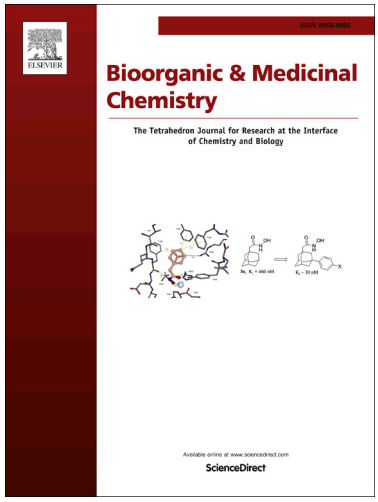
Accepted Manuscript



N-[(2,6-Dimethylphenoxy)alkyl]aminoalkanols - Their Physicochemical and Anticonvulsant Properties

Anna M. Waszkielewicz, Marek Cegła, Ewa Żesławska, Wojciech Nitek, Karolina Słoczyńska, Henryk Marona

PII: S0968-0896(15)00539-8
DOI: http://dx.doi.org/10.1016/j.bmc.2015.06.045
Reference: BMC 12401

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 26 March 2015
Revised Date: 12 June 2015
Accepted Date: 18 June 2015

Please cite this article as: Waszkielewicz, A.M., Cegła, M., Żesławska, E., Nitek, W., Słoczyńska, K., Marona, H., *N*-[(2,6-Dimethylphenoxy)alkyl]aminoalkanols - Their Physicochemical and Anticonvulsant Properties, *Bioorganic & Medicinal Chemistry* (2015), doi: http://dx.doi.org/10.1016/j.bmc.2015.06.045

N-[(2,6-Dimethylphenoxy)alkyl]aminoalkanols - Their Physicochemical and Anticonvulsant Properties

Anna M. Waszkielewicz[a]*, Marek Cegła[b], Ewa Żesławska[c], Wojciech Nitek[d], Karolina Słoczyńska[a,e], and Henryk Marona[a]

[a] Department of Bioorganic Chemistry, Chair of Organic Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland

[b] Department of Organic Chemistry, Chair of Organic Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland

[c] Department of Chemistry, Institute of Biology, Pedagogical University, Podchorążych 2, 30-084 Krakow, Poland

[d] Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow, Poland

[e] Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland

*e-mail address awaszkie@cm-uj.krakow.pl

Tel. +4812 620 55 76

Fax +4812 620 54 05

ABSTRACT

Twenty four new *N*-[(dimethylphenoxy)alkyl]aminoalkanols have been synthesized and evaluated for anticonvulsant activity in a series of *in vivo* tests: the maximum electroshock (MES), 6 Hz, and subcutaneous metrazole (ScMet). The compounds were also evaluated for possible neurotoxicity in the rotarod test. The majority of the achieved compounds exhibit quantified anticonvulsant activity. The most active compound **4**: *R*-(-)-2*N*-[(2,6-dimethylphenoxy)ethyl]aminopropan-1-ol is active in MES with ED_{50} =5.34 (male mice, *i.p.*), 22.28 (female mice, *i.p.*), 51.19 (male mice, *p.o.*), 7.43 (rats, *i.p.*), and 28.60 (rats, *p.o.*). Thermal analysis proved that its hydrochloride (**4a**) can exist in polymorphic forms. The compound binds to σ, 5-HT_{1A}, and α_2 receptors as well as 5-HT transporter and it does not exhibit mutagenic properties.

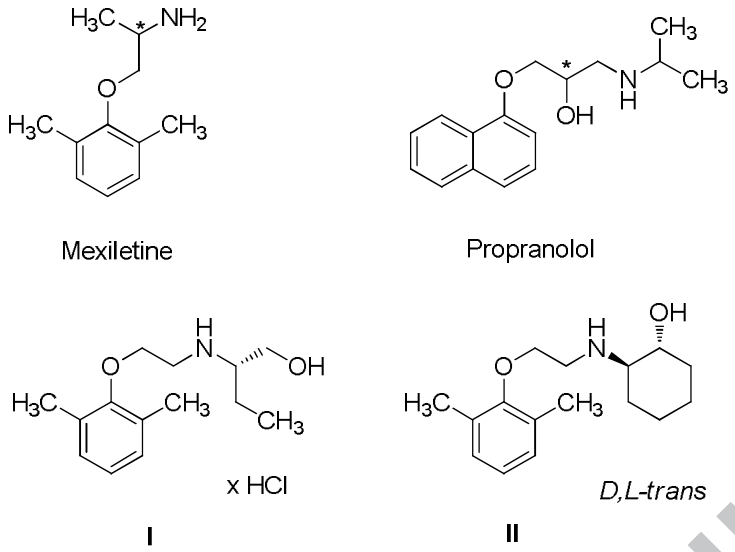
Keywords: 6 Hz, Ames test, aminoalkanols, anticonvulsant activity, crystal structure, DSC, MES, mutagenicity, rotarod, seizures, TG, TOX

1. Introduction

Epilepsy is one of the most common neurological disorders, characterized by hipersynchronous activity of neurons. Being caused by multiple factors such as genes, trauma, neurodegeneration, or intoxication, it concerns over 50 million people worldwide. Approximately 35% seizures are considered pharmacoresistant, therefore, there exist premises for further drug discovery process for this indication. Search for new drugs is continuously based on *in vivo* screening, basically performed within the Antiepileptic Drug Development program (ADD, National Institute of Neurological Disorders and Stroke, Bethesda, USA).[1] Due to the fact that all current antiepileptic drugs have been associated with this program, in order to improve effectiveness of new drugs, the program has evolved for epileptogenesis and psychomotor seizure *in vivo* tests (6 Hz). Up to date, many papers on new anticonvulsant agents base on the program, concerning various chemical entities.[2-4]

Anticonvulsant properties of cardiovascular drugs containing aroxyalkyl or aminoalkanol moieties, such as mexiletine and propranolol, respectively,[5-6] have been premise for our research. On this basis we have found some promising anticonvulsant activity among aroxyalkyl (and also aroxyacetyl) derivatives of aminoalkanols.[7-11] We already described synthesis and evaluation of pharmacological properties of appropriate derivatives of 4-methylphenol, 2,6-dimethylphenol,[7-9] 4-chlor-3-methylphenol and 2-chlor-5-methylphenol,[10] as well as 4-chlor-2-methylphenol.[11] One of them, *e.g.* (*S*)-(+)-2*N*-[(2,6-dimethylphenoxy)ethyl]aminobutan-1-ol hydrochloride displayed protection against MES-induced seizures and low toxicity (mice, *i.p.*) with an ED_{50}=7.57 mg / kg and PI=4.55.[8] Another compound, (*D,L*)-*trans*-2*N*-[(2,6-dimethylphenoxy)ethyl]aminocyclohexan-1-ol exhibited ED_{50} = 7.73 mg/kg b.w. and PI (MES, mice, *i.p.*) = 3.90 (Figure 1).[7] Both protective indices in the MES test in mice are higher than that of valproate (PI=1.7) and similar to that of carbamazepine (PI=4.9). From the preliminary assay data, it was ascertained that anticonvulsant activity was associated with aminoalkanol type and configuration. Such activity drew our attention onto influence of position and type of substituents within the phenyl ring as well as length of the alkyl linker between the phenoxy and the aminoalkanol groups. In case of active racemates, enantiomeric structures have been achieved.

Figure 1. Chemical structures of reference compounds. [2-3, 7-8]



We herein report results of evaluation of biological activity of novel derivatives of 2,6-dimethylphenol, where the linker between phenoxyl and aminoalkanol moieties contains 2-4 methylene groups. Some of the presented compounds – **7**, **9**, and **12**, **15**, **17**, and **19**-**20** – have been published for evaluation of their lipophilicity and screening results.[9] This article completes the documentation concerning their physicochemical data, activity profile and structure-activity relationship. Compounds **3**-**6**, **8**, **10**, **12**-**15** and **17**-**22** were subject to intellectual property rights protection as a group of potential anticonvulsant drug candidates, in our team, and therefore they were assigned CAS numbers.[12]

The choice of substituents in the phenyl ring was based on our previous results and it seemed optimal to use two methyl substituents in positions 2,6. Within the group of 2,6-dimethylphenoxyalkyl derivatives, the chosen aminoalkanol moieties represent examples of our experience as well – the 1,2-aminoalkanol configuration has proved the optimal for anticonvulsant activity. However, exceptions also have been achieved and evaluated, *e.g.* derivatives of 3-amino-1-propanol (compound **2**), 5-amino-1-pentanol (**8**), or *D,L*-valinol (**10**). Moreover, it was decided to choose as simple moiety as possible, in order to keep lipophilicity in the required range of clogP=2-3, for possible distribution in the nervous system instead of cardiovascular one. Therefore, any potential of adverse events related with similar mechanisms of action would be minimized.

The length of the linker between both moieties was also an important parameter, since using ethylene group resulted in good activity and acceptable lipophilicity, and then propylene linker diminished activity and enlarged neurotoxicity at higher lipophilicity values. Next, use of butylene linker was dictated by interest in structure-activity relationship for the most active compounds in the investigated group. It was also interesting for the fact that clogP did not differ much for both propylene and butylene derivatives.

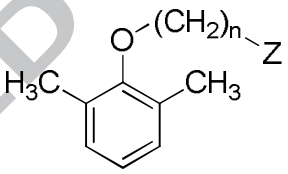
2. Results and discussion

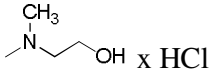
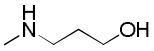
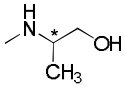
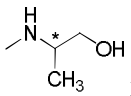
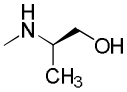
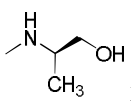
2.1. Chemistry

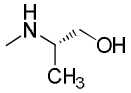
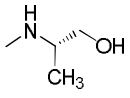
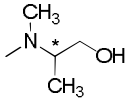
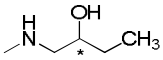
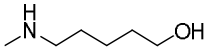
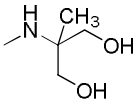
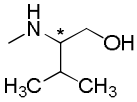
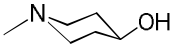
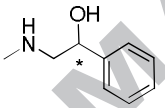
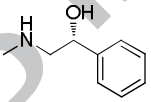
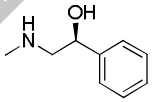
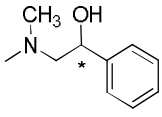
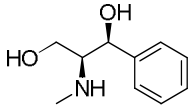
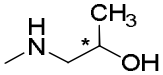
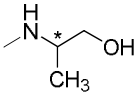
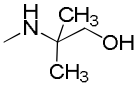
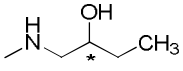
Twenty four new compounds have been achieved by *N*-alkylation of appropriate (2,6-dimethylphenoxy)ethyl, -propyl or -butyl bromides and the procedures have been published.[7-8, 13] The structures and clogP are presented in Table 1. It can be observed that with this group of derivatives the lipophilicity parameter clogP ranges 2.31-4.03 which is acceptable for anticonvulsants.[14]

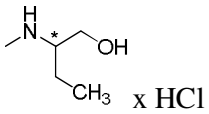
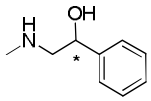
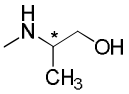
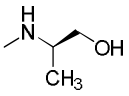
Table 1

Chemical structures of the title compounds



n	Compd.	Z	Configuration	clogP
2	**1**	 x HCl	-	2.74
	2		-	2.39
	3		*R,S*	2.40
	3a	 x HCl	*R,S*	2.40[a)]
	4		*R*	2.40
	4a	 x HCl	*R*	2.40[a)]

	5		*S*	2.40
	5a	 x HCl	*S*	2.40[a)]
	6	 x HCl	*R,S*	3.05[a)]
	7		*R,S*	2.93
	8	 x HCl	-	2.69[a)]
	9		-	2.45
	10		*D,L*	3.10
	11			2.31
	12		*R,S*	3.66
	13		*R*	3.66
	14		*S*	3.66
	15	 x HCl	*R,S*	4.31[a)]
	16		*L-threo*	3.57
3	**17**		*R,S*	2.76
	18		*R,S*	2.76
	19		-	3.16
	20		*R,S*	3.29

	21		*R,S*	3.29[a)]
	22		*R,S*	4.03
4	**23**		*R,S*	2.74
	24		*R*	2.74

[a)] Parameter calculated for base with use of ChemBioDraw Ultra 12.0 computer program.

During synthetic work, the pure compound **4a** was achieved by saturation of **4** in acetone solution with gaseous HCl, resulting in crystals with melting point 107-109 °C. However, double recrystallization resulted in achievement of crystals with melting point 115-117 °C. The higher melting point was also achieved if the base was saturated with gaseous HCl in ethyl acetate/EtOH (4:1) solution instead of acetone. For this reason, the salts are marked as **4a1** for the lower mp. and **4a2** for the higher one and they have been evaluated separately. However, it was observed, that **4a2** during storage at room temperature can reform into **4a1**. The same was observed for **5a** – the melting points ranged 106-108 °C and 115-117 °C (**5a1** and **5a2**, respectively). Due to the above observations, thermal analysis was performed for **3a-5a**. The premise was that polymorphism – the ability of a molecule to crystallize into more than one crystal form, influences many parameters such as solubility, shelf life, and formulation processes. Pseudopolymorphism resulting from solvatation is related with different amount of solvent molecules included in the crystal, and this fact may influence accuracy of drug dosing.[15] Differential scanning calorimetry (DSC) and thermogravimetry (TG) was performed for compounds **3a-5a**. The results for **3a** and **4a2** are presented in Fig. 2-3, respectively. It can be noticed from the DSC curve that **3a** can exist in one crystalline form since two separate endothermic peaks exist: one at the temperature corresponding to melting point measured with criometer, and one more at ca. 300 °C indicating deterioration in these conditions, confirmed by thermogravimetry (TG) and its derivative (DTG) curves. The results obtained for **4a** and **5a** indicate that they both can exist in polymorphic forms. The thermal curves for **4a** and **5a** indicate that they both deteriorate at ca. 300 °C. None of the polymorphic forms of **4a** contains water whereas both polymorphic forms of **5a** contain small amount of water. The mass of **5a1** during heating to 120 °C diminished by 1.68% and for **5a2** the mass diminished by 5.5%. The DSC curve for enantiomer **5a** contains two endothermic

peaks: for **5a1** the peak at 109.9 °C correlates with melting point whereas the other endothermic peak indicates transformation of **5a1** to **5a2** with mp. 115-117 °C (Fig. **5a2**).

Figure 2. DSC, TG, and TDG curves of compound **3a**.

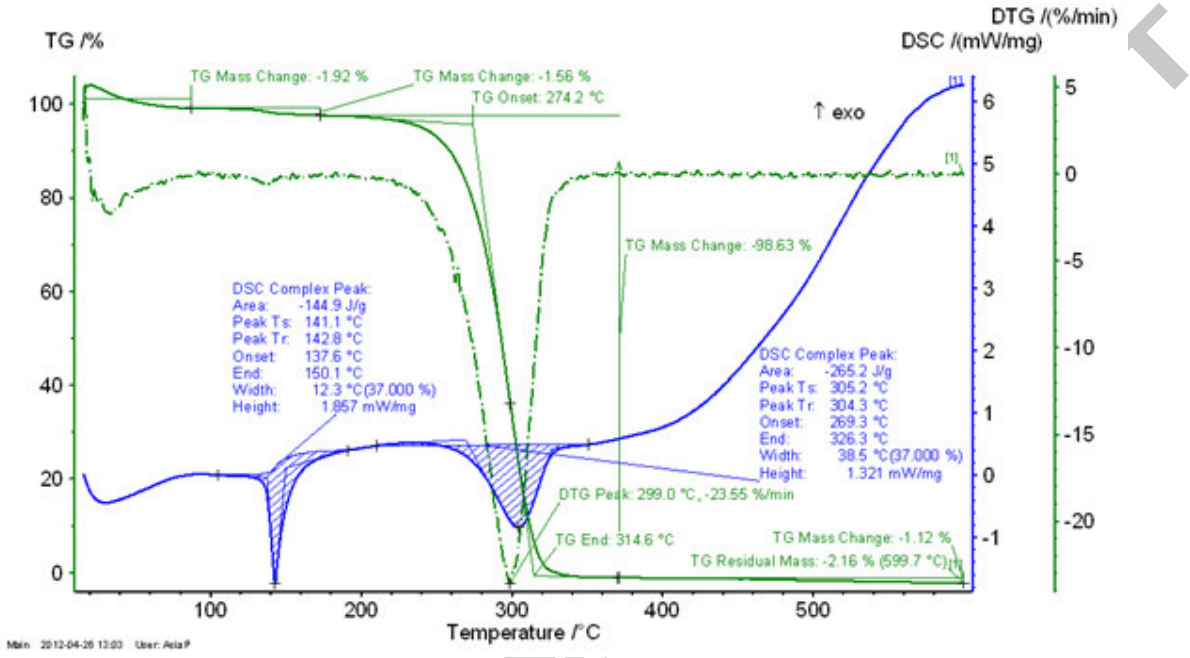
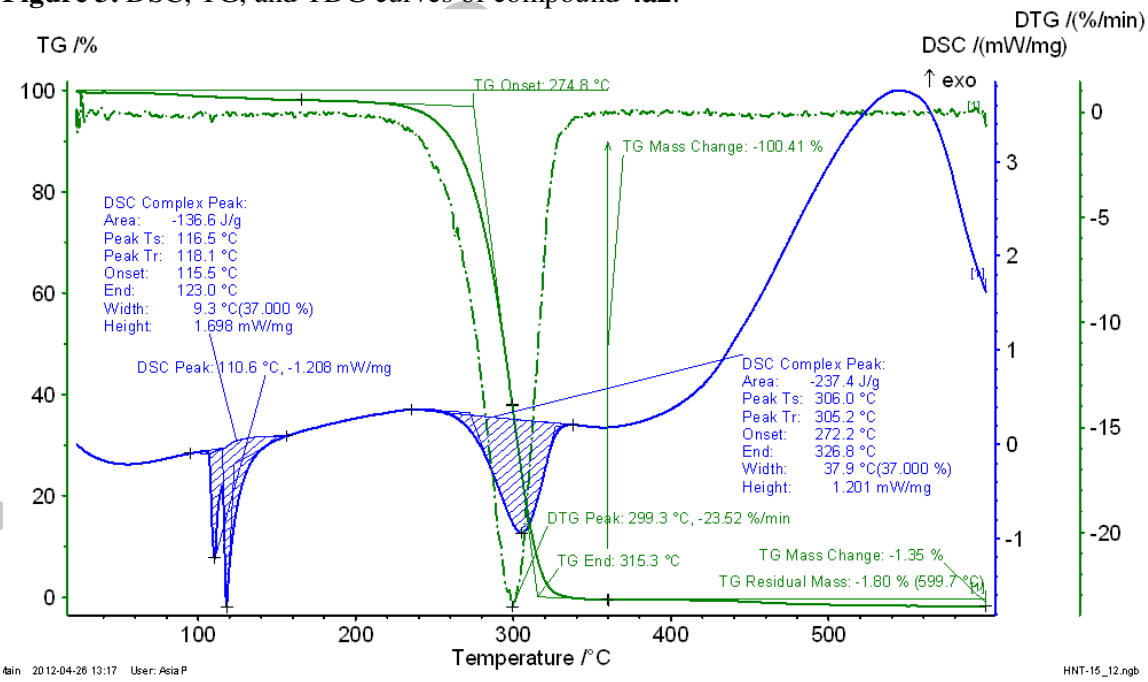


Figure 3. DSC, TG, and TDG curves of compound **4a2**.



*Crystal structure of **4a2***

An overview of the asymmetric unit of **4a2** with the atom numbering is presented in Fig.7. The crystal structure of **4a2** confirms *R* configuration at C2. The bond lengths and bond angles have typical values. The geometry of this molecule was compared to the earlier determined crystal structure of mexiletine.[16] Both compounds contain 2-(2,6-dimethylphenoxy)ethylamine moiety. **4a2** has longer side chain than mexiletine and its conformation is bent. The arrangement of the ether oxygen atom (O2) and the protonated nitrogen atom shows synclinal conformation (gouche). This conformation is also observed in mexiletine. In **4a2** the hydroxyl oxygen atom (O1) and nitrogen atom also has synclinal conformation. The comparison of selected parameters is shown in Table 2. The nitrogen atom of **4a2** has different orientation in comparison to the known structure of mexiletine.

Figure 4. The molecular structure of **4a2** showing the atom numbering scheme. Displacement ellipsoids are drawn at the 50% probability level.

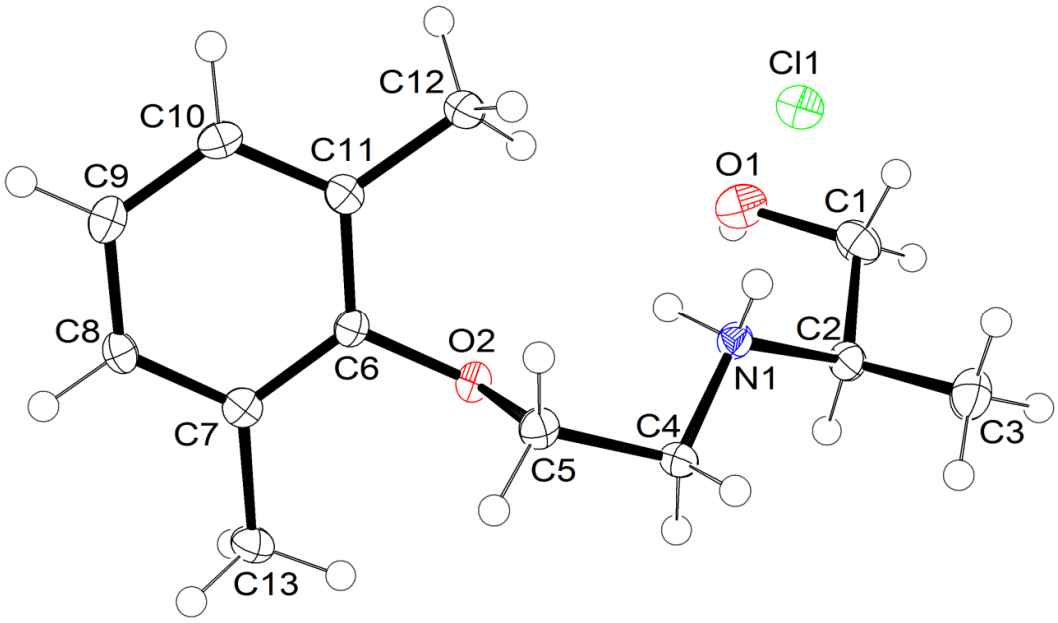


Table 2
The selected bond lengths (Å), bond and torsion angles (°) in the crystal structure of **4a2** and mexiletine

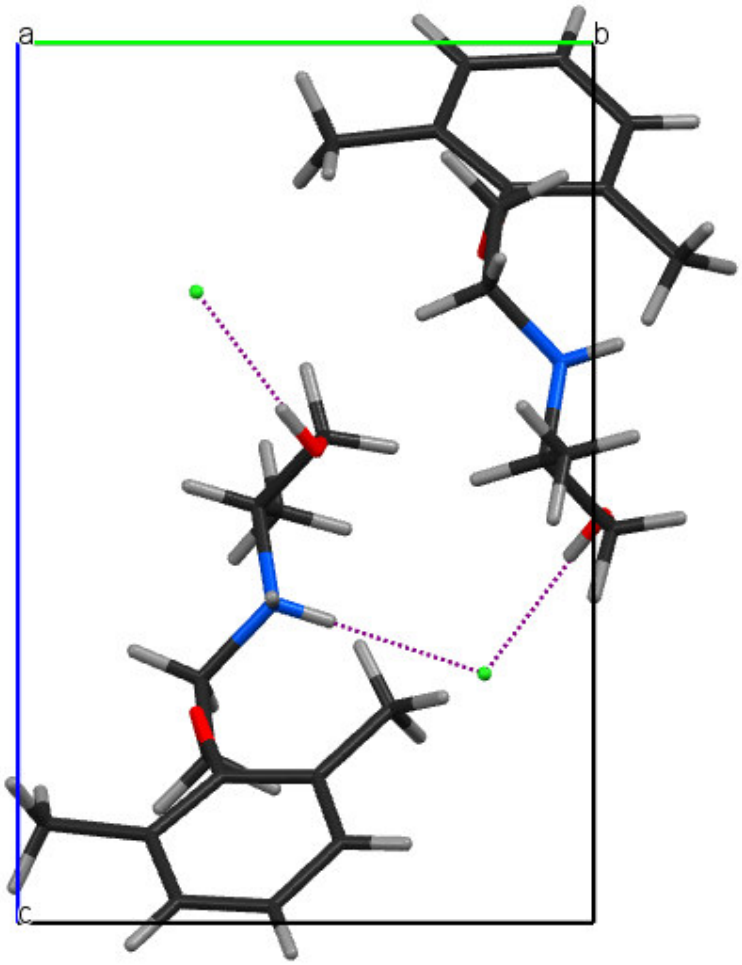
	4a2	Mexiletine[17]
C6-O2-C5	111.52	114.69
C6-O2-C5-C4	-169.22	- 174.49
O2-C5-C4-N1	57.11	-60.55
O2-C5-C4-H4B	-63.22	56.24

The unit cell consists of two molecules of **4a2**. The packing of the molecules in the unit cell can be characterized by the intermolecular interaction listed in Table 3. The chlorine anion connects two molecules *via* hydrogen bonds between protonated nitrogen atom of one molecule and hydroxyl group of another molecule (Fig. 8). The crystal structure is also stabilized by weak C-H··O and C-H··Cl interactions.

Table 3
Intermolecular interactions for **4a2**

D-H···A	H···A (Å)	D···A (Å)	D-H-A (°)	Symmetry code
N1-HN2B···Cl1	2.29(4)	3.127(2)	174(3)	
O1-H1···Cl1	2.23(4)	3.034(2)	176(4)	-x+1, y-1/2, -z+1
C2-H2···O1	2.58	3.462(3)	147.5	-x+1, y-1/2, -z+1
C3-H3B···Cl1	2.91	3.858(3)	163.0	-x, y-1/2, -z+1
C4-H4B···O1	2.60	3.450(3)	144.5	-x+1, y-1/2, -z+1

Figure 5. Packing of molecules in the unit cell of the crystalline **4a2** projected along [100] direction. Dashed lines indicate hydrogen bonds.



2.2. Pharmacology

The anticonvulsant activity and neurotoxicity of all synthesized compounds have been evaluated according to standard protocols within the National Institutes of Neurological Disorders and Stroke (NINDS, Rockville, USA).[1] Currently three tests are used for screening the compounds for their anticonvulsant activity: MES (maximal electroshock seizures), ScMet (subcutaneous metrazol), 6 Hz (6 Hertz test). MES represents *grand-mal* epilepsy, ScMet – *petit-mal* epilepsy, and 6 Hz – psychomotor seizures and epileptogenesis. The results are confronted with rotarod test used for evaluation of neurotoxicity (TOX).

Comparing the screening results of compounds **1-24** (Table 4), it can be noticed that 2-amino-1-propanol derivatives exhibit the strongest activity. Moreover, *R* enantiomers have proved better potency than *S*, which is not consistent with reference structure II. In particular, 2*N*-

(2,6-dimethylphenoxy)ethyl]amino-1-propanol (**3**-**5**) seems the optimal compound of all title group, especially the *R* enantiomer (**4**), active at 10 mg/kg b.w. 0.5 h after administration. It is interesting that the racemate (**3**) is more toxic than any of the enantiomers (**4**, **5**). The *N*-methyl derivative (**6**) is visibly more toxic than its analog **4a**.

Table 4
Screening results of the tested compounds in mice, *i.p.*

Compd.	Dose [mg/kg b.w.]	Time [h]						ASP [c]
		MES [a]		ScMet [a]		TOX [b]		
		0.5	4	0.5	4	0.5	4	
3	3	0/4				0/4		1
	10	0/4				0/4		
	30	1/1	0/1	0/1	0/1	2/4	0/2	
	100					8/8		
	300					4/4		
3a	3	0/4				0/4		4
	10	0/4				0/4		
	30	1/1	0/1	0/1		3/4	0/2	
	100	1/1		0/1		8/8[D]	0/4	
	300					4/4[D]		
4	3	0/4				0/4		1
	10	1/4				0/4		
	30	1/1	0/1	0/1	0/1	0/4	0/2	
	100	2/2	1/2	0/1	0/1	8/8 [D]	1/3	
	300					4/4 [D]		
4a2	30	0/1	0/1	0/1	0/1	0/4	0/2	1
	100	3/3	0/3	0/1	0/1	8/8	0/4	
	300					4/4	1/1	
5	3	0/4				0/4		1
	10	2/4				0/4		
	30	1/1	0/1	0/1	0/1	0/4	0/2	
	100					8/8[D]		
	300					4/4[D]		
6	3	0/4				0/4		4
	10	0/4				0/4		
	30	1/1	0/1	0/1	0/1	1/4	0/2	
	100	1/1	0/1			8/8	0/4	
	300					4/4		
8	30	0/1	0/1	0/1	0/1			1
	100	3/3	0/1	0/1	0/1	7/8		
	300				0/1	4/4	1/1	
10	30	0/1	0/1	0/1	0/1	0/4	0/2	1
	100	2/2	0/2	0/1 [D]		7/8 [D]	0/2	
	300					4/4		
13	3	0/4				0/4		1
	10	0/4				0/4		
	30	1/1	0/1	0/1	0/1	0/4	0/2	
	100					8/8 [D]		
	300					4/4 [D]		
14	3	0/4				0/4		1
	10	0/4				0/4		

	30	1/1	0/1	0/1	0/1	0/4	0/2	
	100	3/3	0/3	0/1		7/8 [D]	0/3	
	300					4/4 [D]		
16	30	0/1	0/1	0/1	0/1	0/4	0/2	-
	100			0/1 [D]		7/8 [D]		
	300			0/1 [D]		3/4 [D]		
18	3	0/4				0/4		1
	10	0/4				0/4		
	30	1/1	0/1	0/1	0/1	2/4	0/2	
	100	3/3	0/1	0/1	0/1	8/8	1/3	
	300					4/4		
21	3	0/4				0/4		1
	10	0/4				0/4		
	30	1/1	0/1	0/1	0/1	0/4	0/2	
	100	3/3	0/2		0/1	8/8 [D]	0/3	
	300					4/4 [D]		
22	30	0/1	0/1 [D]	0/1	1/1	2/4	0/2	4
	100	1/1	0/1	0/1		8/8 [D]	0/1	
	300					4/4		

[a)]Number of animals protected/number of animals tested in the MES and ScMet tests; [b)]Number of animals displaying motor impairment/number of animals used in the rotarod test; [c)] ASP classification: 1 – anticonvulsant activity at doses 100 mg/kg or less; 2 – anticonvulsant activity at doses greater than 100 mg/kg; 3 – compound inactive at 300 mg/kg; 4 – compound either active or inactive but toxic at doses of 30 mg/kg; "-" the compound was not tested in the particular case.;* number of animals which died during test are stated in brackets; [D] - death.

The most active compounds have been advanced to observations of the activity and neurotoxicity in time in order to reach time of peak effect (TPE) (Table 5), which was needed for evaluation of ED_{50} at the particular time – for most compounds it was 15 min after administration. Then, ED_{50}s and TD_{50}s with proper confidence intervals (CI) for activity or neurotoxicity doses have been determined (Table 6). It can be observed that among 2-amino-1-propanol derivatives of (2,6-dimethylphenoxy)butyl (**3**-**5**), that racemate's ED_{50} in MES, mice, *i.p.*, seems larger than any of the enantiomers' activity; however, the confidence interval is broader.

Table 5
Observation of anticonvulsant activity and neurotoxicity in mice

Compd.	Route of administration	Test	Dose [mg/kg]	Time [h] 0.25	0.5	1.0	2.0	4.0	6.0	24.0	TPE [h]
2	*i.p.*	6 Hz (32 mA)	100	4/4	3/4	3/4	0/4	0/4			0.25
		TOX	100	4/4	3/4	0/4	0/4	0/4			0.25
4	*i.p.*	MES	15	8/8	7/8						0.25
			20	4/4	4/4	0/4	0/4	0/4			
		6 Hz (32 mA)	55	4/4	4/4	2/4	1/4	0/4			0.25
		6 Hz (44 mA)	50	4/4	3/4	0/4	0/4	0/4			0.25
		TOX	50	8/8	4/8	1/8	1/8	0/8	0/8	1/8	0.25
			55	8/8	7/8						
	p.o.	MES	40	4/4	3/4	0/4	0/4				0.25
		TOX	200	6/8	5/8	3/8	0/8	0/8			0.25
5	*i.p.*	MES	15	4/4	2/4	0/4	0/4	0/4			0.25
		TOX	40	5/8	0/8	0/8	0/8				0.25
11	*i.p.*	MES	25	2/4	2/4	0/4					0.25
			50	4/4	4/4	3/4	0/4	0/4			
		6 Hz (32 mA)	50	3/4	3/4	0/4	0/4	0/4			0.25
			100	4/4	4/4	2/4	0/4	0/4			
		TOX	50	0/8	0/8	0/8	0/8	0/4			0.25
			85	6/8	0/8	0/8	0/8	0/8			
			100	4/4	4/4	0/4	0/4	0/4			
12	*i.p.*	6 Hz (32 mA)	30	4/4	0/4	0/4	0/4	0/4			0.25
			60	4/8							
23	*i.p.*	6 Hz (32 mA)	50	3/4	2/3	0/4	0/4	0/4			0.25
		TOX	50	0/4	1/3	0/4	0/4	1/4			0.5
24	*i.p.*	6 Hz (32 mA)	30	0/4	0/4	0/4					0.25
			100	4/4	3/3	3/3	3/4	0/3			
		TOX	30	0/4	0/4	0/4					0.25
			100	4/4	4/4	4/4	0/4	1/4			

[a)] Number of animals protected / number of animals tested; [b)] number of animals exhibiting toxicity / number of animals tested in the rotarod test TPE – time of peak effect.

Table 6
Quantitative evaluation of activity in mice, *i.p.*

Compd.	Route of administration	Test	Dose [mg/kg]	Activity at TPE [a]	TPE ED_{50} [mg/kg] (Confidence Interval)
3	*i.p.*	MES [c]	2	0/8	TPE=0.5 h
			4	3/8	ED_{50}=10.08 (5.62-18.64)
			8	3/8	
			16	5/8	
			32	7/8	
4	*i.p.*	MES	2.5	1/8	TPE=0.25 h
			5	3/8	ED_{50}=5.34 (3.50-7.40)
			10	7/8	
			15	8/8	
	i.p.	MES [c]	5	0/8[n]	TPE=0.25 h
			15	0/8	ED_{50}=22.28 (19.55-24.7)
			20	2/8	
			25	6/8	
			30	8/8	
	p.o.	MES	20	0/8	TPE=0.25 h
			40	3/8	ED_{50}=51.19 (36.52-70.16)
			58	5/8	
			75	6/8	
	i.p.	ScMET	6.3	0/2	ED_{50}>50
			12.5	0/2	
			25	0/2	
			50	0/2	
	i.p.	6 Hz	30	0/8	TPE=0.25 h
		(22 mA)	37	1/8	ED_{50}=19.5 (10.3-30.5)
			45	9/16	
			60	8/8	
	i.p.	6 Hz [c]			TPE=0.25 h
		(22 mA)			ED_{50}=7.06 (2.25-15.81)
	i.p.	6 Hz	23	0/8	TPE=0.25 h
		(32 mA)	27	4/16	ED_{50}=30.90 (28.81-33.48)
			31	3/8	
			35	13/16	
			55	8/8	
	i.p.	6 Hz [a]	12.5	0/8	TPE=0.25 h
		(44 mA)	25	2/8	ED_{50}=30.02 (24.38-33.81)
			30	3/8	
			35	13/16	
			50	15/16	
	i.p.	6 Hz [c]	15	0/8	TPE=0.25 h
		(44 mA)	30	2/8	ED_{50}=41.3 (31.24-51.29)
			45	3/8	
			60	8/8	
	i.p.	FRINGS	2.5	1/8	TPE=0.25 h

			5	2/8	ED_{50}=5.39 (3.71-6.98)
			7.5	6/8	
			10	8/8	
	i.p.	BIC	24	1/8	TPE=0.25h
			40	2/8	ED_{50}>75
			75	0/8	
	i.p.	PIC	25	1/8	TPE=0.25h
			40	4/8	ED_{50}>75
			57	2/8	
			75	4/8	
	i.p.	TOX	15	1/8	TPE=0.25 h
			25	2/8	TD_{50}=29.48 (21.76-37.87)
			38	5/8	
			50	8/8	
	i.p.	TOX [b) c)]	15	0/8	TPE=0.25 h
			30	1/8	TD_{50}=38.71 (31.67-43.53)
			45	12/16	
			60	8/8	
	p.o.	TOX	70	0/8	TPE=0.25 h
			100	1/8	TD_{50}=144.28 (120.69-185.38)
			120	3/8	
			150	5/8	
			200	6/8	
4a2	*i.p.*	MES			TPE=0.25 h ED_{50}(MES)=22.28 TD_{50}=38.71
5	*i.p.*	MES	6	0/8	TPE=0.25 h
			7.5	4/8	ED_{50}=8.59 (7.23-10.03)
			9	5/8	
			12	6/8	
			15	8/8	
	i.p.	ScMET	20	0/8	ED_{50}>52
			30	0/8	
			40	0/3	
			52	0/3	
	i.p.	TOX	30	0/8	TPE=0.25 h
			35	3/8	TD_{50}=37.66 (34.67-42.36)
			40	5/8	
			52	8/8	
11	*i.p.*	MES	8	1/8	TPE=0.25 h
			12	0/8	ED_{50}=17.31 (13.47-21.62)
			17	6/8	
			20	5/8	
			25	5/8	
			40	8/8	
	i.p.	ScMET	60	0/8	TPE=0.25 h
			70	0/8	ED_{50}>70.0

	i.p.	TOX	50	0/8	TPE=0.25 h
			60	3/8	TD_{50}=71.26 (62.05-81.71)
			70	4/8	
			85	6/8	
			100	7/8 [D]	
12	*i.p.*	6Hz	30	0/8	
		(32 mA)	60	4/8 [M]	
Carbamazepine	*i.p.*	MES			ED_{50}=7.81 (6.32-8.45) [18]
		6 Hz			Max. 75% protection at 40 and 80
		FRINGS			ED_{50}=11.2 (7.73-16.2)
		TOX			TD_{50}=45.4 (32.9-54.4)
Propranolol	*i.p.*	MES			ED_{50}=15-20 [6]
Allopregnanolone	*i.p.*	MES			TPE=0.17 h ED_{50}>100 [19]
		ScMET			TPE=0.17 h ED_{50}=13.7 (10.1-18.7)
		6 Hz (32 mA)			TPE=0.17 h ED_{50}=14.2 (10.3-19.4)

[a)]Number of animals protected/number of animals tested; [b)]Number of animals displaying motor impairment/number of animals used in the rotarod test; [c)] female mice; BIC – bicuculline; PIC – picrotoxin; [n]- not included in probit; [M] – minimal motor impairment.

Taking a closer look at the activity of **4**, it is active in MES, 6 Hz (22-42 mA), and audiogenic seizures, and the protective indices *vs.* rotarod test (TD_{50}/ED_{50}) are 5.5 (MES and Frings, mice, *i.p.*) or >17 (MES, rats, *p.o.*). Activity in 6 Hz tests is observed at higher doses than MES, from 10.3 mg/kg b.w. up to 30 mg/kg b.w. which is the very TD_{50} value (Table 6).

Similar procedure as in mice has been repeated in rats mainly after oral administration with some adjustments. For compounds first active in mice, observation of anticonvulsant activity at various doses in time has been performed (Table 7). The experiments resulted in preliminary dose range and TPE, which was used for determination of ED_{50} or TD_{50}, for relevant compounds and tests (Table 8).

Also in the above procedure, the superior activity of 2-amino-1-propanol derivatives (**3-5**) is clearly visible. Among them, the *R* enantiomer (**4**) is more active than the racemate **3** – the CI of activity in MES is 24.84-70.47 mg/kg for **3** (higher values can be relevant to *S*-enantiomer in the racemate) and 20.44-34.64 mg/kg for **4**.

In both mice and rats, the peak effect is observed 15 min after *i.p.* administration. Oral administration elongates onset of peak activity to 30 min for rats only (Table 7). In terms of activity profile, the confidence intervals of ED_{50}s cover for MES and audiogenic seizures (mice, *i.p.*). Moreover, considering the ratio between oral and intraperitoneal ED_{50}, it can be

presumed that bioavailability could range 10% in mice (Table 6) and 25% in rats (Table 8). These presumptions should be confirmed with pharmacokinetic studies. Moreover, it can be observed that the confidence intervals in the same tests but for various sex of animals do not cover: in MES CI for males is 3.50-7.40, for females ranges 19.55-24.7 mg/kg. The CI for 6 Hz (22 mA) in males is 40.1-48.29 and in females is 2.25-15.81 mg/kg. At 42 mA the CI for males is 24.38-33.81 and for females 31.24-51.29 mg/kg. Such differences in sex for neurological activity could be explained by possible interactions with sex hormones, especially that allopregnanolone is known for its activity in MES (Table 6).[19]

Table 7.
Observation of anticonvulsant activity and neurotoxicity in rats

Compd.	Route of Administration	Test	Dose [mg/kg b.w.]	Time [h] 0.25	0.5	1.0	2.0	4.0	6.0	8.0	24.0	TPE
3	*i.p.*	MES	10	3/4	2/4	1/4	1/4	1/4				0.5 h
			12	3/4	4/4	1/4	1/4	0/4				
			32	1/8	7/8							
		TOX	12	0/4	0/4	0/4	0/4	0/4				
			50	1/8	1/8	1/8	I	I				
			100	8/8	8/8	8/8	8/8	7/8	7/8		7/8	
	p.o.	MES	30	1/4	0/4	1/4	1/4	0/4				1 h
			60	1/4	1/4	2/4	0/4	0/4				TD_{50}<500
		TOX	30	0/4	0/4	0/4	0/4	0/4				
			400	2/8	2/8	0/8	0/8	4/8	4/8		1/8	
			500	4/5	4/5	3/5	1/5	2/5 D	2/5	2/5	1/5	
4	*i.p.*	MES	12	4/4	3/4	2/4	2/4	2/4				0.25 h
			50	4/4	4/4	4/4	4/4	2/4				
		TOX	12	0/4	0/4	0/4	0/4	0/4				
			50	4/4	3/4	2/4	0/4	0/4				
			100	8/8 D	8/8	8/8	8/8	8/8	7/8	7/8	7/8	
	p.o.	MES	30	1/4	1/4	0/4	0/4	1/4				0.5 h
			60	3/4	4/4							
			80	3/4	3/4	2/4	1/4	1/4				
		TOX	30	0/4	0/4	0/4	0/4	0/4				TD_{50}>500
			62.5	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	
			125	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	
			250	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	
			500	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	
5	*i.p.*	MES	8	3/4	4/4	0/4	0/4					0.5 h
			12	3/4	3/4	3/4	2/4	0/4				
		TOX	12	0/4	0/4	0/4	0/4	0/4				0.25 h
			50	5/8	1/8	0/8	0/8	0/8	0/8		0/8	
	p.o.	MES	12.5	1/4	2/4	2/4	2/4	2/4				
		TOX	12.5	0/4	0/4	0/4	0/4	0/4				
6	*p.o.*	MES	30	0/4	0/4	0/4	0/4	0/4				
		TOX	30	0/4	0/4	0/4	0/4	0/4				
7	*i.p.*	TOX	10	0/2	0/2	0/2	0/2	0/2				

			30	1/2	0/2	0/2	0/2	0/2	
			100	2/2 [D]					
	p.o.	MES	12.5	2/4	2/4	1/4	1/4	1/4	1 h
		TOX	12.5	0/4	0/4	0/4	0/4	0/4	TD_{50}>500
10	*p.o.*	MES	30	0/4	0/4	0/4	0/4	1/4	
		TOX	30	0/4	0/4	0/4	0/4	0/4	
11	*i.p.*	MES	30	4/4	3/4	2/4	1/4	0/4	0.25 h
		TOX	30	0/4	0/4	0/4	0/4	0/4	
12	*i.p.*	TOX	30	0/2	0/2	0/2	0/2	0/2	
			100	2/2	2/2	2/2	1/2	0/2	
	p.o.	TOX	62.5	0/2	0/2	0/2	0/2	0/2	1 h
			125	0/2	0/2	0/2	0/2	0/2	TD_{50}>500
			250	0/2	0/2	0/2	0/2	0/2	
			500	0/2	0/2	0/2	0/2	0/2	
18	*p.o.*	MES	30	0/4	0/4	0/4	1/4	0/4	
		TOX	30	0/4	0/4	0/4	0/4	0/4	

[a)] Number of animals protected / number of animals tested; [b)] number of animals exhibiting toxicity / number of animals tested in the rotarod test; [D] – death; TPE – time of peak effect.

Table 8
Quantitative analysis in rats

Compd.	Route of administration	Test	Dose [mg/kg b.w.]	Activity	TPE ED_{50} [mg/kg b.w.] (Confidence Interval)
3	*i.p.*	MES	2	0/8	TPE=0.5 h
			4	3/8	ED_{50}=10.08
			8	3/8	(5.62-18.64)
			16	5/8	
			32	7/8	
	p.o.	MES	20	2/8	TPE=1 h
			40	2/8	ED_{50}=47.03
			60	4/8	(24.84-70.47)
			70	6/8	
			80	7/8	
4	*i.p.*	MES			TPE=0.5 h
					ED_{50}=7.43
					(3.76-11.7)
	p.o.	MES	12.5	0/8	TPE=0.5 h
			25	3/8	ED_{50}=28.60
			37.5	6/8	(20.44-34.64)
			50	8/8	
		ScMET	31.3	0/2	TPE=0.5 h
			62.5	0/2	ED_{50}>250.0
			125.0	1/10	
			250	0/2	
5	*i.p.*	MES	2.5	1/8	TPE=0.5 h
			5	2/8	ED_{50}=6.50
			7.5	4/8	(4.30-10.33)

			10	7/8	
	i.p.	ScMET	7.8	0/2	TPE=0.5 h
			15.6	0/2	ED_{50}>62.5
			31.3	0/2	
			62.5	0/2	
	i.p.	TOX	12.5	1/8	TPE=0.25 h
			25	2/8	TD_{50}=35.81
			50	5/8	(22.28-58.09)
			100	6/6 [D]	
7					ED_{50}=34.86
12	*p.o.*	MES	7.5	2/8	TPE=1 h
			15	2/8	ED_{50}=21.06
			23	4/8	(9.51-41.57)
			30	6/8	
			45	6/8	
		ScMET	31.3	0/2	ED_{50}>250
			62.5	0/2	
			125	0/2	
			250	0/2	
Carbamazepine [18]	*p.o.*	MES			ED_{50}=5.35 (3.26-7.62)
		ScMET			ED_{50}>250
		TOX			TD_{50}=364 (223-500)

[a] Number of animals protected / number of animals tested; [b] number of animals exhibiting toxicity / number of animals tested in the rotarod test; [D] – death; TPE – time of peak effect.

Compound **4** was also evaluated for activity in the murine model of status epilepticus – pilocarpine test (Table 9). As a result, TD_{95} was determined as 64.99 mg/kg *i.p.*

Table 9
Pilocarpine test results in mice, *i.p.*

Compd.	Test	Dose	Time [h]						TD_{95}
		[mg/kg b.w.]	0.0	0.25	0.5	1.0	2.0	4.0	[mg/kg b.w.]
4	TOX [a]	65	1/8						64.99
24	TOX [a]	300		0/2	0/2	1/2	2/2	2/2	

[a] Number of animals exhibiting toxicity / number of animals tested in the rotarod test.

Some anticonvulsant drugs cause higher susceptibility of patients to seizures. Therefore, compound **4** has been tested for its potential proconvulsant activity in the intravenous metrazol test (ivMet) in mice, after intraperitoneal administration. Typically, metrazol causes seizures after timed intravenous administration (time and dose are measured). If compound administered intraperitoneally prior to the test diminishes the dose (or shortens the time), its

proconvulsant activity can be assumed. For compound **4** administered at doses ca. ED_{50} (MES, mice, *i.p.*) 5 mg/kg and TD_{50} (rotarod, mice, *i.p.*) 30 mg/kg, the doses of Met causing seizures are 31.18 (negative control), 30.11 (at ED_{50}) and 29.26 mg/kg (at TD_{50}) (Table 10).

Table 10
Influence on seizure threshold – *i.v.* metrazol in mice, *i.p.*

Compd.	Time [h]	Dose [mg/kg b.w.]	Weight [g] ± SEM	Time to twitch [min] ± SEM	Dose at twitch [mg/kg b.w.] ± SEM	Time to clonus ± SEM	Dose at clonus [mg/kg b.w.] ± SEM
4	0.25	0	27.3 ± 0.2	30.07 ± 1.23	31.18 ± 1.18	34.38 ± 0.94	35.67 ± 0.91
		5	27.3 ± 0.2	29.04 ± 1.30	30.11 ± 1.26	33.06 ± 1.23	34.32 ± 1.25
		30	27.6 ± 0.3	28.50 ± 1.46	29.26 ± 1.55	32.70 ± 1.28	33.62 ± 1.53
7	0.25	0	27.3 ± 0.3	29.93 ± 0.87	31.11 ± 1.04	33.75 ± 0.96	35.05 ± 1.05
		6	27.0 ± 0.2	25.87 ± 0.91	27.14 ± 0.92	30.82 ± 0.95	32.32 ± 0.90
		33	27.0 ± 0.1	25.57 ± 1.00	26.82 ± 1.02	29.28 ± 0.86	30.71 ± 0.86
12	0.5	0	30.3 ± 0.38	31.45 ± 0.75	29.44 ± 0.75	36.54 ± 0.92	34.18 ± 0.81
		29	30.4 ± 0.41	33.43 ± 1.48	31.26 ± 1.43	36.96 ± 1.58	34.54 ± 1.49
		46	29.8 ± 0.54	31.23 ± 1.44	29.70 ± 1.29	35.95 ± 2.04	34.18 ± 1.82
Carbamazepine [18]	0.25	0		35.2 ± 0.7		46.2 ± 1.5	
		11		36.1 ± 1.3		48.6 ± 4.2	
		28		33.5 ± 0.8		42.7 ± 2.1	
		48		36.5 ± 1.1		54.5 ± 3.8	
Valproic acid [18]	0.25	0		34.8 ± 1.8		43.4 ± 1.8	
		60		37.1 ± 1.1		45.0 ± 2.3	
		120		40.4 ± 2.0		55.2 ± 1.9	

Data is average of 10 animals in group; $p>0.05$.

Another model for psychomotor seizures is hippocampal kindling model in rats *i.p.*, where compound **4** exhibits activity at 30 mg/kg b.w. (Table 11-12). Some activity is also visible in

a rat model of drug-resistant seizures – the lamotrigine-resistant amygdala kindling test already at 30 mg/kg (*i.p.*) where the compound diminishes seizures into the state referring to aura in humans - stage 1 in the Racine's scale (Table 13).

Table 11

Results of preliminary hippocampal kindling screen in rats, *i.p.*

Compd.	Dose [mg/kg b.w.]	Time [h]	Animal No.	Seizure score		Afterdischarge duration [s]		TPE [h]
				Pre-drug	Drug	Pre-drug	Drug	
4	30	0	Rat #1	5	5		35	
			Rat #2		5		100	
		0.25	Rat #1		5		38	
			Rat #2		5		90	
		1	Rat #1		5		43	
			Rat #2		5		140	
		2	Rat #1		5		81	
			Rat #2		5		65	
		4	Rat #1		5			
			Rat #2		5		48	
5	10	0.25-	Rat #1	4-5	4-5	50-82	46-137	
		2.25	Rat #2	5	5	37-75	43-95	
7	20	0.25-	Rat #1	5	4-5	68-77	53-88	
		2.25	Rat #2	4-5	4-5	79-100	57-67	
12	30	0	Rat #1		5		44	0.75
			Rat #2		5		33	
		0.25	Rat #1		2		14	
			Rat #2		5		42	
		1	Rat #1		5		57	
			Rat #2		5		40	
		2	Rat #1		5		62	
			Rat #2		5		57	
		4	Rat #1		5		55	
			Rat #2		5		56	
	50	2.25	Rat #1	5	4	70-74	57	
			Rat #2	5	1	49-70	17	

Seizures are scored according to the following criteria (Racine's scale): stage 1 – mouth and facial clonus; stage 2 – stage 1 plus head nodding; stage 3 – stage 2 plus forelimb clonus; stage 4 – stage 3 plus rearing; stage 5 – stage 4 plus repeated rearing and falling [20]; [#] significantly different from control.

Table 12
Activity in hippocampal kindling in rats, *i.p.*

Compd.	Dose [mg/kg b.w.]	Time [h]	Seizure score ± SEM [a)]	Duration [s] ± SEM	No. animals protected/ tested	ED_{50} (Confidence interval) [mg/kg b.w.]
4	30	0	4.88 ± 0.13	74.57 ± 8.29		TPE=0.25
		0.25	4.00 ± 0.33 [#]	63.00 ± 7.11		ED_{50}>30
		0.75	4.75 ± 0.16	62.88 ± 4.43		
		1.25	4.63 ± 0.18	84.75 ± 10.99		
		1.75	4.25 ± 0.62	60.75 ± 11.29		
		2.25	4.88 ± 0.13	82.50 ± 4.89		
	60	0	4.9 ± 0.1	49 ± 4		
		0.25	2.7 ± 0.6 [#]	41 ± 4		
		0.75	3.6 ± 0.2 [#]	35 ± 1 [#]		
		1.25	3.6 ± 0.4 [#]	43 ± 3		
		1.75	3.7 ± 0.5 [#]	55 ± 4		
		2.25	4.4 ± 0.2	58 ± 3		
12	12.5	0.75	4.67 ± 0.21	60.50 ± 5.25	0/6	30.63
	25	0.75	2.50 ± 0.76 [#]	30.75 ± 7.51 [#]	4/8	(18.52-53.46)
	50	0.75	2.00 ± 0.63 [#]	28.50 ± 9.00 [#]	6/8	

[a)] Racine's scale as in Table 11; [#] significantly different from control

Table 13
Lamotrigine-resistant amygdala kindling in rats, *i.p.*

Compd.	Dose [mg/kg b.w.]	Time [h]	Seizure score ± SEM [a)]	Duration [s] ± SEM	No. animals protected/ tested
4	Control	0.5	5.0 ± 0.0	104.60 ± 23.57	0/5
	30	0.5	3.6 ± 0.6	158.40 ± 21.60	3/5
	Control	0.5	5.0 ± 0.0	109.40 ± 19.56	0/5
	45	0.5	1.4 ± 0.6 [#]	124.80 ± 32.05	5/5

[a)] Racine's scale as in Table 11; [#] Statistically significant

An interesting pharmacological profile can be observed also for the 4-piperidinol derivative – compound **11**. It has undergone procedures involving 4 h observations of activity at 6 Hz, MES and TOX. The activity is observed already in 25 mg/kg in MES and in 50 mg/kg in 6 Hz, both at 0.25 h after administration (Table 5). Its ED_{50} in MES is 17.31 and TD_{50} is 71.26 mg/kg (mice, *i.p.*), and the confidence intervals do not cover (Table 6). Activity in corneal kindling of mice is observed with ED_{50} 57.52 mg/kg (Table 14). Compound **11** is also most active in rats, 15 min after *i.p.* administration.

Table 14
Corneal kindling in mice, *i.p.*

Compd.	Dose [mg/kg b.w.]	Time [h]	Individual seizure scores [a)]	Average seizure score	ED_{50} [mg/kg b.w.]
11	17	0.25	3, 5, 0, 3	2.75	57.52

[a)] Racine's scale as in Table 11.

The final interesting compound in this group is an *R,S*-2-amino-1-phenylethanol derivative – compound **12**. In subsequent assays it proved higher potency than its enantiomers **13** (*R*) and **14** (*S*) which were not advanced to further assays after screening (Table 4). ED_{50} of **12** in rats in MES after oral administration is 21.06 mg/kg (Table 8) and the compound does not have a clear influence on seizure threshold in mice (Table 10). Taking into account activity in psychomotor seizures reflected by hippocampal kindling in rats (*i.p.*) – the activity is documented with ED_{50}=30.63 mg/kg (Table 12).

Among the title compounds, **4** has been selected for verification of possible mechanism of action, with use of radioligand binding studies as the high throughput profile at Cerep, France.[21] The study included binding of the compound to 82 targets at a single concentration. First, it was chosen to examine the base – compound **4**, at a large concentration 100 μM, in order to receive an overview of possible local adverse reactions after injection of its solution. The result is presented in Table 15. However, 50% binding at such concentration may show more of adverse events than of the activity, therefore, IC_{50} values were measured as well. In order to receive the exact data of the chosen form, the assay was performed for the hydrochloride – **4a2**. The results are presented in Table 16. As it can be observed, **4a2** binds to four targets at 10^{-6} M: σ, 5-HT_{1A}, 5-HT transporter, and α_2. Among the observed pharmacological profile, the activity can be attributed to these receptors and, as a result, some possible adverse reactions could be further explored due to the shown binding. Moreover, functional assays may be another step in understanding the observed activity.

Table 15

Receptor binding of compound **4**

Receptor	Binding at 10^{-4} M of compound **4**			Study conditions			Reference	
	% Inhibition of Control Specific Binding	Mean / % of Control Specific Binding	SEM % Control	Origin of receptor	Radioligand	Radioligand type	Compound	IC_{50} [M]
A_1	-33	133.1	2.1	human recombinant CHO cells	[^{3}H]DPCPX	Antagonist	DPCPX	$5.0*10^{-10}$
A_{2A}	30	69.8	9.0	human recombinant HEK-293 cells	[^{3}H]CGS 21680	Agonist	NECA	$3.7*10^{-8}$
A_3	-13	113.0	4.2	human recombinant HEK-293 cells	[^{125}I]ABMECA	Agonist	IB-MECA	$6.0*10^{-10}$
α_1	73	26.6	1.0	rat cerebral cortex	[^{3}H]prazosin	Antagonist	prazosin	$2.5*10^{-10}$
α_2	70	29.9	1.8	rat cerebral cortex	[^{3}H]RX 821002	Antagonist	yohimbine	$9.6*10^{-8}$
β_1	15	85.2	10.7	human recombinant HEK-293 cells	[^{3}H](-)CGP12177	Agonist	atenolol	$2.1*10^{-7}$
β_2	8	91.9	3.9	human recombinant CHO cells	[^{3}H](-)CGP12177	Agonist	ICI 118551	$7.4*10^{-10}$
AT_1	-33	132.7	3.2	human recombinant HEK-293 cells	[^{125}I] [Sar^1,Ile^8]-ATII	antagonist	saralasin	$4.0*10^{-10}$
AT_2	-3	102.8	0.8	human recombinant HEK-293 cells	[^{125}I]CGP42112A	agonist	angiotensin-II	$8.0*10^{-11}$
BZD (central)	-10	109.5	2.7	rat cerebral cortex	[^{3}H]flunitrazepam	Agonist	diazepam	$6.8*10^{-9}$
BZD (peripheral)	-8	107.6	3.0	rat heart	[^{3}H]PK 11195	Antagonist	PK 11195	$1.6*10^{-9}$
BB (non-selective)	4	96.5	1.5	rat cerebral cortex	[^{125}I][Tyr^4]bombesin	Agonist	bombesin	$3.4*10^{-10}$

B_2	-12	112.3	4.4	human recombinant CHO cells	[^{3}H]bradykinin	Agonist	NPC 567	$1.4*10^{-8}$
CGRP	-21	121.3	1.2	human recombinant CHO cells	[^{125}I]hCGRPα	Agonist	hCGRPalpha	$3.2*10^{-11}$
CB_1	-3	103.3	5.7	human recombinant CHO cells	[^{3}H]CP 55940	Agonist	CP 55940	$9.0*10^{-10}$
CCK_1 (CCK_A)	-21	121.2	7.7	human recombinant CHO cells	[^{125}I]CCK-8s	Agonist	CCK-8s	$1.3*10^{-10}$
CCK_2 (CCK_B)	-19	119.2	1.5	human recombinant CHO cells	[^{125}I]CCK-8s	Agonist	CCK-8s	$1.2*10^{-10}$
D_1	22	78.2	1.2	human recombinant CHO cells	[^{3}H]SCH23390	Antagonist	SCH 23390	$3.1*10^{-10}$
D_{2S}	36	64.0	0.6	human recombinant HEK-293 cells	[^{3}H]methylspiperone	Antagonist	(+)butaclamol	$1.1*10^{-9}$
D_3	58	42.2	2.6	human recombinant CHO cells	[^{3}H]methylspiperone	Antagonist	(+)butaclamol	$1.6*10^{-9}$
$D_{4.4}$	55	44.7	2.3	human recombinant CHO cells	[^{3}H]methylspiperone	antagonist	clozapine	$4.1*10^{-8}$
D_5	27	73.2	5.6	human recombinant GH4 cells	[^{3}H]SCH23390	Antagonist	SCH 23390	$5.6*10^{-10}$
ET_A	-39	139.3	1.9	Human recombinant CHO cells	[^{125}I]endothelin-1	Agonist	endothelin-1	$2.3*10^{-11}$
ET_B	-19	119.3	2.1	human recombinant CHO cells	[^{125}I]endothelin-1	Agonist	endothelin-3	$6.3*10^{-12}$
GABA (non-selective)	5	94.9	2.3	rat cerebral cortex	[^{3}H]GABA	Agonist	GABA	$2.1*10^{-8}$
GAL_1	-9	108.5	6.6	human recombinant HEK-293 cells	[^{125}I]galanin	Agonist	galanin	$7.9*10^{-11}$
GAL_2	4	96.2	6.4	human recombinant CHO cells	[^{125}I]galanin	Agonist	galanin	$2.6*10^{-9}$
PDGF	7	93.0	10.8	Balb/c 3T3 cells	[^{125}I]PDGF BB	Agonist	PDGF BB	$4.7*10^{-11}$
CXCR2 (IL-	-16	116.0	4.5	human recombinant	[^{125}I]IL-8	Agonist	IL-8	$5.1*10^{-11}$

8B)				HEK-293 cells				
CCR1	-2	102.0	3.5	human recombinant HEK-293 cells	[^{125}I]MIP-1α	Agonist	MIP-1alpha	$2.1*10^{-11}$
TNF-α	1	98.5	5.4	U-937 cells	[^{125}I]TNF-α	Agonist	TNF-alpha	$9.9*10^{-11}$
H_1	40	60.2	0.8	human recombinant HEK-293 cells	[^{3}H]pyrilamine	antagonist	pyrilamine	$1.4*10^{-9}$
H_2	-4	104.2	3.5	human recombinant CHO cells	[^{125}I]APT	Antagonist	cimetidine	$1.0*10^{-6}$
MC_4	4	96.1	2.0	human recombinant CHO cells	[^{125}I]NDP-α-MSH	Agonist	NDP-alpha - MSH	$1.5*10^{-10}$
MT_1 (ML_{1A})	27	73.1	3.9	human recombinant CHO cells	[^{125}I]2-iodomelatonin	Agonist	melatonin	$1.1*10^{-10}$
M_1	11	88.9	4.4	human recombinant CHO cells	[^{3}H]pirenzepine	Antagonist	pirenzepine	$2.1*10^{-8}$
M_2	1	98.8	1.3	human recombinant CHO cells	[^{3}H]AF-DX384	Antagonist	methoctramine	$2.2*10^{-8}$
M_3	18	81.9	3.6	human recombinant CHO cells	[^{3}H]4-DAMP	Antagonist	4-DAMP	$4.1*10^{-10}$
M_4	20	79.6	1.2	human recombinant CHO cells	[^{3}H]4-DAMP	Antagonist	4-DAMP	$4.9*10^{-10}$
M_5	29	71.4	2.0	human recombinant CHO cells	[^{3}H]4-DAMP	Antagonist	4-DAMP	$3.2*10^{-10}$
NK_1	-5	105.3	0.5	U-373MG cells (endogenous)	[^{125}I]BH-SP	Agonist	[Sar9,Met(O2)11]-SP	$1.1*10^{-10}$
NK_2	-5	104.9	0.2	human recombinant CHO cells	[^{125}I]NKA	Agonist	[Nleu10]-NKA (4-10)	$3.5*10^{-9}$
NK_3	3	96.7	1.8	human recombinant CHO cells	[^{3}H]SR142801	Antagonist	SB 222200	$6.7*10^{-9}$
Y_1	-19	118.9	1.6	SK-N-MC cells (endogenous)	[^{125}I]peptideYY	Agonist	NPY	$2.1*10^{-10}$
Y_2	-26	126.1	13.2	KAN-TS cells	[^{125}I]peptideYY	Agonist	NPY	$2.8*10^{-11}$
NTS_1 (NT_1)	-12	112.1	11.8	human recombinant	[^{125}I]Tyr3-	Agonist	neurotensin	$4.9*10^{-10}$

				CHO cells	neurotensin			
δ_2 (DOP)	2	97.7	2.3	human recombinant CHO cells	[^{3}H]DADLE	Agonist	DPDPE	$1.3*10^{-9}$
κ (KOP)	33	66.7	1.2	rat recombinant CHO cells	[^{3}H]U 69593	Agonist	U 50488	$6.4*10^{-10}$
μ (MOP)	16	84.0	3.3	human recombinant HEK-293 cells	[^{3}H]DAMGO	Agonist	DAMGO	$4.3*10^{-10}$
NOP (ORL1)	1	98.7	1.6	human recombinant HEK-293 cells	[^{3}H]nociceptin	Agonist	nociceptin	$1.3*10^{-9}$
PAC_1 (PACAP)	0	99.7	3.8	human recombinant CHO cells	[^{125}I]PACAP1-27	Agonist	PACAP1-38	$4.0*10^{-11}$
$PPAR_\gamma$	7	93.1	7.5	human recombinant (*E. coli*)	[^{3}H]rosiglitazone	Agonist	rosiglitazone	$1.0*10^{-8}$
PCP	26	74.2	4.1	rat cerebral cortex	[^{3}H]TCP	Antagonist	MK 801	$4.6*10^{-9}$
EP_2	5	95.3	5.6	human recombinant HEK-293 cells	[^{3}H]PGE_2	agonist	PGE2	$2.6*10^{-9}$
IP (PGI_2)	-1	101.3	1.3	human recombinant HEK-293 cells	[^{3}H]iloprost	Agonist	iloprost	$1.0*10^{-8}$
P2X	-9	108.6	1.2	rat urinary bladder	[^{3}H]α,β-MeATP	Agonist	alpha ,beta - MeATP	$1.9*10^{-9}$
P2Y	4	96.5	1.3	rat cerebral cortex	[^{35}S]dATPαS	Agonist	dATPalpha S	$2.9*10^{-8}$
5-HT_{1A}	94	6.2	0.5	human recombinant HEK-293 cells	[^{3}H]8-OHDPAT	Agonist	8-OH-DPAT	$5.3*10^{-10}$
5-HT_{1B}	15	85.5	5.1	rat cerebral cortex	[^{125}I]CYP (+30 μM isoproterenol)	Antagonist	serotonin	$4.9*10^{-9}$
5-HT_{2A}	25	75.3	9.1	human recombinant HEK-293 cells	[^{3}H]ketanserin	Antagonist	ketanserin	$3.5*10^{-10}$
5-HT_{2B}	85	15.3	1.2	human recombinant CHO cells	[^{125}I](±)DOI	Agonist	(±)DOI	$2.5*10^{-9}$
5-HT_{2C}	39	61.4	5.7	human recombinant HEK-293 cells	[^{3}H]mesulergine	Antagonist	RS 102221	$1.5*10^{-9}$
5-HT_3	6	94.2	2.7	human recombinant	[^{3}H]BRL43694	Antagonist	MDL 72222	$6.9*10^{-9}$

				CHO cells				
5-HT_{5A}	13	87.1	9.1	human recombinant HEK-293 cells	[^{3}H]LSD	Agonist	serotonin	$1.8*10^{-7}$
5-HT_6	-11	110.8	0.2	human recombinant CHO cells	[^{3}H]LSD	Agonist	serotonin	$1.2*10^{-7}$
5-HT_7	57	42.6	0.6	human recombinant CHO cells	[^{3}H]LSD	Agonist	serotonin	$2.5*10^{-10}$
σ (non-selective)	99	1.1	4.5	Jurkat cells (endogenous)	[^{3}H]DTG	Agonist	haloperidol	$1.7*10^{-8}$
sst (non-selective)	15	84.9	1.5	AtT-20 cells	[^{125}I]Tyr11-somatostatin-14	Agonist	somatostatin-14	$7.4*10^{-11}$
GR	-17	116.7	10.5	IM-9 cells (cytosol)	[^{3}H]dexamethasone	Agonist	dexamethasone	$3.8*10^{-9}$
$VPAC_1$ (VIP_1)	-19	119.0	7.2	human recombinant CHO cells	[^{125}I]VIP	Agonist	VIP	$1.1*10^{-10}$
V_{1a}	3	96.7	7.3	human recombinant CHO cells	[^{3}H]AVP	Agonist	[d(CH2)51,Tyr(Me)2]-AVP	$9.9*10^{-10}$
Ca^{2+} channel (L, verapamil site)	32	67.9	2.9	rat cerebral cortex	[^{3}H]D888 Antagonist		D 600	$1.5*10^{-8}$
K_V channel	-3	103.4	2.8	rat cerebral cortex	[^{125}I]α-dendrotoxin	antagonist	alpha-dendrotoxin	$5.7*10^{-10}$
SK_{Ca} channel	4	95.9	2.2	rat cerebral cortex	[^{125}I]apamin	antagonist	apamin	$1.6*10^{-11}$
Na^+ channel (site 2)	60	40.2	4.5	rat cerebral cortex	[^{3}H]batrachotoxinin	antagonist	veratridine	$4.0*10^{-6}$
Cl^- channel (GABA-gated)	-2	102.1	2.6	rat cerebral cortex	[^{35}S]TBPS	antagonist	picrotoxinin	$7.7*10^{-8}$
norepinephrine transporter (h) (antagonist radioligand)	24	76.3	3.1	human recombinant CHO cells	[^{3}H]nisoxetine Antagonist	antagonist	protriptyline	$2.8*10^{-9}$
dopamine	58	42.0	1.7	human recombinant	[^{3}H]BTCP	antagonist	BTCP	$2.8*10^{-9}$

transporter				CHO cells	Antagonist			
5-HT	93	6.5	1.9	human recombinant	[^{3}H]imipramine	antagonist	imipramine	$8.8*10^{-10}$
transporter				CHO cells	Antagonist			

Table 16

IC_{50} results for **4a2**

Receptor	IC_{50} [M][a)]	K_i [M][a)]
α_1	$3.9*10^{-5}$	$1.0*10^{-5}$
α_2	$1.8*10^{-5}$	**$7.9*10^{-6}$**
D_3	$7.5*10^{-5}$	$1.7*10^{-5}$
$D_{4.4}$	$1.0*10^{-4}$	$3.9*10^{-5}$
5-HT_{1A}	**$2.8*10^{-6}$**	**$1.7*10^{-6}$**
5-HT_{2B}	$1.0*10^{-5}$	$5.2*10^{-6}$
5-HT_7	$7.0*10^{-5}$	$2.6*10^{-5}$
Σ	**$1.9*10^{-6}$**	**$1.5*10^{-6}$**
Na^+	$>1.0*10^{-4}$	-
Dopamine transporter	$4.0*10^{-5}$	$2.1*10^{-5}$
5-HT transporter	**$7.0*10^{-6}$**	**$3.2*10^{-6}$**

[a)] Results achieved for 5 concentrations.

Mutagenicity evaluation

With the used strains of *Salmonella typhimurium*, neither compounds **3a** and **4**, nor their metabolites exhibited mutagenicity compared to known mutagens (Table 17-18).

Table 17
Mutagenic evaluation of compound **3a** in *Salmonella typhimurium* strains TA100 and TA1535

Compd.	Concentration [µg per plate]	Number of revertants *Salmonella typhimurium*			
		TA100		TA1535	
		-S9 Mean ± SD	+S9 Mean ± SD	-S9 Mean ± SD	+S9 Mean ± SD
3a	0.4	120 ± 18	172 ± 6	10 ± 4	19 ± 8
	4.0	131 ± 13	150 ± 16	10 ± 8	18 ± 7
	40.0	148 ± 4	153 ± 21	8 ± 1	16 ± 8
	80.0	147 ± 7	166 ± 6	12 ± 1	14 ± 6
	160.0	131 ± 6	158 ± 19	10 ± 4	20 ± 5
DMSO		114 ± 18	101 ± 10	11 ± 2	22 ± 5
Positive control	5.0	1039 ±26	961 ± 38	452 ± 33	407 ± 32

For assays without metabolic activation (-S9) the positive control was sodium azide (SA), whereas for assays with metabolic activation (+S9) the positive control was 2-aminoanthracene (2AA); mean values of three independent experiments ± standard deviation are presented.

Table 18
Effect of compound **4** in the Ames mutagenicity assay

Compd.	Concentration [mM]	% of control [a)]			
		+ NADPH		- NADPH	
		Sample 1	Sample 2	Sample 1	Sample 2
4	0.1	4.3	6.2	4.7	4.5
	0.2	6.0	7.6	6.3	4.2
	0.5	6.3	8.5	4.0	5.1
None		7.6	3.7		
DMSO		7.6	3.9		
Benzo[a]pyrene	0.02	100	100	5.3	3.1
Acridine orange	0.02	120	101	13.3	14.1

[a)] The number of colonies is presented as % of the number generated by 0.02 mM benzo[a]pyrene.

3. Conclusions

Twenty four new derivatives of aminoalkanols have been synthesized and evaluated for their anticonvulsant activity and neurotoxicity in mice (male and in some cases female) and rats, after intraperitoneal or oral administration. The highest potency is observed for *R*-2*N*-(2,6-dimethylphenoxy)ethyl]aminopropan-1-ol (**4**), its 4-piperidinol analog (**11**) and *R,S*-2-amino-1-phenylethanol analog (**13**). As far as the linker between xylenoxyl and aminoalkanol group is concerned, ethylene is the most favorable as it is present in all the most active compounds **4**, **11** and **12** as well as in the reference compounds **I** and **II**. Compound **4a** exerts its activity through binding to σ, 5-HT_{1A}, and α_2 receptors as well as 5-HT transporter.

Compounds **3-5** were achieved also in the form of hydrochlorides **3a-5a** for improvement of their solubility for intravenous administration in further research. The enantiomers revealed two forms **4a1**, **4a2**, **5a1**, and **5a2**, respectively, with various melting points, which by means of thermal analysis (TG, DSC) was proved to be an effect of polymorphism. Moreover, crystallography analysis has been completed for **4a2**.

Considering the observations described in this paper, in the light of premises shown in the introduction, the group of aroxyalkylaminoalkanols should be further explored for possible anticonvulsant activity. Moreover, there are premises for the most active compounds for exploration of their pharmacological profiles and pharmacokinetics. Especially information regarding concentrations of **4a** in brain at time points of peak activity may confirm activity on the achieved receptor panel in the nervous tissue.

4. Experimental protocols

4.1. Chemistry

The synthesis of compounds **1**-**24** was performed according to formerly published procedures.[7, 13] The aminoalkanols used for synthesis of compounds **3**, **6-7**, **10, 12, 15, 17-18, 20-23** were racemic (Table 1). 1-amino-2-butanol was achieved according to formerly published procedures.[8] Other reagents were purchased from Alfa Aesar GmbH&Co KG (Karlsruhe, Germany) and Merck Sp. z o.o. (Warszawa, Poland) and solvents were commercially available materials of reagent grade.

Melting points (mp.) were determined using a Büchi SMP-20 apparatus and are uncorrected. Analyses of C, H, N were within ± 0.4% of the theoretical values. Analytical TLC was carried out on precoated plates (silica gel, 60 F-254 Merck). Spots were visualized with UV light. ^{1}H NMR and ^{13}C NMR spectra for compounds **1, 3-4a, 5a, 8-10, 13-18, 20-22,** were recorded at Faculty of Chemistry, Jagiellonian University (Krakow, Poland), on a Bruker AVANCE III 600 (resonance frequencies 600.20 MHz for ^{1}H and 150.94 MHz for ^{13}C) equipped a 5-mm probehead: PABBO with z-gradient or TBI with XYZ gradients. The ^{1}H spectra were recorded with 16 scans, 1 s relaxation delay, 4 s acquisition time, 128 kW FID size, with 16234 Hz spectral width. The ^{13}C spectra were recorded with WALTZ-16 ^{1}H broadband decoupling, a few thousands scans, 2 s relaxation delay, 0.9 s acquisition time, 64 kW FID size, 36 057 Hz spectral width. Standard pulse sequences from Bruker library were used for 2D spectra. Gradient enhanced sequences were used for the homo- and heteronuclear 2D experiments. All processing and analysis were performed using Bruker's TopSpin 3.0 software suite.

^{1}H NMR spectra for compounds **2**, **5**-7, **11**-**12**, **19**, **23**-**24** were recorded at Faculty of Pharmacy, Jagiellonian University Medical College (Krakow, Poland) with a Varian Mercury-VX 300 NMR spectrometer at 29˚C. Chemical shifts were referenced against solvent lock signal. Standard Varian pulse sequences were used for 2D experiments.

Results are presented in the following format: chemical shift δ (ppm), multiplicity, *J* values 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), ddd (doublet of doublets of doublets), dt (doublet of triplets), ddq (doublet of doublets of quintets), hd (heptet of doublets), t (triplet), qn (quintet), m (multiplet).

The IR spectra were recorded on a Jasco FT/IR 410 spectrometer (KBr pellets). Measurement of optical rotation ($[\alpha]_{589}$ and/or $[\alpha]_{546}$) was carried out using Jasco 2000 (λ = D (589 nm)). For mass spectrometry analysis samples were prepared in acetonitrile/water (10/90 v/v)

mixture. The LC/MS system consisted of a Waters Acquity UPLC, coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). All the analyses were carried out usingan Acquity UPLC BEH C18, 1.7 lm, 2.1 _ 100 mm column. A flow rate of 0.3 mL/min and a gradient of (5‑95)% B over 10 min and then 100% B over 2 min was used. Eluent A: water/0.1% HCO_2H; eluent B: acetonitrile/0.1% HCO_2H. LC/MS data were obtained by scanning the first quadrupole in 0.5 s in a mass range from 50 to 1000 Da; eight scans were summed up to produce the final spectrum.

2N-[(2,6-dimethylphenoxy)ethyl]-2N-methylaminoethan-1-ol (1)

$C_{13}H_{22}NO_2Cl$; 259.77; Mp. 100-102 °C; ^{1}H NMR (DMSO-d_6, δ ppm): 10.88 (bs, 1H, NH^+); 7.02 ($\underline{A}_2B$, *J*=7.4; 2H, H-3, H-5); 6.92 ($A_2\underline{B}$, *J*=7.4, 1H, H-4); 5.41 (bs, 1H, CH_2-O$\underline{H}$); 4.19 (t, *J*=5.6, 2H, Ar-O-CH_2-); 3.85 (t, *J*=5.3, 2H, -C$\underline{H}_2$-OH); 3.57 (bs, 2H, Ar-O-CH_2-C$\underline{H}_2$-N<); 3.32 (bs, 2H, N-C$\underline{H}_2$, -CH_2-OH); 2.94 (s, 3H, N-CH_3); 2.26 (s, 6H, CH_3-Ar (2,6)); ^{13}C NMR (δ ppm) 154.8 (C-1); 130.1 (C-2, 6); 128.7 (C-3, 5); 124.2 (C-4); 65.8 (Ar-O-CH_2-); 57.6 (N-$\underline{C}H_2$, -CH_2-OH); 55.2 (-CH_2-OH); 55.1 (Ar-O-CH_2-$\underline{C}H_2$-N<); 40.7 (N-CH_3); 16.1 (CH_3-Ar (3,6)); LC-MS $[M+H]^+$ m/z: 224.10, 99%.

3N-[(2,6-dimethylphenoxy)ethyl]aminopropan-1-ol (2)

$C_{13}H_{21}NO_2$; 223.31; Mp. 95-97 °C; IR (KBr, cm^{-1}) *v*: 3419, 3112, 3033, 2954, 2853, 2766, 2725, 2542, 2441, 2404, 1604, 1484, 1406, 1264, 1204, 1039; ^{1}H NMR (DMSO-d_6, δ ppm): 9.18 (bs, 2H, NH_2^+); 7.06-6.99 (m, 2H, H-3, H-5); 6.97-6.89 (m, 1H, H-4); 4.83 (bs, 1H, OH); 4.01 (t, *J*=5.39, 2H, Ar-O-C$\underline{H}_2$-CH_2-NH_2^+); 3.50 (t, 2H, *J*=5.64, -C$\underline{H}_2$-OH); 3.32 (bs, Ar-O-CH_2-C$\underline{H}_2$-NH_2^+); 3.07 (bs, 2H, NH_2^+-C$\underline{H}_2$-CH_2-CH_2OH); 2.24 (s, 6H, Ar-CH_3); 1.91-1.76 (m, 2H, NH_2^+-CH_2-C$\underline{H}_2$-CH_2OH); LC-MS $[M+H]^+$ m/z: 224.10, 100%.

R,S-2N-[(2,6-dimethylphenoxy)ethyl]aminopropan-1-ol (3)

$C_{13}H_{21}NO_2$; 223.31; Mp. 66-68 °C; R_f=0.56 (CH_3OH/ethyl acetate 1:1); IR (KBr, cm^{-1}) *v*: 3291, 3147, 3068, 2982, 2964, 2930, 2843, 2593, 1473, 1201, 1041, 757, 434; ^{1}H NMR (DMSO-d_6, δ ppm): 7.00 (d, *J*=7.3, 1H, H-3 or H-5); 7.00 (d, *J*=7.6, 1H, H-3 or H-5); 6.88 (dd, *J*=7.6, *J*=7.3, 1H, H-4); 4.55 (dd, *J*=5.4, *J*=4.8, 1H, OH); 3.79 (ddd, *J*=9.3, *J*=5.7, *J*=5.0, 1H, C$\underline{H}$H-O-Ar); 3.77 (ddd, *J*=9.3, *J*=6.8, *J*=4.9, 1H, CH$\underline{H}$-O-Ar); 3.31 (ddd, *J*=10.4, *J*=5.0, *J*=4.8, 1H, C$\underline{H}$H-O-H); 3.24 (ddd, *J*=10.4, *J*=6.8, *J*=5.4, 1H, CH$\underline{H}$-O-H); 2.91 (ddd, *J*=12.2, *J*=5.7, *J*=4.9, 1H, C$\underline{H}$H-N); 2.86 (ddd, *J*=12.2, *J*=6.8, *J*=5.0, 1H, CH$\underline{H}$-N); 2.67 (ddq, *J*=6.8, *J*=6.4, *J*=5.0, 1H, CH); 2.22 (s, 6H, CH_3-Ar(2,6)); 1.97 (bs, 1H, N-H); 0.93 (t, *J*=6.4, 3H, CH-

$\underline{C}H_3$). ^{13}C NMR (δ ppm): 155.4 (C-1), 130.2 (C-2, C-6); 128.6 (C-3, C-5); 123.5 (C-4); 71.8 (CH_2-O-Ar); 65.5 (CH_2-OH); 54.4 (CH); 46.7 (CH_2-N); 17.1 (CH-$\underline{C}H_3$); 15.9 (CH_3-Ar(2.6)); LC-MS $[M+H]^+$ m/z: 224.26, 100%.

R,S-2N-[(2,6-dimethylphenoxy)ethyl]aminopropan-1-ol hydrochloride (3a)

$C_{13}H_{22}NO_2Cl$; 259.77; Mp. 137-139 °C; [$\%^{calc.}/_{analyzed}$] $C^{60.10}/_{59.74}$, $H^{8.54}/_{8.67}$, $N^{5.40}/_{5.23}$; ^1H NMR (DMSO-d_6, δ ppm): 9.06 (bs, 2H, NH_2^+); 7.04 (AB_2X_3 d, *J*=7.5, *J*=0.7, 1H, H-3, H-5); 6.94 (AB_2X_3, *J*=7.5, 1H, H-4); 5.42 (dd, *J*=5.4, *J*=4.8, 1H, OH); 4.07 (t, *J*=5.7, 2H, CH_2-O-Ar); 3.71-3.66 (m, 1H, CH$\underline{H}$-O-H); 3.63-3.58 (m, 1H, C$\underline{H}$H-O-H); 3.36 (t, *J*=5.7, 2H, CH_2-N); 3.35-3.30 (m, 1H, CH); 2.26 (AB_2X_3, *J*=0.7, 6H, CH_3-Ar(2,6)); 1.27 (d, *J*=6.7, 3H, CH-CH_3); ^{13}C NMR (δ ppm): 154.6 (C-1), 130.3 (C-2, C-6); 128.7 (C-3, C-5); 124.1 (C-4); 66.8 (CH_2-O-Ar); 61.0 (CH_2-OH); 55.0 (CH); 44.0 (CH_2-N); 15.9 (CH-$\underline{C}H_3$); 13.2 (CH_3-Ar(2,6)).

R-2N-[(2,6-dimethylphenoxy)ethyl]aminopropan-1-ol (4)

$C_{13}H_{21}NO_2$; 223.31; Mp. 81-82 °C; R_f=0.56 (CH_3OH/ethyl acetate 1:1); ^1H NMR (DMSO-d_6, δ ppm): 7.07-6.96 (m, 2H, H-3, H-5); 6.95-6.84 (m, 1H, H-4); 4.57 (t, *J*=5.2, 1H, OH); 3.85-3.71 (m, 2H, Ar-O-CH_2); 3.33-3.19 (m, 2H, $C\underline{H_2}$-OH); 2.97-2.80 (m, 2H, CH_2-N); 2.74-2.61 (m, 1H, CH); 2.22 (s, 6H, 2(Ar(CH_3)); 1.97 (bs, 1H, NH); 0.93 (d, *J*=6.2, 3H, CH_3); LC-MS $[M+H]^+$ m/z: 224.26, 100%; (c=1%, $CHCl_3$) $[\alpha]_{589}^{24.1}$= -36.6°; $[\alpha]_{546}^{22.7}$= -38.0°.

R-2N-[(2,6-dimethylphenoxy)ethyl]aminopropan-1-ol hydrochloride (4a)

$C_{13}H_{22}NO_2Cl$; 259.77; **4a1** Mp. 107-109 °C or **4a2** 115-117 °C; **4a2**: [$\%^{calc.}/_{analyzed}$] $C^{60.10}/_{60.36}$, $H^{8.54}/_{8.19}$, $N^{5.39}/_{5.36}$; ^1H NMR (DMSO-d_6, δ ppm): 9.27 (bs, 1H, NH^+); 9.00 (bs, 1H, NH); 7.04 (d, *J*=7.0, 1H, H-3 or H-5); 7.04 (d, *J*=6.9, 1H, H-3 or H-5); 6.95 (dd, *J*=7.0, *J*=6.9, 1H, H-4); 5.44 (t, *J*=5.2, 1H, OH); 4.08 (t, *J*=5.7, 2H, CH_2-O-Ar); 3.71-3.67 (m, 1H, C$\underline{H}$H-O-H); 3.64-3.59 (m, 1H, CH$\underline{H}$-O-H); 2.91 (t, *J*=5.7, 1H, CH_2-N); 3.36-3.33 (m, 1H, >CH); 2.26 (s, 6H, CH_3-Ar(2,6)); 1.28 (d, *J*=6.8, 3H, CH-C$\underline{H_3}$); ^{13}C NMR (δ ppm): 154.7 (C-1); 130.3 (C-2, C-6); 128.7 (C-3, C-5); 124.1 (C-4); 66.7 (CH_2-O-Ar); 61.0 (CH_2-OH); 55.1 (CH); 43.9 (CH_2-N); 15.9 (CH-$\underline{C}H_3$); 13.2 (CH_3-Ar(2,6)); LC-MS $[M+H]^+$ m/z: 224.26, **4a1** 100%, **4a2** 99.45%; (c = 2%, CH_3OH) $[\alpha]_{589}^{25.0}$= -7.96°; $[\alpha]_{546}^{24.0}$= -6.0°.

S-2N-[(2,6-dimethylphenoxy)ethyl]aminopropan-1-ol (5)

$C_{13}H_{21}NO_2$; 223.31; Mp. 83-85 °C; R_f= 0.56 (CH_3OH/ethyl acetate 1:1); ^1H NMR (DMSO-d_6, δ ppm): 7.04-6.88 (m, 3H, H-Ar); 5.0-4.2 (bb, 1H, OH); 3.78 (t, *J*=5.5, 2H, Ar-O-CH_2); 3.32 (dd, *J*=5.2, *J*=10.4, 1H, $\underline{H}$CH-OH); 3.23 (dd, *J*=6.8, *J*=10.4, 1H, HC$\underline{H}$-OH); 2.99-2.72 (m,

2H, CH_2N); 2.75-2.58 (m, 1H, CH); 2.28 (s, 6H, $Ar(CH_3)_2$); 0.93 (d, *J*=7.4, 3H, CH_3); LC-MS $[M+H]^+$ m/z: 224.26, 100%; (c=1%, $CHCl_3$) $[\alpha]_D^{22.7}$= +37.7°; $[\alpha]_{546}^{24.1}$= +35.0°.

S-2N-[(2,6-dimethylphenoxy)ethyl]aminopropan-1-ol hydrochloride (5a)

$C_{13}H_{22}NO_2Cl$; 259.77; Mp. **5a1** 106-108 °C or **5a2** 115-117 °C; ^{1}H NMR **5a2** (DMSO-d_6, δ ppm): 9.10 (bs, 2H, NH_2^+); 7.07-7.00 (m, 2H, H-3, H-5); 6.99-6.91 (m, 1H, H-4); 5.42 (bs, 1H, -OH); 4.07 (t, *J*=5.8, 2H, Ar-O-CH_2-); 3.74-3.54 (m, 2H, -CH_2-OH); 3.36 (t, *J*=5.8, 2H, -CH_2-N); 3.37-3.26 (m, 1H, CH); 2.26 (s, 6H, 2(Ar-CH_3)); 1.28 (d, *J*=6.7, 3H, -CH_3); LC-MS $[M+H]^+$ m/z: 224.10, **5a1** 100%, **5a2** 100%; (c=2%, CH_3OH) $[\alpha]_{589}^{25.1}$= +8.04º, $[\alpha]_{589}^{24.0}$= +6.0º.

R,S-2N-[(2,6-dimethylphenoxy)ethyl]-2N-methylaminopropan-1-ol hydrochloride (6)

$C_{14}H_{24}NO_2Cl$; 273.80; Mp. 130-132 ºC (EtOH/ethyl acetate (1:3)); [$\%^{calc.}/_{analyzed}$] $C^{61.41}/_{61.27}$, $H^{8.83}/_{8.82}$, $N^{5.11}/_{5.01}$; R_f = 0.63 (CH_3OH/ethyl acetate (1/1)); IR (KBr, cm^{-1}) *v*: 3356, 3021, 2967, 2930, 2674, 2647, 2360, 2342, 1475, 1199, 771; ^{1}H NMR (DMSO-d_6, δ ppm): 11.45 (bs, 1H, NH^+); 11.12 (bs, 1H, NH^+) 7.04 (d, *J*=7.5, 2H, H-3, H-5); 6.95 (dd, *J*=7.5, *J*=7.5, 1H, H-4); 5.54 (bs, 1H, OH); 5.48 (bs, 1H, OH) 4.26-4.12 (m, 2H, C<u>H</u>$_2$-OH); 4.16-4.08 (m, 1H, CH); 3.72-3.59 (m, 1H, CH<u>H</u>-O); 3.59-3.47 (m, 1H, C<u>H</u>H-O); 3.30-3.00 (m, 2H, CH_2-N); 2.96, 2.93 (bs, 3H, CH_3-N); 2.26 (s, 6H, (CH_3-Ar)$_2$; 1.14 (d, *J*=6.3, 3H, CH_3-R); LC-MS $[M+H]^+$ m/z: 238.12, 100%.

1N-[(2,6-dimethylphenoxy)ethyl]amino-2-butanol (7)

$C_{14}H_{23}NO_2$; 237.34; Mp. 52-54 ºC (hexane); [$\%^{calc.}/_{analyzed}$] $C^{70.85}/_{70.85}$, $H^{9.77}/_{9.55}$, N, $^{5.48}/_{5.80}$; R_f=0.45 (CH_3OH/ethyl acetate (1/1)); ^{1}H NMR ($CDCl_3$, δ ppm): 7.02-6.90 (m, 3H, H-Ar); 3.88 (t, *J*=5.1; 2H, -O-CH_2); 3.62-3.54 (m, 1H, C<u>H</u>-OH); 3.09-2.96 (m, 2H, -O-CH_2-C<u>H</u>$_2$-NH); 2.85 (dd, *J*=3.1, *J*=12.1, 1H, -NH-C<u>H</u>H-CH-OH); 2.53 (dd, *J*=9.4, *J*=11.9, 1H, -NH-CH<u>H</u>-CHOH); 2.28 (s, 6H, $Ar(CH_3)_2$); 1.55-1.45 (m, 2H, -C<u>H</u>$_2$-CH_3); 0.99 (t, *J*=7.4, 3H, -CH_2-CH_3); LC-MS $[M+H]^+$ m/z: 238.22, 99.84%.

5N-[(2,6-dimethylphenoxy)ethyl]amino-1-pentanol hydrochloride (8)

$C_{15}H_{26}NO_2Cl$; 287.83; Mp. 138-140 ºC (EtOH/ethyl acetate (1:3)); ^{1}H NMR (DMSO-d_6, δ ppm): 9.40 (bs, 2H, NH_2^+); 7.04 (<u>A</u>$_2$B, *J*=7.7, 2H, H-3, H-5); 6.95 (A$_2$<u>B</u>, *J*=7.7, 1H, H-4); 4.45 (bs, 1H, OH); 4.06 (t, *J*=5.6, 2H, Ar-O-CH_2-); 3.42-3.38 (m, 2H, -C<u>H</u>$_2$-OH); 3.32-3.28 (m, 2H, Ar-O-CH_2-C<u>H</u>$_2$-); 3.00-2.94 (m, 2H, Ar-O-CH_2-CH_2-CH_2-NH-C<u>H</u>$_2$); 2.26 (s, 6H, 2 x Ar–CH_3); 1.75-1.67 (m, 2H, Ar-O-CH_2-CH_2-NH-CH_2-C<u>H</u>$_2$); 1.48-1.40 (m, 2H, -C<u>H</u>$_2$-CH_2-OH);

1.40-1.31 (m, 2H, -C<u>H$_2$</u>-CH$_2$-CH$_2$-OH); ^{13}C NMR (δ ppm): 154.6 (C-1); 130.3 (C-2,6); 128.7 (C-3,5); 124.1 (C-4); 66.6 (Ar-O-<u>C</u>H$_2$-); 60.2 (-CH$_2$-OH); 47.1 (Ar-O-CH$_2$-CH$_2$-NH-<u>C</u>H$_2$); 46.5 (Ar-O-CH$_2$-<u>C</u>H$_2$-); 31.8 (-<u>C</u>H$_2$-CH$_2$-OH); 25.0 (<u>C</u>H$_2$-CH$_2$-CH$_2$-CH$_2$-OH); 22.6 (-<u>C</u>H$_2$-CH$_2$-CH$_2$-OH); 15.9 (2x Ar–<u>C</u>H$_3$); LC-MS [M+H]$^+$ m/z: 252.14, 100%.

2N-[(2,6-dimethylphenoxy)ethyl]amino-2-methylpropan-1,3-diol (9)

$C_{14}H_{23}NO_3$; 253.34; Mp. 84-85 ºC (hexane); [%$^{calc.}$/$_{analyzed}$] C$^{66.37}$/$_{66.17}$; H$^{9.15}$/$_{9.26}$; N$^{5.53}$/$_{5.54}$; R_f=0.52 (CH$_3$OH/ethyl acetate (1/1)); ^{1}H NMR (DMSO-d$_6$, δ ppm): 7.00 (<u>A$_2$</u>B, *J*=7.4, 2H, H-3; H-5); 6.89 (A$_2$<u>B</u>; *J*=7.4, 1H, H-4); 4.37 (t, *J*=5.3, 2H, 2 x -CH$_2$-O<u>H</u>); 3.76(t, *J*=5.6, 2H, Ar-O-CH$_2$-); 3.27 (d, *J*=5.3, 4H, 2 x -C<u>H$_2$</u>-OH); 2.85 (t, *J*=5.6, 2H, Ar-O-CH$_2$-C<u>H$_2$</u>-N<); 2.22 (s, 6H, CH$_3$-Ar (2,6)); 1.85 (bs, 1H, NH); 0.92 (s, 3H, -CH$_3$); ^{13}C NMR (δ ppm) 156.0 (C-1); 130.8 (C-2, 6); 129.1 (C-3, 5); 124.0 (C-4); 73.2 (Ar-O-CH$_2$-); 65.0 (-CH$_2$-OH); 56.8 (N-C$_q$); 41.9 (Ar-O-CH$_2$-<u>C</u>H$_2$-N<); 19.0 (-CH$_3$); 16.5 (CH$_3$-Ar (3,6)); LC-MS [M+H]$^+$ m/z: 254.13, 100%.

R,S-2N-[(2,6-dimethylphenoxy)ethyl]amino-3-methylbutan-1-ol hydrochloride (10)

$C_{15}H_{25}NO_2$; 251.36; Mp. 170-172 ºC (EtOH/acetone (1:3)); [%$^{calc.}$/$_{analyzed}$] C$^{62.59}$/$_{62.24}$, H$^{9.10}$/$_{9.01}$, N$^{4.87}$/$_{4.89}$; R_f = 0.69 (CH$_3$OH/ethyl acetate (1/1)); IR (KBr, cm^{-1}) *v*: 3379, 2963, 2874, 2822, 2740, 2463, 2360, 2343, 1597, 1584, 1466, 1200, 1044, 770; ^{1}H NMR (DMSO-d$_6$, δ ppm): 8.98 (bs, 2H, NH$_2^+$); 7.04 (<u>A$_2$</u>B, *J*=7.7, 2H, H-3, H-5); 6.95 (A$_2$<u>B</u>, *J*=7.7, 1H, H-4); 5.41 (t, *J*=4.5, 1H, OH); 4.13 (t, *J*=5.9, 2H, Ar-O-CH$_2$-); 3.82-3.67 (m, 2H, -C<u>H$_2$</u>-OH); 3.52-3.38 (m, 2H, -CH$_2$-N); 3.17-3.07 (m, 1H, N-CH); 2.26 (s, 6H, 2 x Ar–CH$_3$ (2,6)); 2.20 (hd, *J*=6.8, *J*=5.3, 1H, CH$_3$-C<u>H</u>–CH$_3$); 1.03 (d, *J*=6.8, 3H, C<u>H$_3$</u>-CH–CH$_3$); 0.99 (d, *J*=6.8, 3H, CH$_3$-CH–C<u>H$_3$</u>); ^{13}C NMR (δ ppm): 154.8 (C-1); 130.2 (C-2,6); 128.7 (C-3,5); 124.1 (C-4); 66.7 (Ar-O-<u>C</u>H$_2$-); 64.0 (N-<u>C</u>H); 56.9 (-CH$_2$-OH); 45.1 (-CH$_2$-N); 27.1 (CH$_3$-<u>C</u>H–CH$_3$); 19.4 (<u>C</u>H$_3$-CH–CH$_3$); 17.3 (CH$_3$-CH–<u>C</u>H$_3$); 16.0 (2 x Ar–<u>C</u>H$_3$ (2,6)); LC-MS [M+H]$^+$ m/z: 252.21, 100%.

R,S-4N-[(2,6-dimethylphenoxy)ethyl]amino-1-piperidinol (11)

$C_{16}H_{25}NO_2$; 263.38; Mp. 68-70 ºC (heptane); [%$^{calc.}$/$_{analyzed}$] C$^{72.25}$/$_{72.12}$, H$^{9.30}$/$_{9.47}$, N$^{5.62}$/$_{5.57}$; R_f= 0.50 (CH$_3$OH/ethyl acetate 1:1); IR (KBr, cm^{-1}) *v*: 3149, 3019, 2946, 2925, 2874, 2828, 2667, 2359, 1473, 1198, 1077; ^{1}H NMR (CDCl$_3$, δ ppm): 7.02-6.97 (m, 2H, H-3, H-5); 6.94-6.87 (m, 1H, H-4); 3.89 (t, *J*=6.03, 2H, Ar-O-CH$_2$); 3.78-3.67 (m, 1H, C<u>H</u>-OH); 2.96-2.84 (m, 2H, N-CHpip); 2.79 (t, *J*=6.03, 2H, Ar-O-CH$_2$-C<u>H$_2$</u>-N); 2.29 (s, 6H, Ar-CH$_3$); 2.01-1.86 (m, 2H, pip); 1.72-1.57 (m, 2H, pip); LC-MS [M+H]$^+$ m/z: 250.26, 100%.

R,S-2N-[(2,6-dimethylphenoxy)ethyl]amino-1-phenylethan-1-ol (12)

$C_{18}H_{23}NO_2$; 285.38; Mp. 110-111 °C (toluene/heptane (1/1)); [$\%^{calc.}/_{analyzed}$] $C^{75.76}/_{75.29}$; $H^{8.12}/_{8.23}$; $N^{4.91}/_{4.83}$; R_f = 0.62 (CH_3OH); IR (KBr, cm^{-1}) *v*: 3424, 3305, 3061, 3028, 2926, 2895, 2734, 1453, 1427, 1199, 1066, 758; ^{1}H NMR (DMSO-d_6, δ ppm): 7.41-7.26 (m, 5H, H'-Ar), 7.02-6.90 (m, 3H, H-Ar), 4.76 (dd, J=8.6, J=3.0, 1H, -CHOH-), 3.89 (t, J=4.9, 2H, -O-CH2-), 3.10-3.00 (m, 3H, -CH2-NH-CHH-CHOH-Ar), 2.83 (dd, J=11.8, J=9.2, 1H, -CHH-CHOH-Ar), 2.27 (s, 6H, Ar(CH3)2), 1.78 (bs, 1H, -NH-); LC-MS $[M+H]^+$ m/z: 286.17, 100%.

R-(-)-2N-[(2,6-dimethylphenoxy)ethyl]amino-1-phenylethanol (13)

$C_{18}H_{23}NO_2$; 285.38; Mp. 78-80 °C (toluene/heptane (1/1)); R_f = 0.62 (CH_3OH); ^{1}H NMR (DMSO-d_6, δ ppm): 7.38-7.21 (m, 5H, H-2', H6'); 7.0 (dd, *J*=7.9, 1H, H-3 or H-5); 7.0 (dd, *J*=8.0, 1H, H-3 or H-5); 6.89 (dd, *J*=8.0, *J*=7.9, 1H, H-4); 5.30 (d, *J*=4.2, 1H, -OH); 4.68-4.64 (m, 1H, CH); 3.80-3.76 (m, 2H, Ar-O-CH_2-); 2.94-2.86 (m, 2H, -CH_2-N); 2.72 (d, *J*=6.3, 2H, N-CH_2-); 2.20 (s, 6H, CH_3-Ar (2,6)); 1.97 (bs, 1H, -NH); ^{13}C NMR (δ ppm): 155.4 (C-1); 144.5 (C-1'); 130.2 (C-2, 6); 128.6 (C-3, 5); 127.8 (C-3', 5'); 126.7 (C-4'); 125.8 (C-2',6'); 123.4 (C-4); 71.5 (CH); 71.5 (Ar-O-CH_2-); 57.6 (N-CH_2-); 49.0 (-CH_2-N); 15.8 (CH_3-Ar (3,6)); LC-MS $[M+H]^+$ m/z: 286.17, 100%; (c=1%, CH_3OH) $[\alpha]_D^{22.7}$= -23.80º; $[\alpha]_{546}^{24.1}$= -23.0º.

S-(+)-2N-[(2,6-dimethylphenoxy)ethyl]amino-1-phenylethanol (14)

$C_{18}H_{23}NO_2$; 285.38; Mp. 78-80 °C (toluene/heptane (1/1)); R_f = 0.62 (CH_3OH); ^{1} H NMR the same as (*R*); LC-MS $[M+H]^+$ m/z: 286.17, 100%; (c=1%, CH_3OH): $[\alpha]_D^{22.7}$= + 23.31º; $[\alpha]_{546}^{24.1}$= + 22.5º.

R,S-2N-[(2,6-dimethylphenoxy)ethyl]-2N-methylamino-1-phenylethan-1-ol hydrochloride (15)

$C_{19}H_{26}NO_2Cl$; 335.87; Mp. 130-132 ºC (EtOH/acetone (1/3)); [$\%^{calc.}/_{analyzed}$] $C^{67.95}/_{67.84}$, $H^{7.80}/_{7.77}$, $N^{4.17}/_{3.99}$; R_f = 0.69 (CH_3OH); IR (KBr, cm^{-1}) *v*: 3220, 3019, 2950, 2806, 2718, 1477, 1465, 1363, 1200, 1092, 909, 701; ^{1}H NMR (DMSO-d_6, δ ppm): 10.45 (bs, 1H, NH^+); 10.06 (bs, 1H, NH^+); 7.49-7.36 (m, 4H, H-Ar); 7.34-7.30 (m, 1H, H-Ar); 7.04 (d, *J*=7.4; 2H, H-3, H-5); 6.95 (t, *J*=7.4, 1H, H-4); 6.33-6.28 (bs, 1H, OH); 5.28-5.11 (m, 1H, CH); 4.21-4.10 (m, 2H, O-CH_2); 3.80-3.55 (m, 2H, O-CH_2-CH_2-N); 3.50-3.25 (m, 2H, N-CH_2-CH); 3.04 (s, 3H, N-CH_3); 2.26 (s, 6H, Ar-$(CH_3)_2$); LC-MS $[M+H]^+$ m/z: 300.12, 100%.

L-threo-2N-[(2,6-dimethylphenoxy)ethyl]amino-1-phenyl-1,3-propandiol (16)

$C_{19}H_{25}NO_3$; 315.41; Mp. 74-76 ºC (heptane); ^{1}H NMR (DMSO-d_6, δ ppm): 7.40-7.20 (m, 5H, H-2', H6'); 7.00 ($\underline{A}_2B$; *J*=7.4, 2H, H-3, H-5); 6.89 ($A_2\underline{B}$, *J*=7.4, 1H, H-4); 5.18 (bs, 1H, CH-O$\underline{H}$); 4.57 (d, *J*=6.1, 1H, Ar-CH); 4.44 (t, *J*=5.0, 1H, CH_2-O$\underline{H}$); 3.73 (t, *J*=5.5, 2H, Ar-O-CH_2-); 3.46-3.40 (m, 1H, C$\underline{H}$H-OH); 3.19-3.13 (m, 1H, CH$\underline{H}$-OH); 2.97-2.90 (m, 1H, -C$\underline{H}$H-N); 2.84-2.78 (m, 1H, -CH$\underline{H}$-N); 2.70-2.65 (m, 1H, N-CH-); 2.20 (s, 6H, CH_3-Ar (2,6)); 2.18 (bs, 1H, -NH); ^{13}C NMR (δ ppm): 156.0 (C-1); 144.4 (C-1'); 130.8 (C-2, 6); 129.1 (C-3, 5); 128.2 (C-3', 5'); 127.2 (C-4'); 127.0 (C-2',6'); 124.0 (C-4); 72.5 (CH); 72.2 (Ar-O-CH_2-); 65.9 (N-CH-); 60.7 (-CH_2-OH); 48.2 (-CH_2-N); 16.4 (CH_3-Ar (3,6)); LC-MS $[M+H]^+$ m/z: 316.14, 100%; (c=2%, CH_3OH): $[\alpha]_D^{20.00}$= + 8.77º.

R,S-1N-[(2,6-dimethylphenoxy)propyl]aminopropan-2-ol (17)

$C_{14}H_{23}NO_2$; 237.34; Mp. 76-78 °C (heptane); [$\%^{calc.}/_{analyzed}$] $C^{70.85}/_{70.79}$, $H^{9.77}/_{9.96}$, $N^{5.90}/_{5.86}$; R_f=0.28 (CH_3OH/ethyl acetate (1/1)); IR (KBr, cm^{-1}) *v*: 3260, 3106, 2968, 2963, 2936, 2923, 2863, 2817, 2769, 1464, 1204, 1057, 762; ^{1}H NMR (DMSO-d_6, δ ppm): 7.05-6.95 (m, 2H, H-3, H-5), 6.94-6.84 (m, 1H, H-4), 4.41 (d, *J*=4.2 Hz, 1H, -OH), 3.77 (t, *J*=6.3Hz, 2H, -CH_2-O-Ar), 3.71-3.61 (m, 1H, -CH-), 2.72 (t, *J*=6.9 Hz, 2H, -CH_2-C$\underline{H}_2$-NH-), 2.43 (d, *J*=6.1 Hz, 2 H, -C$\underline{H}_2$-CH-), 2.21 (s, 6H, Ar-$(CH_3)_2$), 1.6 (bs, 1H, -NH-), 1.04 (d, *J*=6.3 Hz, 3 H, -CH-C$\underline{H}_3$); LC-MS $[M+H]^+$ m/z: 238.18, 98.65%.

R,S-2N-[(2,6-dimethylphenoxy)propyl]aminopropan-1-ol (18)

$C_{14}H_{23}NO_2$; 237.34; Mp. 84-86 °C (heptane); [$\%^{calc.}/_{analyzed}$] $C^{70.85}/_{70.87}$, $H^{9.77}/_{9.44}$, $N^{5.90}/_{5.85}$; R_f=0.25 (CH_3OH); IR (KBr, cm^{-1}) *v*: 3307, 3134, 2972, 2948, 2930, 2876, 2838, 2680, 2361, 2341, 1473, 1207, 1053, 782; ^{1}H NMR (DMSO-d_6, δ ppm): 6.99 (d, *J*=7.3, 2H, H3, H5); 6.88 (t, *J*=7.3, 1H, H4); 4.47 (bs, 1H, OH); 3.77 (t, *J*=6.4, 2H, Ar-O-CH_2); 3.30-3.21 (m, 2H, C$\underline{H}_2$-OH); 2.79 (dt, *J*=11.4, *J*=7.0, 1H, C$\underline{H}$H-N); 2.68 (dt, *J*=11.4, *J*=6.7, 1H, CH$\underline{H}$-N); 2.65-2.57 (m, 1H, CH); 2.21 (s, 6H, 2*(Ar-CH_3)); 1.85 (qn, *J*=6.8, 2H, R-CH_2-R); 1.55 (bs, 1H, NH); 0.92 (d, *J*=6.4, 3H, R-CH_3); LC-MS $[M+H]^+$ m/z: 238.22, 100%.

2N-[(2,6-dimethylphenoxy)propyl]amino-2-methylpropan-1-ol (19)

$C_{15}H_{25}NO_2$; 251.37; Mp. 73-75 °C (heptane); [$\%^{calc.}/_{analyzed}$] $C^{71.67}/_{71.60}$, $H^{10.02}/_{10.10}$, $N^{5.57}/_{5.44}$; R_f= 0.27 (CH_3OH/ethyl acetate (1/1)); IR (KBr, cm^{-1}) *v*: 3272, 3071, 2988, 2970, 2942, 2902, 2870, 2817, 2757, 1592, 1477, 1210, 1073, 764; ^{1}H NMR ($CDCl_3$, δ ppm): 7.02-6.89 (m, 3H, H-3, H-4, H-5), 3.84 (t, *J*=6.2, 2H, -CH_2-O-Ar), 3.30 (s, 2H, -$\underline{CH}_2$-OH), 2.77 (t, *J*=6.6, 2H, -

O-CH_2-CH_2-$\underline{CH_2}$-NH-), 2.28 (s, 6H, Ar-$(CH_3)_2$), 1.95 (qn, *J*=6.4, 2H, -O-CH_2-$\underline{CH_2}$-CH_2-NH-), 1.06 (s, 6H, (-C-$CH_3)_2$); LC-MS $[M+H]^+$ m/z: 252.14, 100%.

R,S-1N-[(2,6-dimethylphenoxy)propyl]aminobutan-2-ol (20)

$C_{15}H_{25}NO_2$; 251.37; Mp. 62-64 °C (heptane); [$\%^{calc.}/_{analyzed}$] $C^{71.67}/_{71.68}$, $H^{10.02}/_{10.41}$, $N^{5.57}/_{5.52}$; R_f=0.26 (CH_3OH/ethyl acetate 1:1); IR (KBr, cm^{-1}) *v*: 3259, 3122, 2972, 2960, 2917, 2850, 2820, 2776, 2348, 1911, 1594, 1476, 1461, 1204, 1064; ^{1}H-NMR (DMSO-d_6, δ ppm): 7.00 (d, *J*=7.5, 2H, H-3, H-5); 6.88 (dd, *J*=7.5, *J*=7.5, 1H, H-4); 4.38 (bs, 1H, OH); 3.77 (t, *J*=6.2, 2H, CH_2-OAr); 3.46-3.37 (m, 1H, CH); 2.78-2.67 (m, 2H, CH_2-$C\underline{H_2}$-N); 2.55 (dd, *J*=11.4, *J*=4.4, 1H, NH-CH$\underline{H}$-CH); 2.42 (dd, *J*=11.4, *J*=7.5, 1H, NH-C$\underline{H}$H-CH); 2.21 (s, 6H, CH_3-Ar(2,6)); 1.89-1.82 (m, 2H, R-$C\underline{H_2}$-R); 1.63 (bs, 1H, NH); 1.47-1.37 (m, 1H, C$\underline{H}$H(Et)); 1.35-1.25 (m, 1H, CH$\underline{H}$(Et)); 0.86 (t, *J*=7,4, 3H, CH_3(Et)); LC-MS $[M+H]^+$ m/z: 252.21, 100%.

R,S-2N-[(2,6-dimethylphenoxy)propyl]aminobutan-1-ol hydrochloride (21)

$C_{15}H_{26}NO_2Cl$; 287.82; Mp. 126-128 °C (EtOH/ethyl acetate (1:3)); [$\%^{calc.}/_{analyzed}$] $C^{62.60}/_{62.39}$, $H^{9.10}/_{8.94}$, $N^{4.87}/_{4.88}$; R_f=0.26 (CH_3OH/ethyl acetate 1:1); IR (KBr, cm^{-1}) *v*: 3301, 3020, 2970, 2921, 2845, 2813, 2670, 2504, 2355, 1460, 1202, 761; ^{1}H NMR (DMSO-d_6, δ ppm): 8.84 (bs, 1H, NH^+); 8.72 (bs, 1H, NH); 7.05-6.99 (m, 2H, H-3; H-5); 6.96-6.88 (m, 1H, H-4); 5.37 (t, *J*=5.1, 1H, OH); 3.81 (t, *J*=6.1, 2H, Ar-O-CH_2); 3.74 (ddd, *J*=12.2, *J*=5.1, *J*=3.2, 1H, C$\underline{H}$H-OH); 3.58 (ddd, *J*=12.2, *J*=5.1, *J*=4.7, 1H, CH$\underline{H}$-OH); 3.26-3.11 (m, 2H, CH_2N); 3.10-2.99 (m, 1H, CH); 2.22 (s, 6H, 2(Ar-CH_3)); 2.20-2.06 (m, 2H, R-(CH_2)-R); 1.81-1.53 (m, 2H, -CH_2-Et); 0.93 (t, *J*=7.3, 3H, -CH_3); LC-MS $[M+H]^+$ m/z: 252.14, 100%.

R,S-2N-[(2,6-dimethylphenoxy)propyl]amino-1-phenylethan-1-ol (22)

$C_{19}H_{25}NO_2$; 299.41; Mp. 90-92 °C (hexane); [$\%^{calc.}/_{analyzed}$] $C^{76.21}/_{76.64}$, $H^{8.42}/_{8.76}$, $N^{4.68}/_{4.68}$; R_f=0.39 (CH_3OH/ethyl acetate 1:1); IR (KBr, cm^{-1}) *v*: 3308, 3063, 3031, 2928, 2896, 2837, 2769, 2733, 1454, 1199, 759, 699; ^{1}H NMR (DMSO-d_6, δ ppm): 7.36-7.28 (m, 4H, H-2', H-3', H-5', H-6'), 7.22 (m, 1H, H-4'), 6.99 ($\underline{A_2}$B, J=7.3, 2H, H-3, H-5), 6.89 ($A_2\underline{B}$, *J*=7.3, 1H, H-4), 5.20 (d, *J*=3.8, 1H, -OH), 4.65-4.60 (m, 1H, -CH-), 3.76 (t, *J*=6.3, 2H, Ar-O-CH_2-), 2.81-2.71 (m, 2H, -O-CH_2-CH_2-$C\underline{H_2}$-NH-), 2.19 (s, *J*=11.4, *J*=4.4, 1H, -NH-CH$\underline{H}$-CH-), 2.42 (dd, *J*=11.4, *J*=7.5, 1H, -NH-C$\underline{H}$H-CH-), 2.21 (s, 6H, Ar-$(CH_3)_2$), 1.85 (p, 2H, R-$C\underline{H_2}$-R), 1.76 (bs, 1H, -NH-); LC-MS $[M+H]^+$ m/z: 300.19, 100%.

R,S-2N-[(2,6-dimethylphenoxy)butyl]aminopropan-1-ol (23)

$C_{15}H_{25}NO_2$; 251.36; Mp. 60-62 °C (hexane); [$\%^{calc.}/_{analyzed}$] $C^{71.67}/_{71.80}$, $H^{10.03}/_{10.10}$, $N^{5.57}/_{5.54}$; R_f=0.17 (CH_3OH/ethyl acetate 1:1); IR (KBr, cm^{-1}) *v*: 3278, 3087, 2966, 2912, 2805, 2578, 1465, 1377, 1263, 1059; ^1H NMR ($CDCl_3$, δ ppm): 7.01-6.90 (m, 2H, H-Ar); 6.93-6.88 (m, 1H, H-4Ar); 3.78 (t, *J*=6.4, 2H, O-CH_2); 3.59 (dd, *J*=4.1, *J*=10.3, 1H, -C<u>H</u>H-OH); 3.24 (dd, *J*=7.0, *J*=10.4, 1H, -CH<u>H</u>-OH); 2.86-2.76 (m, 2H, CH_2-N); 2.66-2.59 (m, 1H, C<u>H</u>-CH_3); 2.27 (s, 6H, Ar(CH_3)$_2$); 1.90-1.80 (m, 2H, N-CH_2-CH_2-C<u>H</u>$_2$-CH_2-O); 1.78-1.68 (m, 2H, N-CH_2-C<u>H</u>$_2$-CH_2-CH_2-O); 1.07 (d, *J*=6.4, 3H, CH-C<u>H</u>$_3$); LC-MS $[M+H]^+$ m/z: 252.25, 100%.

R-2N-[(2,6-dimethylphenoxy)butyl]aminopropan-1-ol (24)

$C_{15}H_{25}NO_2$; 251.36; Mp. 77-79 °C (hexane); [$\%^{calc.}/_{analyzed}$] $C^{71.67}/_{71.90}$, $H^{10.03}/_{10.08}$, $N^{5.57}/_{5.53}$; R_f=0.20 (CH_3OH/ethyl acetate 1:1); IR (KBr, cm^{-1}) *v*: 3255, 3095, 2962, 2919, 2853, 2692, 2585, 1584, 1260, 760; ^1H NMR ($CDCl_3$, δ ppm): 7.04-6.97 (m, 2H, H3,5-Ar); 6.95-6.87 (m, 1H, H-4Ar); 3.77 (t, *J*=6.28, 2H, O-CH_2); 3.59 (dd, *J*=4.23, *J*=10.39, 1H, -C<u>H</u>H-OH); 3.24 (dd, *J*=7.18, *J*=10.52, 1H, -CH<u>H</u>-OH); 2.88-2.72 (m, 2H, CH_2-N); 2.62 (dt, *J*=7.05, =11.28, 1H, C<u>H</u>-CH_3); 2.27 (s, 6H, Ar(CH_3)$_2$); 1.92-1.79 (m, 2H, O-CH_2-C<u>H</u>$_2$); 1.78-1.65 (m, 2H, N-CH_2-C<u>H</u>$_2$); 1.06 (d, *J*=6.41, 3H, CH-C<u>H</u>$_3$); LC-MS $[M+H]^+$ m/z: 252.14, 100%; (c=1%, CH_3OH): $[\alpha]_D^{20.0}$= - 18.36º.

Thermogravimetry (TG) and Differential Scanning Calorimetry (DSC) analysis for **3a-5a** was performed on STA 449F3 Jupiter Netzsch (Selb, Germany). Samples (ca. 5 mg) were heated from 20 °C to 600 °C with heating rate 10 °C/min in atmosphere of argon (flow rate 12 mL/min). Aluminum (III) dioxide was used as reference.

Crystals suitable for X-ray diffraction analysis were obtained by slow evaporation of propyl acetate solution of **4a2**. Within three weeks colorless crystals appeared at 20 °C. Diffraction data for single crystal were collected at 120K using Oxford Diffraction SuperNova four circle diffractometer, equipped with the Mo (0.71069 Å) Kα radiation source, graphite monochromator and Oxford CryoJet system for measurements at low temperature. Cell refinement and data reduction was performed using firmware.[17]

Crystal and structure refinement data are summarized in Table 19. Positions of non-hydrogen atoms were determined by direct methods using SIR-97.[22] All non-hydrogen atoms were refined anisotropically using weighted full-matrix least-squares on F^2. All hydrogen atoms

joined to carbon atoms were positioned with an idealized geometry and refined using a riding model with $U_{iso}(H)$ fixed at 1.2 U_{eq} of C and 1.5 U_{eq} for methyl groups. Hydrogen atoms joined to oxygen and nitrogen atoms were found from the difference Fourier map and refined without any restraints. Refinement and further calculations were carried out using SHELXL-97.[23] For molecular graphics ORTEP [24] and MERCURY [25] programs were used.

Table 19

Crystal and structure refinement data for **4a2**

Identification code	**4a2**
Empirical formula	$C_{13}H_{21}NO_2 \cdot HCl$
Formula weight	259.76
Temperature (K)	120(2)
Wavelength (Å)	0.71073
Crystal system	monoclinic
Space group	P 2_1
Unit cell dimensions a, b, c (Å) α, β, γ (°)	a = 7.0280 (2) α = 90 b = 7.9682 (2) β = 103.579(3) c = 12.5261 (6) γ = 90
Volume ($Å^3$)	681.86(3)
Z, Calculated density (Mg/m^3)	2, 1.265
Absorption coefficient (mm^{-1})	0.272
F(000)	394
Crystal size (mm)	0.384 x 0.253 x 0.189
Theta range for data collection (°)	3.055 to 28.638
Reflections collected/unique	9054/3160 [R(int) = 0.0395]
Reflections [I>2σ(I)]	2552
Data / restraints / parameters	3160 / 1 / 170
Goodness-of-fit on F^2	1.066
Final R indices [I>2σ(I)]	R1 = 0.0350, wR2 = 0.0698
Largest diff. peak and hole (e/ $Å^3$)	0.244 and -0.221

CCDC-1046749 contains the supplementary crystallographic data. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

4.2. Pharmacology

Antiepileptic activity and neurological toxicity assays were carried out by the Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institute of Health in Rockville, USA.[1] Compounds were injected as suspensions in 0.5% methylcellulose at three dosage levels (30,

100 and 300 mg/kg b.w.) intraperitoneally (*i.p.*) into mice, and orally. The preliminary evaluation was a qualitative assay which used small groups of animals (1-8) and included three tests: maximal electroshock seizure (MES), subcutaneous pentylenetetrazol (ScMet), and neurotoxicity (rotarod), noted at 30 min and 4 h after administration.[18]

The MES were elicited by 60 Hz alternating current at 50 mA (mice) or 150 mA (rats) delivered for 0.2 s via corneal electrodes. A drop of 0.9% NaCl solution was placed into each eye prior to applying the electrodes. Protection in the MES test was defined as the abolition of the hindlimb tonic extension component of the seizure.

The ScMet was conducted by administration 85 mg/kg of pentylenetetrazole dissolved in 0.9% NaCl solution into the posterior midline of mice. A minimal time of 30 min subsequent to subcutaneous administration of pentylenetetrazole was used for seizure detection. A failure to observe even a threshold seizure (a single episode of clonic spasm of at least 5 s in duration) was regarded as protection.

Neurological deficit was measured in mice by the rotarod test. The mouse was placed on a 1 inch diameter knurled plastic rod rotating at 6 rpm. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials. In rats, neurological deficit was indicated by ataxia and loss of placing response and muscle tone.

The 6 Hz model test was carried out according to the protocol originally described by Brown *et al.* [26] and more recently by Barton *et al.* [27] and Kamiński *et al.* [19] Corneal stimulation (0.2 ms duration monopolar rectangular pulses at 6 Hz for 3 s) was delivered by a constant current device. During the stimulation, mice were manually restrained and released into the observation cage immediately after application of current. The seizures were often preceded by a brief period of intense locomotor agitation. The animals then exhibited a “stunned” posture associated with rearing, forelimb automatic movements and clonus, twitching of the vibrissae, and Straub-tail. The duration of the seizure activity ranged from 60 to 120 s in untreated animals. In the end of the seizure, animals resumed their normal exploratory behavior. The experimental endpoint was protection against the seizure. The animal was considered to be protected if it resumed its normal exploratory behavior within 10 s from the stimulation.

The pilocarpine test involves determination whether the investigated compound can halt acute pilocarpine-induced status. A challenge dose of pilocarpine (50 mg/kg) is administered *i.p.* and animals are observed until the first convulsive (*e.g.* stage 3, 4, or 5) seizure (time zero). The seizure severity is determined using the Racine scale.[20] At this point a minimally toxic

dose of the candidate drug is administered to a group of 8 male albino Sprague Dawley rats (150-180 g) *i.p.* Efficacy is defined by the ability of an investigational drug to halt the further expression of pilocarpine induced convulsive seizures (*e.g.* stage 3, 4, or 5).

Hippocampal kindling test determines whether the compound can be effective in treating complex partial or psychomotor seizures, and in preventing the process of spreading seizures from focus into further generalization.[28] A bipolar stimulating electrode was stereotactically implanted in the ventral hippocampus (AP -3.6, ML 4.9, DV -5.0 from dura, incisor bar +5) of adult male Sprague Dawley rats (250-300 g) under ketamine-xylazine anesthesia. Three anchor screws were attached to the skull with dental acrylic cement. After the incision is colosed with sutures, the animal receives a single dose of Bicillin (60,000 units, *i.m.*) and is returned in his home cage to the animal quarters. Animals were kindled according to the procedure of Lothman and Williamson.[29] After 1 week animals are stimulated with suprathreshold trains for 200 μA for 10 s, 50 Hz, every 30 min for 6 h on alternate days until they are fully kindled. One week later a single dose of test substance (50 mg/kg, *i.p.*) on the behavioral seizure score and afterdischarge duration was assessed in a single group of kindled rats (n=6-8) at 15, 45, 75, 105, 135, 165, and 195 min. after drug administration. Results obtained at the various points were compared with the last control stimulus delivered 15 min prior to drug administration. Thus, each animal serves as its own control. When a drug treatment is observed to significantly lower seizure score and decrease afterdischarge, a dose-response study is initiated. For this study, the ability of candidate substance to block afterdischarge and reduces seizure severity is quantified by varying the dose between 0 and 100% effect. Often the same group of kindled rats is used for a multiple dose study. Under this condition, animals are permitted to dry-out for 4-5 days before being subjected to the second dose.

The timed *i.v.* infusion of metrazol (ivMet) test provides measure of a test substance's ability to raise or lower seizure threshold. At least two doses of the test compound are employed, the MES ED_{50} and the TD_{50} (mice, *i.p.*). Randomly selected mice are injected *i.p.* 2 min apart with either the vehicle or the two test drug doses, maintaining the same order of dosing until 30 mice have been injected. At the previously determined TPE, 0.5% heparinized metrazol solution is infused at a constant rate of 0.34 mL/min into a lateral tail vein of an unrestrained mouse. Infuse is by means of a Sage syringe pump (model 341A) and a 10 mL B-D plastic syringe connected to a length of No. 20 P.E. tubing. The rate selector is set on 4, and the switch is set at mL/min. At the start of the infusion a hemostat clamped to the tubing to

prevent backflow is removed, the infusion started, and two stopwatches started. The time from the start to the appearance of the "first twitch" and the onset of sustained clonus is recorded in seconds. The times to each endpoint are converted to mg/kg b.w. of metrazol for each mouse as follows: mg/kg b.w. Met = (inf. Time [s] x rate of inf. [mL/min] x mg Met/min x 1000 g)/ (60 [s] x weight of animal [g]). The mean and standard error for each group and the significance of the difference between the test groups and the control are calculated. An increase in mg/kg to first twitch or first clonus indicates the test substance increases seizure threshold, whereas a decrease indicates that the test substance decreases seizure threshold and may be proconvulsant.

Anticonvulsant quantification, *i.e.* the doses of drug required to cause the biological responses in 50% of animals (ED_{50}), and the respective 95% confidence intervals, were determined for selected compounds displaying sufficient anticonvulsant activity and low neurotoxicity in the above primary evaluations, by means of a computer program using probit analysis.

4.3. Mutagenicity assays

For testing of compound **3a** *Salmonella typhimurium* test strains TA100 and TA1535 were obtained from Dr. T. Nohmi, Division of Genetics and Mutagenesis, National Institute of Hygienic Sciences, Tokyo, Japan.

The assay was performed by a preincubation method following previously described protocols.[30] Briefly, bacteria cultures were inoculated 12 h prior to performing the assay. Next, 100 μL of the overnight culture of the *Salmonella* strain (approx. 1-2x 10^8 bacteria per tube), 50 μL of a test compound at different concentrations, and 500 μL of buffer solution (or liver S9 fraction in the experiment with metabolic activation) were added to 2 mL of the top agar supplemented with histidine and biotin. Subsequently, the mixture was mixed and poured onto the surface of GM agar plates. The plates were incubated at 37°C for 48 h, and the number of colonies able to grow without histidine was determined. Positive control (SA in classical experiment and 2AA in the experiment with metabolic activation) and negative control (DMSO) were performed alongside. Three independent repetitions were performed for the compound. A sample was classified mutagenic when the mutant frequency was at least two-fold above the spontaneous revertant frequency, in each strain.

In the ADD program, for testing of compound **4**, *S. typhimurium* strain TA98 was used.[31] Compound **4** was dissolved in dimethylsulfoxide (DMSO) and mixed with 20% cytochrome P_{450}-enriched rat liver S9 post-mitochondrial fraction (20% w/v liver homogenate in 0.25 M sucrose) in a phosphate buffer and added to an overnight culture of *S. typhimurium* strain TA98. This mixture was divided into two samples and NADPH added to one of the samples

to determine if the test compound requires cytochrome P_{450} bioactivation to be mutagenic. Both reactions (+NADPH and –NADPH) were incubated at 37°C for 30 min. and then added to a top agar solution containing 0.6% agar, 0.05 mM histidine (sufficient only for growth initiation), and 0.05 mM biotin and plated on minimal glucose agar plates. Revertant *S. typhimurium* colonies were counted after a 48-hour incubation at 37°C. Results are reported as a percentage of the number of revertant colonies obtained with 0.02 mM benzo[a]pyrene incubated with NADPH. Acridine orange was also used as positive control.

ACKNOWLEDGEMENTS

The authors thank Prof. Jeff Jiang, Prof. James Stables, Prof. Steve White, Prof. Harvey Kupferberg, and Tracy Chen Ph.D. from National Institute of Neurological Disorders and Stroke (NIH, Rockville, USA) for providing the results of anticonvulsant evaluation and Professor Katarzyna Kieć-Kononowicz for coordination of the cooperation between the National Institute of Neurological Disorders and Stroke and the Faculty of Pharmacy Jagiellonian University Medical College.

The research was supported by the European Regional Development Fund in the framework of the Polish Innovative Economy Operational Program.

REFERENCES

1. Stables, J. P.; Kupferberg, H. J. The NIH Anticonvulsant Drug Development (ADD) Program: Preclinical Anticonvulsant Screening Project. In *Molecular and Cellular Targets for Anti-epileptic Drugs*; Avanzini, G.; Regesta, P.; Tanganelli, A.; Avoli, M., Eds.; John Libbey & Company Ltd: London, 1997; Vol. 23129, pp. 191–198.

2. He, X.; Qiu, G.; Yang, J.;Xiao, Y., Wu, Z.,Qiu, G., Hu, X. *Eur. J. Med. Chem.* **2010**, *45*, 3818-3830.

3. He, X.; Zhong, M.; Zhang, T.; Yang, J.; Wu, Z.;Xiao, Y.; Guo, H.; Qiu, G.; Hu, X. *Eur. J. Med. Chem.* **2012**, *48*, 338-346.

4. Li, J.; Lou, J.; Wang, Z.; Wang, T.; Xiao, Y.; Hu, X.; Liu, P.; Hong, X. Eur. J. Med. Chem. **2015**, *92*, 370-376.

5. Chew, C.; Collett, J.; Singh, B. *Drugs* **1979**, *17*, 161–181.

6. Fischer, W. *Seizure J. Br. Epilepsy Assoc.* **2002**, *11*, 285–302.

7. Pękala, E.; Waszkielewicz, A. M.; Szneler, E.; Walczak, M.; Marona, H. *Bioorg. Med. Chem.* **2011**, *19*, 6927–6934.

8. Marona, H.; Antkiewicz-Michaluk, L. *Acta Pol. Pharm.* **1998**, *55*.

9. Waszkielewicz, A.; Szkaradek, N.; Pękala, E.; Galzarano, F.; Marona, H. *Biomed. Chromatogr.* **2010**, *24*, 1365–1372.

10. Marona, H.; Waszkielewicz, A. M.; Szneler, E. *Acta Pol. Pharm.* **2005**, *62*, 345–353.

11. Waszkielewicz, A. M.; Szneler, E.; Cegła, M.; Marona, H. *Lett. Drug Des.Discov.* **2013**, *10*, 34–42.

12. Marona, H.; Waszkielewicz, A.; Kieć-Kononowicz, K. Derivatives of Aminoalkanols, Method of Obtaining of Aminoalkanols and Their Use. *PCT/PL2009/000004, 2008, WO 2009/093916A3*.

13. Augstein, J.; Austin, W.; Boscott, R.; Green, S.; Worthing, C. *J Med Chem* **1965**, *8*, 356–367.

14. Pajouhesh, H.; Lenz, G. R. *NeuroRx* **2005**, *2*, 541–553

15. Giron, D. *J Therm Anal Calorim* **2001**, *64*, 37–60.

16. Sivý, J.; Kettmann, V.; Frešová, E. *Acta Crystallogr. Sect. C Cryst. Struct. Commun.* **1991**, *47*, 2695–2696.

17. Oxford Diffraction (2010). CrysAlis PRO. Oxford Diffraction Ltd, Yarnton, England.

18. White, S. H.; Woodhead, J. H.; Wilcox, K. S.; Kupferberg, H. J.; Wolf, H. H. General Principles of Discovery and Preclinical Development of Antiepileptic Drugs. In *Antiepileptic Drugs, fifth ed.*; Levy, R. H.; Mattson, R. H.; Meldrum, B. S.; E., P., Eds.; Lippincott Williams & Wilkins: Philadelphia, 2002; pp. 36–48.

19. Kaminski, R. M.; Livingood, M. R.; Rogawski, M. A. *Epilepsia* **2004**, *45*, 864–867.

20. Racine, R. J. *Electroencephalogr. Clin. Neurophysiol.* **1972**, *32*, 281–294.

21. www.cerep.fr

22. Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115–119.

23. Sheldrick, G. M. *Acta Crystallogr. A.* **2008**, *64*, 112–122.

24. Farrugia, L. J. *J. Appl. Crystallogr.* **1997**, *30*, 565–565.

25. Macrae, C. F.; Edgington, P. R.; McCabe, P.; Pidcock, E.; Shields, G. P.; Taylor, R.; Towler, M.; van de Streek, J. *J. Appl. Crystallogr.* **2006**, *39*, 453–457.

26. Brown, W.; Schiffman, D. *J. Pharmacol. Exp. Ther.* **1953**, *107*, 273–283.

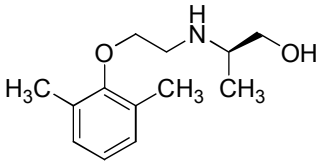
27. Barton, M. E.; Klein, B. D.; Wolf, H. H.; White, H. S. *Epilepsy Res.* **2001**, *47*, 217–227.

28. Lothman, E. W.; Salerno, R. a; Perlin, J. B.; Kaiser, D. L. *Epilepsy Res.* **1988**, *2*, 367–379.

29. Lothman, E. W.; Williamson, J. M. *Brain Res.* **1994**, *649*, 71–84.

30. Mortelmans, K.; Zeiger, E. *Mutat. Res.* **2000**, *455*, 29–60.

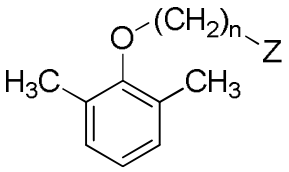
31. Maron, D. M.; Ames, B. N. *Mutat. Res.* **1983**, *113*, 173–215.

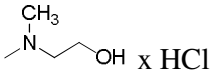
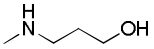
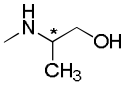
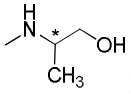
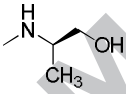
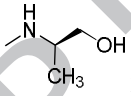
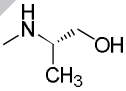
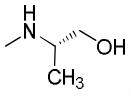
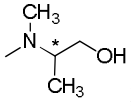
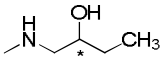
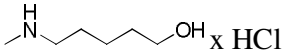
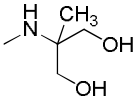
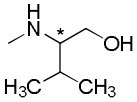
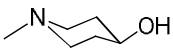


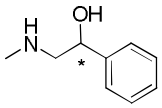
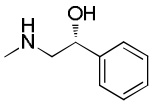
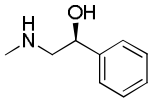
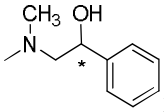
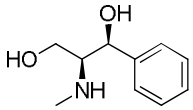
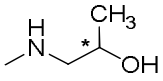
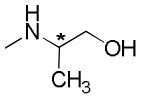
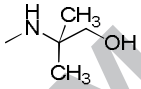
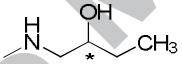
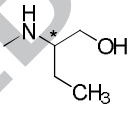
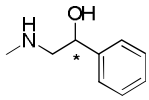
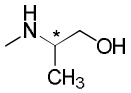
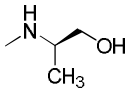
MES ED_{50} = 5.34 mg/kg b.w. (mice, *i.p.*, 0.25 h)
22.28 mg/kg b.w. (female mice, *i.p.*, 0.25 h)
6 Hz ED_{50} = 19.5 mg/kg (mice, *i.p.*, 0.25 h)
7.06 mg/kg (female mice, *i.p.*, 0.25 h)
FRINGS ED_{50} = 5.39 mg/kg (mice, *i.p.*, 0.25 h)
σ $IC_{50} = 1.9*10^{-6}$ M
5-HT_{1A} $IC_{50} = 2.8*10^{-6}$ M
5-HT transporter $IC_{50} = 7.0*10^{-6}$ M

Table 1

Chemical structures of the title compounds



n	Compd.	Z	Configuration	clogP
2	**1**	 x HCl	-	2.74
	2		-	2.39
	3		*R,S*	2.40
	3a	 x HCl	*R,S*	2.40[a)]
	4		*R*	2.40
	4a	 x HCl	*R*	2.40[a)]
	5		*S*	2.40
	5a	 x HCl	*S*	2.40[a)]
	6	 x HCl	*R,S*	3.05[a)]
	7		*R,S*	2.93
	8	 x HCl	-	2.69[a)]
	9		-	2.45
	10		*D,L*	3.10
	11			2.31

	12		*R,S*	3.66
	13		*R*	3.66
	14		*S*	3.66
	15	 x HCl	*R,S*	4.31[a)]
	16		*L-threo*	3.57
3	**17**		*R,S*	2.76
	18		*R,S*	2.76
	19		-	3.16
	20		*R,S*	3.29
	21	 x HCl	*R,S*	3.29[a)]
	22		*R,S*	4.03
4	**23**		*R,S*	2.74
	24		*R*	2.74

[a)] Parameter calculated for base with use of ChemBioDraw Ultra 12.0 computer program.

Table 2
The selected bond lengths (Å), bond and torsion angles (°) in the crystal structure of **4a2** and mexiletine

	4a2	Mexiletine[17]
C6-O2-C5	111.52	114.69
C6-O2-C5-C4	-169.22	- 174.49
O2-C5-C4-N1	57.11	-60.55
O2-C5-C4-H4B	-63.22	56.24