Full Paper

Synthesis, Physicochemical and Anticonvulsant Properties of New *N*-(Pyridine-2-yl) Derivatives of 2-Azaspiro[4.4]nonane and [4.5]decane-1,3-dione. Part II

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A series of *N*-(pyridine-2-yl) derivatives of 2-azaspiro[4.4]nonane- (**1a – e**), 2-azaspiro[4.5]decane- (**2a – e**) and 6-methyl-2-azaspiro[4.5]decane-1,3-dione (**3a – e**) were synthesized and tested for their anticonvulsant activity in the maximum electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) seizure threshold tests. To explain the possible mechanism of action, the most active compounds *N*-(3-methylpyridine-2-yl)-2-azaspiro[4.4]nonane-1,3-dione (**1b**), *N*-(3-methylpyridine-2-yl)-2-azaspiro[4.5]decane-1,3-dione (**2b**), *N*-(4-methylpyridine-2-yl)-2-azaspiro[4.5]decane-1,3-dione (**2c**), and *N*-(3-methylpyridine-2-yl)-6-methyl-2-azaspiro[4.5]decane-1,3-dione (**3b**) were tested *in vitro* for their influence on voltage-sensitive calcium channel receptors, however, they revealed low affinities. For all synthesized compounds the lipophilicity was determined by use of RP-TLC method. The correlation between the lipophilicity and anticonvulsant activity was obtained – the higher the lipophilicity the stronger the anticonvulsant efficacy.

Keywords: 2-Azaspiro[4.4]nonane-1,3-dione / 2-Azaspiro[4.5]decane-1,3-dione / Anticonvulsant activity / Calcium channel receptors affinity / Lipophilicity

Received: October 5, 2005; accepted: November 14, 2005

DOI 10.1002/ardp.200500219

Introduction

Epilepsy is one of the most frequent neurological disorders characterized by spontaneous recurrent seizures arising from excessive electrical activity in a portion of the brain. Clinically available anticonvulsant drugs exert satisfactory control in 60 – 70% of patients, however many of them also produce undesirable harmful side-effects. Therefore, there is a growing necessity for novel specific and low-toxicity anticonvulsant molecules [1, 2].

In the course of developing new potentially anticonvulsant active agents, our attention has been focused on the group of pyrrolidine-2,5-dione derivatives (succinimides) variously substituted at the nitrogen atom as well as at the 3-position of the imide ring [3 – 5].

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In our earlier studies, we described anticonvulsant activity of many *N*-(pyridine-2-yl)-3-phenyl-pyrrolidine-2,5-diones which displayed protection against both MES and scPTZ tests [6 – 8]. In this series the most active compound was *N*-(3-methylpyridine-2-yl)-3-(3-bromophenyl)-pyrrolidine-2,5-dione with ED_{50} = 54.2 mg/kg (MES) and ED_{50} = 110.3 mg/kg (scPTZ). The structure-activity relationship analysis indicated that their anticonvulsant activity was closely connected with the conformational and electronic properties of those molecules and highly depended on the position of the methyl group at the pyridine moiety [9, 10].

Among the classes of compounds with well documented anticonvulsant activity are spirosuccinimide derivatives [11, 12]. Based on these facts in our recent studies, we replaced the phenyl moiety at the 3-position of pyrrolidine-2,5-dione ring with a cycloalkyl fragment to obtain a new series of *N*-benzyl and *N*-aminophenyl 2-azaspiro-[4.4]nonane- and 2-azaspiro[4.5]decane-1,3-dione derivatives, among which *N*-aminophenyl-2-azaspiro[4.4]-

nonane-1,3-dione was the most active with ED_{50} = 72.61 mg/kg in the MES test [13].

In view of these data, we designed and synthesized in this work a new series of 2-azaspiro[4.4]nonane-, 2-azaspiro[4.5]decane- and 6-methyl-2-azaspiro[4.5]decane-1,3-dione derivatives with pyridine-2-yl moiety at the nitrogen atom. The compounds were evaluated for their anticonvulsant activity and neurotoxic properties within the antiepileptic drug development (ADD) program (Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NINCDS), Bethesda).

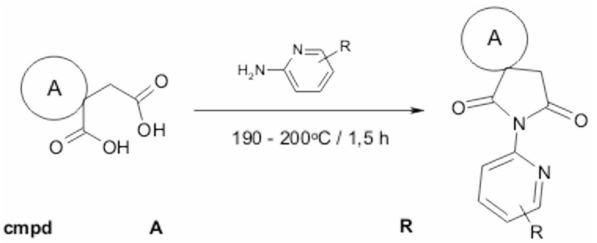
It is well known that several antiepileptic drugs are thought to interact with voltage-gated Ca^{2+} [14]. Taking this fact into account, to explain the possible mechanism of action for selected active compounds their influence on voltage-gated calcium channels were investigated.

Lipophilicity, the inherent property of the chemical compound is widely used as structural descriptor in rational drug design. Therefore, in this study we decided to evaluate the influence of the lipophilicity on anticonvulsant activity of the investigated derivatives.

Results and discussion

Chemistry

Compounds **1a–e**, **2a–e**, and **3a–e** were synthesized according to Scheme 1. The starting dicarboxylic acids were prepared as reported previously [15]. First, in a reaction of cyclopentanone, cyclohexanone, or 2-methylcyclohexanone with ethyl cyanoacetate, the corresponding cycloalkylidene cyanoethyl esters were obtained. Treatment of the intermediate products with potassium cyanide, followed by a hydrolysis, furnished the respective acids. The final *N*-substituted spirosuccinimides (**1a–e**, **2a–e**, and **3a–e**) were obtained in a one-pot cyclization reaction of the prepared acids and appropriately substituted 2-aminopyridines by heating them at ca. 190–200°C



cmpd	A		R
1a -1e	cyclopentane	a	H
2a -2e	cyclohexane	b	3-CH_3
3a -3e	2-methylcyclohexane	c	4-CH_3
		d	6-CH_3
		e	4,6-CH_3

Scheme 1. Synthesis and structures of the title compounds.

for 1.5 h. Compounds **1a** and **2a** have been previously described by El-Talbany *et al.* [16, 17] (no ^{1}H-NMR data available), but none of them were tested for their anticonvulsant activity. All other compounds are new ones.

Anticonvulsant activity

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

The investigated compounds **1a–e**, **2a–e**, and **3a–e** showed diversified anticonvulsant properties from active at a dose 100 mg/kg or less (class 1) (**2b**, **2c**, **2d**, **3d**, and **3e**) to active at a dose of 300 mg/kg (class 2) (**1b–e**, **2e**, **3b**, **3c**) and inactive ones (class 3) (**1a**, **2a**, **3a**). The results of anticonvulsant identification studies in mice are shown in Table 1.

Except for compounds **2c** and **2e**, effective only in the scPTZ test, at doses of 100 and 300 mg/kg, respectively, all other derivatives revealed protection in both MES and scPTZ screens. The most active *N*-(3-methylpyridine-2-yl)-2-azaspiro[4.5]decane-1,3-dione (**2b**) exhibited anti-scPTZ activity at a dose of 30 mg/kg and anti-MES protection at a dose of 100 mg/kg. The 6-methyl isomer **2d** was less active and inhibited the seizures at doses of 100 and 300 mg/kg in the MES and scPTZ test, respectively. Similar results were obtained for the *N*-(4,6-dimethypyridine-2-yl)-6-methyl-2-azaspiro[4.5]decane-1,3-dione (**3e**), whereas **3d** with one methyl group at 6-position of the pyridine-2-yl moiety was more potent in the scPTZ screen (100 mg/kg) as compared to its anti-MES activity (300 mg/kg). Compounds **3b**, **3c** as well as methyl analogues of *N*-(pyridine-2-yl)-2-azaspiro[4.4]nonane-1,3-dione (**1b–1e**), regardless on the number and position of the methyl substituents at the pyridine-2-yl moiety, revealed protection against both screens at doses of 300 mg/kg. The unsubstituted compounds **1a**, **2a**, and **3a** were found to be inactive.

In the neurotoxicity screen only compounds **2a**, **2b**, **2c**, and **3b** were devoid of toxicity at maximum administrated dose (300 mg/kg). The other derivatives were found to be toxic at a dose of 100 mg/kg (**1e**, **2d**, **2e**, **3c–e**) or 300 mg/kg (**1a–d**, **3a**).

Compounds **2a**, **2d**, and **3b**, randomly selected from derivatives active or inactive 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 obtained results are shown in Table 2. As can be seen from these data, only compound **3b** was moderately

Table 1. Anticonvulsant, neurotoxicity screening results and R_{M0} values of investigated compounds.

Compound	Intraperitoneal injection in mice[a]						R_{M0}[c]	ASP[d] class
	MES		scPTZ		TOX[b]			
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h		
1a	–	–	–	–	300	–	0.78	3
1b	300	–	300	–	300[14]	–	0.85	2
1c	300	–	300	–	300[14]	–	0.84	2
1d	300	–	300	–	300[14]	–	0.95	2
1e	300	–	300	–	100[14]	–	0.97	2
2a	–	–	–	–	–	–	0.95	3
2b	100	–	30	–	–	–	1.28	1
2c	–	–	100	–	–	–	1.12	1
2d	100	–	300	–	100	–	1.14	1
2e	–	–	300	–	100[14]	–	1.17	2
3a	–	–	–	–	300	–	0.90	3
3b	300	–	300	–	–	–	1.25	2
3c	300	–	300	–	100[14, 33]	–	1.05	2
3d	300	–	100	–	100	–	1.15	1
3e	100	–	300	–	100[14]	–	1.32	1

a) Doses of 30, 100, and 300 mg/kg were administrated. The figures in the Table indicate the minimum dose (mg/kg), whereby bioactivity was demonstrated. The dash (–) indicates an absence of activity at maximum dose administrated.

b) Toxicity screen: the minimum dose of compound whereby toxicity was exhibited. Response comments: [14] unable to grasp rotorod, [33] tremors.

c) The relative retention parameters, calculated according to equation: $R_M = R_{M0} + ac$, where c is the concentration of *n*-propanol in a mobile phase and a is the slope of the straight line.

d) 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.

Table 2. The results in rats after oral administration at a dose of 30 mg/kg.

Compound	Oral administration to rats									
	MES[a]					TOX[b]				
	0.25 h	0.5 h	1 h	2 h	4 h	0.25 h	0.5 h	1 h	2 h	4 h
2a	1/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
2d	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
3b	2/4	1/4	2/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4

a) Maximal electroshock test, number of animals protected/number of animals tested.

b) Rotorod test for neurological toxicity, number of animals exhibiting toxicity/number of animals tested.

Table 3. Results of calcium-channels binding studies.

Compound	IC_{50} [μM]
1b	181.2
2b	199.4
2c	168.0
3b	101.3

active in rat MES oral screen and revealed the time of onset of anticonvulsant activity at 0.25 h and two peaks of 50% protection at time points 0.25 h and 1 h. In addition, the tested substance protected 25% of animals at 0.5, 2 and 4 h. The total duration of satisfactory action of this compound appeared to be quite short within 0.25 to 1 h. The derivative **2a** showed marginal protection, whereas **2d** was inactive in rats. No evidence of neurological toxicity was observed at the dose of 30 mg/kg administrated orally.

Several anticonvulsants i.e. valproate, ethosuximide, that are known as effective drugs in absence epilepsy exert their activity by the interaction with voltage-gated Ca^{2+} channels [18, 19]. As described above, the results of preliminary pharmacological screening revealed that the majority of investigated derivatives exhibited protection in the scPTZ screen, recognized as the experimental model for early identification of molecules affording protection against absence seizures. Therefore, to search for possible mechanism of action, the chosen active compounds, **1b**, **2b**, **2c**, and **3b** were *in-vitro* evaluated on voltage-sensitive calcium channels affinity, by their ability to displace [^{3}H]nitrendipine in rat cortical tissue. The radioligand binding experiments, however, revealed low affinities; IC_{50} ranged from 101.3 to 199.4 μM (Table 3). These preliminary results suggest that anticonvulsant activity is rather not related with voltage-gated calcium channels.

Lipophilicity

Lipophilicity is a fundamental physicochemical property of bioactive compounds that play 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 measurement of the lipophilic properties of different chemical molecules is nowadays the chromatographic techniques in reversed-phase system. In consequence, the correlation between lipophilicity and biological activity can be based on chromatographic retention parameters [20–23]. In our previous studies [24], the correlation between anticonvulsant activity and chromatographic R_{M0} parameters was obtained. Based on these facts, in the presented work we determined the lipophilicity of all compounds by use of the RP-TLC method.

The examination of the chromatographic behaviour in the RP-TLC system showed a linear correlation between

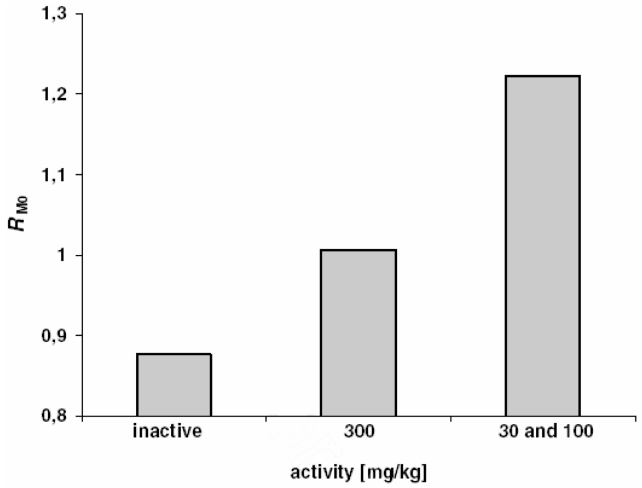


Figure 1. Comparison of mean R_{M0} parameters for active and inactive compounds.

R_M values and *n*-propanol concentration (v/v) in a mobile phase. The relative retention parameters R_{M0} were determined by the extrapolation method, and are listed in Table 1.

As expected, the lowest R_{M0} values were observed for unsubstituted derivatives of **1a**, **2a**, and **3a**. The change of the ring size from cyclopentane (**1a–e**) to cyclohexane (**2a–e**) increased the lipophilicity. Surprisingly, the presence of the additional methyl group at the cyclohexane fragment (**3a–e**) caused the increase of R_{M0} parameters only for compounds **3d** and **3e** in comparison to the unsubstituted analogues **2d** and **2e**. Moreover, the R_{M0} data proved that the introduction of the methyl groups into pyridine ring enhanced the lipophilicity.

As it is shown in Figure 1, the mean R_{M0} parameter for inactive compounds was lower in comparison to active ones (30, 100, and 300 mg/kg). Furthermore, in general, the higher the lipophilicity (R_{M0}) the more potent anticonvulsant activity was demonstrated. The applied statistical analysis (Mann-Whitney test) revealed that the differences between R_{M0} values of active and inactive derivatives were statistically significant ($p < 0.05$).

In conclusion, the most active were compounds with cyclohexane ring as spiro nucleus and methyl groups especially in 3- or 6-position of pyridine-2-yl moiety. It was in agreement with our earlier experiments [6, 9, 10, 13] indicating that the presence of methyl substituent at the 3-position of pyridine-2-yl ring is important for the anticonvulsant activity. The change of the size of a spirocycloalkyl unit to cyclopentane as well as the introduction of a methyl substituent into 6-position of 2-azaspiro-[4.5]decane-1,3-dione fragment decreased the activity and also increased the toxicity. The role of the lipophilicity in the anticonvulsant activity was proved, the higher the lipophilicity, the stronger anticonvulsant efficacy.

The obtained results revealed that a number of novel *N*-(pyridine-2-yl) derivatives of spirosuccinimides were moderately effective in MES as well as scPTZ screens, however none of them was more potent than molecules with an aryl moiety at the 3-position of pyrrolidine-2,5-dione [6-8]. Thus, the presence of an aromatic area at the 3-position of an imide ring seems to be the most important factor for anticonvulsant activity.

The authors wish to thank Dr James Stables for providing them with 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).

Experimental

Chemistry

All 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 the developing system chloroform : acetone (9 : 1). Spots were detected by their absorption under UV light (λ = 254 nm) and by visualization with 0.05 mol I_2 in 10% HCl. The chemical structures were confirmed by elemental and spectral analyses (^{1}H-NMR). ^{1}H-NMR spectra were obtained in a Varian Mercury 300 MHz spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$, with TMS as an internal standard. Chemical shifts are reported in δ values (ppm) and J values in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), d (doublet), t (triplet), td (triplet of doublets), dt (doublet of triplets), m (multiplet). Elemental analyses for C, H, N were carried out with an Elementar Vario EL III (Hanau, Germany).

*General procedure for the preparation of the **1a–e**, **2a–e**, and **3a–e***

A total of 0.02 mol of the appropriate substituted 2-aminopyridines were dissolved in 20 mL of water and 0.02 mol of cyclopentane- acid, cyclohexane- acid or 1-(2-methylcyclohexane-1-carboxy-1-acetic acid was gradually added. The mixture was heated in an oil bath with simultaneous distillation of water. After the water was completely removed, the temperature of the reaction mixture was raised up to 180°C and maintained for 1.5 h. The crude products were recrystallized from methanol.

N-Pyridine-2-yl-2-azaspiro[4.4]nonane-1,3-dione ***1a***

Compound **1a** was obtained as white solid (yield 70%), m.p. 143 – 145°C (130°C [16]). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.76 – 2.30 (m, 8H, cyclopentane), 2.78 (s, 2H, imide), 7.29 – 7.37 (m, 2H, H-4, H-5, pyridine), 7.85 (td, 1H, H-3, *J* = 7.70 Hz, pyridine), 8.66 (dt, 1H, H-6, *J* = 4.86 Hz, pyridine). Anal. calcd. for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.62; H, 6.02; N, 12.10.

N-(3-Methylpyridine-2-yl)-2-azaspiro[4.4]nonane-1,3-dione ***1b***

Compound **1b** was obtained as white solid (yield 55%), m.p. 135 – 137°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.78 – 2.34 (m, 8H, cyclopentane), 2.38 (s, 3H, CH_3), 2.79 (s, 2H, imide), 7.28 – 7.32 (m, 1H, H-5, pyridine), 7.65 – 7.69 (m, 1H, H-4, pyridine), 8.47 (dt, 1H, H-6, *J* = 4.77 Hz, pyridine). Anal. calcd. for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.72; H, 6.41; N, 11.27.

N-(4-Methylpyridine-2-yl)-2-azaspiro[4.4]nonane-1,3-dione ***1c***

Compound **1c** was obtained as white solid (yield 61%), m.p. 97 – 99°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.70 – 2.28 (m, 8H, cyclopentane), 2.40 (s, 3H, CH_3), 2.77 (s, 2H, imide), 7.10 – 7.17 (m, 2H, H-3, H-5, pyridine), 8.49 (d, 1H, H-6, *J* = 5.22 Hz, pyridine). Anal. calcd. for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.80; H, 6.53; N, 11.38.

N-(6-Methylpyridine-2-yl)-2-azaspiro[4.4]nonane-1,3-dione ***1d***

Compound **1d** was obtained as white solid (yield 63%), m.p. 78 – 80°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.80 – 2.32 (m, 8H, cyclopentane), 2.63 (s, 3H, CH_3), 2.81 (s, 2H, imide), 7.10 (d, 1H, H-5, *J* = 7.70 Hz, pyridine), 7.25 (d, 1H, H-4, J = 7.70 Hz, pyridine), 7.77 (t, 1H, H-3, *J* = 7.84 Hz, pyridine). Anal. calcd. for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.62; H, 6.49; N, 11.35.

N-(4,6-Dimethylpyridine-2-yl)-2-azaspiro[4.4]nonane-1,3-dione ***1e***

Compound **1e** was obtained as white solid (yield 59%), m.p. 100 – 102°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.80 – 2.32 (m, 8H, cyclopentane), 2.39 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 2.80 (s, 2H, imide), 6.92 (s, 1H, H-5, pyridine), 7.07 (s, 1H, H-3, pyridine). Anal. calcd. for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.70; H, 6.98; N, 10.73.

N-Pyridin-2-yl-2-azaspiro[4.5]decane-1,3-dione ***2a***

Compound **2a** was obtained as white solid (yield 72%), m.p. 148 – 150°C (133°C [17]). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.40 – 2.02 (m, 10H, cyclohexane), 2.79 (s, 2H, imide), 7.30 – 7.42 (m, 2H, H-4, H-5, pyridine), 7.89 (td, 1H, H-3, *J* = 7.70 Hz, pyridine), 8.68 – 8.70 (m, 1H, H-6, pyridine). Anal. calcd. for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.75; H, 6.58; N, 11.40.

N-(3-Methylpyridine-2-yl)-2-azaspiro[4.5]decane-1,3-dione ***2b***

Compound **2b** was obtained as white solid (yield 56%), m. p. 166 – 168°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δd (ppm): 1.36 – 1.95 (m, 10H, cyclohexane), 2.18 (s, 3H, CH_3), 2.77 (s, 2H, imide), 7.30 – 7.33 (m, 1H, H-5, pyridine), 7.66 – 7.69 (m, 1H, H-4, pyridine), 8.46 – 8.48 (m, 1H, H-6, pyridine). Anal. calcd. for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.62; H, 7.00; N, 10.79.

N-(4-Methylpyridine-2-yl)-2-azaspiro[4.5]decane-1,3-dione ***2c***

Compound **2c** was obtained as white solid (yield 64%), m.p. 138 – 140°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.33 – 1.97 (m, 10H, cyclohexane), 2.41 (s, 3H, CH_3), 2.74 (s, 2H, imide), 7.09 – 7.17 (m, 2H, H-3, H-5, pyridine), 8.49 (d, 1H, H-6, *J* = 5.22 Hz, pyridine). Anal. calcd. for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.71; H, 7.01; N, 10.80.

N-(6-Methylpyridine-2-yl)-2-azaspiro[4.5]decane-1,3-dione ***2d***

Compound **2d** was obtained as white solid (yield 60%), m.p. 151 – 153°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.38 – 1.98 (m, 10H, cyclohexane), 2.59 (s, 3H, CH_3), 2.75 (s, 2H, imide), 7.07 (d, 1H, H-5, *J* = 7.69 Hz, pyridine), 7.22 (d, 1H, H-4, *J* = 7.70 Hz, pyridine), 7.75 (t, 1H, H-3, *J* = 7.84 Hz, pyridine). Anal. calcd. for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.60; H, 6.92; N, 10.72.

N-(4,6-Dimethylpyridine-2-yl)-2-azaspiro[4.5]decane-1,3-dione ***2e***

Compound **2e** was obtained as white solid (yield 55%), m.p. 99 – 101°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.39 – 1.99 (m, 10H, cyclohexane), 2.39 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.76 (s, 2H, imide), 6.88 (s, 1H, H-5, pyridine), 7.07 (s, 1H, H-3, pyridine). Anal. calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.49; H, 7.25; N, 10.18.

N-(Pyridine-2-yl)-6-methyl-2-azaspiro[4.5]decane-1,3-dione ***3a***

Compound **3a** was obtained as white solid (yield 67%), m.p. 81 – 83°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δd (ppm): 1.06 (d, 3H, *J* = 6.88 Hz, CH3), 1.82 – 2.41 (m, 9H, cyclohexane), 2.52 (d, 1H, *J* = 18.43 Hz, imide), 2.91 (d, 1H, *J* = 18.43 Hz, imide), 7.31 – 7.41 (m, 2H, H-4, H-5, pyridine), 7.89 (td, 1H, H-3, *J* = 7.70 Hz, pyridine), 8.68 – 8.71 (m, 1H, H-6, pyridine). Anal. calcd. for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.63; H, 6.93; N, 10.74.

N-(3-Methylpyridine-2-yl)-6-methyl-2-azaspiro[4.5] decane-1,3-dione ***3b***

Compound **3b** was obtained as white solid (yield 57%), m.p. 175 – 177°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 0.96 (d, 3H, *J* = 6.92 Hz, CH_3), 1.02 – 2.01 (m, 8H, cyclohexane), 2.04 – 2.15 (m, 1H, cyclohexane), 2.22 (s, 3H, CH_3), 2.58 (d, 1H, *J* = 18.46 Hz, imide), 2.78 (d, 1H, *J* = 18.43 Hz, imide), 7.28 – 7.32 (m, 1H, H-5, pyridine), 7.67 (t, 1H, H-4, *J* = 6.28 Hz, pyridine), 8.48 (d, 1H, H-6, *J* = 4.62 Hz, pyridine). Anal. calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.42; H, 7.32; N, 10.18.

N-(4-Methylpyridine-2-yl)-6-methyl-2-azaspiro[4.5] decane-1,3-dione ***3c***

Compound **3c** was obtained as white solid (yield 64%), m.p. 80 – 82°C. Yield: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.06 (d, 3H, *J* = 6.60 Hz, CH_3), 1.36 – 2.40 (m, 9H, cyclohexane), 2.45 (s, 3H, CH_3), 2.50 (d, 1H, *J* = 18.43 Hz, imide), 2.90 (d, 1H, *J* = 18.15 Hz, imide), 7.12 – 7.13 (m, 1H, H-5, pyridine), 7.19 – 7.21 (m, 1H, H-3, pyridine), 8.54 (d, 1H, H-6, *J* = 5.22 Hz, pyridine). Anal. calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.39; H, 7.39; N, 10.10.

N-(6-Methylpyridine-2-yl)-6-methyl-2-azaspiro[4.5]decane-1,3-dione ***3d***

Compound **3d** was obtained as white solid (yield 58%), m.p. 146 – 148°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 0.94 (d, 3H, *J* = 6.87 Hz, CH_3), 1.36 – 1.98 (m, 8H, cyclohexane), 2.06 – 2.14 (m, 1H, cyclohexane), 2.58 (d, 1H, *J* = 18.43 Hz, imide), 2.62 (s, 3H, CH_3), 2.80 (d, 1H, *J* = 18.43 Hz, imide), 7.05 (d, 1H, H-5, *J* = 7.70 Hz, pyridine), 7.24 (d, 1H, H-4, *J* = 7.70 Hz, pyridine), 7.75 (t, 1H, H-3, *J* = 7.84 Hz, pyridine). Anal. calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.51; H, 7.31; N, 10.16.

N-(4,6-Dimethylpyridine-2-yl)-6-methyl-2-azaspiro[4.5]decane-1,3-dione ***3e***

Compound **3d** was obtained as white solid (yield 60%), m.p. 115 – 117°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.05 (d, 3H, *J* = 6.87 Hz, CH_3), 1.21 – 1.98 (m, 8H, cyclohexane), 2.00 – 2.19 (m, 1H, cyclohexane), 2.39 (s, 3H, CH_3), 2.50 (d, 1H, *J* = 18.43 Hz, imide), 2.57 (s, 3H, CH_3), 2.88 (d, 1H, *J* = 18.43 Hz, imide), 6.88 (s, 1H, H-5, pyridine), 7.20 (s, 1H, H-3, pyridine). Anal. calcd. for $C_{17}H_{22}N_2O_2$: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.20; H, 7.62; N, 9.70.

Pharmacology

Anticonvulsant screening

The anticonvulsant evaluations were performed using procedures described elsewhere [25, 26]. Phase I studies of the investigated compounds involved three testes: maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ), and a rotorod 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 at 0.5 and 4 h time periods. The results are presented in Table 1. Selected compounds from phase I (**2a**, **2d**, and **3b**) 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 rotorod 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 2.

Calcium channel binding

The experiment was performed as described previously [27]. The rat brain was rapidly removed after decapitation, placed on an ice-chilled porcelain plate, and the cerebral-cortices dissected out. The tissues were homogenized using a Polyrton disintegrator (setting 4, 10 s) at 0 °C in 20 volumes 50 mmol/L Tris-HCl buffer (pH 7.6). The homogenate was centrifuged at 0°C and 1000 × *g* for 10 min, the supernatant was decanted and recentrifuged at 0°C and 25.000 × *g* for 30 min, and the resulting pellet was resuspended in the buffer and recentrifuged under the same conditions. The pellet thus obtained (fraction P2) was stored at −18°C for no longer than 48 h. For incubation it was reconstituted in the Tris-HCl buffer to give a final protein concentration (measured according to Lowry *et al*. [28]) of approximately 1.2 mg/mL. The radioligand [^{3}H]nitrendypine (NEN, specific activity 87.0 Ci/mmol), for estimating voltage-dependent Ca^{2+} channels was used. The incubation mixture (final volume 550 mL) consisted of 450 mL membrane suspension, 50 mL of radioligand solution (0.8 nM), and 50 mL buffer containing seven concentrations (0.1 nM – 100 mM) of tested compounds. For measuring unspecific binding, nifedipine was used at a concentration of 10 mM. Incubation was carried out in duplicate, in a shaking water bath; at 25°C for 30 min. Addition of a ligand initiated the incubation, which was terminated by rapid filtration through GF/C Whatman fiberglass filters. The filters were then rinsed twice with 5 mL portions of ice-cold incubation buffer and placed in plastic scintillation minivials. A scintillation fluid (aquascint) was added and the samples were counted for radioactivity in a Beckman LS 3801 scintillation counter. Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, Version 3.0 – San Diego, CA, USA). The results are shown in Table 3.

Lipophilicity

The lipophilicity of all compounds was measured by reversed phase thin layer chromatography (RP-TLC). TLC was performed on 20 × 20 cm RP-18F_{254S} precoated silica plates (Merck). Solutions (10 mg mL^{-1}) of the tested compounds were prepared in chloroform, and 10 mL volumes were spotted onto the plates, 2 cm apart. Mixtures of the *n*-propanol content ranging between 30 – 65% (v/v) in a 5% increment and TRIS buffer (pH = 7.0) were used as a mobile phases. The chromatography chamber was saturated with the mobile phase for 30 min before use. After the development of chromatograms (180 mm), the plates were dried in the air at a room temperature and were inspected under UV light (λ = 254 nm). R_F (retardation factor) values were estimated as mean values of the results obtained from three determinations. The retention parameters (R_M) were calculated by means of the equation:

$$R_M = \log (1/R_f - 1)$$

The R_M values were extrapolated to 0% of organic modifier concentration to estimate the relative retention parameters (R_{M0}) by use of the equation:

$$R_M = R_{M0} + ac$$

c is the concentration of *n*-propanol and a the slope of the straight line [29].

The correlation coefficients were always > 0.97. The relative retention parameters (R_{M0}) are listed in Table 1.

Statistical analysis

Statistical analyses were performed using the computer program Statistica version 5, 1997 Edition, Copyright© Statsoft, Inc. 1984 – 1997. Data with $p < 0.05$ value were considered to be significant.

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