Bioorganic & Medicinal Chemistry 19 (2011) 6927–6934

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and anticonvulsant activity of *trans*- and *cis*-2-(2,6-dimethylphenoxy)-*N*-(2- or 4-hydroxycyclohexyl)acetamides and their amine analogs

Elżbieta Pękala [a], Anna M. Waszkielewicz [b,*], Edward Szneler [c], Maria Walczak [d], Henryk Marona [b]

[a] Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University-Medical College, 30-688 Krakow, Poland
[b] Department of Bioorganic Chemistry, Chair of Organic Chemistry, Faculty of Pharmacy, Jagiellonian University-Medical College, 30-688 Krakow, Poland
[c] Faculty of Chemistry, Jagiellonian University, 30-060 Krakow, Poland
[d] Department of Pharmacokinetics and Physical Pharmacy, Faculty of Pharmacy, Jagiellonian University-Medical College, 30-688 Krakow, Poland

ARTICLE INFO

Article history:
Received 30 May 2011
Revised 25 August 2011
Accepted 8 September 2011
Available online 14 September 2011

Dedicated to the memory of Professor Marian Eckstein

Keywords:
Aminocyclohexanol
New amides
Epilepsy
Seizures
Anticonvulsant
NIH
MES
Preclinical

ABSTRACT

A group of *trans*- and *cis*-2-(2,6-dimethylphenoxy)-*N*-(2-hydroxycyclohexyl)acetamides (**1**–**7**) and -ethylamines (**8**–**9**) have been synthesized and investigated for their anticonvulsant activity. One of them, racemic *trans*-2-(2,6-dimethylphenoxy)-*N*-(2-hydroxycyclohexyl)acetamide proved to be the most effective in MES (mice, ip), exhibiting ED_{50} = 42.97 mg/kg b.w. and TD_{50} = 105.67 mg/kg b.w. It also proved protection in focal seizures (electric kindling, rats, ip) and it raises seizure threshold. The mechanism of action is inhibition of voltage-gated sodium currents and enhancement of GABA effect. Safety pharmacology assay on threshold tonic extension revealed no lowering of the seizure threshold.

1. Introduction

Epilepsy is one of the major neurological disorders. Antiepileptic drugs (AEDs) exert their action by various mechanisms, for example, they can influence the inhibitory or excitatory neurotransmitter systems (GABA or glutamic and aspartic acid, respectively), or the ion transport across cell membranes. Conventional antiepileptic drugs: valproate, phenytoin, carbamazepine, or benzodiazepines, are widely used but exhibit an unfavorable side effect profile and fail to control seizures adequately. In the recent years, several new drugs, such as: lamotrigine,[1] pregabaline,[2] brivaracetam,[3] or lacosamide[4] have been added to the list of therapeutic agents against epilepsy. However, there is a significant group of patients (up to 30%) who are resistant to the available antiepileptic drugs.[5] Hence, there is an unmet need to develop new AEDs exhibiting more selective activity and lower toxicity. Chemical diversity and various mechanisms of action of anticonvulsants make it difficult to find a common way of identifying new drugs. Novel anticonvulsant agents are discovered through conventional screening and/or structure modification rather than a mechanism–driven design. Drug identification is conducted via in vivo screening tests, on the basis of seizure type.

Numerous new compounds are synthesized and screened for their anticonvulsant activities each year. In order to make the discovery of new AEDs more rational, several investigators identify structural fragments that may enhance anticonvulsant properties and allow orientating the synthesis of novel compounds, in which some of these active fragments can appear.[6] One of the structural features that play a significant role in relation to antiepileptic activity is an amide group.[7–9]

Lately, new AEDs have come up, exhibiting the above mentioned amide group. Among many examples there are valpromide with its new generation valrocemide and valnoctamide,[9–12] levetiracetam with brivaracetam and seletracetam,[13–16] as well as retigabine,[17,18] ameltolide,[19,20] and rufinamide[21] (Fig. 1).

Structure–activity studies performed by our team have shown that anticonvulsant activity could be derived from N-substituted derivatives of appropriate chiral 1,2-aminoalkanols. As an example, appropriate derivatives of (*S*)-(+)-amino-1-butanol[22] and (*R*)-(−)-2-amino-1-propanol[23] displayed anti-MES activity

* Corresponding author. Tel./fax: +48 12 6205594.
E-mail address: awaszkie@cm-uj.krakow.pl (A.M. Waszkielewicz).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.09.014

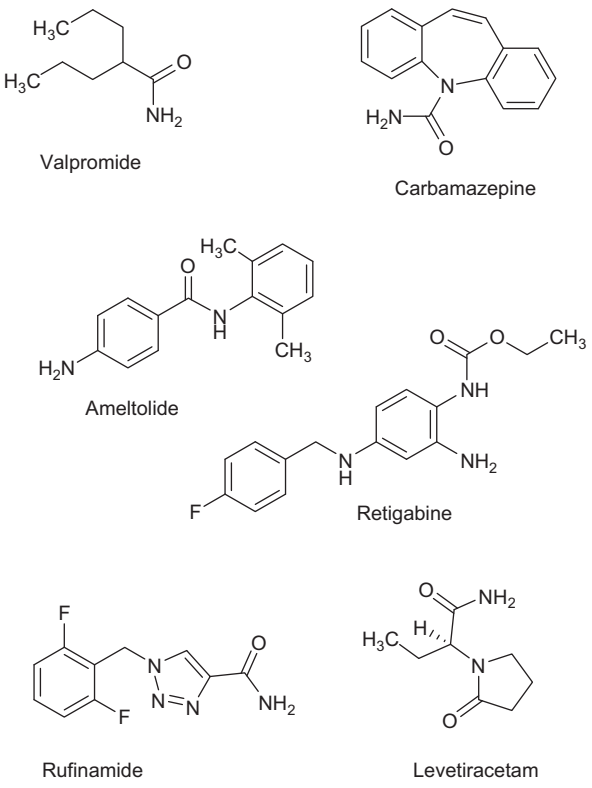


Figure 1. Structures of known anticonvulsants with an amide group.

with protective index (TD_{50}/ED_{50}) of 4.55 and 6.23, respectively. The results correspond to those for phenytoin, carbamazepine, and valproate.[24] With respect to the anticonvulsant activity of 1,2-aminoalkanol derivatives the aim of the study was to test whether binding of this moiety into a cyclic ring would enhance or diminish anticonvulsant activity. Such modification resulted in stereochemical variability, an important aspect in optimizing biological activity of drug candidates. Moreover, elongation of the alkyl moiety between the amine and hydroxyl groups from 2 to 4 carbons could provide an interesting discussion on structure–activity relationship.

We herein report synthesis and anticonvulsant activity of new compounds which are amide derivatives of chiral (racemic and enantiomeric) *trans*- or *cis*-2-amino-1-cyclohexanol (**1–6**) as well as achiral 2-amino-4-cyclohexanol (**7**), which contain the common 2,6-dimethylphenoxyacetyl core. In order to compare the potential anticonvulsant activities, their amine analogs have been synthesized, too (**8**–**9**).

2. Results and discussion

2.1. Chemistry

The title compounds **1**–**3** (Scheme 1) were prepared in our laboratory as a result of acylation of *trans* racemic, (1*R*,2*R*)- or (1*S*,2*S*)-2-amino-1-cyclohexanol, using the appropriate acid chloride in biphasic medium (water–toluene). K_2CO_3 was used as a proton acceptor. Racemic *trans*-2-amino-1-cyclohexanol was obtained from cyclohexene oxide in the reaction with 25% ammonia.[25] The racemic *trans*-2-amino-1-cyclohexanol was separated into its optically active *S*-(+) and *R*-(−) enantiomers, using diastereoisomeric pairs of salts (hydrogen (+)-tartrates). The process was possible due to the difference in solubility of the both isomers in ethanol and methanol.[26] Compounds **4–6** were obtained from appropriate **1**–**3** with use of $SOCl_2$. The reaction of *trans*-2-aminocyclohexanol is performed on the asymmetric center and leads to an oxazolidine derivative, due to presence of the rigid cyclohexane moiety. Cyclization reactions in this case do not cause racemization nor retention, however, they cause isomerization. Closing the ring is performed through *cis*-oxazoline, which after hydrolysis opens to an appropriate ester. Then the acyl group is rearranged from the oxygen atom (in the carboxyl group) to the amine group and as a result appropriate *cis*-amides (**4**–**6**) are formed. The above reaction proceeds as the effect of anchimeric support of the adjacent group.[25] Compounds **8** and **9** were achieved in two ways: by reduction of the amide **1** and **7**, respectively, using $LiAlH_4$ in diethyl ether solution under nitrogen atmosphere at room temperature (yield 50%) and by N-alkylation of *trans*-2-amino-1- or *trans*-2-amino-4-cyclohexanol with 2,6-dimethylphenoxyethyl bromide (yield 65%). Physico-chemical data of **1**–**9** are presented in Table 1.

2.2. Pharmacology

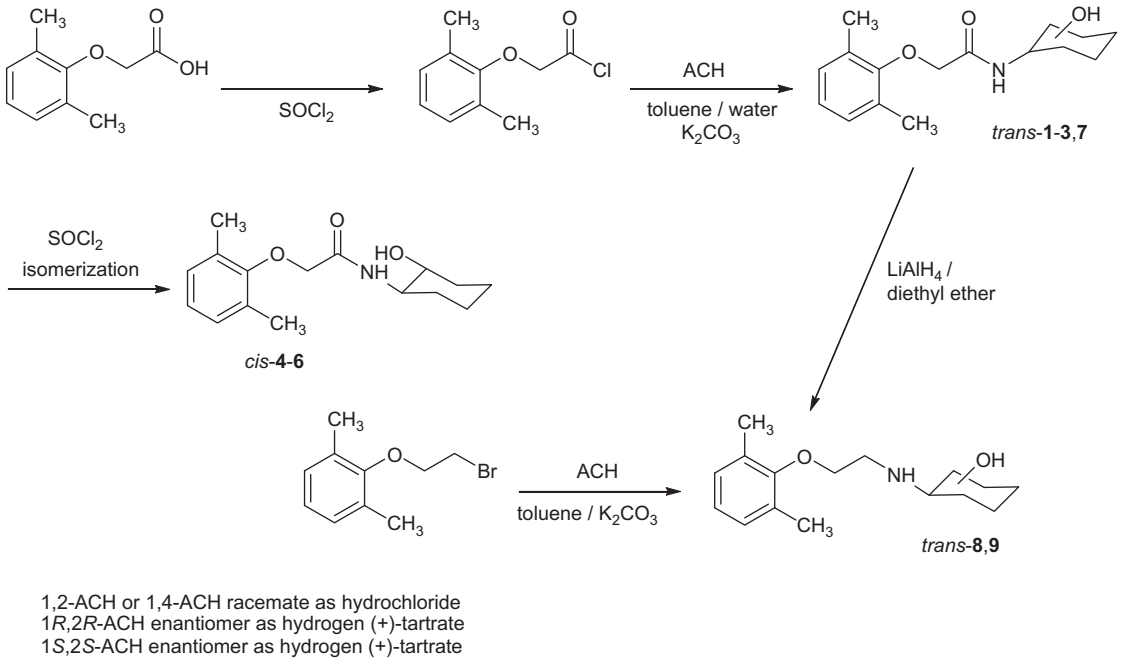
Molecular chirality is a phenomenon that plays fundamental important role in biological processes. A wide range of biological and physical functions are generated through precise molecular recognition because enzymes, receptors, and other natural binding sites within biological systems interact with different enantiomers in decisively diverse ways. As a result of such chiral recognition, drug enantiomers may differ in their pharmacokinetic handling and their pharmacological and/or toxicological profiles. For a drug with a single stereogenic center, both enantiomers may be pharmacologically active. However, if the main pharmacological effect is observed only for one enantiomer, several possibilities exist for the other one: inactivity, a qualitatively different effect, an antagonistic effect, or severe toxicity. For this reason we designed and studied the racemic form (*trans*-**1**), their enantiomers (*trans*-**2,3**) and corresponding geometric isomers (*cis*-**4, 5, 6**).

The new compounds **1**–**9** were tested *in vivo* by using three screens (mice, rats): the MES, ScMet (anticonvulsant tests) and TOX (neurotoxicity, rotarod) (Tables 2 and 3). For the selected compounds (**1**, **2**, **4**, and **8**) advanced quantitative tests (ED_{50} and TD_{50}) in mice and/or rats were performed (Table 2).

Compounds **1**, **2**, **4**, **7**, and **8** have been evaluated in the time-to-peak-effect (TPE) assay in rats (Table 4). Among them, **1**, **2**, **4**, and **8** are the most active 15 min after administration (TPE = 0.25 h) which can serve as a premise that the compound itself and not a metabolite is biologically active. As seen in the TPE assay, **1** has not revealed any neurotoxicity in rats given the doses up to 500 mg/kg b.w. po, observed for 24 h. This can be interpreted as no neurotoxicity of the compound nor its potential metabolites.

The most interesting anticonvulsant results were observed for *trans*-racemic-2-(2,6-dimethylphenoxy)-*N*-((1*R*,2*R*)-2-hydroxycyclohexyl)acetamide (**1**) and its amine derivative (**8**). Compound **1** displayed anti-MES activity with protective index (TD_{50}/ED_{50} of 2.47 (mice, ip) and 21.21 (rats, po). **8** exhibited TD_{50}/ED_{50} of 3.90 (mice, ip) and 19.06 (rats, po) corresponding with PI for valproate (1.70 and 2.2, respectively).[3]

Since compounds **2** and **3** are enantiomers of **1**, it could be expected that one of the enantiomers should be chosen for further development. The screening results in MES, mice, ip (Table 2) reveal that compound **2** (1*S*,2*S*) is the one active and less neurotoxic than **3** (1*R*,2*R*) in the dose of 100 mg/kg b.w., 0.5 h after administration, giving premises for choice of **2** for development. However, further in quantitative analysis of ED_{50}, TD_{50} and PI (Table 2) it turned out that although **2** has more favorable ED_{50} than **1** (35.47 and 42.97 mg/kg b.w., respectively), the PI of **1** is more favorable than **2**, proving that the racemate (**1**) would be slightly safer to use. Moreover, considering the above results, costs of providing of the enantiomer would not be justified compared to costs of synthesizing the racemate (**1**).



Scheme 1. Synthesis of alkanoloamides **1**–**7** and alkanoloamines **8**–**9**.

Table 1
Physico-chemical data of *N*-acyl (**1**–**7**) and *N*-alkyl (**8**–**9**) derivatives of 2-amino-1- or 2-amino-4-cyclohexanol

Compds.	Stereoisomer	Configuration	Mp [°C] crystal. solvent	Yield [%]	TLC R_f	$[\alpha]^D$ c [%]	Elemental analysis		
							C calc./found	H calc./found	N calc./found
1	Racemic	*trans*	106–108 *n*-hexane/toluene (1:1)	62	0.68[a]	-	69.24 69.01	8.36 8.44	5.05 4.94
2	(1*S*,2*S*)	*trans*	110–111 *n*-hexane/toluene (1:1)	55	0.68[a]	3.176 5, $CHCl_3$	69.24 69.07	8.35 8.34	5.05 5.19
3	(1*R*,2*R*)	*trans*	108–110 *n*-hexane/toluene (1:1)	57	0.68[a]	-3.259 5, $CHCl_3$	69.24 69.26	8.35 8.65	5.05 4.94
4	Racemic	*cis*	88–90 *n*-hexane	60	0.79[a]	-	69.24 69.42	8.35 8.52	5.05 5.17
5	(1*R*,2*S*)	*cis*	125–127 *n*-hexane	48	0.80[a]	-31.186 5, $CHCl_3$	69.24 69.19	8.35 8.41	5.05 5.23
6	(1*S*,2*R*)	*cis*	126–128 *n*-hexane	52	0.80[a]	31.06 5, $CHCl_3$	69.24 69.38	8.35 8.57	5.05 5.93
7	Racemic	*trans*	160–162 *n*-hexane	57	0.4[b]	-	69.42 69.50	8.35 8.36	5.05 5.06
8[22]	Racemic	*trans*	90–91 *n*-heptane	65	0.52[c]	-	64.09 63.87	8.74 8.47	4.69 4.86
8a	Racemic	*trans*	174–176	87	0.61[c]		72.94 72.83	9.57 9.39	5.34 5.41
9	Racemic	*trans*	84–86 *n*-heptane	64	0.62[c]	-	64.09 63.88	8.74 8.75	4.69 4.49

Molecular weight for compounds: **1**–**7**: 277.34 g ∗ mol^{-1} ($C_{16}H_{23}NO_3$); **8**–**9**: 263.37 g ∗ mol^{-1} ($C_{16}H_{25}NO_2$); **8a** 299.83 g ∗ mol^{-1} ($C_{16}H_{25}NO_2$ *x*HCl).
[a] $CHCl_3$/acetone (7:3).
[b] Toluene/acetone (5:1).
[c] Toluene/methanol (5:1).

Therefore, among **1**–**3**, compound **1** was chosen for quantitative assays (ED_{50}, TD_{50}, PI) in rats, po (Table 2). Additionally, quantitative studies in rats, po have been performed for compound **8** – the amide analog of **1** (Table 2). Comparing the ED_{50}s between **1** and **8**, it can be noticed that there is no real difference between them (23.56 and 26.23 mg/kg/b.w., respectively), TD_{50}s are >500 mg/kg b.w. in both cases. Therefore, both compounds were advanced to further testing.

Due to the structure similarity of **1** and **8** (common dimethylphenol moiety) to mexiletine – a known antiarrythmic drug which lowers seizure threshold – it is necessary to perform safety pharmacology assays in order to exclude such effect from antiepileptic drug candidates.[27] Therefore, threshold tonic extension (TTE) test was performed for **1** and **8**, in order to assess whether the compounds would enhance susceptibility to seizures. The results are shown in Figures 2 and 3, respectively.

Table 2
Anticonvulsant activity of *N*-acyl (**1**–**7**) and *N*-alkyl (**8**–**9**) derivatives of 2-amino-1- or 2-amino-4-cyclohexanol

Compds.	Dose mg/kg mice, ip	MES 0.5 h	MES 4 h	ScMET 0.5 h	ScMET 4 h	TOX 0.5 h	TOX 4 h	ASP class[A]	TD_{50}[B]	ED_{50}[C]	$PI_{(MES)}$[D]
1[31]	30	0/1	0/1	0/1	0/1	0/4	0/2	1	105.61[a]	42.97[a]	2.475[a]
	100	2/3	0/3	3/5	0/1	2/8	0/4		>500[b]	23.56[b]	>21.218[b]
	300		0/1	1/1		4/4	0/2				
2[31]	30	0/1	0/1	0/1	0/1	0/4	0/2	1	68.70[a]	35.47[a]	1.937[a]
	100	1/3	0/3	3/5	0/1	0/8	0/4				
	300	1/1	0/1	0/1	0/1	0/4	0/2				
3[31]	30	0/1	0/1	0/1	0/1	0/4	0/2	2	nd	nd	nd
	100	0/3	0/3	0/1	0/1	2/8	0/4				
	300	1/1	0/1	0/1	0/1	2/4	0/2				
4	30	0/1	0/1	0/1	0/1	0/4	0/2	1	117.73[a]	78.83[a]	1.493[a]
	100	0/3	0/3	0/1	0/1	0/8	0/4				
	300	1/1	0/1	0/1	0/1	0/4	0/2				
5	30	0/1	0/1	0/1	0/1	0/4	0/2	1	nd	nd	nd
	100	1/3	0/3	0/1	0/1	0/8	0/4				
	300	1/1	0/1	0/1	0/1	0/4	0/2				
6	30	0/1	0/1	0/1	0/1	0/4	0/2	1	nd	nd	nd
	100	2/3	0/3	0/1	0/1	0/8	0/4				
	300	1/1	0/1	1/1	0/1	2/4	0/2				
7	30	0/1	0/1	0/1	0/1	0/4	0/2	1	nd	nd	nd
	100	3/3	0/3	0/1	0/1	7/8	0/4				
	30	1/1	0/1	1/1	0/1	4/4	0/2				
8	3	0/4				0/4		1	28.36[a]	7.73[a]	3.90[a]
	10	3/4				0/4			>500[b]	26.23[b]	>19.06[b]
	30	1/1	0/1	0/1	0/1	4/4	0/2				
	100			0/1		8/8					
						4/4					
9	30	0/1	0/1	0/1	0/1	0/4	0/2	1	nd	nd	nd
	100	3/3	0/3	0/1	0/1	7/8	0/4				
	300					4/4					
PHT[E]									34.45[a]	6.48[a]	6.60[a]
									>500[b]	32.2[b]	>22[b]
CBZ[E]									47.8[a]	9.85[a]	4.90[a]
									361[b]	3.57[b]	101[b]
VPA[E]									483[a]	287[a]	1.70[a]
									859[b]	395[b]	2.2[b]

MES – maximal electroshock seizure: number of animals protected/number of animals tested; ScMET – subcutaneous pentylenetetrazole seizure: number of animals protected/number of animals tested; TOX – neurotoxicity screen: number of animals exhibiting toxicity/number of animals tested in the rotorod test.
nd, not determined; PHT, phenytoin; CBZ, carbamazepine; VPA, valproate.
[A] The ASP classification (Anticonvulsant Screening Project) is as follows: 1 – anticonvulsant activity at 100 mg/kg or less; 2 – anticonvulsant activity at doses greater than 100 mg/kg; 3 – compound inactive at 300 mg/kg; 4 – toxicity at doses 30 mg/kg.
[B] TD_{50} – dose (mg/kg) eliciting evidence of minimal neurological toxicity in 50% of animals; 95% confidence interval is shown in parentheses; the slope regression line is shown in brackets.
[C] ED_{50} – dose (mg/kg) eliciting the MES protection in 50% of animals.
[D] PI – neurotoxic dose/median effective dose (TD_{50}/ED_{50}) for an anticonvulsant test. In each case 32 mice or 32 rats were used for determination of ED_{50} and toxicity (TOX).
[E] Data from Ref.[3]
[a] Values for mice after ip administration.
[b] Values for rats after po administration.

Table 3
Anticonvulsant Screening Project: phase VIa. The results in rats (dose 30 mg/kg, po)

Compds.	Test	Time [h]				
		0.25	0.5	1.0	2.0	4.0
1	MES[a]	3/4	3/4	2/4	2/4	2/4
	TOX[a]	0/4	0/4	0/4	0/4	0/4
2	MES	1/4	3/4	2/4	2/4	1/4
	TOX	0/4	0/4	0/4	0/4	0/4
3	MES	0/4	1/4	1/4	1/4	1/4
	TOX	0/4	0/4	0/4	0/4	0/4
4	MES	2/4	0/4	0/4	0/4	0/4
	TOX	0/4	0/4	0/4	0/4	0/4
7	MES	4/4	4/4	3/4	3/4	1/4
	TOX	0/4	0/4	0/4	0/4	0/4
8	MES	1/4	3/4	1/4	1/4	0/4
	TOX	0/4	0/4	0/4	0/4	0/4

[a] see Table 1.

It can be observed in Figure 2 that the more dose of compound **1** is administered, the more pentylenetetrazole is needed to cause a twitch or a clonus, although the dependence is not linear. Nevertheless, it can be stated that compound **1** does not have the side effect of lowering seizure threshold. The doses tested were equivalent to ED_{50} in MES (mice, ip) = 43 mg/kg and TD_{50} in MES (mice, ip) = 106 mg/kg. It is interesting that compound **8** exhibits the opposite effect – it lowers seizure threshold (Fig. 3), and the difference between the structures is in the presence of the amide group. Lowering seizure threshold may cause increasing susceptibility of patients to seizures, therefore such effect eliminated compound **8** from further development.

Compound **1** was advanced for further assays as the most promising one. It was investigated for prevention of expression and acquisition of focal seizures in the hippocampal kindling test in rats (Fig. 4). The greatest activity was observed 15 and 45 min after administration (the Racine score of 5 decreased below 3 which is considered protection).[28] Therefore, compound **1** can be termed 'antiepileptogenic'.[29] The activity 15 min after administration is consistent with TPE in the MES test, in spite of different routes of administration.

In order to have the first insight into molecular mechanisms of action, compound **1** was tested in electrophysiological studies. The

Table 4
Anticonvulsant Screening Project: time to peak effect for **1**, **2**, **4**, **7** and **8**

Compds.	Test	Dose (mg/kg) Animals (route of administration)	Time [h]							
			0.25	0.50	1.0	2.0	4.0	6.0	8.0	24
	MES[a]	15 rats (po)	3/4	1/4						
	MES	30 rats (po)	4/4	4/4	1/4	1/4	1/4			
1	TOX[a]	62.50 rats (po)	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	TOX	125 rats (po)	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	TOX	250 rats (po)	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	TOX	500 rats (po)	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	MES	90 mice (ip)	8/8	7/8						
	MES	130 mice (ip)	4/4	4/4	2/4	0/4	0/4			
	TOX	130 mice (ip)	8/8	0/8	0/8	0/8				
2	MES	60 mice (ip)	8/8	1/8						
	MES	90 mice (ip)	4/4	4/4	0/4	1/4	0/4			
	TOX	90 mice (ip)	7/8	4/8	3/8	2/8	2/8			0/8
	MES	70 mice (ip)	3/4	0/4	0/4	0/4				
4	TOX	110 mice (ip)	3/8	3/8						
	TOX	300 mice (ip)	8/8	8/8	5/8	0/8	0/8			
7	TOX	100 rats (ip)	7/8	8/8	7/8	5/8				
8	MES	30 rats (ip)	4/4	4/4	4/4	0/4	0/4			
	TOX	30 rats (ip)	3/4	2/4	0/4	0/4	0/4			

[a] see Table 1.

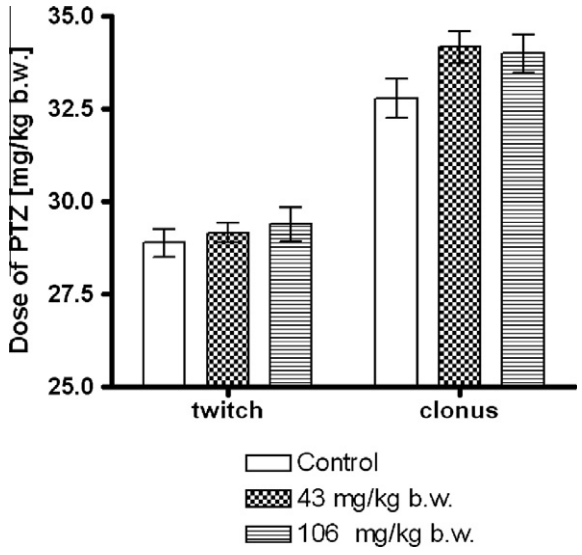


Figure 2. Results of threshold tonic extension test for **1**. After compound **1** (ED_{50} in MES (mice, ip) = 43 mg/kg and TD_{50} in MES (mice, ip) = 106 mg/kg) is administered intraperitoneally to mice, infusion of pentylenetetrazol is started and lasts until the first twich and the first clonus. The doses of administered PTZ were calculated from the speed of infusion. As it can be seen, the higher dose of **1**, the more PTZ is needed to cause seizures. Therefore, **1** raises seizure threshold. Results are presented as average of eight animals ± S.E.M.

purpose for the assays was to correlate with the data from the animal seizure models. The assays evaluate compound's interactions with sodium, glutamate or GABA ion channels.[30] The methodology uses whole cell patch-clamp electrophysiological measurement on single neurons.

The assays performed on neuroblastoma N1E-115 cells revealed voltage-gated sodium channel blocking effect at 100 μmol concentration (Table 5), when voltage of −60 or −90 mV was used as holding potential. The results show that activity measured as percent of control stimulation (samples without the tested compound) fall in the range for known antiepileptic sodium channel blockers such as carbamazepine, lamotrigine, and phenytoin, with statistical significance p <0.05. The shown activity is consistent with in vivo activity in MES of compound **1**.

Studies on murine cortical neurons examined neuronal response to stimuli of inhibitory (GABA), as well as excitatory (NMDA and kainate) ion channels in the presence of the tested compound. The results presented in Table 6 proved enhancement of GABA effect as another mechanism of action of **1** (100 μmol concentration).

It is shown in the results that structural modification of compounds containing 2,6-dimethylphenoxy group (present also in antiarrythmic and anticonvulsant mexiletine) may reduce mexiletine's adverse event of lowering seizure threshold. Moreover, significant anticonvulsant activity can be observed among these compounds. Therefore, further investigation within this group of derivatives and further structural modifications of compound **1** are justified.

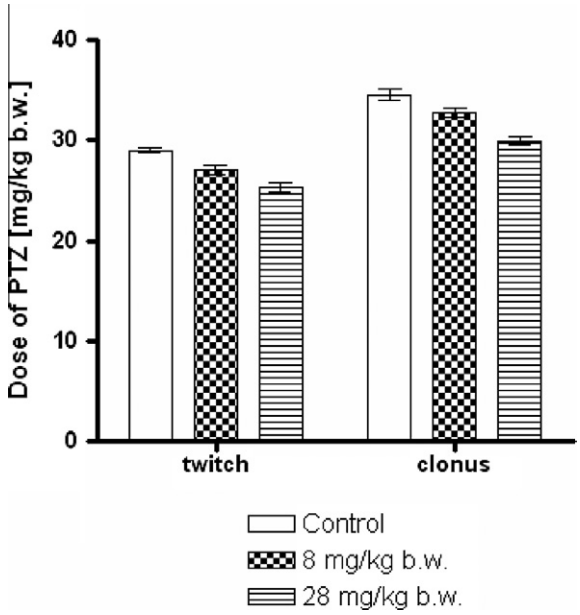


Figure 3. Results of threshold tonic extension test for **8**. After compound **8** (ED_{50} in MES (mice, ip) = 8 mg/kg and TD_{50} in MES (mice, ip) = 28 mg/kg) is administered intraperitoneally to mice, infusion of pentylenetetrazol is started and lasts until the first twitch and the first clonus. The doses of administered PTZ were calculated from the speed of infusion. As it can be seen, the higher dose of **8**, the less PTZ is needed to cause seizures. Therefore, **8** lowers seizure threshold. Results are presented as average of 8 animals ± S.E.M.; p value <0.05 at 28 mg/kg b.w.

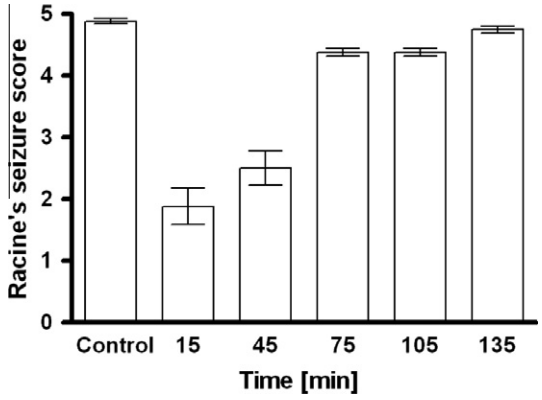


Figure 4. Compound **1** was administered ip to fully kindled rats (score 5 in the Racine's scale) at the dose 100 mg/kg b.w. Seizures were induced 15, 45, 75, 105, and 135 min after administration. Scores below 3 are considered protection. Therefore, the compound is effective 15–45 min after administration.

Table 5
Anticonvulsant Screening Project: electrophysiology studies for **1**. Anticonvulsant drugs on voltage dependent sodium currents in N1E-115 neuroblastoma cells. Part I

Compds.	Holding potential [mV]	No. of cells	% of control (±SEM)
1	−60	8	39 ± 2*
1	−90	7	85 ± 5*
CBZ	−90	5	78 ± 2**
LMG	−90	8	67 ± 5**
PHT	−90	6	78 ± 2**

Drugs concentration: 100 μmol.
The results from several individual cells were averaged, the SEM was calculated and statistical significance was determined using the Student's t-test.
* Significantly different from control, p <0.05.
** Significantly different from control, p <0.001.

Table 6
Anticonvulsant Screening Project: electrophysiology studies for compound **1**. Effect on whole cell currents of mouse cortical neurons (14–21 days, in vitro). Part II

Effect evoked by:	No. of cells	% of control (±SEM)
1 mmol GABA	5	179 ± 15*
10 μmol NMDA +1 μmol glycine	6	104 ± 6
100 μmol kainate	6	98 ± 3

Holding potential −70 mV; compound concentration:100 μmol.
* Significantly different from control, p <0.05; potent GABA enhancement.

2.3. Conclusions

The seven new compounds with an amide group and their two amine analogs derived from chiral or achiral aminocyclohexanol have been synthesized and tested for anticonvulsant activity. The results of anticonvulsant screening revealed that all derivatives were effective in the MES screen. The quantitative studies in mice after ip administration showed that two compounds (**1** and its amine derivative **8**) were more potent than valproate in the maximal electroshock test (MES). The highest activity was observed for **1** with ED_{50} of 42.97 mg/kg (MES) and **8** with ED_{50} of 7.73 mg/kg (MES). Compound **1** can also be termed 'antiepileptogenic' and it raises seizure threshold. Its observed mechanisms of action are block of sodium currents and enhancement of GABA effect.

3. Experimental

3.1. Chemistry

3.1.1. General

^1H and ^{13}C NMR spectra were recorded on a Bruker spectrometer 500 or 125 MHz, respectively using the signal from DMSO in DMSO-d_6 and TMS in $CDCl_3$ as internal standards. The IR spectra were recorded on a Perkin Elmer or Jasco FT/IR 410 spectrometer (KBr pellets). Melting points 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) using the solvent systems: [a]$CHCl_3$/acetone (7:3), [b]toluene/acetone (5:1) and [c]toluene /methanol (5:1). Spots were visualized with UV light. Measurements of optical rotation ($[\alpha]_D$) were carried out on Jasco DIP-1000 (λ = D (546 nm)).

The reagents and solvents – except for the described above *R*-(−) and *S*-(+)-*trans*-2-amino-1-cyclohexanol – were commercially available materials of reagent grade.

Isomeric *trans*-2-amino-1-cyclohexanols $[\alpha]_{546}^{20}$: *R*-(−) = −40.2°; *S*-(+) = +40.2° and isomeric hydrogen (+)-tartrates of *trans*-2-amino-1-cyclohexanol $[\alpha]_{546}^{20}$: *R*-(−) = −4.3°; *S*-(+) = +38.96° were obtained according to literature.[26] The initial 2,6-dimethylpheno xyacetic acid (mp 128–130 °C) was obtained by a classical method using the appropriate sodium phenolate and chloroacetic acid. 2, 6-dimethylphenoxyethyl bromide (bp 121–123 °C) was synthesized from 2,6-dimethylphenoxyethanol with tribromophosphine.

3.1.2. General procedure for *trans*-amides (1–3, 7)

The appropriate salt of *trans*-2-amino-1- or *trans*-2-amino-4-cyclohexanol (20 mmol) and 5.6 g (40 mmol) of powdered anhydrous K_2CO_3 was suspended in 50 mL of cooled water. Next, a solution of 25 mmol of 2,6-dimethylphenoxyacetic chloride in 50 mL of dry toluene was added with vigorous stirring at 10–12 °C. The reaction mixture was stirred for 2 h at room temperature, next it was heated to the boiling point and then left to cool down. The precipitated amide deposit was filtered off, then it was

stirred with a 10% solution of $NaHCO_3$, and after drying it was recrystallized from *n*-hexane or -hexane/toluene.

3.1.2.1. (±)-*Trans*-2-(2,6-dimethylphenoxy)-N-(2-hydroxycyclohexyl)acetamide (1). IR (cm^{-1}): 3300, 1650, 1260; ^1H NMR (500.13 MHz, DMSO-d_6): δ 7.71 (1H, d, J = 7.8 Hz, NH), 7.03 (2H, $AA'BX_3X'_3$, J_{AB} = 7.5 Hz; J_{AX} = 0.6 Hz, H-3, H-5), 6.94 (1H, $AA'BX_3X'_3$, J_{AB} = 7.5 Hz, H-4), 4.85 (1H, d, J = 5.3 Hz, OH), 4.21 (1H, d, J = 14.2 Hz, Ar–O–C*H*H–), 4.16 (1H, d, J = 14.2 Hz, Ar–O–CH*H*), 3.54–3.46 (1H, m, *H* –C–NH), 3.42–3.32 (1H, m, *H* –C–OH), 2.22 (6H, dt, J = 0.6 Hz; J = 0.2 Hz, Ar–CH_3), 1.92–1.79 (2H, m, H-6′ (eq), H-3′ (eq)), 1.68–1.54 (2H, m, H-5′ (eq), H-4′ (eq)), 1.30–1.14 (4H, m, H-3′ (ax), H-4′ (ax), H-5′ (ax), H-6′ (ax)); ^{13}C NMR (500.13 MHz, DMSO-d_6): δ 167.40 (CO), 154.93 (C-1), 130.24 (C-2, C-6), 128.69 (C-3, C-5), 124.02 (C-4), 70.76 (Ar–O–CH_2–), 70.76 (C-1′ (C–OH cyclohexane)), 54.28 (C-2′ (C–NH cyclohexane)), 34.27 (C-6′), 30.87 (C-5′), 24.16 (C-4′),23.83 (C-3′), 15.90 (2*x* CH_3).

3.1.2.2. (1S,2S)-*Trans*-2-(2,6-dimethylphenoxy)-*N*-(2-hydroxycyclohexyl)acetamide (2). IR (cm^{-1}): 3420, 3380, 1650, 1265; ^1H NMR (500.13 MHz, DMSO-d_6): δ 7.71 (1H, d, J = 7.7 Hz, NH), 7.02 (2H, $A_2BX_3Y'_3$, J_{AB} = 7.5 Hz; J_{AX} = 0.6 Hz, J_{AY} = 0.7 Hz, H-3, H-5), 6.94 (1H, $A_2'BX_3Y'_3$, J_{AB} = 7.5 Hz; H-4), 4.57 (1H, d, J = 5.2 Hz, OH), 4.22 (2H, d, J = 14.2 Hz, Ar–O–C*H*H–), 4.15 (2H, d, J = 14.2 Hz, Ar–O–CH*H* –), 3.56–3.43 (1H, m, *H*–C–NH), 3.43–3.33 (1H, m, *H*–C–OH), 2.22 (3H, d, J = 0.6 Hz, Ar–CH_3), 2.22 (3H, d, J = 0.7 Hz, Ar–CH_3) 1.91–1.80 (2H, m, H-6′ (eq), H-3′ (eq)), 1.67–1.54 (2H, m, H-5′ (eq), H-4′ (eq)), 1.29–1.12 (4H, m, H-3′ (ax), H-4′ (ax), H-5′ (ax), H-6′ (ax)); ^{13}C NMR (500.13 MHz, DMSO-d_6): δ 167.40 (CO), 154.93 (C-1), 130.24 (C-2, C-6), 128.69 (C-3, C-5), 124.02 (C-4), 70.76 (Ar–O–CH_2–), 70.76 (C-1′ (C–OH cyclohexane)), 54.28 (C-2′ (C–NH cyclohexane)), 34.28 (C-6′), 30.87 (C-5′), 24.17 (C-4′), 23.84 (C-3′), 15.91 (2*x* CH_3).

3.1.2.3. (1R,2R)-*Trans*-2-(2,6-dimethylphenoxy)-*N*-(2-hydroxycyclohexyl)acetamide (3). IR (cm^{-1}): 3290, 1650, 1260; ^1H NMR (500.13 MHz, DMSO-d_6): δ 7.76 (1H, d, J = 7.9 Hz, NH), 7.03 (2H, $AA'BX_3X'_3$, J_{AB} = 7.5 Hz; J_{AX} = 0.8 Hz, H-3, H-5), 6.94 (1H, $AA'BX_3X'_3$, J_{AB} = 7.5 Hz; H-4), 4.61 (1H, d, J = 5.4 Hz, OH), 4.22 (2H, d, J = 14.2 Hz, Ar–O–C*H*H–), 4.16 (2H, d, J = 14.2 Hz, Ar–O–CH*H* –), 3.53–3.46 (1H, m, *H* –C–NH), 3.41–3.33 (1H, m, *H*–C–OH), 2.22 (6H, d, J = 0.8 Hz, Ar–CH_3), 1.91–1.80 (2H, m, H-6′ (eq)), H-3′ (eq)), 1.68–1.54 (2H, m, H-5′ (eq), H-4′ (eq)), 1.30–1.13 (4H, m, H-3′ (ax), H-4′ (ax), H-5′ (ax), H-6′ (ax)); ^{13}C NMR (500.13 MHz, DMSO-d_6): δ 167.40 (CO), 154.93 (C-1), 130.24 (C-2, C-6), 128.69 (C-3, C-5), 124.01 (C-4), 70.76 (Ar–O–CH_2–), 70.76 (C-1′ (C–OH cyclohexane)), 54.28 (C-2′ (C–NH cyclohexane)), 34.27 (C-6′), 30.87 (C-5′), 24.16 (C-4′), 23.84 (C-3′), 15.90 (2*x* CH_3).

3.1.2.4. (±)-*Trans*-2-(2,6-dimethylphenoxy)-*N*-(4-hydroxycyclohexyl)acetamide (7). IR (cm^{-1}): 3374, 3285, 1652, 1265, 1238; ^1H NMR (500.13 MHz, DMSO-d_6): δ 7.80 (1H, d, J = 8.2 Hz, NH), 6.97–7.06 (2H, m, H-3, H-5), 6.90–6.96 (1H, m, H-4), 4.49 (1H, d, J = 4.4 Hz, OH), 4.16 (2H, s, O–CH_2–C=O), 3.57–3.70 (1H, m, C-4′ (*H*–C–OH (cyclohexane))), 3.33–3.43 (1H, m, C-1′ (*H*–C–OH (cyclohexane))), 2.22 (6H, s, 2*x* Ar–CH_3), 1.79–1.86 (2H, m, C-3′ (eq), C-5′ (eq)), 1.71–1.79 (2H, m, C-2′ (eq), C-6′ (eq)), 1.32–1.43 (2H, m, C-3′ (ax), C-5′ (ax)), 1.16–1.27 (2H, m, C-2′ (ax), C-6′ (ax)); ^{13}C NMR (500.13 MHz, DMSO-d_6): δ 167.35 (CO), 155.49 (C-1), 130.72 (C-2, C-6), 129.20 (C-3, C-5), 124.52 (C-4), 71.28 (Ar–O–CH_2–), 68.54 (C-4′ (CH–OH cyclohexane)), 47.45 (C-1′ (CH–NH cyclohexane)), 34.49 (C-3′), 30.87 (C-5′), 30.54 (C-2′,C-6′), 16.36 (2*x* CH_3).

3.1.3. General procedure for *cis*-amides (4–6)

The appropriate *trans*-2-(2,6-dimethylphenoxy)-*N*-(2-hydroxycyclohexyl)acetamide (10 mmol) was added in small portions to 5 mL of thionyl chloride (cooled to 0 °C). The mixture was allowed to warm to room temperature and left for 3 h. Then the mixture was carefully poured into ca. 100 mL of distilled water. The resulting aqueous solution was filtered and the filtrate was refluxed for 10 min. Then the solution was cooled in an ice-salt-bath, filtered, and potassium hydroxide (6 mol/L) was added to the filtrate in order to yield the final *cis*-amide, which afterward was recrystallized from n-hexane.

3.1.3.1. (±)-*Cis*-2-(2,6-dimethylphenoxy)-*N*-(2-hydroxycyclohexyl)acetamide (4). IR (cm^{-1}): 3420, 1640, 1260; ^1H NMR (500.13 MHz, DMSO-d_6): δ 7.50 (1H, d, J = 8.2 Hz, NH), 7.02 (2H, A_2B, J = 7.4 Hz; J = 7.4 Hz, H-3, H-5), 6.95 (1H, A_2B, J = 7.4 Hz; H-4), 4.89 (1H, d, J = 4.0 Hz, OH), 4.24 (2H, d, J = 14.3 Hz, Ar–O–C*H*H–), 4.20 (2H, d, J = 14.3 Hz, Ar–O–CH*H* –), 3.75–3.79 (1H, m, *H* –C–NH), 3.71–3.75 (1H, m, *H*–C–OH), 2.22 (6H, s, 2*x* Ar–CH_3), 1.22–1.73 (8H, m, cyclohexane), ^{13}C NMR (500.13 MHz, DMSO-d_6): δ 166.72 (CO), 154.75 (C-1), 130.12 (C-2, C-6), 128.76 (C-3, C-5), 124.13 (C-4), 70.58 (Ar–O–CH_2–), 66.50 (C-1′ (C–OH cyclohexane)), 50.21 (C-2′ (C–NH cyclohexane)), 31.84 (C-6′), 26.54 (C-5′), 23.71 (C-4′), 19.12 (C-3′), 15.91 (2*x* CH_3).

3.1.3.2. (1R,2S)-*Cis*-2-(2,6-dimethylphenoxy)-*N*-(2-hydroxycyclohexyl)acetamide (5). IR (cm^{-1}): 3420, 1650,1260; ^1H NMR (500.13 MHz, DMSO-d_6): δ 7.50 (1H, d, J = 8.2 Hz, NH), 7.02 (2H, A_2B, J = 7.5 Hz; J = 7.4 Hz, H-3, H-5), 6.95 (1H, A_2B, J = 7.5 Hz; H-4), 4.88 (1H, d, J = 4.0 Hz, OH), 4.25 (2H, d, J = 14.3 Hz, Ar–O–C*H* H–), 4.20 (2H, d, J = 14.3 Hz, Ar–O–CH*H* –), 3.75–3.80 (1H, m, *H* –C–NH), 3.71–3.75 (1H, m, *H* –C–OH), 2.22 (6H, s, 2*x* Ar–CH_3), 1.22–1.73 (8H, m, cyclohexane), ^{13}C NMR (500.13 MHz, DMSO-d_6): δ 166.72 (CO), 154.74 (C-1), 130.13 (C-2, C-6), 128.76 (C-3, C-5), 124.14 (C-4), 70.56 (Ar–O–CH_2–), 66.48 (C-1′ (C-OH cyclohexane)), 50.20 (C-2′ (C–NH cyclohexane)), 31.84 (C-6′), 26.54 (C-5′), 23.72 (C-4′), 19.11 (C-3′), 15.92 (2*x* CH_3).

3.1.3.3. (1S,2R)-*Cis*-2-(2,6-dimethylphenoxy)-*N*-(2-hydroxycyclohexyl)acetamide (6). IR (cm^{-1}): 3420, 3360, 1650, 1260; ^1H NMR (500.13 MHz, DMSO-d_6): δ 7.48 (1H, d, J = 8.1 Hz, NH), 7.03 (2H, A_2BX_6, J_{AB} = 7.4 Hz; J_{AX} = 0.6 Hz; J_{BX} = 0.2, H-3, H-5), 6.94 (1H, A_2BX_6, J_{AB} = 7.4 Hz; J_{AX} = 0.6 Hz; J_{BX} = 0.2; H-4), 4.85 (1H, d, J = 4.3 Hz, OH), 4.26 (2H, d, J = 14.3 Hz, Ar–O–C*H*H–), 4.18 (2H, d, J = 14.3 Hz, Ar–O–CH*H*), 4.69–4.81 (2H, m, *H*–C–NH + *H*–C–OH), 2.22 (6H, td, J = 0.6 Hz; J = 0.2 Hz, 2*x* Ar–CH_3), 1.20–1.75 (8H, m, cyclohexane), ^{13}C NMR (500.13 MHz, DMSO-d_6): δ 166.72 (CO), 154.74 (C-1), 130.12 (C-2, C-6), 128.76 (C-3, C-5), 124.13 (C-4), 70.58 (Ar–O–CH_2–), 66.50 (C-1′ (C–OH cyclohexane)), 50.21 (C-2′ (C–NH cyclohexane)), 31.84 (C-6′), 26.55 (C-5′), 23.71 (C-4′), 19.12 (C-3′), 15.91 (2*x* CH_3).

3.1.4. General procedure for *trans*-alkanoloamines (8–9)

2,6-Dimethylphenoxyethyl bromide (20 mmol) was dissolved in 80 mL of toluene. Then 24 mmol of appropriate *trans*-2-amino-1- or *trans*-2-amino-4-cyclohexanol was added and refluxed for 5–6 h in presence of 16 mmol of anhydrous K_2CO_3. The inorganic salt precipitate was filtered from the hot mixture and washed with hot toluene (2 × 10 mL). The precipitate was separated from the cooled filtrate, filtered off and recrystallized from *n*-heptane. Compound **8** was converted into salt (hydrochloride – **8a**) in *n*-propanol with excess of EtOH saturated with HCl.

3.1.4.1. (±)-*Trans*-2-(2-(2,6-dimethylphenoxy)ethylamino)cyclohexanol (8). IR (cm^{-1}): 3293, 3176, 3021, 1288, 1265, 1234, 1207; ^1H NMR (500.13 MHz, DMSO-d_6): δ 7.00 (2H, d, J = 7.5 Hz, H-3, H-5), 6.89 (1H, d, J = 7.5 H-4), 4.42 (1H, d, J = 5.1 Hz, OH), 3.75–3.84 (2H, m, Ar–O–CH_2), 3.08–3.17 (1H, m, *H*–C–OH (cyclohexane)), 2.88–2.95 (1H, m, *H* –C–NH), 2.78–2.86 (1H, m, CH*H*–N), 2.36 (1H, bbs, NH), 2.22–2.27 (1H, m, N–CH), 2.22 (6H, s, 2*x* Ar–CH_3), 1.87–1.96 (1H,

m, cyclohexane), 1.75–1.86 (1H, m, (cyclohexane (ax))), 1.55–1.68 (1H, m, 0.97–1.22, (cyclohexane (ax))), 1.09–1.27 (2H, m, (cyclohexane (ax))), 0.87–1.00 (3H, m, (cyclohexane (eq))), 0.88–0.89 (1H, m, (cyclohexane (eq))); ^{13}C NMR (500.13 MHz, DMSO-d_6): δ 155.50 (C-1), 130.30 (C-2, C-6), 128.67 (C-3, C-5), 123.54 (C-4), 72.72 (Ar–O–CH_2–), 71.79 (C-1′ (CH–OH cyclohexane)), 63.08 (C-2′ (CH–NH cyclohexane)), 46.73 (–CH_2–N) 34.11 (C-6′), 29.92 (C-5′), 24.26 (C-4′), 24.14 (C-3′), 15.99 (2*x* CH_3).

3.1.4.2. (±)-*Trans*-4-(2-(2,6-dimethylphenoxy)ethylamino)cyclohexanol (9). IR (cm^{-1}): 3258, 3083, 3036, 1265, 1228, 1210; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 6.99 (2H, d, J = 7.4 Hz, H-3, H-5), 6.88 (1H, d, J = 7.4 H-4), 4.42 (1H, d, J = 3.4 Hz, OH), 3.75 (2H, t, J = 5.7 Hz, Ar–O–CH_2–), 3.32–3.44 (1H, m, *H*–C–NH (cyclohexane)), 2.21 (6H, s, 2*x* Ar–CH_3), 1.74–1.89 (4H, m, (C-3′ (eq), C-5′ (eq), C-2′ (eq), C-6′ (eq)), 1.61 (1H, bs, NH), 1.22–1.11 (2H, m, C-3′ (ax), C-5′ (ax)), 1.09–0.97 (2H, m, C-2′ (ax), C-6′ (ax)); ^{13}C NMR (500.13 MHz, DMSO-d_6): δ 155.98 (C-1), 130.67 (C-2, C-6), 129.08 (C-3, C-5), 123.92 (C-4), 72.35 (Ar–O–CH_2–), 69.24 (C-4′ (CH–OH cyclohexane)), 56.12 (C-4′ (CH–NH cyclohexane)), 47.06 (–CH_2–N) 34.27 (C-3′, C-5′), 31.39 (C-2′, C-6′), 16.35 (2*x* CH_3).

3.2. Pharmacology

Antiepileptic activity and neurotoxicity assays were carried out by the Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health in Rockville, USA. Compounds were injected as suspensions in 0.5% methylcellulose at three dosage levels (30, 100 and 300 mg/kg b.w.) intraperitoneally (ip) to mice, and orally (po) to rats at dosage rates of 30 mg/kg b.w. Phase I of the evaluation was a qualitative assay which used small groups of animals (**1**–**8**) and included three tests: maximal electroshock seizure (MES), subcutaneous pentylenetetrazol test (ScMet), and neurotoxicity (rotarod), noted at 30 min and 4 h after administration. The MES seizures 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. Anticonvulsant quantification, that is, the doses of drug required to produce the biological responses in 50% of animals (ED_{50}), and the respective 95% confidence intervals, were determined for selected compounds displaying sufficient antiepileptic activity and low neurotoxicity in the above primary evaluations, by means of a computer program using probit analysis. Inhibition of focal seizures was tested using hippocampal kindling in rats (ip). Rat's hippocampus was kindled with 50 Hz alternating current at 200 mA per 1 s for 10 s every 0.5 h, for 6 h (12 cycles per day). This procedure was repeated every other day so that within 10 days the rat received 60 cycles, and as a result the animal developed 5th stage of seizures described by Racine from 1 (corresponding with aura in human) to 5 (corresponding with full tonic–clonic seizures). A tested substance was administered ip and after the TPE time the animal was given another electrical stimulus of the mentioned parameters. The behavior of the animal is again described by the scale of Racine.[5] Electrophysiology studies were performed on single N1E-115 cells and the murine cortical neurons were of patch-clamp type. The cells were obtained from cultures, and the whole cell recordings were performed on primary cultured neurons using borosilicate glass electrodes as described by Hamill et al.[30]

The activity of studied compounds is presented in Tables 2–5 and Figures 2–4.

Acknowledgments

The authors are grateful to J. Stables Ph.D for providing the pharmacological data through the Antiepileptic Drug Development program at the National Institutes of Neurological Disorders and Stroke (Rockville, USA) and would like to thank Professor K. Kieć-Kononowicz for coordination of the cooperation between the National Institutes of Neurological Disorders and Stroke and the Faculty of Pharmacy, Jagiellonian University-Medical College.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.09.014.

References and notes

1. Holmes, G. L.; Frank, L. M.; Sheth, R. D.; Philbrook, B.; Wooten, J. D.; Vuong, A.; Kerls, S.; Hammer, A. E.; Messenheimer, J. *Epilepsy Res.* **2008**, *82*, 124.
2. Gil-Nagel, A.; Zaccara, G.; Baldinetti, F.; Leon, T. *Seizure* **2009**, *18*, 184.
3. Johannessen Landmark, C.; Johannessen, S. I. *Drugs* **2008**, *68*, 1925.
4. Errington, A. C.; Stohr, T.; Heers, C.; Lees, G. *Mol. Pharmacol.* **2008**, *73*, 157.
5. Remy, S.; Beck, H. *Brain* **2006**, *129*, 18.
6. McNamara, O. J. Drugs effective in the therapy of the epilepsies. In *The Pharmacological Basis of Therapeutics*; Hardman, O. J., Limbird, L. E., Gilman, A. G., Eds.; McGraw-Hill: New York, 2001; pp 521–548.
7. Löscher, W.; Schmidt, D. *Epilepsy Res.* **2006**, *69*, 183.
8. Cramer, A. J.; Mintzer, S.; Wheless, J.; Mattson, R. H. *Expert Rev. Neurother.* **2010**, *10*(6), 885.
9. Isoherranen, N.; Yagen, B.; Blotnik, S.; Spiegelstein, O.; Wilcox, K. S.; Woodhead, J.; Finnell, R. H.; White, H. S.; Bialer, M. *Br. J. Pharmacol.* **2003**, *138*, 602.
10. Isoherranen, N.; White, H. S.; Klein, B.; Roeder, M.; Woodhead, J. H.; Schurig, V.; Yagen, B.; Bialer, M. *Pharm. Res.* **2003**, *20*, 1293.
11. Spiegelstein, O.; Yagen, B.; Levy, R. H.; Finnell, R. H.; Bennett, G. D.; Roeder, M.; Schurig, V.; Bialer, M. S. *Pharm. Res.* **1999**, *16*, 1582.
12. Trojnar, M. K.; Wierzchowska-Cioch, E.; Krzyżanowski, M.; Jargiełło, M.; Czuczwar, S. J. *Pol. J. Pharmacol.* **2004**, *56*, 283.
13. Klitgaard, H.; Matagne, A.; Gobert, J. *Eur. J. Pharmacol.* **1998**, *353*, 191.
14. Genton, P.; Van Vleymen, B. *Epileptic Disord.* **2000**, *2*, 99.
15. White, J. R.; Walczak, T. S.; Leppik, I. E.; Rarick, J.; Tran, T. J.; Beniak, T. E.; Matchinsky, D. J.; Gumnit, R. J. *Neurology* **2003**, *61*, 1218.
16. Bialer, M. *Expert Opin. Invest. Drugs* **2006**, *15*, 637.
17. Wickenden, A. D.; Yu, W.; Zou, A.; Jegla, T.; Wagoner, P. K.; Retigabine *Mol. Pharmacol.* **2000**, *58*, 591.
18. Hetka, R.; Rundfeldt, C.; Heinemann, U.; Schmitz, D. *Eur. J. Pharmacol.* **1999**, *386*, 165.
19. Clark, C. R.; Wells, M. J.; Sansom, R. T.; Norris, G. N.; Dockens, R. C.; Ravis, W. R. *J. Med. Chem.* **1984**, *27*, 779.
20. Vamecq, J.; Lambert, D.; Poupaert, J.; Masereel, B.; Stables, J. P. *J. Med. Chem.* **1998**, *41*, 3307.
21. Rogawski, M. A. *Epilepsy Res.* **2006**, *69*, 273.
22. Marona, H.; Antkiewicz-Michaluk, L. *Acta Pol. Pharm. Drug-Res.* **1998**, *55*, 487.
23. Marona, H.; Pękala, E.; Antkiewicz-Michaluk, L.; Walczak, M.; Szneler, E. *Bioorg. Med. Chem.* **2008**, *16*, 7234.
24. Mulzac, D.; Scott, K. R. *Epilepsia* **1993**, *34*, 1141.
25. Winstein, S.; Boschan, R. *J. Am. Chem. Soc.* **1950**, *72*, 4669.
26. Newman, P. Optical Resolution Procedures for Chemical Compounds. *Volume 1: Amines and Related Compounds*. Optical Resolution Information Center, Manhattan College Riverdale: New York, 1984; p 10471.
27. Orlof, M. J.; Williams, H. L.; Pfeiffer, C. C. *Proc. Soc. Exp. Biol. Med.* **1949**, *70*, 254.
28. Racine, R. J. *Electroencephalogr. Clin. Neurophysiol.* **1972**, *32*, 281.
29. Stables, J. P.; Kupferberg, H. J. The NIH anticonvulsant drug development (ADD) program: preclinical anticonvulsant screening project. In *Molecular and Cellular Targets for New Antiepileptic DRUGS*; Avanzini, G., Ed.; John Libbey Eurotext: London, 1997; pp 191–198 (Chapter 16).
30. Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. *Pflugers Arch.* **1981**, *391*, 85.
31. Pękala, E.; Marona, H. *Biomed. Chromatogr.* **2009**, *23*, 543.