (6 a.m.–6 p.m.) and were allowed free access of food and water. In the in vivo experiments the animals were deprived of food on the night before the oral administration of the test compound. Phenelzine sulphate 4 mg/kg s.c. in the experiment with serotonin (5-HT) and noradrenaline (NA) and 2.5 mg/kg s.c. in the experiment with dopamine (DA) was injected 1 h after the test compound. The rats were then resupplied with food.

5.2.2. Tissue preparation in in vitro experiments

The rats were killed by decapitation and the whole brain, except the cerebellum was dissected out on ice. The mitochondria were prepared as described by Ask et al. [16].

5.2.3. Tissue preparation in ex vivo experiments

The rats were killed by decapitation 48 h after the administration of the test compounds when the effect of these were presumed to have disappeared. The hypothalami and striata were rapidly dissected out, weighed, and homogenized in 20 volumes of ice-chilled 0.25 M sucrose in small all-glass homogenizers. The homogenates were centrifuged at 800 g for 10 min and the supernatants were used in the MAO assay.

5.2.4. MAO activity in vitro

The incubation medium consisted of 50 mL of mitochondrial suspension, 100 mL of the test compound or distilled water, 820 mL of 0.11 M sodium phosphate buffer pH 7.4 and after 10 min preincubation 25 mL of the substrate, either [^{14}C]5-HT (50 μM final concentration) or [^{14}C]PEA (2.5 μM final concentration) [17]. The incubation was continued for 5 min, the reaction was stopped by the addition of 1 mL of 1 M HCl. The acid metabolites were extracted into 6 mL of ethyl acetate by vigorous shaking in a multitube vortex mixer (Model 2601, Scientific Manufactoring Industries). After centrifugation 4 mL of ethyl acetate was transferred to a counting vial containing 1 mL of ethanol and 10 mL of liquid scintillation cocktail (Ultima Gold, Packard). The radioactivity was measured in a liquid scintillation counter (LS 6000TA, Beckman).

5.2.5. MAO activity ex vivo

The deamination of [^{14}C]5-HT, [^{3}H]NA and [^{14}C]DA by the synaptosomal preparation was determined as described previously by [7]. After a 10-min preincubation of 50 mL of the synaptosome-rich supernatants in 925 mL of Krebs–Henseleit's buffer, pH 7.4, containing 5.6 mM glucose, 1.1 mM ascorbic acid, and 0.13 mM disodium edetate, the inkubation was continued for a further 10 min at 37 °C with [^{14}C]5-HT (0.1 μM) or [^{14}C]DA (0.25 μM) in the absence and presence of 0.12 μM citalopram (5-HT) or 0.1 μM GBR 12909 (DA). In the [^{3}H]NA (0.25 μM) experiments 200 mL of the hypothalamic supernatants was used in the absence and presence of maprotiline (3 μM). The deaminated products were extracted into ethyl acetate in accordance with the in vitro experiments, and the monoamine oxidase activities were calculated from the radioactivities.

The MAO inhibition inside and outside the aminergic neurons was estimated from the protection against the

irreversible action of phenelzine as described by Green and El Hait [13]. The percent inhibition produced by the reversible inhibitors was calculated according to the following formula:

$$[1 - \ln(100/t) / \ln(100/p)] \times 100$$

in which t is the MAO activity in the synaptosomes from the animals treated with the test compound + phenelzine, and p is that from the rats treated with saline + phenelzine, the activity expressed in percentage of that in the control animals. The ED_{50} values were estimated from log–response curves based on at least three doses with four rats in each dose group.

5.2.6. Determination of 5-HT, NA, DA and metabolites

The concentrations of 5-HT, 5-HIAA, NA, DA, DOPAC and HVA in hypothalamus, hippocampus and striatum were determined by use of high performance liquid chromatography with electrochemical detection according to the method of Magnusson et al. [18] The mobile phase was 0.1 M phosphate buffer (pH 2.5): methanol: acetonitrile (89:9:2 v/v) containing 1 mM octyl sulphate. The frozen samples were weighed and homogenized in 0.1 M perchloric acid, containing 2.5 mM sodium bisulphite, 1 mM ethylendiaminetetraacetic acid (EDTA) and isoprenaline as internal standard. The supernatants were injected directly onto a Supelcosil C 18 (3 μM) column, connected to a detector (ESA Coulochem 5100), set to 0.05/0.30 V.

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