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PRELIMINARY EVALUATION OF CENTRAL NERVOUS SYSTEM ACTIVITY OF (*E*)-*N*-2-METHYL-3-PHENYLPROP-2-ENYL ((*E*)-*N*- α-METHYLCINNAMYL) DERIVATIVES OF SELECTED AMINOALKANOLS

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Abstract: A series of (*E*)-α-methylcinnamyl derivatives of selected aminoalkanols was synthetized and evaluated for activity in central nervous system. All compounds were tested as anticonvulsants and one additionally in antidepressant- and anxiolytic-like assays. The compounds possessed pharmacophoric elements regarded as beneficial for anticonvulsant activity: hydrophobic unit and two hydrogen bonds donor/acceptor features. The compounds were verified in mice after intraperitoneal (*i.p.*) administration in maximal electroshock (MES) and subcutaneous pentetrazole (scPTZ) induced seizures as well as neurotoxicity assessments. Eight of the tested substances showed protection in MES test at the dose of 100 mg/kg. The derivative of 2-aminopropan-1-ol was also tested in 6-Hz test in mice *i.p.* and showed anticonvulsant activity but at the same time the neurotoxicity was noted. The derivative of 2-amino-1-phenylethanol which possessed additional hydrophobic unit in aminoalkanol moiety was tested in other *in vivo* assays to evaluate antidepressant- and anxiolytic-like activity. The compound proved beneficial properties especially as anxiolytic agent remaining active in four-plate test in mice at the dose of 2.5 mg/kg (*i.p.*). *In vitro* biotransformation studies of 2-amino-1-phenylethanol derivative carried out in mouse liver microsomal assay indicated two main metabolites as a result of aliphatic and aromatic hydroxylation or aliphatic carbonylation. To identify possible mechanism of action, we evaluated serotonin receptors (5-HT_{1A}, 5-HT_6 and 5-HT_7) binding affinities of the compounds but none of them proved to bind to any of tested receptors.

Keywords: aminoalkanol, anticonvulsant, antidepressant, anxiolytic, cinnamyl, biotransformation

Searching for new anticonvulsant agents constitutes an important issue for modern medicinal chemistry. Despite broad availability of antiepileptic drugs (AEDs) about 30% of diagnosed epileptic seizures remain resistant to pharmacotherapy by means of one or more drugs (1). Moreover, antiepileptic drugs are likely to cause several adverse effects influencing the compliance among epilepsy patient and sometimes even threating their lives (2). There are multiple features which new anticonvulsant drugs should possess to overcome currently used AEDs such as better efficacy in resistant seizures, inhibition of epileptogenesis process, possible usage in other central nervous system disorders, less number of adverse effects, better tolerability and better pharmacokinetic properties (3, 4).

In order to identify new more favorable molecules several strategies could be utilized. Screening in different animal models of seizures is a common way of searching for new anticonvulsants. Although it enables rather serendipitously discovery of active compounds, it is used not only for newly synthetized molecules but also for substances with known mechanism of action and derivatives of previously identified anticonvulsant agents (5). On the other hand, there are several ways of rational drug design of new anticonvulsants

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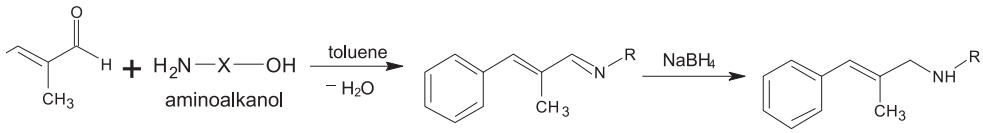
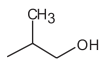
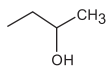
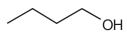
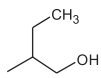
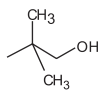
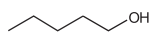
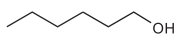
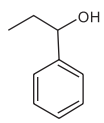
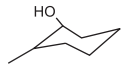
including identification of new molecular targets and design their ligands according to receptor-based pharmacophore models (6). In order to design active compound when the exact three-dimensional structure of the target is not known ligand-based pharmacophore model could be used. Investigations made in the group of antiepileptic drugs and compounds in pre-clinical development possessing anticonvulsant activity showed that some structural elements are common and may be relevant for certain activity. Those elements arranged in appropriate manner in three-dimensional structure were at least one hydrophobic unit (e.g., ring), one or two electron donor atoms, and/or an NH group. Some authors indicated the importance of so called hydrogen bonding domain, which is formed by electron donor atom in close distance to NH group (7-9).

Table 1. Synthesis and chemical structures of the tested compounds [**I-X**].



Compound	R	Base/salt	Configuration
I		base	*R*,*S*
II		base	*R*,*S*
III		HCl	-
IV		base	*R*,*S*
V		base	-
VI		HCl	-
VII		HCl	-
VIII		base	*R*,*S*
VIIIa		HCl	*R*,*S*
IX		base	*trans*, *D*,*L*
X		base	*trans*

Cinnamamide derivatives containing at least two pharmacophoric domains (hydrophobic unit and hydrogen bonding domain) proved anticonvulsant and antidepressant activity (10-12). Previously, we have also investigated *trans*-cinnamyl derivatives of aminoalkanols (13). Cinnamyl moiety ensured the presence of hydrophobic unit but the compounds were devoid of hydrogen bonding domain, however the derivatives showed moderate anticonvulsant activity in maximal electroshock test in mice after intraperitoneal administration.

In the current paper, we report a series of modified (*E*)-cinnamyl derivatives of aminoalkanols. The designed compounds possess at least four pharmacophoric domains including two hydrophobic units and two hydrogen bonds donor/acceptor features. We aimed to investigate how an additional methyl group in cinnamyl domain would influence pharmacological properties of active compounds. The compounds were tested for their anticonvulsant activity. The derivative which possessed additional hydrophobic unit in aminoalkanol moiety was additionally tested in antidepressant- and anxiolytic-like assays. We also performed *in vitro* biotransformation of that compound in liver microsomal assay. Additionally, *in silico* studies were employed to predict the major cytochrome P450 (CYP450) isoenzymes involved in its metabolism. Moreover, we investigated the affinities to serotonin 5-HT_{1A}, 5-HT_6 and 5-HT_7 receptors to identify possible mechanism of action and other possible activities in central nervous system.

EXPERIMENTAL

Chemistry

Apparatus and reagents

Reagents were manufactured by Alfa Aesar (Karlsruhe, Germany). Solvents were commercially available materials of reagent grade. Melting points (m.p.) were determined using a Büchi SMP-20 apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. Elemental analyses were performed on a Vario EI III elemental analyzer (Hanau, Germany). The ^{1}H NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 by means of a Varian Mercury-VX 300 NMR spectrometer (Varian Inc., Palo Alto, USA) with TMS or DMSO as internal standard, respectively. Results of ^{1}H NMR are presented in the following format: chemical shift δ in ppm, multiplicity, coupling constant *J* in Hertz (Hz), number of protons, protons' position. Multiplicities are showed as the abbreviations: s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), m (multiplet). The IR spectra were recorded on a Jasco FT/IR 410 spectrometer (Gross-Umstadt, Germany) using KBr pellets and are reported in cm^{-1}. The purity of obtained compounds was confirmed by the thin-layer chromatography (TLC), carried out on pre-coated aluminum sheets (silica gel 60 F-254, Merck, Darmstadt, Germany) using mixtures of chloroform/methanol (9 : 1 v/v[a]), (5 : 1, v/v[b]), methanol/ethyl acetate (1 : 1, v/v[c]). Spots were visualized by UV light. Theoretical values of the partition coefficient (LogP) of the tested compounds were calculated by means of ACDLabs 12.0 program (ACD/LogP) and on-line molinspiration tool (miLogP) (14).

Synthesis of the tested compounds

Trans-D,L-2-aminocyclohexanol was synthesized in reaction of cyclohexene oxide with 25% ammonia (15). The tested compounds were obtained in two step reaction presented in Table 1. At first, 0.02 mole (2.92 g) of α-methylcinnamaldehyde (predominantly *E,* 97%) were dissolved in 70 mL of toluene in round-bottom flask. Then, 0.02 mole of appropriate aminoalkanol was added. The reagents were heated in Dean-Stark apparatus for 2 h. Extensive water loss was observed and the reaction mixture was gradually changing color to become dark orange what indicated formation of Schiff bases. After that time, solvent was evaporated under reduced pressure. The obtained oily residues were dissolved in 40 mL of methanol. Then, a solution of sodium borohydride (0.04 mole, 1.8 g in 50 mL of water) was being added dropwise for 30 min. The reaction mixture was constantly mixed in water bath containing ice-cold water. Then, methanol was evaporated under reduced pressure. The residue was acidified by means of 10% HCl, activated carbon was added and the suspension was filtered. The filtrate was extracted with 30 mL of dichloromethane. After separation of two phases 10% NaOH was added to water phase. In case of compounds **VIII**, **IX**, and **X**, a precipitate was observed, which was filtered, dried and crystalized from *n*-hexane. For other compounds, mixtures were extracted with two portions of dichloromethane (50 mL + 30 mL), extracts were combined together and dried over anhydrous Na_2SO_4. Then, the solvent was evaporated under reduced pressure. The residues were crystallized with *n*-hexane yielding the solids of compounds **I**, **II**, **IV** and **V**. In case of compounds **III**, **VI** and **VII** the crystallization failed and oily residues were converted into hydrochlorides in ethyl acetate with an excess of ethanol saturated with HCl. Compound **VIII** was also converted into hydrochloride in similar conditions yielding compound **VIIIa**

which was easily soluble in water. All compounds were recrystallized, bases from *n*-hexane, hydrochlorides from acetone.

Physicochemical properties of the compounds

***R,S*-2-{[(2*E*)-2-methyl-3-phenylprop-2-en-1-yl]amino}propan-1-ol [I]**

White solid (yield 68%), m.p. 69-71°C. M.w. = 205.30. IR (KBr, cm^{-1}): 3319, 3103, 2964, 2888, 1492, 1444, 1363, 1052, 902, 701. ^{1}H-NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.12 (d, *J* = 6.4 Hz, 3H, CH-C$\underline{H}_3$), 1.93 (d, *J* = 1.4 Hz, 3H, C-CH_3), 2.22 (bs, 2H, OH, NH), 2.81-2.93 (m, 1H, C$\underline{H}$(CH_3)-CH_2), 3.26-3.35 (m, 2H, C$\underline{H}$H-OH, C$\underline{H}$H-NH), 3.45 (dd, *J* = 13.6 Hz, *J* = 1.2 Hz, 1H, CH$\underline{H}$-NH), 3.63 (dd, *J* = 10.6 Hz, *J* = 4.0 Hz, 1H, CH$\underline{H}$-OH), 6.47 (s, 1H, Ar-CH=), 7.17-7.37 (m, 5H, Ar-H R_f = 0.31[a]; 0.15[b]; 0.31[c]; miLogP = 2.694; ACD/LogP = 1.70 ± 0.33. Analysis: calcd. for $C_{13}H_{19}NO$: C, 76.06; H, 9.33; N, 6.82%; found: C, 75.75; H, 9.49; N, 6.79%.

***R,S*-1-{[(2*E*)-2-methyl-3-phenylprop-2-en-1-yl]amino}propan-2-ol [II]**

White solid (yield 71%), m.p. 52-54°C. M.w. = 205.30. IR (KBr, cm^{-1}): 3421, 3309, 3083, 2959, 2931, 1445, 750, 698. ^{1}H-NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.17 (d, *J* = 6.2 Hz, 3H, CH-C$\underline{H}_3$), 1.91 (d, *J* = 1.3 Hz, 3H, C-CH_3), 2.41-2.48 (m, 3H, OH, NH, NH-CH$\underline{H}$), 2.75 (dd, *J* = 3.1 Hz, *J* = 9.0 Hz, 1H, NH-C$\underline{H}$H), 3.29-3.40 (m, 2H, C$\underline{H}_2$-NH-CH_2), 3.80-3.85 (m, 1H, CH_2-C$\underline{H}$(OH)), 6.44 (s, 1H, Ar-CH=), 7.18-7.36 (m, 5H, Ar-H). R_f = 0.37[a]; 0.23[b]; 0.26[c]; miLogP = 2.377; ACD/LogP = 1.70 ± 0.33. Analysis: calcd. for $C_{13}H_{19}NO$: C, 76.06; H, 9.33; N, 6.82%; found: C, 75.88; H, 9.55; N, 6.77%.

3-{[(2*E*)-2-methyl-3-phenylprop-2-en-1-yl]amino}propan-1-ol hydrochloride [III]

White solid (yield 63%), m.p. 110-112°C. M.w. = 241.76. IR (KBr, cm^{-1}): 3423, 2947, 2797, 1446, 1061, 701. ^{1}H-NMR (DMSO-d_6, 300 MHz, δ, ppm): 1.78-1.86 (m, 2H, CH_2-C$\underline{H}_2$-CH_2), 1.93 (s, 3H, C-CH_3), 2.95 (bs, 2H, NH-C$\underline{H}_2$), 3.48 (t, *J* = 5.9 Hz, 2H, CH_2-OH), 3.65 (bs, 2H, C$\underline{H}_2$-NH), 4.78 (bs, 1H, OH), 6.68 (s, 1H, Ar-CH=), 7.20-7.42 (m, 5H, Ar-H), 9.0 (bs, 2H, NHH^+). R_f = 0.57[a]; 0.14[b]; 0.18[c]; miLogP = 2.284; ACD/LogP = 1.41 ± 0.29. Analysis: calcd. for $C_{13}H_{20}NOCl$: C, 64.59; H, 8.34; N, 5.79%; found: C, 64.27; H, 8.06; N, 5.68%.

***R,S*-2-{[(2*E*)-2-methyl-3-phenylprop-2-en-1-yl]amino}butan-1-ol [IV]**

White solid (yield 71%), m.p. 48-50°C. M.w. = 219.33. IR (KBr, cm^{-1}): 3324, 3163, 2965, 2931, 1454, 1050, 761, 702. ^{1}H-NMR ($CDCl_3$, 300 MHz, δ, ppm): 0.95 (t, *J* = 7.6 Hz, 3H, CH_2-C$\underline{H}_3$), 1.39-1.66 (m, 2H, CH-C$\underline{H}_2$-CH_3), 1.93 (d, *J* = 1.3 Hz, 3H, C-CH_3), 2.21 (bs, 2H, OH, NH), 2.60-2.69 (m, 1H, NH-C$\underline{H}$), 3.29 (dd, *J* = 13.7 Hz, *J* = 1.0, 1H, C$\underline{H}$H-NH), 3.37 (dd, *J* = 10.8 Hz, *J* = 4.2 Hz, 1H, C$\underline{H}$H-OH), 3.42 (dd *J* = 13.5 Hz, *J* = 1.2 Hz, 1H, CH$\underline{H}$-NH) 3.67 (dd, *J* = 10.5 Hz, *J* = 4.0 Hz, 1H, CH$\underline{H}$-OH), 6.46 (s, 1H, Ar-CH=), 7.18-7.37 (m, 5H, Ar-H). R_f = 0.41[a]; 0.30[b]; 0.39[c]; miLogP = 3.23; ACD/LogP = 2.23 ± 0.33. Analysis: calcd. for $C_{14}H_{21}NO$: C, 76.67; H, 9.65; N, 6.39%; found: C, 76.55; H, 9.81; N, 6.39%.

2-Methyl-2-{[(2*E*)-2-methyl-3-phenylprop-2-en-1-yl]amino}propan-1-ol [V]

White solid (yield 75%), m.p. 47-49°C. M.w. = 219.33. IR (KBr, cm^{-1}): 3293, 3161, 2960, 2869, 1489, 1477, 1446, 1069, 694. ^{1}H-NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.13 (s, 6H, $C(CH_3)_2$), 1.91 (s, 3H, C-CH_3), 3.21 (s, 2H, C$\underline{H}_2$-NH), 3.33 (s, 2H, C$\underline{H}_2$-OH), 6.47 (s, 1H, Ar-CH=), 7.20-7.35 (m, 5H, Ar-H). R_f = 0.23[a]; 0.20[b]; 0.24[c]; miLogP = 3.174; ACD/LogP = 2.05 ± 0.34. Analysis: calcd. for $C_{14}H_{21}NO$: C, 76.67; H, 9.65; N, 6.39%; found: C, 76.41; H, 9.35; N, 6.28%.

4-{[(2*E*)-2-methyl-3-phenylprop-2-en-1-yl]amino}butan-1-ol hydrochloride [VI]

White solid (yield 69%), m.p. 126-128°C. M.w. = 255.79. IR (KBr, cm^{-1}): 3407, 2940, 2797, 1436, 1036, 701. ^{1}H-NMR (DMSO-d_6, 300 MHz, δ, ppm): 1.42-1.50 (m, 2H, NH-CH_2-C$\underline{H}_2$-CH_2-CH_2-OH), 1.67-1.77 (m, 2H, NH-CH_2-CH_2-C$\underline{H}_2$-CH_2-OH), 1.98 (s, 3H, C-CH_3), 2.88 (t, *J* = 7.8 Hz, 2H, NH-C$\underline{H}_2$-CH_2-CH_2-CH_2-OH), 3.41 (t, *J* = 6.2 Hz, 2H, NH-CH_2-CH_2-CH_2-C$\underline{H}_2$-OH), 3.62 (s, 2H, C$\underline{H}_2$-NH), 4.60 (bs, 1H, OH), 6.66 (s, 1H, Ar-CH=), 7.24-7.40 (m, 5H, Ar-H), 9.07 (bs, 2H, NHH^+). R_f = 0.28[a]; 0.15[b]; 0.20[c]; miLogP = 2.555; ACD/LogP = 1.71 ± 0.28. Analysis: calcd. for $C_{14}H_{22}NOCl$: C, 65.74; H, 8.67; N, 5.48%; found: C, 65.42; H, 8.60; N, 5.43%.

5-{[(2*E*)-2-methyl-3-phenylprop-2-en-1-yl]amino}pentan-1-ol hydrochloride [VII]

White solid (yield 65%), m.p. 129-131°C. M.w. = 269.81. IR (KBr, cm^{-1}): 3420, 2937, 2796, 1447, 1435, 1058, 1027, 701. ^{1}H-NMR (DMSO-d_6, 300 MHz, δ, ppm): 1.28-1.47 (m, 4H, -CH_2-CH_2-), 1.62-1.72 (m, 2H, C$\underline{H}_2$-CH_2-OH), 1.94 (s, 3H, C-CH_3), 2.86 (t, *J* = 7.6 Hz, 2H, NH-C$\underline{H}_2$), 3.35-3.41 (m, 2H, C$\underline{H}_2$-OH), 3.63 (s, 2H, C$\underline{H}_2$-NH), 4.42 (t, *J* = 5.1 Hz, 1H, OH), 6.66 (s, 1H, Ar-CH=), 7.25-

7.41 (m, 5H, Ar-H), 8.99 (bs, 2H, NHH^+). R_f = 0.39[a]; 0.12[b]; 0.19[c]; miLogP = 3.06; ACD/LogP = 1.94 ± 0.28. Analysis: calcd. for $C_{15}H_{24}NOCl$: C, 66.78; H, 8.97; N, 5.19%; found: C, 66.51; H, 8.96; N, 5.17%.

***R,S*-2-{[(2*E*)-2-methyl-3-phenylprop-2-en-1-yl]amino}-1-phenylethanol [VIII]**

White solid (yield 78%), m.p. 78-80°C. M.w. = 267.36. IR (KBr, cm^{-1}): 3297, 3049, 3029, 2992, 1557, 1156, 618. ^{1}H-NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.91 (s, 3H, C-CH_3), 2.76 (dd, *J* = 8.7 Hz, *J* = 12.2 Hz, 1H, NH-CHH), 2.96 (dd, *J* = 3.6 Hz, *J* = 12.3 Hz, 1H, NH-CHH), 3.38 (s, 2H, CH_2-NH), 4.75 (dd, *J* = 3.6 Hz, *J* = 8.9 Hz, Ar-CH-OH), 6.43 (s, 1H, Ar-CH=), 7.18-7.41 (m, 10H, Ar-H). R_f = 0.64[a]; 0.48[b]; 0.56[c]; miLogP = 3.459; ACD/LogP = 3.05 ± 0.39. Analysis: calcd. for $C_{18}H_{21}NO$: C, 80.86; H, 7.92; N, 5.24%; found: C, 81.20; H, 7.94; N, 5.24%.

***R,S*-2-{[(2*E*)-2-methyl-3-phenylprop-2-en-1-yl]amino}-1-phenylethanol hydrochloride [VIIIa]**

White solid (yield 65%), m.p. 199-201°C. M.w. = 303.83.

***D,L-trans*-2-{[(2*E*)-2-methyl-3-phenylprop-2-en-1-yl]amino}cyclohexanol [IX]**

White solid (yield 80%), m.p. 65-67°C. M.w. = 245.37. IR (KBr, cm^{-1}): 3309, 3160, 2942, 2855, 1448, 1331, 1107, 1062, 895, 746. ^{1}H-NMR ($CDCl_3$, 300 MHz, δ, ppm): 0.98-2.35 (m, 9H, cyclohex.), 1.94 (s, 3H, C-CH_3), 3.20-3.30 (m, 1H, CH-OH), 3.26 (d, *J* = 13.0 Hz, 1H, CHH-NH), 3.52 (d, *J* = 13.0 Hz, 1H, CHH-NH), 6.46 (s, 1H, Ar-CH=), 7.18-7.36 (m, 5H, Ar-H). R_f = 0.42[a]; 0.37[b]; 0.44[c]; miLogP = 3.751; ACD/LogP = 2.35 ± 0.30. Analysis: calcd. for $C_{16}H_{23}NO$: C, 78.32; H, 9.45; N, 5.71%; found: C, 77.92; H, 9.63; N, 5.68%.

***trans*-4-{[(2*E*)-2-methyl-3-phenylprop-2-en-1-yl]amino}cyclohexanol [X]**

White solid (yield 82%), m.p. 104-106°C. M.w. = 245.37. IR (KBr, cm^{-1}): 3259, 3246, 3098, 2930, 2860, 1374, 1106, 1070, 745, 701. ^{1}H-NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.14-1.36 (m, 4H, cyclohex.), 1.45 (bs, 2H, NH, OH), 1.89 (s, 3H, C-CH_3), 1.93-2.01 (m, 4H, cyclohex.), 2.45-2.54 (m, 1H, NH-CH), 3.41 (s, 2H, CH_2-NH), 3.57-3.68 (m, 1H, CH-OH), 6.43 (s, 1H, Ar-CH=), 7.17-7.36 (m, 5H, Ar-H). R_f = 0.40[a]; 0.16[b]; 0.19[c]; miLogP = 3.282; ACD/LogP = 2.17 ± 0.29. Analysis: calcd. for $C_{16}H_{23}NO$: C, 78.32; H, 9.45; N, 5.71%; found: C, 78.21; H, 9.66; N, 5.69%.

Pharmacology

Anticonvulsant activity assays

Pharmacological tests concerning anticonvulsant activity were performed at Epilepsy Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health (NIH, Bethesda, USA) within Anticonvulsant Screening Program (ASP). Pharmacological evaluation was accepted by Institutional Animal Care and Use Committee. All procedures were previously published (16-18) as well as they are available for public at NIH website (19). In the tests, albino mice Carworth Farms No.1 or rats Sprague-Dawley were used. The tested compounds were dissolved or suspended in 0.5% methylcellulose. Brief descriptions of the experiments are presented below.

MES test

Seizures were induced by delivering for 0.2 s alternating current at 60 Hz and 50 mA (mice) or 150 mA (rats) *via* corneal electrodes. Protection was defined as the abolition of the hindlimb tonic extension of the seizures.

scPTZ test

Seizures were elicited by subcutaneous injection of pentetrazol dissolved in 0.9% NaCl at the dose of 85 mg/kg (mice) or 70 mg/kg (rats). Protection was scored if there was not observed episode of clonic spasm of at least 5 s.

Neurotoxicity (TOX)

In mice, the neurological deficit was measured by rotarod test in which a one inch diameter knurled plastic rod rotating at 6 rpm was used. Each mouse was placed on the rod. If it was not able to maintain on the rod for at least 1 min in each of three trials then neurotoxicity was scored. In rats, neurological deficit was measured in behavioral tests and scored when observed ataxia, loss of placing response and muscle tone.

6-Hz test

Seizures were induced in mice by delivering 0.2 ms pulses of electric current at 6 Hz and 32 mA for 3 s *via* corneal electrodes. The mouse was released into an observation cage immediately after stimulation. In untreated animals the duration of the seizure activity ranges between 60 and 120 s and afterwards they resume their exploratory behavior. When the compounds were tested, the experiment endpoint was protection against the seizures. The protection was scored if the mouse resumed its normal activity within 10 s from the stimulation.

Behavioral studies

Pharmacological tests concerning behavioral studies were performed at Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College. In each experiment adult male CD-1 mice (18-21 g) were used. Mice were kept in plastic cages at room temperature (22 ± 2°C), on 12/12 h light/dark cycle. The animals had free access to chow and water. All experiments were carried out between 9 a.m. and 2 p.m. Each experimental group consisted of 10 randomly selected animals. Mice were used only once in each test and killed by cervical dislocation immediately after the experiment. Behavioral studies were fully accepted by the First Local Ethics Committee on Animal Testing at the Jagiellonian University in Krakow and carried out in accordance with the Guide to the Care and Use of the Experimental Animals.

Forced swim test in mice

The experiment was carried out according to the method of Porsolt et al. (20). The CD-1 mice were individually placed in a glass cylinder (height: 25 cm; diameter: 10 cm) containing 10 cm of water (temperature: 23–25°C), and were left there for 6 min. A mouse was regarded as immobile when it remained floating on the water, making only small movements to keep its head above it. The total duration of immobility was recorded during the last 4 min of a 6-min test session.

Four-plate test in mice

The four-plate apparatus (BIOSEB, Vitrolles) consisted of a plastic cage floored by four identical rectangular metal plates separated from one another by a gap, and at the top covered by a transparent Perspex lid that prevents escape behavior. The plates were connected to a device that can generate electric shocks. After a 15 s habituation period, every time the CD-1 mouse moved from one plate to another it was punished with an electric foot shock (0.8 mA, 0.5 s), which was followed by a 3 s shock interval (21). The number of punished crossings received by mice was recorded during 1 min session.

Spontaneous locomotor activity in mice

The locomotor activity in mice was measured with photoresistor actometers (Ugo Basile) connected to a counter for the recording of light-beam interruptions. The CD-1 mice were individually placed in plastic cages for 30 min habituation period, and then the number of light-beam crossings was counted for 1 min or from 2nd to 6th min (time equal to the observation period in the four-plate test or the forced swim test, respectively).

Biotransformation studies

***In vitro* mouse liver microsomal biotransformation**

The incubation systems were composed of **VIIIa** (20 μM in 100 mM potassium phosphate buffer, pH 7.4), microsomes (0.8 mg/mL, mouse liver microsomes), NADPH-regenerating system (NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase in 100 mM potassium phosphate buffer, pH 7.4), and potassium phosphate buffer (100 mM, pH 7.4). Firstly, the mixture reactions composed of microsomes, the tested compound and a buffer were preincubated at 37°C for 15 min before the addition of the NADPH-regenerating system. The resulting mixture was incubated for 15 or 30 min at 37°C in a thermoblock and shaken during the incubation. Then, an internal standard (levallorphan, 20 μM) was added to the incubation system. The incubation was terminated at different time points with perchloric acid (69-72%, by volume). Thereafter, the samples were centrifuged and the supernatant was analyzed using UPLC/MS in order to determine the specific metabolic profile of **VIIIa**. The tests without NADPH-regenerating system were conducted in parallel. All samples were prepared in duplicate (22, 23). Liver microsomal fractions, NADPH regenerating system components and levallorphan were obtained from Sigma Aldrich (Poznań, Poland).

The UPLC-MS/MS system was composed of Waters ACQUITY® UPLC® (Waters Corporation, Milford, MA, USA) coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). Chromatographic separations were done with an Acquity UPLC BEH (bridged ethyl hybrid) C_{18} column; 2.1 × 100 mm, and 1.7 μm particle size. The column was maintained at 40°C, and eluted under gradient conditions using from 95% to 0% of eluent A over 10 min, at a flow rate of 0.3 mL/min. Eluent A: water/formic acid (0.1%, v/v). 10 μL of each sample were injected. Chromatograms were recorded using a Waters λ PDA detector. Spectra were analyzed in 200-700 nm range with 1.2 nm resolution and a sampling rate 20 points/s.

MS detection settings of the Waters TQD mass spectrometer were as follows: source temperature 150°C, desolvation temperature 350°C, desolvation gas flow rate 600 L/h, cone gas flow 100 L/h, capillary potential 3.00 kV, cone potential 20 V. Nitrogen was used as both nebulizing and drying gas. The data

were obtained in a scan mode ranking from 50 to 1000 m/z in 0.5 s time intervals; 8 scans were summed up to get the final spectrum. Collision activated dissociation (CAD) analyses were carried out with energy of 20 eV, and all the fragmentations were observed in the source. The ion spectra were obtained by scanning from 50 to 500 m/z range. The data acquisition software was MassLynx V 4.1 (Waters).

In silico prediction of metabolism

MetaSite, Molecular Discovery Ltd., v. 5.0.3 is a computational model that enables the prediction of CYP450-dependent metabolism in phase I biotransformations. The software indicates the atoms in a molecule structure that are mostly vulnerable to metabolic changes with cytochromes. Validation procedures have proved that in 85% of the analyzed structures the indicated metabolic sites agreed in top three rankings with experimental data (24, 25). Additionally, MetaSite predicts the chemical structures of the most probable metabolites and enables the identification of cytochrome isoform(s) responsible for the metabolism of substrates (26). The probability of a specific atom *i* to be a site of metabolism catalyzed by the heme moiety of CYP450 depends on several factors, such as the accessibility of atom *i* towards the heme (indicated as E*i*); the chemical reactivity of atom *i* in the specific reaction mechanism (described as R*i*) and the relative probability of a reaction mechanism under consideration occurring (called M*i*). Thus, the probability function (P_{SMi}) for a specific atom *i* can be defined by the following equation (24, 25):

$$P_{SMi} = Ei \times Ri \times Mi$$

Serotonin 5-HT receptors binding assays

Cell culture and preparation of cell membranes

HEK293 cells with stable expression of human serotonin 5-HT_{1A}R, 5-HT_6 or 5-HT_{7b}R (prepared with the use of Lipofectamine 2000) were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were grown in Dulbecco's modified Eagle's medium containing 10% dialyzed fetal bovine serum and 500 μg/mL G418 sulfate. For membranes preparations, cells were subcultured in 10 cm diameter dishes, grown to 90% confluence, washed twice with prewarmed to 37°C phosphate buffered saline (PBS) and were pelleted by centrifugation (200 × g) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparations pellets were stored at –80°C.

Radioligand binding assays

Cell pellets were thawed and homogenized in 20 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35 000 × g for 20 min at 4°C, with incubation for 15 min at 37°C in between. The composition of the assay buffers was as follows: for 5-HT_{1A}R: 50 mM Tris–HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate; for 5-HT_6R: 50 mM Tris–HCl, 0.5 mM EDTA and 4 mM $MgCl_2$, for 5-HT_{7b}R: 50 mM Tris–HCl, 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate.

All assays were incubated in a total volume of 200 μL in 96-well microtitre plates for 1 h at 37°C, except for 5-HT_{1A}R which were incubated at room temperature for 1 h. The process of equilibration is terminated by rapid filtration through Unifilter plates with a 96-well cell harvester and radioactivity retained on the filters was quantified on a Microbeta plate reader. For displacement studies the assay samples contained as radioligands: 1.5 nM [^{3}H]-8-OH-DPAT (187 Ci/mmol) for 5-HT_{1A}R; 2 nM [^{3}H]-LSD (85.2 Ci/mmol for 5-HT_6R or 0.6 nM [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT_7R. Non-specific binding was defined with 10 μM of 5-HT in 5-HT_{1A}R and 5-HT_7R binding experiments, whereas 10 μM methiothepine was used in 5-HT_6R assays.

Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M). The inhibition constants (K_i) were calculated from the Cheng-Prusoff equation (27). Results were expressed as means of at least two separate experiments. Membrane preparation and general assay procedures for cloned receptors were adjusted to 96-microwell format based on protocols described by us previously (28, 29).

RESULTS

Chemistry

Synthetic route and structures of the tested compounds are shown in Table 1. Compounds were obtained in simple two-steps procedure consisting of condensation of (*E*)-α-methylcinnamaldehyde and appropriate aminoalkanol followed by reduction of obtained Schiff bases by means of sodium borohydride.

The search in SciFinder database indicated that some of the structures are already known, anyway, CAS numbers are not available for single *E* enantiomers but for compounds with undefined stereoisomeric function. For **I** (CAS: 1603924-12-4), **II** (CAS: 1563930-71-1), and **VII** (CAS: 1562359-97-0 for base) only calculated physicochemical data are available but there are neither references nor biological activity results available. For **III** (CAS: 722551-27-1 for base, CAS: 1375800-64-8 for enantiomer *E* as base) method of synthesis was

published (30) but there is no information about biological activity.

All presented compounds did not show any violations from Lipinski's "rule of five" (31) making them potentially promising drug-like agents. The partition coefficient (log P) ranged from 1.41 ± 0.29 to 3.05 ± 0.39 when calculated by means of ACDLabs and from 2.284 to 3.751 when calculated by means of molinspiration on-line tool (for details see Experimental section).

Anticonvulsant activity

Anticonvulsant activity and neurotoxicity were evaluated within Anticonvulsant Screening Program

Table 2. Anticonvulsant activity of the tested compounds in mice after *i.p.* administration.

Compound	Dose [mg/kg b.w.]	MES[a]		scPTZ[a]		TOX[b]	
		0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
I	3	0/4	-	-	-	0/4	-
	10	0/4	-	-	-	0/4	-
	30	**1/1**	0/1	0/1	0/1	0/4	0/2
	100	**1/3**	0/3	0/1	0/1	0/8	0/4
	300	-	-	-	-	**4/4(4)**	-
II	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	**3/3**	0/3	0/1	0/1	**4/8(1)**	0/4
	300	-	-	-	-	**4/4(4)**	-
III	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	-	-	0/1	-	**3/4(3)**	-
IV	30	0/1	0/1	0/1	0/1	1/4	0/2
	100	**1/3**	**1/3**	-	0/1	**1/8(1)**	0/4
	300	**1/1**	-	-	-	**3/4(3)**	-
V	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	**3/3**	0/3	0/1	0/1	0/8	0/4
	300	**1/1**	-	0/1	0/1	**4/4(1)**	0/1
VII	30	01	0/1	0/1	0/1	0/4	0/2
	100	**2/3**	0/3	0/1	0/1	**3/8**	0/4
	300	-	-	-	-	**4/4(4)**	-
VIII	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	**2/2**	0/1	0/1	-	**8/8(4)**	**1/2(1)**
	300	-	-	-	-	**4/4(4)**	-
IX	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	**2/2**	0/3	-	-	**6/8(3)**	0/3
	300	-	-	-	-	**4/4(4)**	-
X	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	**3/3**	0/3	0/1	0/1	**4/8**	0/4
	300	-	-	-	-	**4/4(4)**	-

[a] Number of animals protected/number of animals used in the MES or scPTZ test; [b] Number of animals displaying neurotoxicity/number of animals used in the rotarod test, number in parentheses indicates number of observed deaths; - the compound was not tested in this particular case.

Table 3. Anticonvulsant activity in 6-Hz test of compounds **I** and **VI** (mice, *i.p.*, 32 mA).

Compound	Test	Dose [mg/kg b.w.]	Time [h]				
			0.25	0.5	1.0	2.0	4.0
I	6-Hz[a]	100	**4/4**	**3/3**	**1/3**	0/3	0/4
	TOX[b]	100	**4/4**	**2/3**	**1/4(1)**	**1/4(1)**	0/4
VI	6-Hz[a]	50	0/4	0/4	0/4	0/4	0/4
	TOX[b]	50	0/4	0/4	0/4	0/4	0/4

[a] Number of animals protected/number of animals tested in the 6-Hz test; [b] Number of animals displaying neurotoxicity/number of animals used in the rotarod test, number in parentheses indicates deaths.

Table 4. Antidepressant-like activity of compound **VIIIa** in the forced swim test in mice.

Compound	Dose [mg/kg b.w.]	Immobility time Mean ± SEM [s]
Vehicle	-	166.9 ± 13.1
VIIIa	10 20 30	155.9 ± 14.9 94.3 ± 13.3** 111.3 ± 16.1*
Vehicle	-	165.2 ± 11.7
Mianserin	5 10	136.3 ± 10.8 125.9 ± 10.8*

Compound **VIIIa** and mianserin were dissolved in 0.9% NaCl, and administered *i.p.* 30 min before the test. The control groups received 0.9% NaCl. Statistical analysis: one-way ANOVA (Newman-Keuls *post hoc* test) *p < 0.05, **p < 0.01 *vs.* respective vehicle-treated group; n = 10 mice per group.

Table 5. Anxiolytic-like activity of compound **VIIIa** in the four-plate test in mice.

Compound	Dose [mg/kg b.w.]	Number of punished crossings Mean ± SEM
Vehicle	-	3.2 ± 0.3
VIIIa	1.25 2.5 5 10 20 30	4.0 ± 0.4 5.0 ± 0.5* 5.5 ± 0.4** 5.4 ± 0.4** 4.9 ± 0.4* 7.3 ± 0.4****
Vehicle	-	3.1 ± 0.4
Medazepam	1 2	4.3 ± 0.4 6.5 ± 0.7***

Compound **VIIIa** and medazepam were dissolved in 0.9% NaCl, and administered *i.p.* 30 and 60 min before the test, respectively. The control groups received 0.9% NaCl. Statistical analysis: one-way ANOVA (Newman-Keuls *post hoc* test) *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 *vs.* respective vehicle-treated group; n = 10 mice per group.

(ASP) at National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, USA (19). Nine compounds [**I**-**V**, **VII**-**X**] were subjected to standard procedures including three tests: maximal electroshock (MES) and subcutaneous pentetrazole (scPTZ) tests for anticonvulsant activity as well as neurotoxicity evaluation by means of rotarod test. The evaluations were performed in mice after 0.5 and 4 h of intraperitoneal (*i.p.*) administration of the compounds. Compound **VI** was qualified to modified protocol involving prior evaluation in 6-Hz test for anti-seizure activity and rotarod for neurotoxicity while MES and scPTZ tests were omitted for it. Compound **I** was tested in 6-Hz after successful screen in MES. In addition, compounds **I**, **IV** and **VII** were evaluated in rats after oral administration.

Most of the compounds proved their activity in mice after *i.p.* administration in MES, especially at 100 mg/kg, compound **I** additionally at 30 mg/kg. The compounds were not active in scPTZ model. Among the series relatively high neurotoxicity was observed, both at 300 mg/kg and 100 mg/kg (Table 2). Compound **I** was chosen for evaluation in 6-Hz test in mice and proved beneficial protective activity at the dose of 100 mg/kg. The activity was accompanied by neurotoxicity at the same time points and dose. Compound **VI** did not show neither activity nor neurotoxicity in this model (Table 3). Compounds **I**, **IV** and **VII** were tested in rats after oral administration but none of them proved any beneficial properties (data not shown).

Antidepressant-like activity of compound VIIIa in mice

Compound **VIIIa** at the doses 20 and 30 mg/kg (but not 10 mg/kg) significantly decreased immobil-

ity in mice by 43.5% and 33.3%, respectively ($F(3,36) = 5.866$, $p < 0.01$). Mianserin, used as a reference drug, administered at the dose 10 mg/kg (but not 5 mg/kg) statistically decreased immobility in mice by 23.8% ($F(2,27) = 3.367$, $p < 0.05$). The results are presented in Table 4.

Anxiolytic-like activity of compound VIIIa in mice

Compound **VIIIa** at the doses 2.5, 5, 10, 20, and 30 mg/kg (but not 1.25 mg/kg) significantly increased the number of punished crossings in mice by 56.3, 71.9, 68.8, 53.1 and 128.1%, respectively ($F(6,63) = 9.529$, $p < 0.0001$). The reference anxiolytic drug - medazepam administered at a dose 2 mg/kg (but not 1 mg/kg) significantly increased the number of punished crossings in mice by 109.7% ($F(2,27) = 10.63$, $p < 0.001$). The results are presented in Table 5.

The influence of VIIIa on locomotor activity in mice

In the 4-min session, compound **VIIIa** injected at the doses 20 and 30 mg/kg (i.e., doses active in FST in mice) did not influence locomotor activity in mice ($F(2,27) = 0.2109$, ns). In 1-min session compound **VIIIa** administered at the doses 2.5-30 mg/kg (i.e., doses active in four-plate test in mice) did not affect locomotor activity in mice ($F(5,54) = 0.6500$, ns). The results are presented in Table 6.

***In vitro* and *in silico* prediction of metabolism of compound VIIIa**

The metabolism of compound **VIIIa** in mouse liver microsomes was studied *in vitro* at two different time points i.e., after 15 and 30 min. After 15 min of incubation, two major metabolites (M1 and M2) were found. Moreover, after 30 min of incubation, additional two metabolites (M3 and M4) were observed. The chemical structures of metabolites were proposed on the basis of ion fragmentation and liquid chromatography results (Table 7). MetaSite software indicated that CYP2D6, 2C9, 2C19 and 3A4 were primarily involved in the formation of metabolites M1 and M4, whereas CYP2C9 and 3A4 were predicted to contribute to the formation of M2 and M3 metabolites (Fig. 1).

Table 6. The effect of compound **VIIIa** administered at the doses active in forced swim test and four-plate test on locomotor activity in mice.

Compound	Locomotor activity: number of crossings during:		
	Dose [mg/kg b.w.]	1 min Mean ± SEM	4 min Mean ± SEM
Vehicle	-	32.1 ± 5.6	226.1 ± 16.9
VIIIa	2.5	31.8 ± 4.8	-
	5 2	4.7 ± 5.1	-
	10	26.5 ± 5.0	-
	20	36.1 ± 6.4	214.2 ± 19.2
	30	28.5 ± 4.5	200.2 ± 41.7

Compound **VIIIa** was dissolved in 0.9% NaCl and administered *i.p.* 30 before the test. The control groups received 0.9% NaCl. Statistical analysis: one-way ANOVA (Newman-Keuls *post hoc* test); n = 10 mice per group.

Table 7. Retention times, mass characteristics and elemental composition of the $[M + H]^+$ of compound **VIIIa** and its four metabolites.

Compound	Retention time [min]	$[M + H]^+$	Metabolic reaction	% content among metabolites
VIIIa	4.52	268	-	-
M1	3.09	300	Aliphatic and aromatic hydroxylation	71.7
M2	3.45	284	Aliphatic carbonylation	23.3
M3	2.81	316	Aliphatic carbonylation	2.9
M4	2.72	300	Aromatic hydroxylation	2.1

Table 8. Results of binding to serotonin receptors of reference and selected tested compounds.

Compound	K_i [nM]		
	5-HT_{1A}	5-HT_6	5-HT_7
I	14200	10830	16060
II	19590	7232	6793
III	9415	15760	6384
IV	5016	3054	3018
V	20110	15980	20710
VII	1787	13540	17240
VIII	456	5913	5559
IX	4902	11560	4808
X	20008	19720	16910
Buspirone	12	-	-
Clozapine	143	4	18

Radioligand binding assays to rats brain tissues using [^{3}H]-8-OH-DPAT for 5-HT_{1A}, [^{3}H]-LSD for 5-HT_6, [^{3}H]-5-CT for 5-HT_7. Inhibition constants (K_i) were calculated according to the equation of Cheng and Prusoff (27).

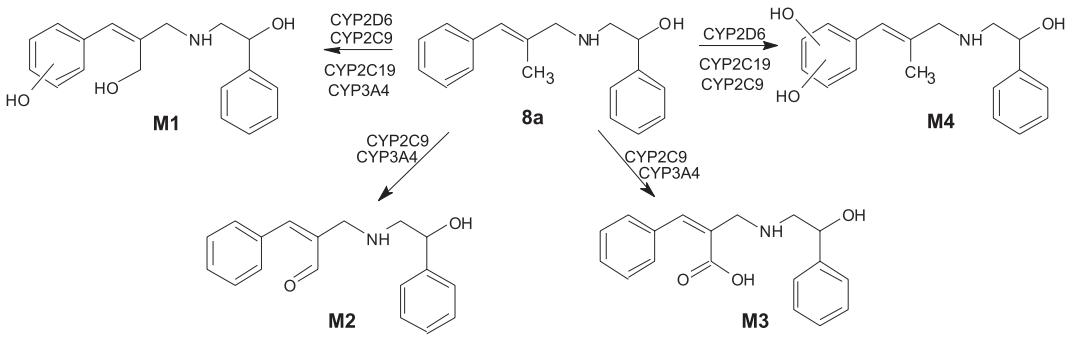


Figure 1. Metabolic pathways of compound **VIIIa** in mouse liver microsomes

Serotonin receptors binding results

The tested compounds did not show significant binding affinities to serotonin receptors 5-HT_{1A}, 5-HT_6, and 5-HT_7 (Table 8). Compound **VIII**, which possesses additional hydrophobic domain in aminoalkanol moiety proved the lowest 5-HT_{1A} inhibition constant among the tested series but still not sufficient for concluding about its molecular mechanism of action.

DISCUSSION AND CONCLUSION

The anticonvulsant activity proved by means of animal models do not depend on particular molecular mechanism of action thus animal models enable efficient screening of relatively large number of compounds. MES - electrically induced seizures and scPTZ - chemically induced seizures tests have been considered as "gold standards" in the discovering of new antiepileptic drugs, they enabled the identification of most of the currently marketed antiepileptic drugs (4). On the other hand, despite introducing of many new antiepileptic drugs, the percentage of failure in epilepsy pharmacotherapy remains over last few decades at constant level of 30% of patients. As the consequence, some models of resistant seizures were introduced at the early stages of antiepileptics development as a hope for identification of new compounds effective in resistant epilepsy in humans. One of such tests is 6-Hz psychomotor seizure model in mice (32, 33). The presented compounds were qualified either to standard anticonvulsant evaluation (MES, scPTZ, rotarod - compounds **I**-**V**, **VII**-**X**) or modified procedures (6-Hz, rotarod - compound **VI**).

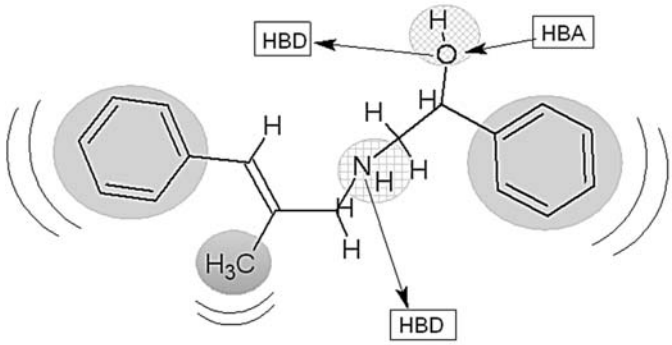


Figure 2. Pharmacophoric features of compound **VIII**. HBA - hydrogen bonds acceptor, HBD - hydrogen bonds donor; aromatic hydrogens were deleted

The reported series of compounds **I-X** possess some domains regarded as beneficial for anticonvulsant activity. Both currently available antiepileptic drugs and compounds in preclinical development may be characterized by possessing pharmacophoric elements such as at least one hydrophobic ring and at least two hydrogen bonding donor/acceptor functions (7). We have previously reported anticonvulsant activity of *trans*-cinnamyl derivatives of aminoalkanols which proved diverse protective activity in MES test (13). Currently reported compounds possess additional methyl substituent in cinnamyl moiety. The compounds in preliminary screening showed some protective properties in MES test but not in scPTZ evaluation. The neurotoxicity was also noted but mostly at the doses higher than active ones. In case of 2-aminopropan-1-ol derivative introduction of methyl substituent caused significant increase of activity in 6-Hz test, which is concerned as the test predictive for resistant seizures in humans.

The derivative of 2-amino-1-phenyletanol differs from other reported substances in possessing additional hydrophobic ring in aminoalkanol moiety (Fig. 2). We have chosen that compound for evaluation of antidepressant- and anxiolytic-like activity and it proved promising properties.

The results of our study indicate that compound **VIIIa** showed antidepressant-like effect in the forced swim test in mice. Interestingly, it produced a U-shaped dose-response curve, which is a very common effect for antidepressants and compounds with antidepressant-like activity (34). It is worth noting that the activity of compound **VIIIa** in this test was weaker than that of mianserin, a reference antidepressant drug. As compound **VIIIa** did not affect locomotor activity in mice at the dose active in the forced swim test, its antidepressant-like activity cannot be attributed to the psychostimulant action. Furthermore, compound **VIIIa** showed a dose-dependent anxiolytic-like activity in the four-plate test in mice. Its effect was comparable in term of active doses to medazepam - a reference anxiolytic drug. Given the fact that compound **VIIIa** administered at the doses active in the four-plate test did not influence locomotor activity in mice during 1-min session, it can be concluded that the observed anxiolytic-like effect was specific.

The current study demonstrated a low stability of compound **VIIIa** as after 30 min of biotransformation there was no remaining parent compound in the incubation mixture and four metabolites were observed. This important metabolic data may help in further understanding the observed pharmacological effects of compound **VIIIa**. However, additional experiments should be performed in order to assess the activity of its metabolites.

Finally, the weak results of binding affinities to serotonin receptors 5-HT_{1A}, 5-HT_6, and 5-HT_7 of the tested compounds are not sufficient for concluding about their molecular mechanism of action. Compound **VIII**, which proved anticonvulsant, antidepressant-, and anxiolytic-like activities showed lowest 5-HT_{1A} inhibition constant among the tested series. That result may suggest beneficial role of additional hydrophobic domain in aminoalkanol moiety in interaction with molecular targets resulting in promising activity in central nervous system.

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