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SYNTHESIS AND ANTICONVULSANT PROPERTIES OF NEW 1-(2-PYRIDINYL)- 3-SUBSTITUTED PYRROLIDINE-2,5-DIONE DERIVATIVES

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Abstract: The synthesis and anticonvulsant properties of new 1-(2-pyridinyl)- succinimides [**I**-**XXII**] differently substituted at the position-3 of imide ring have been described. The profile of pharmacological activity of these compounds was examined by a maximal electroshock (MES) and pentylenetetrazole (*sc*PTZ) tests, whereas their neurotoxicity was determined using a rotarod screen. The results obtained revealed that the anticonvulsant activity depended mainly on the kind of substituents at the position-3 of pyrrolidine-2,5-dione ring. The most active were 3,3-dialkyl-pyrrolidine-2,5-diones [**IX**-**XVIII**] as well as compounds with 3-methylcyclohexane moiety as a spiro nucleus at position-3 of the imide ring [**I**-**IV**]. The 3-cyclohexylsuccinimides [**V**-**VIII**] with cyclohexane ring as a flexible fragment were less active, whereas unsubstituated derivatives [**XIX**-**XXII**] were devoid of activity in both tests applied. In addition, the anti-seizure protection depended on the position of methyl group at the pyridine moiety. The most potent were compounds with the methyl substituent at the position-4 [**II**, **VI**, **XVII**] or -6 [**XI**, **XIV**]. It should be noted, that in the whole series the most active was 1-(4-methyl-2-pyridinyl)-3-cyclohexyl-pyrrolidne-2,5-dione [**VI**], which showed the anti-*sc*PTZ protection at the dose of 30 mg/kg.

Keywords: pyrrolidine-2,5-diones; 1-(2-pyridinyl)-succinimides; anticonvulsant activity

Epilepsy is one of the most common neurological disorders affecting approximately 1% of the population worldwide (1). Despite the progress in understanding the pathogenesis of seizures, the current therapy remains still ineffective in at least 30% of epilepsies.

Moreover, a large number of new antiepileptic drugs (ADEs) marketed during recent years, did not change the proportion of patients responding to the treatment and many of those medications cause serous side effects, which include ataxia, nausea, mental dulling and hepatotoxicity. Thus, new ADEs with higher efficacy, better safety and tolerability are urgently needed (2-5).

The pyridine moiety is known as one of the important structural fragments that appears in the structures of anticonvulsant active molecules (6-9). In our earlier studies on a search for new potential anti-seizure agents, a series of 3-phenyl-pyrrolidine-2,5-diones as well as 2-azaspiro[4.4]nonane- and [4.5]decane-1,3-diones containing the pyridine moiety at the imide nitrogen atom have been described (10-16). Many of these compounds exhibited potent anticonvulsant properties in the maximal electroshock seizure (MES) and/or pentylenetetrazole (*sc*PTZ) tests, recognized as the most widely employed animal seizure models for early identification of candidate anticonvulsants. The structure-activity relationship analysis (SAR) revealed that the most active were compounds with the methyl substituent at position-3 and -6 of the 2-pyridinyl moiety. In line with the above data, as a continuation of our systematic research in this group of derivatives, in the present study we have designed and synthesized a series of 1-(2-pyridinyl)-pyrrolidine-2,5-diones differently substituted at the position-3 of succinimide ring. The aim of these modifications was to establish the most relevant substitution pattern in respect to anticonvulsant activity of that type of derivatives. Furthermore, this work enabled to investigate the role of the spiro nucleus as a structural fragment essential for activity of azaspiranes described previously (16).

All the compounds obtained were evaluated for their anti-seizure activity and neutotoxic properties within the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and

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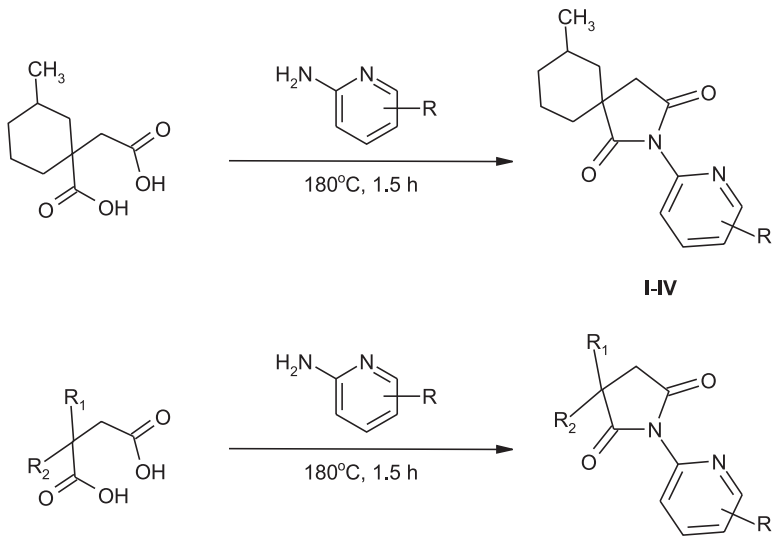
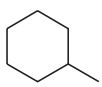
Communicative Disorders and Stroke (NINCDS), Bethesda, USA.

Compounds **I-XXII** were synthesized according to Scheme 1. The starting 1-carboxy-1-(3-methylcyclohexane)acetic acid, 2,2-dimethyl-, 2-ethyl-2-methyl- and 2,2-diethyl-succinic acids were prepared as reported previously (17, 18). The synthetic procedures of 2-cyclohexylsuccinic acid have been described in the literature (19). The final 1-(2-pyridinyl)-succinimides (**I-XVIII**) were obtained in a one-pot cyclization reaction of the prepared acids and appropriately substituted 2-amino-pyridines by heating them at ca. 180°C for 1.5 h. Compounds **XIX-XII** were synthesized in the same manner using the succinic anhydride as a reaction substrate.

The structures of the compounds synthesized were confirmed by the examination of their ^{1}H NMR spectra, which revealed characteristic chemical shifts in agreement with their proposed structures. The chemical shifts of the cyclohexane rings [**I-VIII**] were observed as multiplets within the range of δ 0.95-1.91 ppm [**I-IV**] and δ 1.06-2.13 ppm [**V-VIII**]. The signals due to the methyl substituents at the position-3 of pyrrolidine-2,5-dione appeared at δ 1.41-1.46 ppm, as singlets [**X**, **XI**, **XIII-XV**] or doublets [**XII, XIX**]. The ethyl groups of compounds **XII-XVIII** were observed in two ranges δ 0.96-1.06 and δ 1.65-1.95 that correspond to the signals of methyl and methylene protons, respectively. The methyl substituents at the 2-pyridinyl moiety were recorded as singlets at δ 2.19 ppm to δ 2.59 ppm. The appearance of singlets, doublets or multiplets within the range δ 2.59-3.04 ppm correspond to the protons of imide rings. The methyl groups at position-7 of 2-azaspiro[4.5]decane-1,3-diones [**I-IV**] were recorded as multiplet [**I**] or doublets [**II-IV**]



Cmpd.	V-VIII	IX-XI	XII-XV	XVI-XVIII	XIX-XXII
R_1		CH_3	CH_3	C_2H_5	H
R_2	H	CH_3	C_2H_5	C_2H_5	H

R	3-CH_3	4-CH_3	6-CH_3	4,6-CH_3
Cmpd.	**I**, **V**, **IX**, **XII**, **XVI**, **XIX**	**II**, **VI**, **X**, **XIII**, **XVII**, **XX**	**III**, **VII**, **XI**, **XIV**, **XVIII**, **XI**	**IV**, **VIII**, **XV**, **XXII**

Scheme 1. Synthesis of compounds **I – XXII.**

Table 1. Physicochemical and analytical data for compounds **I-XXII**.

Compound no.	Molecular formula Weight	Yield % M. p. [°C]	Analysis (calc./found)			R_f[a]
			%C	%H	%N	
I	$C_{16}H_{20}O_2N_2$ 272.35	59 103-105	70.56 70.66	7.40 7.52	10.29 10.38	0.80
II	$C_{16}H_{20}O_2N_2$ 272.35	67 102-104	70.56 70.49	7.40 7.42	10.29 10.39	0.74
III	$C_{16}H_{20}O_2N_2$ 272.35	62 190-192	70.56 70.72	7.40 7.49	10.29 10.28	0.85
IV	$C_{17}H_{22}O_2N_2$ 283.38	60 120-122	71.30 71.50	7.74 7.69	9.78 9.60	0.81
V	$C_{16}H_{20}O_2N_2$ 272.35	57 130-132	70.56 70.70	7.40 7.38	10.29 10.39	0.73
VI	$C_{16}H_{20}O_2N_2$ 272.35	63 119-121	70.56 70.42	7.40 7.32	10.29 10.18	0.74
VII	$C_{16}H_{20}O_2N_2$ 272.35	65 96-98	70.56 70.55	7.40 7.38	10.29 10.20	0.76
VIII	$C_{17}H_{22}O_2N_2$ 283.38	60 140-142	71.30 71.19	7.74 7.63	9.78 9.70	0.60
IX	$C_{12}H_{14}O_2N_2$ 218.26	66 88-90	66.04 65.97	6.47 6.40	12.84 12.88	0.58
X	$C_{12}H_{14}O_2N_2$ 218.26	67 134-136	66.04 66.11	6.47 6.53	12.84 12.80	0.56
XI	$C_{12}H_{14}O_2N_2$ 218.26	60 122-124	66.04 66.10	6.47 6.50	12.84 12.82	0.60
XII	$C_{13}H_{16}O_2N_2$ 232.28	59 98-100	67.22 67.15	6.94 6.89	12.04 12.10	0. 77
XIII	$C_{13}H_{16}O_2N_2$ 232.28	65 58-60	67.22 67.27	6.94 6.90	12.04 12.01	0.78
XIV	$C_{13}H_{16}O_2N_2$ 232.28	60 78-80	67.22 67.16	6.94 6.90	12.04 12.08	0.80
XV	$C_{14}H_{18}O_2N_2$ 246.31	62 90-92	68.27 68.32	7.37 7.39	11.37 11.30	0.79
XVI	$C_{14}H_{18}O_2N_2$ 246.31	58 76-78	68.27 68.20	7.37 7.34	11.37 11.30	0.69
XV	$C_{14}H_{18}O_2N_2$ 246.31	61 38-40	68.27 68.19	7.37 7.29	11.37 11.34	0.70
XVIII	$C_{14}H_{18}O_2N_2$ 246.31	62 58-60	68.27 68.17	7.37 7.30	11.37 11.40	0.72
XIX	$C_{10}H_{10}O_2N_2$ 190.20	60 126-128	63.15 63.20	5.30 5.19	14.73 14.64	0.38
XX	$C_{10}H_{10}O_2N_2$ 190.20	57 141-143	63.15 63.07	5.30 5.20	14.73 14.67	0.50
XXI	$C_{10}H_{10}O_2N_2$ 190.20	62 148-150	63.15 63.10	5.30 5.25	14.73 14.70	0.60
XXII	$C_{11}H_{12}O_2N_2$ 204.23	61 195-197	64.69 64.75	5.92 5.60	13.72 13.65	0.63

[a] Developing system: chloroform : acetone (9 : 1, v/v).

within the range of δ 0.95-0.96 ppm. All the aromatic protons were well separated and observed at the expected region. The detailed spectral data of each compound are presented in Tables 2-4.

EXPERIMENTAL

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 the developing system: chloroform : acetone (9 : 1, v/v). 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 on 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), q (quartet), m (multiplet). Elemental analyses for C, H, N were carried out with an Elementar Vario EL III (Hanau, Germany) and were within ± 0.4% of the theoretical values. The physicochemical data, yields, elemental analyses and R_f values are presented in Table 1. The ^{1}H NMR data are shown in Tables 2-4.

General procedure for the preparation of compounds I-XXII

0.01 mol of the appropriately substituted 2-aminopyridine was dissolved in 20 mL of water and 0.01 mol of corresponding 2-substituted succinic acid [**I-XVIII**] or succinic anhydride [**XIX**-X**XII**] 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 rose up to 180°C and was maintained for 1.5 h. The crude products were recrystallized from isopropanol.

Pharmacology

Compounds **I-XXII** were pharmacologically pre-evaluated within the Antiepileptic Drug

Table 2. ^{1}H-NMR data of compounds **I-VIII**.

No.	^{1}H NMR ($CDCl_3$) δ (ppm)
I	0.95-1.08 (m, 4H, 3H, CH_3, 1H, cyclohexane), 1.20-1.91 (m, 8H, cyclohexane), 2.19 (s, 3H, CH_3-pyridine), 2.77 (s, 2H, imide), 7.26-7.33 (m, 1H, pyridine), 7.66-7.69 (m, 1H, pyridine), 8.47 (d, 1H, pyridine *J* = 4.6 Hz)
II	0.95 (d, 3H, CH_3, *J* = 6.1 Hz), 0.98-1.07 (m, 1H, cyclohexane), 1.30-1.88 (m, 8H, cyclohexane), 2.41 (s, 3H, CH_3-pyridine), 2.74 (s, 2H, imide), 7.09-7.18 (m, 2H, pyridine), 8.50 (d, 1H, *J* = 5.1 Hz, pyridine)
III	0.96 (d, 3H, CH_3, *J* = 6.1 Hz), 0.99-1.07 (m, 1H, cyklohexane), 1.30-1.87 (m, 8H, cyclohexane), 2.59 (s, 3H, CH_3-pyridine), 2.74 (s, 2H, imide), 7.04 (d, 1H, pyridine, *J* = 7.6 Hz), 7.20 (d, 1H, pyridine *J* = 7.6 Hz), 7.72 (t, 1H, pyridine, *J* = 7.6 Hz)
IV	0.95 (d, 3H, CH_3, *J* = 6.1 Hz), 1.02-1.07 (m, 1H, cyclohexane), 1.29-1.86 (m, 8H, cyclohexane), 2.35 (s, 3H, CH_3-pyridine), 2.54 (s, 3H, CH_3-pyridine), 2.72 (s, 2H, imide), 6.85 (s, 1H, pyridine), 7.03 (s, 1H, pyridine)
V	1.07-1.40 (m, 5H, cyclohexane), 1.62-1.78 (m, 5H, cyclohexane), 2.03-2.13 (m, 1H, cyclohexane), 2.20 (s, 3H, CH_3-pyridine), 2.67-2.77 (m, 1H, imide), 2.84-3.04 (m, 2H, imide), 7.28-7.33 (m, 1H, pyridine), 7.65-7.69 (m, 1H, pyridine), 8.47 (d, 1H, pyridine, *J* = 4.8 Hz)
VI	1.07-1.39 (m, 5H, cyclohexane), 1.61-1.83 (m, 5H, cyclohexane), 2.02-2.10 (m, 1H, cyclohexane), 2.42 (s, 3H, CH_3-pyridine), 2.65-2.72 (m, 1H, imide), 2.83-2.98 (m, 2H, imide), 7.07 (s, 1H, pyridine), 7.17 (d, 1H, pyridine, *J* = 5.1 Hz), 8.50 (d, 1H, pyridine, *J* = 4.8 Hz,)
VII	1.08-1.39 (m, 5H, cyclohexane), 1.59-1.81 (m, 5H, cyclohexane), 2.01-2.10 (m, 1H, cyclohexane), 2.59 (s, 3H, CH_3-pyridine), 2.65-2.85 (m, 1H, imide), 2.92-2.98 (m, 2H, imide), 7.02 (d, 1H, pyridine, *J* = 7.6 Hz), 7.21 (d, 1H, pyridine, *J* = 7.6 Hz), 7.73 (t, 1H, pyridine, *J* = 7.6 Hz)
VIII	1.06-1.39 (m, 5H, cyclohexane), 1.61-1.81 (m, 5H, cyclohexane), 2.00-2.09 (m, 1H, cyclohexane), 2.36 (s, 3H, CH_3,), 2.54 (s, 3H, CH_3-pyridine), 2.63-2.85 (m, 1H, imide), 2.90-2.97 (m, 2H, imide), 6.83 (s, 1H, pyridine), 7.03 (s, 1H, pyridine)

Table 3. ^{1}H-NMR data of compounds **IX**-**XV**.

No.	^{1}H NMR ($CDCl_3$) δ (ppm)
IX	1.46 (d, 6H, CH_3, CH_3, *J* = 1.2 Hz), 2.19 (s, 3H, CH_3-pyridine), 2.77 (d, 2H, imide, *J* = 2.3 Hz), 7.29-7.33 (dd, 1H, pyridine, *J* = 4.8 Hz), 7.65-7.69 (m, 1H, pyridine), 8.46-8.48 (m, 1H, pyridine)
X	1.44 (s, 6H, CH_3, CH_3), 2.41 (s, 3H, CH_3-pyridine), 2.74 (s, 2H, imide), 7.09-7.17 (m, 2H, pyridine), 8.49 (d, 1H, pyridine, *J* = 4.8 Hz)
XI	1.44 (s, 6H, CH_3, CH_3), 2.59 (s, 3H, CH_3-pyridine), 2.74 (s, 2H, imide), 7.04 (d, 1H, pyridine, *J* = 7.9 Hz), 7.20 (d, 1H, pyridine, *J* = 7.6 Hz), 7.72 (t, 1H, pyridine, *J* = 7.6 Hz)
XII	1.00-1.06 (m, 3H, -CH_2-$\underline{CH_3}$), 1.44 (d, 3H, CH_3 *J* = 2.5 Hz), 1.66-1.95 (m, 2H, -$\underline{CH_2}$-CH_3), 2.20 (s, 3H, CH_3-pyridine), 2.60-2.68 (m, 1H, imide), 2.82-2.89 (m, 1H. imide), 7.28-7.33 (m, 1H, pyridine), 7.66-7.70 (m, 1H, pyridine), 8.48 (d, 1H, pyridine *J* = 4.6 Hz)
XIII	1.00 (t, 3H, -CH_2-$\underline{CH_3}$, *J* = 7.4 Hz), 1.42 (s, 3H, CH_3), 1.65-1.93 (m, 2H, -$\underline{CH_2}$-CH_3), 2.42 (s, 3H, CH_3-pyridine), 2.62-2.67 (m, 1H, imide), 2.81-2.86 (m, 1H, imide), 7.08 (s, 1H, pyridine), 7.17 (d, 1H, pyridine, *J* = 5.1 Hz), 8.50 (d, 1H, pyridine, *J* = 4.8 Hz)
XIV	1.00 (t, 3H, -CH_2-$\underline{CH_3}$, *J* = 7.4 Hz), 1.42 (s, 3H, CH_3), 1.65-1.92 (m, 2H, -$\underline{CH_2}$-CH_3), 2.59 (s, 4H, 3H, CH_3-pyridine, 1H, imide), 2.83 (d, 1H, imide, *J* = 18.2 Hz), 7.03 (d, 1H, pyridine, *J* = 7.6 Hz), 7.20 (d, 1H, pyridine, *J* = 7.6 Hz), 7.72 (t, 1H, pyridine, *J* = 7.6 Hz)
XV	0.99 (t, 3H, -CH_2-$\underline{CH_3}$, *J* = 7.4 Hz), 1.41 (s, 3H, CH_3), 1.65-1.91 (m, 2H, -$\underline{CH_2}$-CH_3), 2.35 (s, 3H CH_3-pyridine), 2.54 (s, 3H, CH_3-pyridine), 2.61 (d, 1H, imide, *J* = 18.2 Hz), 2.78 (d, 1H, imide, *J* = 18.1 Hz), 6.84 (s, 1H, pyridine), 7.03 (s, 1H, pyridine)

Table 4. ^{1}H-NMR data of compounds **XVI-XXII**.

No.	^{1}H NMR ($CDCl_3$) δ (ppm)
XVI	1.02 (q, 6H, -CH_2-$\underline{CH_3}$, -CH_2-$\underline{CH_3}$, *J* = 7.3 Hz), 1.70-1.94 (m, 4H, -$\underline{CH_2}$-CH_3, -$\underline{CH_2}$-CH_3), 2.19 (s, 3H, CH_3-pyridine), 2.73 (s, 2H, imide), 7.27-7.30 (q, 1H, pyridine, *J* = 4.8 Hz), 7.64-7.68 (m, 1H, pyridine), 8.45-8.48 (m, 1H, pyridine)
XVII	0.99 (t, 6H, -CH_2-$\underline{CH_3}$, -CH_2-$\underline{CH_3}$, *J* = 7.4 Hz), 1.65-1.92 (m, 4H, -$\underline{CH_2}$-CH_3, -$\underline{CH_2}$-CH_3), 2.41 (s, 3H, CH_3-pyridine), 2.70 (s, 2H, imide), 7.05 (s, 1H, pyridine), 7.15-7.17 (m, 1H, pyridine), 8.50 (d, 1H, pyridine, *J* = 5.1 Hz)
XVIII	0.96-1.02 (m, 6H, -CH_2-$\underline{CH_3}$, -CH_2-$\underline{CH_3}$), 1.67-1.92 (m, -$\underline{CH_2}$-CH_3, -$\underline{CH_2}$-CH_3), 2.58 (s, 3H, CH_3-pyridine), 2.70 (s, 2H, imide), 7.01 (d, 1H, pyridine, *J* = 7.9 Hz), 7.19 (d, 1H, pyridine, *J* = 7.6 Hz), 7.70 (t, 1H, pyridine, *J* = 7.8 Hz)
XIX	2.21 (s, 3H, CH_3-pyridine), 2.94 (s, 4H, imide), 7.32 (dd, 1H, pyridine, *J* = 4.7 Hz), 7.67-7.70 (m, 1H, pyridine), 8.47 (dd, 1H, pyridine, *J* = 4.8 Hz)
XX	2.41 (s, 3H, CH_3-pyridine), 2.90 (s, 4H, imide), 7.16-7.19 (m, 2H, pyridine), 8.49 (d, 1H, pyridine *J* = 4.8 Hz)
XXI	2.59 (s, 3H, CH_3-pyridine), 2.90 (s, 4H, imide), 7.06(d, 1H, pyridine, *J* = 7.6 Hz), 7.22 (d, 1H, pyridine, J = 7.6 Hz), 7.74 (t, 1H, pyridine *J* = 7.6 Hz)
XXII	2.36 (s, 3H, CH_3-pyridine), 2.53 (s, 3H, CH_3), 2.88 (s, 4H, imide), 6.87 (s, 1H, pyridine), 7.04 (s, 1H, pyridine)

Development (ADD) Program using procedures described elsewhere (20, 21).

Phase I studies of the compounds investigated involved three testes: maximal electroshock seizure (MES), subcutaneous pentylenetrazole seizure (*sc*PTZ) and neurological toxicity (TOX). Male albino mice (CF #1 strain, weighing 18-25 g) were used as experimental animals.

In the MES test, an electrical stimulus (50 mA) of 0.2 s in duration was delivered via corneal electrodes primed with an electrolyte solution containing an anesthetic agent. Mice were tested using the following doses: 30, 100 and 300 mg/kg of compounds investigated. The compounds were injected intraperitoneally as a suspension in a 0.5% methylcellulose/water mixture, in a volume of 0.01 mL/g body weight. Abolition of the hindlimb tonic extensor component indicates the test compound's ability to inhibit MES-induced seizure spread.

The *sc*PTZ test utilizes of pentylenetetrazole (85 mg/kg). This produces clonic seizures lasting for a period of at least 5 s in 97% (CD_{97}) of animals tested. At the anticipated time of testing the pentylenetetrazole was administrated subcutaneously. The compounds tested were dissolved in 0.9% saline and injected intraperitoneally in mice at a vol-

Table 5. Anticonvulsant screening project (ASP) phase I in mice (**I**-**XXII**).

Compound No.	Intraperitoneal injection in mice[a]						ASP Class[c]
	MES		*sc*PTZ		TOX[b]		
	0.5[h]	4[h]	0.5[h]	4[h]	0.5[h]	4[h]	
I	300	-	-	-	300[1]	-	2
II	300	-	100[2]	-	100[1]	-	1
III	-	-	300[2]	-	100	-	2
IV	300	-	-	-	100[1]	-	2
V	300	-	-	-	-	-	2
VI	-	-	30	-	30	30	4
VII	-	-	-	-	300[1]	-	3
VIII	-	-	-	-	100	-	3
IX	300	-	300	-	-	-	2
X[d]	300	-	300	-	300[1]	-	2
XI	300	-	100	-	300[1]	-	1
XII	300	-	-	-	300[1]	-	2
XIII[d]	-	-	300	-	300	-	2
XIV	300	-	100	-	100	-	1
XV	300	-	300	-	100[1]	-	2
XVI	300	-	300	-	-	-	2
XVII	100	-	300	-	100[1]	-	1
XVIII[e]	300	-	300	-	300[1]	-	2
XIX	-	-	-	-	100	-	3
XX	-	-	-	-	-	-	3
XXI	-	-	-	-	-	-	3
XXII	-	-	-	-	-	-	3

[a] Doses of 30, 100 and 300 mg/kg were administrated. The figures in the Table indicate the minimal 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: [1] unable to grasp rotarod, [2] myoclonic jerks.
[c] 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.
[d] Compounds **X** and **XIII** active at a dose of 100 mg/kg in *sc*PTZ test at 0.25 h (**X** and **XIII**) or 1.0 h (**X**).
[e] Compound **XVIII** showed anti-MES protection at a dose of 100 mg/kg at 0.25 h.

ume of 0.01 mL/g body weight. The animals were observed over a 30 min period. Absence of clonic seizures in the observed time period indicated an ability of compounds to abolish the effect of pentylenetetrazole on seizure threshold.

A neurological toxicity test (TOX) induced by a compound was detected in mice using standardized rotarod test. Untreated control mice, when placed on the 6 rpm rotation rod, can maintain their equilibrium for a prolonged period of time. Neurological impairment can be demonstrated by the inability of mice to maintain equilibrium for 1 min in each of three successive trials.

According to the ADD program, the activity of the compounds investigated was classed with the following categories: active at doses of 100 mg/kg or less (class 1), active at doses greater than 100 mg/kg (class 2), inactive at 300 mg/kg (class 3). The results of preliminary screening for compounds **I**-**XXII** are presented in Table 5.

Compound **IX** underwent phase VIa in which was administrated orally into rats using four animals at a fixed dose of 30 mg/kg for both the MES and the rotarod toxicity tests. Rats were tested at five time periods: 0.25, 0.5, 1, 2 and 4 h after substance administration. The ratios of animals protected or toxic to animals tested were determined. The initial identification study in rats provides information as

Table 6. The results in rats after oral administration at a dose of 30 mg/kg.

Compound No.	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]
IX	1/4	1/4	1/4	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] Rotarod test for neurological toxicity, number of animals exhibiting toxicity/number of animals tested.

to whether the test substance is active or toxic at a dose of 30 mg/kg after *p.o.* administration. It discloses also the time of onset, the approximate time of peak effect (TPE) and the duration of anticonvulsant activity or neurotoxicity (Table 6).

Compound **XIII** was tested in the 6-Hz psychomotor seizure screen. For this test twenty mice are pre-treated *i.p.* with 100 mg/kg of the test substance. At time points: 0.25, 0.5, 1, 2 and 4 h after treatment, individual mice (4 in each time point) are challenged with sufficient current (32 mA at 6 Hz for 3 s), delivered through the corneal electrodes to induce a psychomotor seizures. This seizure is typically characterized by a minimal clonic phase followed by stereotyped behaviors. Animals which not display this symptoms are considered protected (22).

RESULTS

The profile of anticonvulsant activity of compounds **I-XXII** was established and the results of anticonvulsant identification studies in mice are shown in Table 5.

The compounds investigated **I-XXII** showed diversified anticonvulsant properties from active at a dose of 100 mg/kg or less (class 1 ASP) [**II**, **XI**, **XIV** and **XVII**], to active at a dose of 300 mg/kg (class 2 ASP) [**I**, **III-V**, **IX**, **X**, **XII**, **XIII**, **XV**, **XVI** and **XVIII**] and inactive ones (class 3 ASP) [**VII**, **VIII** and **XIX-XXII**].

The most potent anti-seizure protection was observed for 3,3-dialkyl-pyrrolidine-2,5-diones [**IX-XVIII**]. In this group of derivatives, except of **XII** and **XIII** active in the MES or *sc*PTZ screen, respectively, all other compounds were effective in both tests applied. In general, the most active were molecules with the methyl substituent at position-4 [**X** and **XVII**] or -6 [**XI, XIV** and **XVIII**] of the 2-pyridinyl moiety. These substances showed anti-MES protection at a dose of 100 mg/kg at 0.25 h [**XVIII**] or 0.5 h [**XVII**] and 300 mg/kg at 0.5 h [**X**, **XI**, **XIV** and **XVIII**]. The anti-*sc*PTZ activity was observed at a dose of 100 mg/kg at 0.25 h [**X** and **XIII**], 0.5 h [**XI** and **XIV**] or 1.0 h [**X**] and 300 mg/kg at 0.5 h [**X**, **XIII**, **XVII** and **XVIII**]. The 3-methyl isomers [**IX** and **XVI**] as well as compound **XV** with two methyl groups at position-4,6 of the 2-pyridinyl ring inhibited MES and *sc*PTZ seizures at a dose of 300 mg/kg at 0.5 h. The further investigations revealed that an introduction of spiro nucleus yielded less active compounds **I-IV**. Among these derivatives, the most effective was 1-(4-methyl-2-pyridinyl)-7-methyl-2-azaspiro[4.5]decane-1,3-dione [**II**] which showed the anti-*sc*PTZ and anti-MES protection at a dose of 100 mg/kg and 300 mg/kg, respectively. The other molecules were active only in MES [**I** and **IV**] or *sc*PTZ [**III**] screen at 0.5 h after intraperitoneal administration.

To evaluate the effect of the cycloalkyl fragment attached to the C3 spiro carbon atom on anticonvulsant activity of spirosuccinimides [**I-IV**] as well as compounds described previously (16), the series of 3-cyclohexyl-pyrrolidine-2,5-dione derivatives [**V-VIII**] has been synthesized. These molecules were designed as the analogs of the respective active spirosuccinimides. The results obtained revealed that the introduction of the cyclohexyl moiety as a flexible fragment at position-3 of the imide ring made the compounds less active [**V**] or inactive [**VII** and **VIII**]. Surprisingly, 1-(4-methyl-2-pyridinyl)-3-cyclohexyl-pyrrolidne-2,5-dione [**VI**] showed protection at a dose of 30 mg/k at 0.5 h in the *sc*PTZ test and was the most active derivative among all compound described herein. This molecule 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. As shown in Table 1, compounds **XIX**-X**XII** without substituents at the 3-position of pyrrolidine-2,5-dione ring were inactive.

In the neurotoxicity screen only compounds **V**, **IX**, **XVI** and **XX-XXI** were devoid of toxicity at a maximal dose administrated (300 mg/kg). The other derivatives were found to be toxic at a dose of 100 mg/kg [**II-IV**, **VIII**, **XIV**, **XV**, **XVII** and **XIX**] or

300 mg/kg [**I**, **VII**, **X**-**XIII**, **XV** and **XVIII**]. Mice were unable to grasp rotarod after administration of compounds **I**, **II**, **IV**, **VII**, **X**-**XII**, **XV**, **XVII** and **XVIII**.

Compound **IX**, randomly selected from derivatives active in *i.p*. screen in mice, was examined for its activity (MES test) and toxicity at a dose of 30 mg/kg after *p.o*. administration into rats. The tested substance showed marginal protection of 25% of animals at 0.25 h, 0.5 h and 1 h. The total duration of action of this compound was quite short within 0.25 h to 1 h. No evidence of neurological toxicity was observed at the dose of 30 mg/kg administrated orally (Table 6).

Compound **XIII** was chosen for the evaluation of anticonvulsant activity in the 6 Hz test. The selection was made randomly as a part of the search of molecules providing anti-6-Hz protection among chemically diversified compounds. The 6-Hz screen described previously as psychomotor seizure model reminiscent of aura of human patients with partial or limbic epilepsy (23-26), more recently has been validated as a model of therapy-resistant epilepsy (27). Furthermore, substances effective in the above test may reveal novel, unknown mechanism of anticonvulsant activity. Compound **XIII** administrated intraperitoneally at a dose of 60 mg/kg into mice protected 100% of animals tested only at 0.25 h, however, at the same time point caused the motor impairment.

In conclusion, the results obtained revealed that a number of new 1-(2-pyridinyl)- derivatives of succinimides were effective in MES and/or *sc*PTZ screens. The most active were compounds with two alkyl substituents at position-3 of pyrrolidine-2,5-dione, which may be recognized as close analogues of ethosuximide (2-ethyl-2-methyl-pyrrolidine-2,5-dione), known as one of the not numerous anticonvulsants effective in the treatment of absence epilepsy (28, 29). 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 for anticonvulsant activity of that type of compounds. The presence of methyl groups especially in position-4 or -6 of 2-pyridinyl moiety seems to be favorable for anti-seizures properties. This was contrary to our earlier experiments (10-16) indicating the important role of the 3-methyl substitution. The lack of any substituents at position-3 of succinimide moiety causes the loss of anticonvulsant activity as well as toxicity.

In summary, none of the compounds described herein was more potent that the molecules with an aryl moiety at position-3 of pyrrolidine-2,5-dione ring (10-12), however, the interesting pharmacological results encourage to continue further investigations in this group of derivatives.

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