Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 53–61
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Synthesis, physicochemical and anticonvulsant properties of new *N*-phenylamino derivatives of 2-azaspiro[4.4]nonane- and [4.5]decane-1,3-diones: Part V

Krzysztof Kamiński [a], Jolanta Obniska [a,*], Małgorzata Dybała [b]

[a] *Department of Pharmaceutical Chemistry, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland*
[b] *Department of Histochemistry and Pharmacobiology, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland*

Received 19 October 2006; received in revised form 20 February 2007; accepted 27 February 2007
Available online 18 March 2007

Abstract

The synthesis, physicochemical and pharmacological properties of new *N*-phenylamino derivatives of 2-azaspiro[4.4]nonane-1,3-dione (**8**–**10**), 2-azaspiro[4.5]decane-1,3-dione (**11**–**18**) and 3-cyclohexyl-pyrrolidine-2,5-dione (**19**, **20**) derivatives were described. The anticonvulsant properties of those compounds were examined by a maximal electroshock (MES) and a pentylenetetrazole (*sc*PTZ) tests, and their neurotoxicity was determined using a rota-rod test. The most active was *N*-[(2,4-dichlorophenyl)-amino]-2-azaspiro[4.4]nonane-1,3-dione (**9**), which exhibited anti-seizure properties in the MES model at a dose of 100 mg/kg in mice and at a dose of 30 mg/kg in rats. To explain the possible mechanism of action, for chosen active derivatives *N*-[(2,4-dichlorophenyl)-amino]-2-azaspiro[4.4]nonane-1,3-dione (**9**), *N*-[(4-bromophenyl)-amino]-2-azaspiro[4.4]nonane-1,3-dione (**10**), *N*-[(2,4-dichlorophenyl)-amino]-2-azaspiro[4.5]decane-1,3-dione (**12**) and *N*-[(4-bromophenyl)-amino]-2-azaspiro[4.5]decane-1,3-dione (**13**) their influence on $GABA_A$ receptors were tested in vitro. Moreover, for all compounds obtained the lipophilic properties were determined by use of RP-HPLC method.

Keywords: 2-Azaspiro[4.4]nonane- and [4.5]decane-1,3-diones; 3-Cyclohexyl-pyrrolidine-2,5-diones; *N*-phenylamino derivatives; Anticonvulsant activity; RP-HPLC

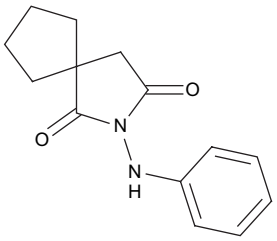
1. Introduction

Epilepsy, a common neurological disorder characterized by recurrent spontaneous seizures arising from excessive electrical activity in some portion of the brain, is a major, worldwide, public health problem, which affects approximately 1% of the population [1]. Despite the increasing understanding of the pathogenesis of seizures and epilepsy, as well as a large number of new anticonvulsant drugs that have been marked during recent years, at least 30% of epilepsies remain uncontrolled. In addition, many of currently used anticonvulsants produce significant undesirable harmful side effects, which may limit their maximal usefulness. For these reasons, there is a growing necessity for novel, specific and low-toxic anticonvulsant drugs [2–4].

In recent years anticonvulsant properties of many 2-azaspiro [4.4]nonane- and [4.5]decane-1,3-diones (spirosuccinimides) with different substituents at the imide nitrogen atom have been described [5–10]. The structure activity relationship studies conducted with these groups of compounds revealed that their efficacy were highly dependent on the size of the cycloalkyl system attached to the C3 spiro carbon atom and the kind of aromatic group at the nitrogen atom of pyrrolidine-2,5-dione ring. Moreover it was proved that the imine (–NH–) linker joining the endocyclic nitrogen atom and aromatic moiety was essential for anticonvulsant activity of those molecules. As a result the most active was *N*-phenylamino-2-azaspiro[4.4]nonane-1,3-dione (**1**) (Fig. 1), with $ED_{50} = 72.61$ mg/kg in the maximal electroshock seizure test (MES) [11].

* Corresponding author. Tel.: +48 12 658 82 16; fax: +48 12 657 02 62.
E-mail address: mfobnisk@cyf-kr.edu.pl (J. Obniska).

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2007.02.024



cmpd **1**
ED_{50} = 72.61 mg/kg
(MES test)

Fig. 1. The structure of lead compound **1**.

Regarding the latter as a lead structure, in effort to obtain compounds with enhanced efficacy, our attention was focused on a group of *N*-phenylamino 3-spirosuccinimides. However, both introduction of methyl substituents at the aromatic ring or enlargement of the cycloalkyl system from cyclopentane to cyclohexane decreased anticonvulsant activity [12]. On the other hand, it was also shown that introduction of the electron-withdrawing substituents (Br or Cl) was essential for the anticonvulsant efficacy for several classes of succinimides [11,13–15].

In line with above data, as a continuation of our systematic research on *N*-phenylamino spirosuccinimides, in the present study we obtained a new series of 2-azaspiro[4.4]nonane- and 2-azaspiro[4.5]decane-1,3-diones as well as their analogues with additional methyl group at position 6, 7, or 8 of 2-azaspiro[4.5]decane-1,3-dione ring, with highly electronegative substituents (Cl or Br) at the phenyl moiety. The aim of these modifications was to examine the influence of such substitution on anticonvulsant activity. On the other hand, to investigate the role of the cycloalkyl system, attached to the imide through the C3 spiro carbon atom, as a structural fragment essential for activity, two analogues with cyclohexyl moiety as a flexible substructure at position-3 of pyrrolidine-2,5-dione ring were synthesized.

The GABA-ergic system is an important component of the pathology of epilepsy and constitutes a vital target in the search of novel antiepileptic drugs. It is also known that several anticonvulsants interact with the specific binding sites of the $GABA_A$ receptor complex [16,17]. Therefore to explain the possible mechanism of action for selected active compounds their $GABA_A$ receptor affinities were investigated.

Lipophilicity, the inherent property of the chemical compound is widely used as structural descriptor in rational drug design [18]. Thus in this study the lipophilic properties of all derivatives were determined by use of RP-HPLC method. We attempted also to evaluate the influence of this parameter on anticonvulsant activity.

2. Chemistry

The synthesis of compounds **8–20** was accomplished as shown in Scheme 1. The starting 1-carboxy-1-cyclopentane (**2**), 1-carboxy-1-cyclohexane (**3**), 1-carboxy-1-(2-methyl-cyclohexane) (**4**), 1-carboxy-1-(3-methyl-cyclohexane) (**5**), and 1-carboxy-1-(4-methyl-cyclohexane) (**6**) acetic acids were prepared as previously reported [19]. The synthetic procedures of 2-cyclohexyl succinic acid (**7**) have been described in a separate literature [20]. The final *N*-substituted spirosuccinimides **8–20** were obtained in a one-pot cyclization reaction of the prepared dicarboxylic acids (**2–7**) and appropriately substituted phenylhydrazines by heating them at ca. 190–200 °C for 1.5–2 h.

The structures of the compounds synthesized were confirmed by the examination of their ^{1}H NMR, MS and IR spectra.

The ^{1}H NMR spectra of the investigated compounds revealed that the characteristic chemical shifts agreed with their proposed structures. The chemical shifts of the cyclopentane and cyclohexane rings were observed as multiplets within the range of δ 1.74–2.37 ppm (**8–10**) and δ 0.99–2.17 ppm (**11–18**). The signals due to pyrrolidine-2,5-dione ring protons appeared at about δ 2.67–2.74 ppm, as singlets (**8–13**, **17**, **18**); δ 2.48–2.73 as doublets (J ca. 18 Hz) (**14–16**) and δ 2.62–2.94 as multiplets (**19** and **20**). Appearance of broad singlets within the range δ 6.07–6.59 ppm corresponds to the one proton of –NH– linker and confirms the formation of imides **8–20**. The methyl groups at positions 6, 7 and 8 of 2-azaspiro[4.5]decane-1,3-dione ring appeared at about δ 0.80–0.96 as doublets. All the aromatic protons were well separated and observed at expected region.

The structures of selected compounds (**9**, **13** and **19**) were further verified by EI-MS spectra (70 eV). The *m/z* values of molecular ion peaks were in complete agreement with calculated molecular weight for individual compounds. The fragmentation of the 2-azaspiro[4.4]nonane- (**9**) and 2-azaspiro[4.5]decane-1,3-dione (**10**) rings was confirmed by the characteristic ions at *m/z* 81, *m/z* 95, *m/z* 109 and *m/z* 123, which were within the range of 10–29% intensity. The ions at *m/z* 83, *m/z* 99 and *m/z* 168 correspond to the fragmentation of 3-cyclohexyl-pyrrolidine-2,5-dione (**19**). The fragmentation of imide moiety was observed as ions at *m/z* 176, *m/z* 202 and *m/z* 204 (**9**); *m/z* 186, *m/z* 212 and *m/z* 214 (**13**); *m/z* 142, *m/z* 144 (**19**). The cleavage of the imine (–NH–) linker confirmed characteristic ions *m/z* 160 (**9**), *m/z* 170 (**13**) and *m/z* 127 (**19**).

For all compounds investigated the IR spectra were recorded. The C=O groups of imide rings were observed within the range ν 1733–1716 cm^{-1}, whereas the aromatic moieties (C=C) appeared between ν 1597 and 1452 cm^{-1}.

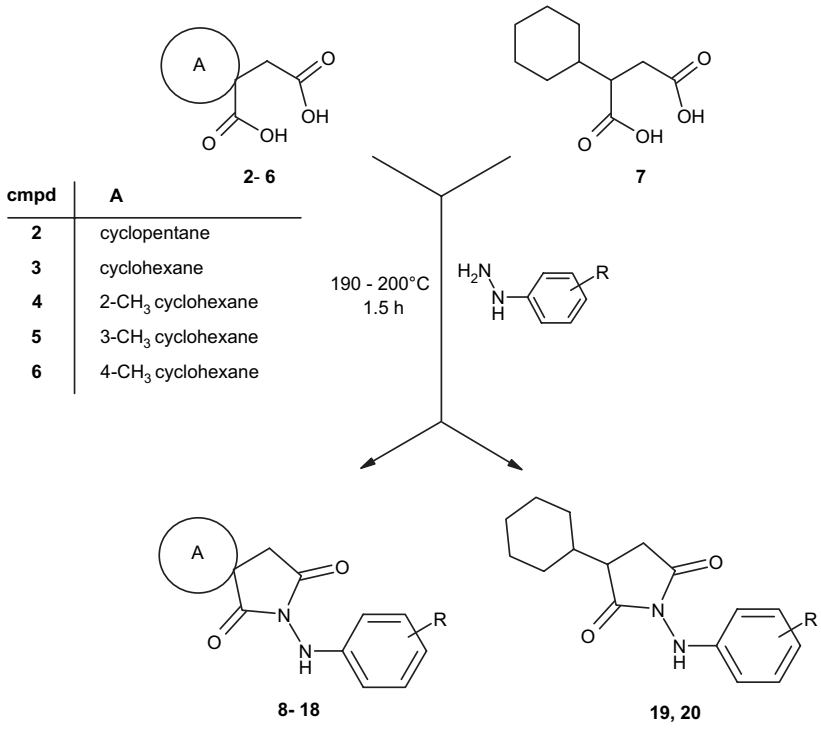
The detailed spectral data of each molecule are presented in Section 7.

3. Pharmacology

3.1. In vivo test

3.1.1. Anticonvulsant screening

The initial anticonvulsant evaluation was performed with the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NINCDS), Bethesda. The profile of anticonvulsant activity



cmpd	A
2	cyclopentane
3	cyclohexane
4	2-CH_3 cyclohexane
5	3-CH_3 cyclohexane
6	4-CH_3 cyclohexane

cmpd	A	R
8	cyclopentane	2-Cl
9	cyclopentane	2,4-Cl
10	cyclopentane	4-Br
11	cyclohexane	2-Cl
12	cyclohexane	2,4-Cl
13	cyclohexane	4-Br
14	2-CH_3-cyclohexane	2-Cl
15	2-CH_3-cyclohexane	2,4-Cl
16	2-CH_3-cyclohexane	4-Br
17	3-CH_3-cyclohexane	2,4-Cl
18	4-CH_3-cyclohexane	2,4-Cl

cmpd	R
19	2-Cl
20	2,4-Cl

Scheme 1. Synthetic protocol of compounds **8**–**20**.

of compounds **8**–**20** was established in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests, after intraperitoneal injection into mice, at doses of 30, 100 and 300 mg/kg. The neurotoxic properties were measured by the minimal motor impairment – rota-rod screen (TOX).

The compounds studied revealed diversified anticonvulsant properties from active at a dose of 100 mg/kg (**9**, **12** and **13**) to active at a dose of 300 mg/kg (**8**, **10**, **15** and **16**) and inactive ones (**11**, **14**, **17**–**20**). The results of anticonvulsant identification studies in mice are shown in Table 2.

Some selected compounds (**8**, **9** and **12**), active in mice were evaluated orally in rats in the MES test at a dose of 30 mg/kg at five time periods ranging from one quarter to 4 h post substance administration (Table 3). Compound **9** was chosen for phase II evaluation for quantification of ED_{50} and TD_{50} values.

3.2. In vitro test

3.2.1. $GABA_A$ binding assays

To search for possible mechanism of action, the affinities of chosen derivatives **9**, **10**, **12** and **13** for $GABA_A$ receptors were assessed in vitro, by their ability to displace [^{3}H]muscimol in rat cortical tissue. These compounds were active only in the MES (**9**, **13**) and both MES and *sc*PTZ tests (**10**, **12**). This selection was based on different molecular mechanisms responsible for the induction of seizures in MES and *sc*PTZ screens. Furthermore it seems that epileptic activity of pentylenetetrazole is probably due to blockade of Cl^- channel of GABA receptor complex [21], thus from this point of view the most interesting are molecules showing anti-*sc*PTZ protection (**10**, **12**). However, the radioligand binding experiments revealed no affinities for all compounds investigated. These preliminary

data suggest that anticonvulsant activity of molecules tested is not related with $GABA_A$ receptors. The results obtained remain in agreement with previous investigations indicating that anticonvulsant activity of compounds with nitrogen five or six member heteroatomic system (usually imide) as core fragment and attached aromatic group involves the influence on voltage-sensitive sodium channels [22,23]. Therefore, it seems to be the most probable mechanism of action of the molecules studied.

4. Lipophilicity

Lipophilicity is a fundamental physicochemical property of bioactive compounds that plays a pivotal role in the transport of a molecule through cellular membranes and influences the localization of compound in the therapeutic site of action. The most widely used measurements of the lipophilic properties of different chemical molecules are nowadays the chromatographic techniques in reversed phase system [24–26]. In consequence, the correlation between lipophilicity and biological activity can be based on chromatographic retention parameters [27–29]. Therefore, in present work we determined the lipophilicity of all compounds by use of RP-HPLC method.

The examination of the chromatographic behaviour in the RP-HPLC system showed a linear correlation between $\log k$ values and acetonitrile concentration (v/v) in a mobile phase. The relative retention parameters $\log k_w$ were determined by the extrapolation method, and are listed in Table 2.

5. Results and discussion

The maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests are claimed to detect compounds affording protection against generalized tonic-clonic seizures and generalized absence seizures, respectively. Thus the MES and *sc*PTZ screens have become the most widely employed seizure models for early identification of candidate anticonvulsants.

The compounds investigated **8–20** showed diversified anticonvulsant properties in the mouse i.p. MES and *sc*PTZ screens. Among all derivatives under study, the most active were 2,4-dichloro substituted compounds **9** and **12**, which revealed protection in the maximal electroshock test (MES) at a dose of 100 mg/kg at 0.5 h and 4 h, respectively. These molecules were also effective at a dose of 300 mg/kg in the same screen at time points 0.5 h (**9**) and 4 h (**9**, **12**). Additionally, compound **12**, exhibited anti-*sc*PTZ activity at a dose of 300 mg/kg at 4 h. The removal of the chloro atom at position-4 of the aryl ring yielded less active derivative **8** which showed protection in both MES and *sc*PTZ screens at a dose of 300 mg/kg at 0.5 h and inactive molecule **11**. The 4-bromo analogues **10** and **13** exhibited anti-MES activity at a dose of 100 mg/kg at time point 2 h (**10**) and 4 h (**13**) and also 300 mg/kg both at 4 h. Compound **13** revealed, however, neurotoxicity at a dose of 30 mg/kg and regardless of its activity according to the procedures of ADD Program was ascribed to 4 ASP class. Moreover derivative **10** showed anti-*sc*PTZ protection at a dose of 300 mg/kg at 0.5 h. The introduction of additional methyl group into position-6 of the 2-azaspiro[4.5]decane-1,3-dione moiety decreased activity, namely the 2,4-dichloro substituted derivative **15** showed anti-MES and anti-*sc*PTZ protection at a dose of 300 mg/kg at 4 h and 0.5 h, respectively, whereas **16** was effective only at MES screen at a dose of 100 mg/kg at 2 h and 300 mg/kg at 4 h. The 2-chloro substituted compound **14** was ineffective in both tests applied. The 7-methyl (**17**) and 8-methyl (**18**) analogues of active anticonvulsant derivative **12** did not show any protection in both screens. To evaluate the role of the cycloalkyl fragment attached to the C3 spiro carbon atom in the aspect of influence on anticonvulsant activity of spirosuccinimides described above, two 3-cyclohexyl-pyrrolidine-2,5-dione derivatives (**19**, **20**) have been synthesized. These molecules were designed as analogues of respective active (**12**) or inactive (**11**) spirosuccinimides. The results obtained revealed that the introduction of the cyclohexyl moiety as a flexible fragment at position-3 of the imide ring made both the compounds inactive.

In the neurotoxicity screen compounds **8**, **11**, **12**, **15**, **17**, **19** and **20** were devoid of toxicity at the maximum dose administrated (300 mg/kg). The other derivatives were found to be toxic at a dose of 30 mg/kg (**13**), 100 mg/kg (**10**, **14**, **18**) or 300 mg/kg (**9**, **16**). The mice were unable to grasp rota-rod after administration of compounds **9** and **16** at a dose of 300 mg/kg.

Compounds **8**, **9** and **12**, randomly selected from derivatives active in i.p. screen in mice, were examined for their activity and toxicity at a dose of 30 mg/kg after p.o. administration into rats. The results obtained are shown in Table 3. As can be seen from these data, only compound **9** was moderately active in rat MES oral screen and revealed the time of onset of anticonvulsant activity at 0.25 h and one peak of 75% protection at time points 2 h. In addition, tested substance protected 50% of animals at 0.25 h, 0.5 h and 1 h. The total duration of satisfactory action of this compound was quite short within 0.25 h–2 h. The derivative **8** showed marginal protection, whereas **12** was inactive in rats. No evidence of neurological toxicity was observed at the dose of 30 mg/kg administrated orally.

Compound **9** was chosen for phase II evaluation for quantification of anticonvulsant activities (ED_{50} and TD_{50}), against MES and *sc*PTZ – induced seizures after oral administration into rats at time point 2 h. The measured median effective dose was greater than 180 mg/kg in the MES test and greater than 125 mg/kg in the *sc*PTZ screen. The tested molecule revealed TD_{50} value to be higher than 250 mg/kg.

The chromatographically determined lipophilicity ($\log k_w$) provided a good basis for evaluation of structure–lipophilicity relationships. As expected, the highest lipophilicities were observed for the 2,4-dichloro derivatives **9**, **12**, **15**, **17**, **18** and **20**, whereas the lowest was shown by the 2-chloro substituted compounds **8**, **11**, **14** and **19**. The change of the ring size from cyclopentane (**8–10**) to cyclohexane (**11–13**) as well as introduction of the additional methyl group into cycloalkyl system (**14–18**) increased the lipophilicity. Furthermore the $\log k_w$ values were higher for 3-cyclohexyl-pyrrolidine-2,5-diones (**19**, **20**) in comparison to respective spirosuccinimide derivatives (**11**, **12**).

In our previous study we determined the lipophilicity of a series of structurally related *N*-phenylamino and *N*-benzyl-2-azaspiro[4.4]nonane- and [4.5]decane-1,3-dione derivatives. The role of lipophilicity in the anticonvulsant activity was proved; the active anticonvulsants were less lipophilic than the inactive ones [27,28]. Thus, the next step of our study was an attempt to evaluate the correlation between the anticonvulsant activity and lipophilicity. In subsequent analysis we used individual and average lipophilicities (i.e. mean log k_w values) separately for plots of compounds active at doses of 100 and 300 mg/kg, in contrast with inactive derivatives. Comparison of lipophilicity for active and inactive molecules is depicted in Fig. 2. As it is shown the ranges for active and inactive molecules overlap, however, mean lipophilicity value for active anticonvulsants is lower compared to inactive ones. The applied statistical analysis (Mann–Whitney test) revealed that the difference between log k_w parameters between these groups is not statistically significant ($U = 11.0$, $p = 0.153$).

In summary, the most active were compounds with cyclopentane and cyclohexane ring as spiro nucleus and two chloro substituents at positions-2 and 4 or bromo atom at position-4 of the aryl ring. Surprisingly, it was not consistent with the data obtained for *N*-pyridine-2-yl-, *N*-phenyl- and especially structurally related *N*-benzyl-spirosuccinimides, where the highest activity was observed for *ortho*-substituted compounds [9–11]. It proves the unique role of the imine (–NH–) linker joining the endocyclic nitrogen atom and aromatic moiety in respect to anticonvulsant properties of such type of derivatives. The introduction of additional methyl group at position 6, 7 or 8 of the 2-azaspiro[4.5]decane-1,3-dione moiety decreased the activity. Furthermore, comparison of results obtained for the spirosuccinimides and compounds with cyclohexyl moiety as a flexible fragment at position-3 of the imide ring, proved an essential role of cycloalkyl system attached through the C3 spiro carbon atom in the aspect of anticonvulsant activity of that type of compounds.

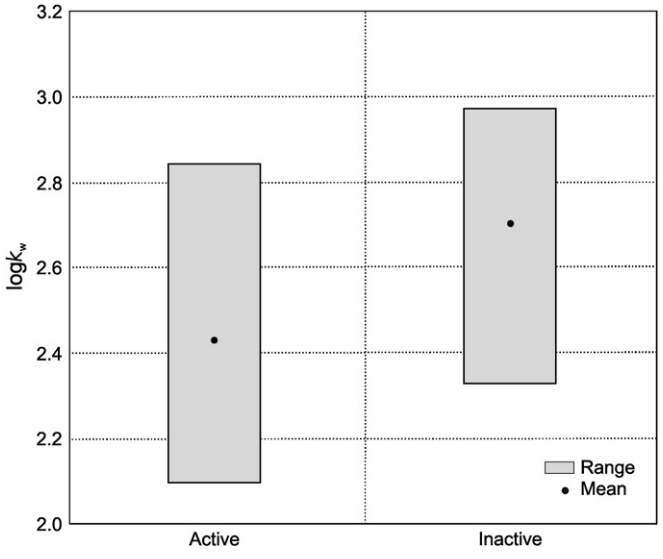


Fig. 2. Comparison of ranges and mean log k_w parameters for active and inactive compounds.

6. Conclusion

The results obtained revealed that number of novel *N*-phenylamino-2-azaspiro[4.4]nonane- and [4.5]decane-1,3-dione derivatives were effective in the MES and *sc*PTZ screens, the most widely employed seizure models for early identification of candidate anticonvulsants. The most active was *N*-[(2,4-dichlorophenyl)-amino]-2-azaspiro[4.4]nonane-1,3-dione, which was chosen for quantification of ED_{50} and TD_{50} values. The anticonvulsant activity profile depended on kind and position of substituents at the aryl moiety as well as the size and manner of attachment of the cyclolkyl system at the position-3 of the pyrrolidine-2,5-dione ring. The $GABA_A$ binding assays suggest that anticonvulsant activity is not related with $GABA_A$ receptors. The influence on voltage-sensitive sodium channels seems to be the most probable mechanism of action of molecules investigated. There was no distinct correlation between the lipophilicity and anticonvulsant efficacy.

7. Experimental protocols

7.1. Chemistry

All the chemicals and solvents were purchased from Merck (Darmstadt, Germany) and were used without further purification. Melting points (m.p.) were determined in open capillaries on a Büchi 353 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The purity of the compounds was confirmed by the thin-layer chromatography (TLC) performed on Merck silica gel 60 F_{254} aluminium sheets (Merck; Darmstadt, Germany), using subsequent developing system: chloroform/acetone (9:1). Spots were detected by their absorption under UV light ($\lambda = 254$ nm) and by visualization with 0.05 mol I_2 in 10% HCl. Elemental analysis for C, H, and N was carried out by a micro method using the elemental Vario EI III Elemental analyser (Hanau, Germany). The results of elemental analyses were within ±0.4% of the theoretical values (Table 1).

Infrared spectra (IR) were recorded in FTIR Spectrometer 1600 (Perkin Elmer). ^{1}H NMR spectra were obtained in a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$, operating at 300 MHz. Chemical shifts are reported in δ values (ppm) relative to TMS $\delta = 0$ (^{1}H), as internal standard. The *J* values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), d (doublet), dd (double doublet), m (multiplet). For chosen compounds **9**, **13** and **19** the mass spectra (MS) were recorded on AMD-604 Mass Spectrometer operating at 70 eV.

7.1.1. General procedure for preparation of compounds 8–20

To a suspension of 1-carboxy-1-cyclopentane (**2**), 1-carboxy-1-cyclohexane (**3**), 1-carboxy-1-(2-methyl-cyclohexane) (**4**),

Table 1
Analytical data for compounds **8**–**20**

Cmpd	Molecular formula, weight	Analysis (calcd/found)		
		%C	%H	%N
8	$C_{14}H_{15}Cl_1N_2O_2$, 278.7	60.33, 60.31	5.42, 5.40	10.05, 10.06
9	$C_{14}H_{14}Cl_2N_2O_2$, 313.2	53.69, 53.70	4.51, 4.49	8.94, 8.95
10	$C_{14}H_{15}Br_1N_2O_2$, 323.2	52.03, 52.02	4.68, 4.66	8.67, 8.66
11	$C_{15}H_{17}Cl_1N_2O_2$, 292.8	61.54, 61.55	5.85, 5.83	9.57, 9.57
12	$C_{15}H_{16}Cl_2N_2O_2$, 327.2	55.06, 55.07	4.93, 4.92	8.56, 8.57
13	$C_{15}H_{17}Br_1N_2O_2$, 337.2	53.43, 53.44	5.08, 5.06	8.31, 8.30
14	$C_{16}H_{19}Cl_1N_2O_2$, 306.8	62.64, 62.62	6.24, 6.25	9.13, 9.12
15	$C_{16}H_{18}Cl_2N_2O_2$, 341.2	56.32, 56.30	5.32, 5.33	8.21, 8.20
16	$C_{16}H_{19}Br_1N_2O_2$, 351.2	54.71, 54.73	5.45, 5.44	7.98, 7.99
17	$C_{16}H_{18}Cl_2N_2O_2$, 341.2	56.32, 56.33	5.32, 5.30	8.21, 8.22
18	$C_{16}H_{18}Cl_2N_2O_2$, 341.2	56.32, 56.30	5.32, 5.31	8.21, 8.20
19	$C_{16}H_{19}Cl_1N_2O_2$, 306.8	62.64, 62.63	6.24, 6.22	9.13, 9.12
20	$C_{16}H_{18}Cl_2N_2O_2$, 341.2	56.32, 56.31	5.32, 5.30	8.21, 8.22

1-carboxy-1-(3-methyl-cyclohexane) (**5**), and 1-carboxy-1-(4-methyl-cyclohexane) (**6**) acetic acids or 2-cyclohexyl-succinic acid (**7**) (0.01 mol) in 10 ml of water, the appropriately substituted phenylhydrazines (0.01 mol) were gradually added. The mixture was heated in an oil bath with simultaneous distillation of water. After complete removal of water the temperature of the reaction mixture was raised up to 190–200 °C and maintained for 1.5 h. The crude products were crystallized from isopropanol. The obtained solid residues were purified by column chromatography on Silica gel 60 (Merck, Darmstadt, Germany) using chloroform/acetone mixture (9:1) as a solvent. After evaporation of the solvents, the oil products were recrystallized from isopropanol to afford the desired compounds.

*7.1.1.1. N-[(2-Chlorophenyl)-amino]-2-azaspiro[4.4]nonane-1,3-dione (**8**).* White powdery crystals. Yield: 72%; m.p. 140–142 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.74–2.37 (m, 8H, cyclopentane), 2.74 (s, 2H, imide), 6.53 (dd, $J = 8.20$ Hz, $J = 1.54$ Hz, 1H, $H_{aromat.}$), 6.59 (br s, 1H, NH), 6.87–6.93 (m, 1H, $H_{aromat.}$), 7.09–7.12 (m, 1H, $H_{aromat.}$), 7.32 (dd, $J = 8.20$ Hz, $J = 1.41$ Hz, 1H, $H_{aromat.}$). IR ν (cm^{-1}): 1730 (C=O), 1594, 1492 (C=C). $C_{14}H_{15}Cl_1N_2O_2$ (278.7).

*7.1.1.2. N-[(2,4-Dichlorophenyl)-amino]-2-azaspiro[4.4]-nonane-1,3-dione (**9**).* White powdery crystals. Yield: 70%; m.p. 154–156 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.74–2.23 (m, 8H, cyclopentane), 2.73 (s, 2H, imide), 6.46 (d, $J = 8.46$ Hz, 1H, $H_{aromat.}$), 6.52 (br s, 1H, NH), 7.12 (dd, $J = 8.72$ Hz, $J = 2.31$ Hz, 1H, $H_{aromat.}$), 7.34 (d, $J = 2.05$ Hz, 1H, $H_{aromat.}$). IR ν (cm^{-1}): 1723 (C=O), 1574, 1490 (C=C). MS *m/z* (%): 316 (10) $[M+4]^+$, 314 (64) $[M+2]^+$, 312 (100.0) $[M]^+$, 204 (30), 202 (48), 176 (54), 160 (24), 109 (29), 81 (28). $C_{14}H_{14}Cl_2N_2O_2$ (313.2).

*7.1.1.3. N-[(4-Bromophenyl)-amino]-2-azaspiro[4.4]nonane-1,3-dione (**10**).* White powdery crystals. Yield: 75%; m.p. 166–168 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73–2.20 (m, 8H, cyclopentane), 2.71 (s, 2H, imide), 6.42 (br s, 1H, NH), 6.63–6.67 (m, 2H, $H_{aromat.}$), 7.33–7.36 (m, 2H, $H_{aromat.}$). IR ν (cm^{-1}): 1704, 1722 (C=O), 1554, 1520, 1492 (C=C). $C_{14}H_{15}Br_1N_2O_2$ (323.2).

*7.1.1.4. N-[(2-Chlorophenyl)-amino]-2-azaspiro[4.5]decane-1,3-dione (**11**).* White powdery crystals. Yield: 69%; m.p. 195–197 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37–1.92 (m, 10H, cyclohexane), 2.70 (s, 2H, imide), 6.50 (dd, $J = 8.20$ Hz, $J = 1.28$ Hz, 1H, $H_{aromat.}$), 6.57 (br s, 1H, NH), 6.87–6.93 (m, 1H, $H_{aromat.}$), 7.09–7.12 (m, 1H, $H_{aromat.}$), 7.32 (dd, $J = 7.97$ Hz, $J = 1.54$ Hz, 1H, $H_{aromat.}$). IR ν (cm^{-1}): 1727 (C=O), 1594, 1492 (C=C). $C_{15}H_{17}Cl_1N_2O_2$ (292.8).

*7.1.1.5. N-[(2,4-Dichlorophenyl)-amino]-2-azaspiro[4.5]-decane-1,3-dione (**12**).* White powdery crystals. Yield: 71%; m.p. 202–204 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37–1.91 (m, 10H, cyclohexane), 2.70 (s, 2H, imide), 6.44 (d, $J = 8.72$ Hz, 1H, $H_{aromat.}$), 6.50 (br s, 1H, NH), 7.10 (dd, $J = 8.72$ Hz, $J = 2.31$ Hz, 1H, $H_{aromat.}$), 7.34 (d, $J = 2.05$ Hz, 1H, $H_{aromat.}$). IR ν (cm^{-1}): 1721 (C=O), 1488, 1452 (C=C). $C_{15}H_{16}Cl_2N_2O_2$ (327.2).

*7.1.1.6. N-[(4-Bromophenyl)-amino]-2-azaspiro[4.5]decane-1,3-dione (**13**).* White powdery crystals. Yield: 67%; m.p. 186–188 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36–1.94 (m, 10H, cyclohexane), 2.67 (s, 2H, imide), 6.07 (br s, 1H, NH), 6.63–6.67 (m, 2H, $H_{aromat.}$), 7.33–7.36 (m, 2H, $H_{aromat.}$). IR ν (cm^{-1}): 1733, 1715 (C=O), 1593, 1492 (C=C). MS *m/z* (%): 338 (100) $[M+2]^+$, 336 (99) $[M]^+$, 214 (22), 212 (22), 186 (38), 170 (10), 123 (10), 95 (17). $C_{15}H_{17}Br_1N_2O_2$ (337.2).

*7.1.1.7. N-[(2-Chlorophenyl)-amino]-6-methyl-2-azaspiro[4.5]-decane-1,3-dione (**14**).* White powdery crystals. Yield: 65%; m.p. 196–198 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (d, $J = 6.67$ Hz, 3H, CH_3), 1.01–1.95 (m, 8H, cyclohexane), 2.04–2.13 (m, 1H, cyclohexane), 2.51 (d, $J = 18.72$ Hz, 1H, imide), 2.73 (d, $J = 18.72$ Hz, 1H, imide), 6.55 (dd, $J = 8.06$ Hz, $J = 1.41$ Hz, 1H, $H_{aromat.}$), 6.59 (br s, 1H, NH), 6.87–6.92 (m, 1H, $H_{aromat.}$), 7.09–7.15 (m, 1H, $H_{aromat.}$), 7.33 (dd, $J = 7.95$ Hz, $J = 1.28$ Hz, 1H, $H_{aromat.}$). IR ν (cm^{-1}): 1726 (C=O), 1597, 1497 (C=C). $C_{16}H_{19}Cl_1N_2O_2$ (306.8).

*7.1.1.8. N-[(2,4-Dichlorophenyl)-amino]-6-methyl-2-azaspiro-[4.5]decane-1,3-dione (**15**).* White powdery crystals. Yield: 60%; m.p. 200–202 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82 (d, $J = 6.92$ Hz, 3H, CH_3), 1.00–1.94 (m, 8H, cyclohexane), 2.00–2.11 (m, 1H, cyclohexane), 2.50 (d, $J = 18.46$ Hz, 1H, imide), 2.72 (d, $J = 18.72$ Hz, 1H, imide), 6.48 (d, $J = 8.72$ Hz, 1H, $H_{aromat.}$), 6.53 (br s, 1H, NH), 7.10 (dd, $J = 8.72$ Hz, $J = 2.31$ Hz, 1H, $H_{aromat.}$), 7.34 (d, $J = 2.31$ Hz, 1H, $H_{aromat.}$). IR ν (cm^{-1}): 1721 (C=O), 1573, 1488 (C=C). $C_{16}H_{18}Cl_2N_2O_2$ (341.2).

*7.1.1.9. N-[(4-Bromophenyl)-amino]-6-methyl-2-azaspiro[4.5]-decane-1,3-dione (**16**).* White powdery crystals. Yield: 60%; m.p. 186–188 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80 (d, $J = 6.92$ Hz, 3H, CH_3), 0.99–1.99 (m, 8H, cyclohexane), 2.01–2.11 (m, 1H, cyclohexane), 2.48 (d, $J = 18.72$ Hz, 1H,

imide), 2.70 (d, $J = 18.72$ Hz, 1H, imide), 6.10 (br s, 1H, NH), 6.65–6.68 (m, 2H, $H_{aromat.}$), 7.32–7.36 (m, 2H, $H_{aromat.}$). IR ν (cm^{-1}): 1724 (C=O), 1594, 1491 (C=C). $C_{16}H_{19}Br_1N_2O_2$ (351.2).

*7.1.1.10. N-[(2,4-Dichlorophenyl)-amino]-7-methyl-2-azaspiro-[4.5]decane-1,3-dione (**17**).* White powdery crystals. Yield: 68%; m.p. 204–206 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 0.96 (d, $J = 6.16$ Hz, 3H, CH_3), 1.02–1.86 (m, 9H, cyclohexane), 2.69 (s, 1H, imide), 6.44 (d, $J = 8.72$ Hz, 1H, $H_{aromat.}$), 6.51 (br s, 1H, NH), 7.09–7.13 (m, 1H, $H_{aromat.}$), 7.34 (d, $J = 2.31$ Hz, 1H, $H_{aromat.}$). IR ν (cm^{-1}): 1723 (C=O), 1576, 1488 (C=C). $C_{16}H_{18}Cl_2N_2O_2$ (341.2).

*7.1.1.11. N-[(2,4-Dichlorophenyl)-amino]-8-methyl-2-azaspiro-[4.5]decane-1,3-dione (**18**).* White powdery crystals. Yield: 68%; m.p. 210–212 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 0.95 (d, $J = 6.67$ Hz, 3H, CH_3), 1.00–2.17 (m, 9H, cyclohexane), 2.68 (s, 1H, imide), 6.44 (d, $J = 8.72$ Hz, 1H, $H_{aromat.}$), 6.51 (br s, 1H, NH), 7.08–7.13 (m, 1H, $H_{aromat.}$), 7.34 (d, $J = 2.31$ Hz, 1H, $H_{aromat.}$). IR ν (cm^{-1}): 1724 (C=O), 1590, 1489 (C=C). $C_{16}H_{18}Cl_2N_2O_2$ (341.2).

*7.1.1.12. N-[(2-Chlorophenyl)-amino]-3-cyclohexyl-pyrrolidine-2,5-dione (**19**).* White powdery crystals. Yield: 60%; m.p. 150–152 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.08–1.82 (m, 10H, cyclohexane), 1.97–2.17 (m, 1H, cyclohexane), 2.62–2.93 (m, 3H, imide), 6.53 (dd, $J = 8.08$ Hz, $J = 1.54$ Hz, 1H, $H_{aromat.}$), 6.57 (br s, 1H, NH), 6.87–6.92 (m, 1H, $H_{aromat.}$), 7.10–7.15 (m, 1H, $H_{aromat.}$), 7.32 (dd, $J = 7.95$ Hz, $J = 1.28$ Hz, 1H, $H_{aromat.}$). IR ν (cm^{-1}): 1720 (C=O), 1595, 1497, 1445 (C=C). MS *m/z* (%): 308 (32) $[M + 2]^+$, 306 (100) $[M]^+$, 168 (8), 144 (10), 142 (34), 127 (43), 99 (11), 83 (11). $C_{16}H_{19}Cl_1N_2O_2$ (306.8).

*7.1.1.13. N-[(2,4-Dichlorophenyl)-amino]-3-cyclohexyl-pyrrolidine-2,5-dione (**20**).* White powdery crystals. Yield: 65%; m.p. 160–162 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.03–1.82 (m, 10H, cyclohexane), 1.96–2.06 (m, 1H, cyclohexane), 2.62–2.94 (m, 3H, imide), 6.46 (d, $J = 8.72$ Hz, 1H, $H_{aromat.}$), 6.50 (br s, 1H, NH), 7.12 (dd, $J = 8.72$ Hz, $J = 2.31$ Hz, 1H, $H_{aromat.}$), 7.34 (d, $J = 2.31$ Hz, 1H, $H_{aromat.}$). IR ν (cm^{-1}): 1716 (C=O), 1489, 1447 (C=C). $C_{16}H_{18}Cl_2N_2O_2$ (341.2).

7.2. Anticonvulsant screening

Compounds **8–20** were pharmacologically pre-evaluated within the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NINCDS), Bethesda, using procedures described elsewhere [30,31].

Phase I studies of the compounds investigated involved three tests: maximal electroshock seizure (MES), subcutaneous pentylenetetrazole seizure (*sc*PTZ) and rota-rod test for neurological toxicity (TOX). All the compounds were injected intraperitoneally into mice as a suspension in 0.5% methylcellulose at the dose levels of 30, 100, and 300 mg/kg with anticonvulsant activity and neurotoxicity assessment at 0.5 h and 4 h intervals after administration. The results are presented in Table 2. Some selected derivatives from phase I (**8**, **9** and **12**) underwent phase VIa in which they were administrated orally into rats using four animals at a fixed dose of 30 mg/kg for both the MES and the rota-rod toxicity tests. Rats were tested at five time periods ranging from one quarter to 4 h post substance administration. The results are shown in Table 3.

Compound **9**, active in mice and rats, was chosen for quantification of the pharmacological parameters (ED_{50} and TD_{50}). The quantitative determination of the median effective dose (ED_{50}) and toxic dose (TD_{50}) was performed at previously estimated time of peak effect after oral administration into rats. Groups of eight rats received various doses of the compound until at least two points were established between the limits of 100% protection and 0% protection or minimal toxicity.

7.3. $GABA_A$ receptor binding assay

The experiment was performed as described previously [32]. Rats' brains were homogenised in 20 volumes of ice-cold 50 mM tris–HCl buffer (pH 7.4) using a ULTRA TURAX homogeniser. The homogenate is then centrifuged at 20 000 × g for 20 min (0–4 °C). The resulting supernatant

Table 2
Anticonvulsant screening project (ASP) phase I results in mice

Cmpd	Intraperitoneal injection in mice						log k_w[d]	ASP[e] class
	MES[a]		*sc*PTZ[b]		TOX[c]			
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h		
8	300	–	300	–	–	–	2.095	2
9	100	300	–	–	300[f]	–	2.419	1
10[g]		300	300	–	100	–	2.115	2
11	–	–	–	–	–	–	2.322	3
12	–	100	–	300	–	–	2.634	1
13	–	100	–	–	30	–	2.337	4
14	–	–	–	–	100	–	2.515	3
15	–	300	300	–	–	–	2.847	2
16[g]	–	300	–	–	–	300[f]	2.561	2
17	–	–	–	–	–	–	2.976	3
18	–	–	–	–	100	100	2.977	3
19	–	–	–	–	–	–	2.536	3
20	–	–	–	–	–	–	2.896	3

The figures in the table indicate the minimum dose whereby bioactivity was demonstrated.

[a] Maximal electroshock test.

[b] Subcutaneous pentylenetetrazole test.

[c] Rota-rod toxicity.

[d] The relative retention parameters calculated according to equation: $\log k = \log k_w - S\varphi$, where S is the slope, φ is the volume fraction of the organic modifier.

[e] The ASP classification is as follows: 1 – anticonvulsant activity at doses of 100 mg/kg or less; 2 – anticonvulsant activity at doses of 300 mg/kg; 3 – compound inactive at doses of 300 mg/kg, 4 – compound active, however, toxic at a dose of 30 mg/kg.

[f] Response comments: unable to grasp rota-rod.

[g] Compounds **10** and **16** revealed additionally anti-MES activity at doses of 100 mg/kg at 2 h.

Table 3
The results in rats after oral administration at a dose of 30 mg/kg

Compd	Oral administration to rats									
	MES[a]					TOX[b]				
	0.25 h	0.5 h	1.0 h	2.0 h	4.0 h	0.25 h	0.5 h	1.0 h	2.0 h	4.0 h
8	1/4	1/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
9	2/4	2/4	2/4	3/4	0/4	0/4	0/4	0/4	0/4	0/4
12	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4

[a] Maximal electroshock test, number of animals protected/number of animals tested.
[b] Rota-rod test for neurological toxicity, number of animals exhibiting toxicity/number of animals tested.

was discarded and the pellet rehomogenised 20 volumes of ice-cold 50 mM tris–HCl buffer (pH 7.4) and centrifuged as above. The pellet was resuspended and centrifuged for further two times. The final pellet was stored at −20 °C for at least 18 h. On the day of the assay, pellet was thawed at room temperature, resuspended in 20 volumes of ice-cold 50 mM tris–HCl buffer (pH 7.4) and centrifuged at 20 000 × g for 28 min (0–4 °C).

Radioligand binding assays were performed in plates (MultiScreen/Millipore). The final incubation mixture (final volume 300 μl) consisted of 240 μl membrane suspension, 30 μl of a 3 nM [^{3}H]muscimol and 30 μl buffer containing eight concentrations (10^{-11}–10^{-4} M) of tested compounds. For measuring unspecific binding 100 μM GABA was applied. The sample was incubated for 10 min at 0 °C. The incubation was terminated by rapid filtration over glass fiber filters (Whatman GF/C) using a vacuum manifold (Millipore). The filters were then washed 2 times with the buffer and placed in scintillation vials with liquid scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA – liquid scintillation counter. All assays were done in duplicates. Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, Version 3.0 – San Diego, CA, USA).

7.4. HPLC measurements

The analytical system consisted of a Waters (Milford, USA) instrument, equipped with 515 HPLC pumps, a 600S flow controller, 486 Tunable Absorbance Detector and injector: Rheodyne 7161 valve (Cotati, CA, USA). The measurements were performed on a LiChrospher C_{18} end-capped column, 250 × 4.6 mm (Merck, Darmstadt, Germany). Acetonitrile for measurements was of gradient grade and was purchased from Merck (Darmstadt, Germany). The water was filtrated under vacuum a 0.45 μm HA Millipore filter (Millipore, Milford, MA, USA) before being mixed with acetonitrile. For chromatographic analysis stock solutions of 1.0 mg/ml of the samples in acetonitrile/water (1:1) were prepared and filtrated through 0.2 μm Millipore filter (Millipore, Milford, MA, USA). These solutions were kept in Eppendorf tubes at room temperature. Isocratic runs were carried out with mixtures containing increasing acetonitrile volume fractions from 40% to 95% v/v in 5% increment. Flow rate was 1 ml/min. The injection volume was 5 μl in all cases. Uracil (Sigma–Aldrich) was used as the unretained compound to determine the dead volume at the different acetonitrile concentrations in the mobile phase. The retention times were measured at room temperature by the UV detector at the λ_{max} of the analytes (214 nm). Three sets of measurements were conducted for each compound and the mean value was used for further calculations. The capacity factors $\log k$ were calculated by us from equation: $k = (t_r - t_0)/t_0$, where t_r and t_0 are the retention times for solute and unretained compound (uracil), respectively. The $\log k$ values were then extrapolated to 100% water content to estimate the relative retention parameters $\log k_w$, according to equation: $\log k = \log k_w - S\varphi$, where S is the slope, φ is the volume fraction of the organic modifier and $\log k_w$ is the chromatographic lipophilicity parameter that characterizes the partition of the compound between a nonpolar hydrocarbon stationary phase and pure water [33]. The $\log k_w$ values better approximate the experimental conditions of lipid phase/water partition system values and are recommended for chromatographic measurement of lipophilicity in QSAR studies [34].

7.5. Statistical analysis

Statistical analyses were performed using the computer program Statistica version 5, 1997 Edition, Copyright© Statsoft, Inc. 1984–1997.

Acknowledgements

The authors wish to thank Dr James Stables for providing them pharmacological data through the Antiepileptic Drug Development Program (Epilepsy Branch, National Institute of Neurological Disorders and Stroke, National Institute of Health, Bethesda, MD, USA).

We are pleased to acknowledge the generous financial support of this work by the Committee for Scientific Research, Poland (Grant No 3 PO5F 024 25).

References

[1] B.M. Kenda, A.C. Matagne, P.E. Talaga, P.M. Pasau, E. Differding, B.I. Lallemand, A.M. Frycia, F.G. Moureau, H.V. Klitgaard, M.R. Gillard, B. Fuks, P. Michel, J. Med. Chem. 47 (2004) 530–549.
[2] J.R. Dimmock, S.C. Vashishtha, J.P. Stables, Eur. J. Med. Chem. 35 (2000) 241–248.
[3] L. Bruno-Blanch, J. Gálvez, R. Garcia-Domenech, Bioorg. Med. Chem. Lett. 13 (2003) 2749–2754.
[4] E. Estrada, A. Pena, Bioorg. Med. Chem. 8 (2000) 2755–2770.
[5] M.L. Traver, J.M. Nicholson, K.R. Scott, J. Pharm. Sci. 74 (1985) 785–787.
[6] I.O. Edafiogho, K.R. Scott, J.A. Moore, V.A. Farrar, J.M. Nicholson, J. Med. Chem. 34 (1991) 387–392.
[7] V.A. Farrar, M. Ciechanowicz-Rutkowska, J. Grochowski, P. Serda, T. Pilati, G. Filippini, C.N. Hinko, A. El-Assadi, J.A. Moore, I.O. Edafiogho, C.W. Andrews, M. Cory, J.M. Nicholson, K.R. Scott, J. Med. Chem. 36 (1993) 3517–3525.

[8] M.S. Alexander, J.P. Stables, M. Ciechanowicz-Rutkowska, M.B. Hursthouse, D.E. Hibbs, I.O. Edafiogho, V.A. Farrar, J.A. Moore, K.R. Scott, Eur. J. Med. Chem. 31 (1996) 787–795.
[9] K. Kamiński, J. Obniska, A. Zagórska, D. Maciąg, Arch. Pharm. Chem. Life Sci. 339 (2006) 255–261.
[10] J. Obniska, K. Kamiński, A. Zagórska, A. Dzierżawska-Majewska, J. Karolak-Wojciechowska, J. Fluorine Chem. 127 (2006) 417–425.
[11] J. Obniska, A. Dzierżawska-Majewska, A. Zagórska, P. Zajdel, J. Karolak-Wojciechowska, Il Farmaco 60 (2005) 529–539.
[12] J. Obniska, K. Kamiński, Acta Polon. Pharm.-Drug Res. 63 (2006) 101–108.
[13] J. Obniska, A. Zejc, J. Karolak-Wojciechowska, Il Farmaco 54 (1996) 423–429.
[14] J. Obniska, A. Zagórska, Il Framaco 58 (2003) 1227–1234.
[15] J. Obniska, K. Kamiński, E. Tatarczyńska, Pharmacol. Rep. 58 (2006) 207–214.
[16] G.H. Fülep, C.E. Hoesl, G. Höfner, K.T. Wanner, Eur. J. Med. Chem. 41 (2006) 809–824.
[17] P. Czapiński, B. Blaszczyk, S.J. Czuczwar, Curr. Top. Med. Chem. 5 (2005) 3–4.
[18] F. Hollósy, T. Lóránd, L. Örfi, D. Erös, G. Kéri, M. Idei, J. Chromatogr. B 768 (2002) 361–368.
[19] K.R. Scott, J.A. Moore, T.B. Zalucky, J.M. Nicholson, J.A.M. Lee, C.N. Hinko, J. Med. Chem. 28 (1985) 413–417.
[20] C.A. Miller, L.M. Long, J. Am. Chem. Soc. 73 (1951) 4895–4898.
[21] W. Löscher, D. Schmidt, Epilepsy Res. 2 (1988) 145–181.
[22] M.G. Wong, J.A. Defina, P.R. Andrews, J. Med. Chem. 29 (1986) 562–572.
[23] K. Unverferth, J. Engel, N. Hofgen, A. Rostock, R. Gunther, H.J. Lankau, M. Menzer, A. Rolfs, J. Liebscher, B. Muller, H.J. Hofmann, J. Med. Chem. 41 (1998) 63–73.
[24] E.H. Kerns, L. Di, S. Petusky, T. Kleintop, D. Huryn, O. McConnell, G. Carter, J. Chromatogr. A 791 (2003) 381–382.
[25] M. Kostecka, A. Niewiadomy, R. Czeczko, Chromatographia 62 (2005) 121–126.
[26] N. Perišić-Janjić, T. Djaković-Sekulić, S. Stojanović, K. Penov-Gaši, Chromatographia 60 (2004) S201–S202.
[27] J. Obniska, K. Kamiński, J. Planar Chromatogr. 18 (2005) 240–243.
[28] J. Obniska, K. Kmiński, Biomed. Chromatogr. 20 (2006) 1185–1191.
[29] C. Sârbu, D. Casoni, M. Darabantu, C. Maiereanu, J. Pharm. Biomed. Anal. 35 (2004) 213–214.
[30] R.L. Krall, J.K. Penry, B.G. White, H.J. Kupferberg, E.A. Swinyard, Epilepsia 19 (1978) 409–428.
[31] H.J. Kupferberg, Epilepsia 30 (Suppl.) (1989) 51–56.
[32] A.R. Green, A. Misra, T.K. Murray, M.F. Snape, A.J. Cross, Neuropharmacology 35 (1996) 1243–1250.
[33] X. Liu, H. Tanaka, A. Yamauchi, B. Testa, H. Chuman, J. Chromatogr. A 1091 (2005) 51–52.
[34] K. Dross, R.F. Rekker, D. Vries, R. Mannhold, Quant. Struct.-Act. Relat. 18 (1999) 549–550.