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Prenylamine derivatives as blockers of the vesicular transporter for dopamine. A quantitative structure–activity study

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Summary — A new series of diphenylalkylamine congeners of prenylamine have been assayed in binding experiments on rat striatal membrane preparations. The aim was to ascertain the influence of structural modifications and lipophilicity on their interaction with the [^{3}H]tyramine-labeled vesicular transporter for dopamine. Thirteen compounds potently inhibited the specific binding of [^{3}H]tyramine, with nanomolar K_i values in the range of those of established markers for the vesicular transporter of dopamine. Less lipophilic compounds displayed higher affinity for the energy-dependent amine transporter.

prenylamine analogue / [^{3}H]tyramine displacement / vesicular transport / quantitative structure–activity relationship / lipophilicity

Introduction

The biogenic amine tyramine has been shown to associate with a high affinity binding site in striatal membrane preparations, putatively located near to, or at the vesicular transporter for dopamine [1] and in this way involves the striatal vesicular release of the same transmitter [2]. This presynaptically located site is, indeed, highly sensitive to the classical vesicular markers reserpine and tetrabenazine, and poorly affected by cocaine, a marker for the neuronal dopamine transporter. Furthermore, kinetic and pharmacological features indicate that [^{3}H]tyramine binding does not represent amine trapped in residual vesicles or resealed membranes [3]. It had been previously shown that among a number of chemically heterogeneous calcium-channel inhibitors, prenylamine and several diphenylalkylamine analogues markedly inhibit [^{3}H]-tyramine binding, showing a competitive-type antagonism at lower concentrations [4].

We have now studied a series of 60 new prenylamine derivatives, in order to assess the structural requirements for their interaction with the [^{3}H]tyramine-labeled, vesicular transporter for dopamine. Furthermore, since the affinity of an amine to the carrier-dependent vesicular transport system depends on its lipophilic character [5], a quantitative structure–activity relationship (QSAR) analysis was run between the affinity (K_i) of prenylamine derivatives for tyramine binding sites, their lipophilicity and certain structural elements.

The routes adopted to synthesize several of the various compounds have been previously described [6–9].

Materials and methods

Chemistry

All prenylamine derivatives used in this study were taken from laboratory stock. They were prepared as published previously [6–9].

Pharmacology. Binding assay of [^{3}H]tyramine

Preparation of the rat striatal membranes and displacement of [^{3}H]tyramine (Dupont-NEN, spec act 36 Ci/mmol) were basically performed as previously described [1] with minor modifications [18]. Appropriate aliquots of incubation buffer (50 mM Tris-HCl containing 120 mM NaCl, 5 mM KCl, 10 μM pargyline and 50 μM ascorbic acid) for a final volume of 1 mL were added to approx 100 μg proteins, followed by 10 μM dopamine, where required to measure non-specific binding, or the competing drugs (10 μL for DMSO-dissolved

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Table I. Prenylamine analogues: structural features, their affinity (log $1/K_i$) for the [^{3}H]tyramine-labeled vesicular transporter for dopamine, and lipophilicity (Σf).

Compound	*Structural features*								K_i[a]	Σf[b]
	X	*Z*	R_1	R_2	R_3	R_4	R_5	*n*		
p-Tyramine[c]									7.87	
VUF9063	S	CH_2	H	H	H	H	H	3	7.74	6.24
VUF9105	O	$(CH_2)_2$	H	H	H	H	H	3	7.63	7.36
VUF8931	=CH	CH_2	H	H	H	H	H	2	7.64	7.28
VUF9004	CH_2	CH_2	H	H	H	H	H	2	7.61	6.80
VUF9036	=CH	$(CH_2)_2$	H	H	H	H	H	3	7.58	7.88
Tetrabenazine[c]									7.51	
VUF8934	=CH	CH_2	H	H	H	CH_3	3-CF_3	2	7.46	8.59
VUF9037	=CH	CH_2	H	H	H	H	H	3	7.46	7.36
MPP+[c]									7.46	
VUF9048	CH_2	CH=CH	H		H	H	H	2	7.44	4.92
VUF9033	=CH	$(CH_2)_2$	H	H	H	H	H	2	7.43	7.79
VUF9064	S	$(CH_2)_2$	H	H	H	H	H	3	7.40	6.77
VUF8924	=CH	CH_2	H	H	H	CH_3	H	2	7.36	7.57
VUF8968	S	$(CH_2)_2$	4-F	4-F	H	H	H	2	7.30	6.72
VUF9027	S	CH_2	4-F	4-F	H	CH_3	H	2	7.26	6.51
Prenylamine									7.23	
VUF8947	CH_2	CH_2	H	H	H	CH_3	H	2	7.20	7.10
VUF8978	S	$(CH_2)_2$	2-F	H	H	CH_3	H	2	7.16	6.78
VUF8943	S	$(CH_2)_2$	H	H	H	H	H	2	7.15	6.25
VUF8946	CH_2	$(CH_2)_2$	H	H	H	CH_3	H	2	7.13	7.62
VUF8975	CH_2	CH=CH	H	H	H	H	H	2	7.10	7.13
VUF9035	=CH	CH=CH	H	H	H	H	H	2	7.09	7.61
VUF8901	S	CH_2	H	H	H	CH_3	H	2	7.04	6.03
VUF8933	S	CH_2	H	H	H	CH_3	3-CF_3	2	7.00	7.04
VUF8948	S	CH_2	4-F	4-F	H	CH_3	3-CF_3	2	6.99	7.52
VUF8977[d]	O	CH_2	H	H	H	CH_3	H	2	6.96	5.48
VUF9034	=CH	CH_2	H	H	H	CH_3	H	3	6.94	7.45
VUF8926	=CH	$(CH_2)_2$	H	H	H	CH_3	H	2	6.94	8.09
VUF8970	S	$(CH_2)_2$	2-CH_3	H	H	CH_3	H	2	6.92	7.06
VUF8932	S	CH=CH	H	H	H	H	H	2	6.92	6.06
VUF9060	S	CH_2	H	*n*-Bu[e]	H	H	H	2	6.91	6.10
VUF9052	S	CH_2	H	H	H	H	2-CF_3	2	6.89	6.74
VUF9001	O	CH_2	4-CH_3	H	H	H	H	2	6.88	5.70
VUF9038	S	$(CH_2)_2$	H	H	CH_3	H	H	2	6.87	6.50
VUF9061	O	CH_2	4-F	4-F	H	CH_3	3-CF_3	2	6.86	6.98
VUF8906	S	$(CH_2)_2$	H	H	H	CH_3	H	2	6.86	6.54
VUF8905	S	CH_2	4-CH_3	H	H	CH_3	H	2	6.79	6.54
VUF9067	O	$(CH_2)_2$	H	H	H	H	H	2	6.76	5.70
VUF8941	S	$(CH_2)_2$	4-F	4-F	H	CH_3	H	2	6.76	7.02
VUF9053	S	CH_2	4-F	4-F	H	H	2-CF_3	2	6.75	7.22
VUF8944	O	CH_2	H	H	H	CH_3	3-CF_3	2	6.75	6.50
VUF9148	=CH	$(CH_2)_2$	H	H	CH_3	H	H	2	6.75	8.25
VUF9047	CH_2	CH_2	H	–	H	H	H	1	6.67	4.59
VUF8929	O	CH_2	4-F	4-F	H	H	H	2	6.63	5.66
VUF9002	S	CH=CH	4-F	4-F	H	H	3-CF_3	2	6.59	7.55
VUF9147	O	$(CH_2)_2$	H	H	CH_3	H	H	2	6.57	5.96
VUF8902	O	CH_2	H	H	H	CH_3	H	2	6.56	5.48
VUF8966	S	CH=CH	4-F	4-F	H	H	H	2	6.61	6.54
VUF8967	S	CH=CH	4-Cl	4-Cl	H	H	H	2	6.57	7.51
VUF8942	S	$(CH_2)_2$	4-Cl	4-Cl	H	CH_3	H	2	6.55	8.00
VUF8940	O	$(CH_2)_2$	H	H	H	CH_3	H	2	6.55	6.00
VUF8903	O	CH_2	4-CH_3	H	H	CH_3	H	2	6.50	6.00

Table I. *Continued.*

Compound	*Structural features*								K_i[a]	Σf[b]
	X	*Z*	R_1	R_2	R_3	R_4	R_5	*n*		
VUF9003	S	CH=CH	H	H	H	H	3-CF_3	2	6.49	7.07
VUF9025	S	$(CH_2)_2$	4-F	4-F	CH_3	CH_3	H	2	6.47	7.28
VUF9062	=CH	–	H	H	H	H	H	2	6.45	5.88
VUF8976	O	CH_2	4-CH_3	4-CH_3	H	CH_3	H	2	6.43	6.52
VUF8904	O	CH_2	4-F	4-F	H	CH_3	H	2	6.40	5.96
VUF8930	O	CH=CH	4-F	4-F	H	H	H	2	6.37	5.99
VUF8928	O	$(CH_2)_2$	4-F	4-F	H	CH_3	H	2	6.35	6.48
VUF9054	S	–	H	H	H	H	H	2	6.35	5.21
VUF9068	O	$(CH_2)_2$	4-F	4-F	CH_3	CH_3	3-CF_3	2	6.27	6.94
VUF8969	SO_2	$(CH_2)_2$	H	H	H	CH_3	H	2	6.23	5.56
VUF9023	S	CH_2	4-F	4-F	CH_3	CH_3	3-CF_3	2	6.22	7.78

[a]High affinity binding was performed on rat striatal membranes using [^{3}H]tyramine (4 nM) as a ligand for the vesicular uptake transporter for dopamine. Dopamine 10 μM was used to characterize non-specific binding. Results are means of two different experiments performed in triplicate, where 100 μg proteins were incubated in the absence or presence of at least six concentrations of DMSO-solubilized prenylamine derivatives for 10 min at 37 °C. Controls contained DMSO as well. [b]Lipophilicity (Σf) was calculated by means of hydrophobic fragmental constants [19]. [c]Values taken from [2]. [d]VUF8977, the *d*-isomer of VUF8902, was left out of the QSAR. [e]The phenyl group on which R_2 is a substituent is as a whole been replaced by an *n*-butyl. This compound was therefore not discussed in the QSAR.

compounds), and [^{3}H]tyramine (4 nM). In displacement experiments at least six concentrations of each drug were used in triplicate. After incubation for 10 min at 37 °C, samples were placed on ice, and diluted with 3 mL of ice-cold 0.9% NaCl. Soon after they were filtered on glass-fiber GF/B filters, and filters were washed with 2 x 3 mL of cold saline. Control samples contained 10 μL of DMSO.

The binding parameters for competition (K_i) assays were calculated with the RADLIG (version 4) program.

Quantitative structure–activity analysis

Lipophilicity of the prenylamine derivatives was calculated using the hydrophobic fragmental constants [19]. Regression analysis (StatWorks version 1.2) was run with the purpose of evaluating the dependency of the binding affinity on lipophilicity and structural properties of the prenylamine derivatives.

Results and discussion

Pharmacology

In present experiments (table I) the inhibitory activity of 60 prenylamine derivatives, and the influence of their lipophilicity, on the high affinity binding process of [^{3}H]tyramine in a striatal preparation containing membranes of broken synaptic vesicles, as well as resealed vesicle ghosts were studied. Specifically bound radioactivity represented to a large extent the association of [^{3}H]tyramine with a site near to or at the membrane vesicular transporter for dopamine, instead of amine having entered the vesicular organelles [3]. Although phenethylamines can penetrate adrenal medulla chromaffin granules [10, 11] as well as brain synaptic vesicles [12] via a carrier-independent, reserpine- and prenylamine-insensitive lipophilic diffusion, tyramine is almost exclusively taken up into caudate synaptic organelles via a translocator energized by an ATP/Mg^{2+}-driven protonic electrochemical gradient [11, 13]. Both [^{3}H]tyramine uptake and binding processes are potently inhibited by the classical, non-transported vesicular markers reserpine and tetrabenazine [3, 13], and by the transported competitor, calmodulin antagonist prenylamine [3, 5, 14]. The latter compound and several additional calcium-channel antagonists would, in fact, affect the proton pump and bioenergetics of catecholamine storage vesicles [16, 17]. It is also conceivable that the inhibition of the inwardly directed H^+-ATPase I (the proton pump) and/or disruption of any of the components of the transmembrane proton electrochemical gradient do strongly inhibit [^{3}H]tyramine [4] and [^{3}H]reserpine [15] binding at striatal synaptic vesicles, whereas diethylpyrocarbonate and amantadine, two inhibitors of the lipophilic membrane permeation, do not [4].

QSAR

All 60 prenylamine derivatives tested consistently inhibited [^{3}H]tyramine binding with nanomolar affinity values (K_i, table I). Thirteen compounds displayed a higher affinity than the lead structure

prenylamine, with K_i's ranging from 18 to 55 nM, thus similar to those of tyramine itself, as well as of the potent vesicular markers tetrabenazine and MPP+ (table I).

Although the best derivative tested (VUF 9063) contains X = S, table I reveals that, based on K_i values of these 13 most active compounds, X = =CH or S and Z = CH_2 or $(CH_2)_2$ (fig 1) increased affinity. The correlation of all 60 prenylamine derivatives tested with their overall lipophilicity (Σf) (table I) was not significant ($r = 0.296$).

Subsequently we arranged the compounds into series and subsets according to their chemical structure, in order to rationalize the contribution of structural parameters. Lipophilicity turns out to be the most important parameter for explaining the affinity for the vesicular transporter of dopamine, although not all subsets gave an equally good correlation. All compounds were racemates, except VUF8977 (the *d*-isomer of VUF8902), which was therefore left out of all further correlation analysis.

For the subseries with X = S the following correlation equation was established, using D (dummy = 0, 1) parameters in order to indicate the contribution of certain structural elements of the prenylamine derivatives.

$$\log 1/K_i = -0.264(\pm0.071)\Sigma f + 0.329(\pm0.099)D_{Z=(CH_2)_2} + 0.271(\pm0.098)D_{Z=CH_2} - 0.362(\pm0.112)D_{R_3} + 8.469(\pm0.496) \quad [1]$$

$n = 22$; $r = 0.835$; $s = 0.171$

This gave an acceptable correlation with the lipophilicity, the contributions of the substitution of Z and R_3 are accounted for with the use of D-parameters. From equation [1], it seems clear that for optimal activity, in the sulphur-series, Z should be saturated, but methylation of the nitrogen (R_3) seems to have a negative contribution to activity. Three sulphur-containing compounds were not included here: VUF9063 and VUF9064 because of their chain length ($n = 3$) and VUF9054 was not taken into account because this compound lacks a substituent at the Z position.

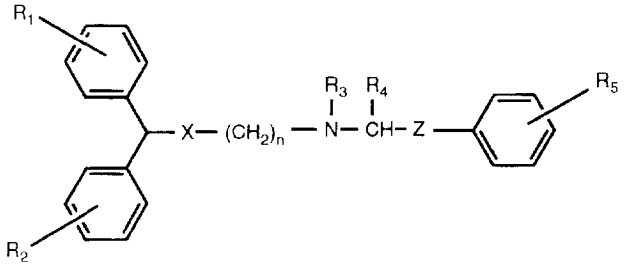

Fig 1. General structure.

For the subseries with X = O only, the following correlation equation can be found:

$$\log 1/K_i = -0.296(\pm0.068)\Sigma f + 8.359(\pm0.430) \quad [2]$$

$n = 5$; $r = 0.929$; $s = 0.083$

Although this is a good correlation, it should be pointed out that the series shows not much variation in substitution pattern and contains only compounds with Z = $(CH_2)_2$, namely VUF9067, VUF9147, VUF8940, VUF8928 and VUF9068. No other subset with even a reasonably good correlation coefficient could be found.

For the subseries with X = =CH no correlation equation is observed as all subsets are too small for studying a structure–activity relationship. The same holds for the subseries with X = CH_2.

We merged the equations [1] and [2] using D = 1 for X = O to give the following equation:

$$\log 1/K_i = -0.263(\pm0.06)\Sigma f + 0.300(\pm0.098)D_{Z=(CH_2)_2} + 0.255(\pm0.098)D_{Z=CH_2} - 0.239(\pm0.094)D_{R_3} - 0.516(\pm0.107)_{D_O} + 8.462(\pm0.456) \quad [3]$$

$n = 27$; $r = 0.848$; $s = 0.171$

From equation [3] it is clear that sulphur in position X is better for affinity than oxygen.

Using a number of different dummy parameters, we tried to obtain a series containing as many compounds as possible. The final equation was:

$$\log 1/K_i = -0.271(\pm0.061)\Sigma f + 0.284(\pm0.081)D_{Z=(CH_2)_2} + 0.253(\pm0.079)D_{Z=CH_2} - 0.255(\pm0.089)D_{R_3} - 0.560(\pm0.081)D_O + 0.517(\pm0.094)D_{=CH} + 0.459(\pm0.102)D_{CH_2} + 0.438(\pm0.103)D_{n=3} + 8.545(\pm0.428) \quad [4]$$

$n = 49$; $r = 0.907$; $s = 0.183$

This equation explains the interaction with the [^{3}H]tyramine sites in terms of lipophilicity and the contribution of the various substitution patterns to this interaction. From equation [4] we conclude that for a high activity the following aspects are important:

- a low overall lipophilicity;
- a saturated chain (Z = $(CH_2)_2$);
- a longer chain ($n = 3$) is better than $n = 2$;
- methyl substitution on R_3 has a negative effect;
- S (X) replaced by O decreases the activity and S replaced by =CH or CH_2 increases the activity; the unsaturated series seems to have the highest activities.

Three compounds (VUF9034, VUF9061 and VUF-8934) turned out to be outliers. As mentioned before VUF8977 (*d*-isomer) was not considered in this equation, as were VUF9054 and VUF9062 (Z = O, only two compounds) and VUF 8969 (X = SO_2, only one compound available).

Based on the equation [4], one could predict which type of compound would give better activity. Optimal prenylamine derivatives for interaction with the vesicular translocator of dopamine should have low lipophilicity, X should preferably be =CH (CH_2 is also good), *n* should be 3, R_3 should not be methylated, and Z should be a saturated chain (optimal Z is $(CH_2)_2$) (fig 1). Of course equation [4] does not give indications about an optimal lipophilicity ($\sum f^2$ as a parameter in the equation is not significant) or about steric hindrance. These predictions should, therefore, be made with caution.

The occurrence of a negative correlation between the affinity of prenylamine derivatives for the [^{3}H]tyramine-labeled dopamine transporter and their lipophilicity (ie, high lipophilicity = large K_i values) is consistent with a similar relationship existing between the affinity value for ATP/Mg^{2+}-dependent uptake at caudate synaptic vesicles, and the lipophilic character of a series of phenylethylamines including dopamine and tyramine (where the more lipophilic compounds had larger K_m values) [5]. This negative correlation is contrary to the one found for the relationship existing between Ca^{2+}-antagonists and calmoduline activity [17], suggesting that, in this respect, relative selective compounds would therefore seem to be possible.

In conclusion, the competitive-type antagonism on [^{3}H]tyramine binding displayed by low concentrations of prenylamine [4] and the high affinity presently shared by most of the less lipophilic prenylamine derivatives comply with their interaction at the level of the vesicular, carrier-mediated transporter system for dopamine, rather than on the lipophilic, non-carrier-mediated permeation of the membrane by [^{3}H]tyramine. The most potent inhibitors of tyramine binding studied here might represent an additional pharmacological tool for studying the vesicular transporter for monoamines.

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