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SYNTHESIS AND ANTICONVULSANT PROPERTIES OF NEW N-PHENYLAMINO DERIVATIVES OF 2-AZASPIRO[4.4]NONANE, 2-AZASPIRO[4.5]DECANE-1,3-DIONE AND 3-CYCLOHEXYL-PYRROLIDINE-2,5-DIONE. PART IV

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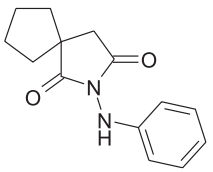
Abstract: To continue our systematic SAR studies a series of N-phenylamino derivatives of 2-azaspiro[4.4]nonane-, 2-azaspiro[4.5]decane-, 6-methyl-2-azaspiro[4.5]decane-1,3-dione and 3-cyclohexylpyrrolidine-2,5-dione were synthesized and tested for their anticonvulsant activity in the maximum electroshock seizure (MES) and subcutaneous metrazole seizure threshold (sc. Met) tests. Among those molecules the most potent were N-(4-methylphenyl)-amino-2-azaspiro[4.4]nonane-1,3-dione [**V**], N-(2-trifluoromethylphenyl)-amino-2-azaspiro[4.4]nonane-1,3-dione [**VI**], N-(3-methylphenyl)-amino-2-azaspiro[4.5]decane-1,3-dione [**VIII**] and N-(4-methylphenyl)-amino-6-methyl-2-azaspiro[4.5]decane-1,3-dione [**XIV**], which inhibited the seizures mainly in the sc. Met test. The obtained results revealed that anticonvulsant activity depended on the presence and the position of the methyl or trifluoromethyl groups at the aryl moiety, as well as the size and the manner of attachment of the cycloalkyl system at the position-3 of the pyrrolidine-2,5-dione ring.

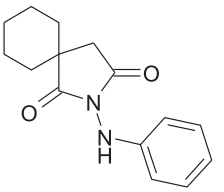
Keywords: anticonvulsant activity; 2-azaspiro[4.4]nonane-1,3-dione; 2-azaspiro[4.5]decane-1,3-dione; 3-cyclohexyl-pyrrolidine-2,5-dione; spirosuccinimides

One of the classes of compounds with well documented anticonvulsant activity are spirosuccinimide derivatives (1-4). Based on this facts, in our earlier studies we have described anticonvulsant properties of many 2-azaspiro[4.4]nonane- and [4.5]decane-1,3-diones differently substituted at the imide nitrogen atom (5-10). The structure-activity relationship studies conducted with these groups of compounds revealed that their activity were highly dependend on the size of the cycloalkyl system attached to the C3 spiro carbon atom and the kind and position of the substituents at the aromatic ring. Moreover, it was proved that the imine (-NH-) linker joining the endocyclic nitrogen atom and aromatic moiety increased the anticonvulsant activity in comparison to respective methylene (-CH_2-) analogues (6). Among those derivatives the most active was N-phenylamino-2-azaspiro[4.4]nonane-1,3-dione **[I]** which structure is presented below:



I
ED_{50} = 76.27 mg/kg
(MES test)

The change of the size of a cycloalkyl unit to cyclohexane resulted in less active N-phenylamino-2-azaspiro[4.5]decane-1,3-dione:



II
300 mg/kg
(sc. Met test)

Regarding the above molecules as lead structures, in this work we have designed, synthesized and tested for their anticonvulsant activity a new series of 2-azaspiro[4.4]nonane-, 2-azaspiro[4.5]decane- and 6-methyl-2-azaspiro[4.5]decane-1,3-diones with phenylamino moiety at the nitrogen atom. On the other hand, to investigate the influence of the cycloalkyl system, attached to the succinimide through the C3 spiro carbon atom, on the anticonvulsant activity, we obtained a series of derivatives with cyclohexyl moiety as a flexible fragment at 3-position of pyrrolidine-2,5-dione ring.

The starting cyclopentane-, cyclohexane-, (2-methylcyclohexane)-1-carboxy-1-acetic acids and 2-cyclohexylsuccinic acid were prepared as reported

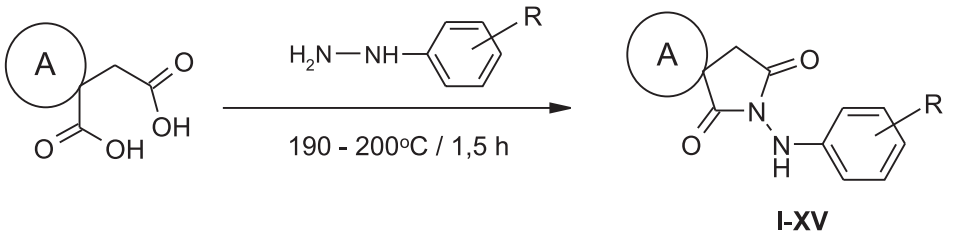
previously (11, 12). The final compounds [**III-XVIII**] were obtained in a one-pot cyclization reaction of the prepared dicarboxylic acids and appropriately substituted phenylhydrazines by heating them at ca. 190-200°C for 1.5 h. The synthetic procedures are shown in Schemes 1 and 2.

The ^{1}H NMR spectra of the compounds synthesized were studied and revealed a few characteristic chemical shifts. The protons of cycloalkyl rings were observed as multiplets within a range of 0.97-2.17 ppm. In the series of 2-azaspiro[4.4]nonane- and 2-azaspiro[4.5]decane-1,3-dione derivatives [**III-X**] the chemical shifts of imide protons were shown as singlets ranging from 2.65 to 2.75 ppm, whereas in a group of 6-methyl-2-azaspiro[4.5] decane-1,3-diones [**XI-XV**] appeared as doublets within a range of 2.46-2.74 ppm. The resonance signals of protons of succinimide moiety [**XVI-XVIII**] were recorded as multiplets within a range of 2.58-2.93 ppm. For all the compounds the protons of -NH- linkers were observed as broad singlets ranging from 6.01 to 6.60 ppm. The resonance signals of protons of methyl groups at the aryl moiety were recorded as singlets, depending on the position within a range of 2.34-2.35 ppm, 2.27-2.28 and at 2.26 ppm for the *ortho*, *meta* and *para* isomers, respectively. The protons of methyl groups at position-6 of the 2-azaspiro[4.5]decane-1,3-dione moiety [**XI-XV**] were shown as doublets ranging from 0.80 to 0.86 ppm. The resonance signals of aromatic protons were well separated and were observed within a range of 6.45-7.56 ppm. For the details see Tables 2, 3 and 4.

EXPERIMENTAL

Chemistry

Melting points (m.p.) were determined with an Electrothermal digital melting point apparatus and are uncorrected. The chemical structures of compounds were confirmed by elemental and spectral analyses. ^{1}H NMR spectra were obtained on a



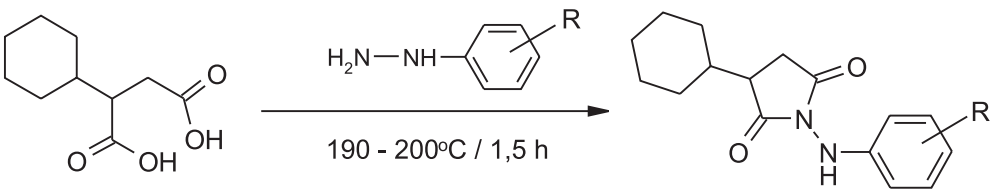
I-XV

Ring A = cyclopentane **I, III-VI**
Ring A = cyclohexane **II, VII-X**
Ring A = metylocyclohexane **XI-XV**

No.	I*, II*, XI	III, VII, XII	IV, VII, XII	V, IX, XIV,	VI, X, XV
R	H	2-CH_3	3-CH_3	4-CH_3	2-CF_3

* For physicochemical and spectral data see reference (6)

Scheme 1.



XVI-XVIII

No.	XVI	VII	VII
R	H	2-CH_3	4-CH_3

Scheme 2.

Varian Mercury 300 MHz spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$. Chemical shifts were reported as parts per million (δ ppm) from $(CH_3)_4Si$ (TMS) as an internal standard. Signal multiplicities are represented by the following abbreviations: s (singlet), brs (broad singlet), d (doublet), t (triplet), m (multiplet).

Elemental analyses for C, H, N were carried out with an Elementar Vario EL III apparatus (Hanau, Germany), and were within ±0.4% of the theoretical values.

The purity of the compounds was checked by the thin-layer chromatography (TLC) performed on Merck silica gel GF_{254} aluminium sheets, using the developing system: chloroform:acetone (9:1, v/v). Spots were detected by their absorption under UV (254 nm) light and by visualization with 0.05 mol I_2 in 10% HCl.

GENERAL PROCEDURE FOR THE PREPARATION OF N-PHENYLAMINO-2-AZASPIRO[4.4]NONANE-, 2-AZASPIRO[4.5]DECANE-, 6-METHYL-2-AZASPIRO[4.5]DECANE-1,3-DIONES [**III-XV**] AND 3-CYCLOHEXYLPYRROLIDINE-2,5-DIONES [**XVI-XVIII**]

To a suspension of cyclopentane-, cyclohexane-, (2-methylcyclohexane)-1-carboxy-1-acetic acid or 2-cyclohexylsuccinic acid (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 and water was simultaneously distilled off. When the water was com-

Table 1. Physicochemical and analytical data for compounds **III-XVIII**

No.	Molecular Formula Weight	Yield mp.[°C]	Analysis (calcd/found)			R_f
			%C	%H	%N	
III	$C_{15}H_{18}N_2O_2$ 258.32	51 170-172	69.74 69.70	7.02 6.98	10.84 10.79	0.66
IV	$C_{15}H_{18}N_2O_2$ 258.32	53 126-128	69.74 69.71	7.02 7.00	10.84 10.80	0.73
V	$C_{15}H_{18}N_2O_2$ 258.32	48 134-136	69.74 69.72	7.02 6.99	10.84 10.78	0.76
VI	$C_{15}H_{15}F_3N_2O_2$ 312.29	47 145-147	57.69 57.60	4.84 4.80	8.97 8.91	0.85
VII	$C_{16}H_{20}N_2O_2$ 272.35	54 154-156	70.56 70.49	7.40 7.32	10.29 10.18	0.71
VIII	$C_{16}H_{20}N_2O_2$ 272.35	50 131-133	70.56 70.49	7.40 7.41	10.29 10.19	0.75
IX	$C_{16}H_{20}N_2O_2$ 272.35	49 156-158	70.56 70.52	7.40 7.42	10.29 10.20	0.80
X	$C_{16}H_{17}F_3O_2N_2$ 326.33	48 191-193	58.89 58.79	5.25 5.30	8.58 8.49	0.92
XI	$C_{16}H_{20}N_2O_2$ 272.35	51 121-123	70.56 70.46	7.40 7.32	10.29 10.25	0.73
XII	$C_{17}H_{22}N_2O_2$ 286.38	53 169-171	71.30 71.21	7.74 7.69	9.78 9.70	0.75
XIII	$C_{17}H_{22}N_2O_2$ 286.38	46 152-154	71.30 71.12	7.74 7.52	9.78 9.69	0.81
XIV	$C_{17}H_{22}N_2O_2$ 286.38	50 128-130	71.30 71.21	7.74 7.69	9.78 9.71	0.75
XV	$C_{17}H_{19}F_3O_2N_2$ 340.35	48 195-197	59.99 59.89	5.63 5.61	8.23 8.15	0.92
XVI	$C_{16}H_{20}N_2O_2$ 272.35	54 168-170	70.56 70.45	7.40 7.42	10.29 10.17	0.83
XVII	$C_{17}H_{22}N_2O_2$ 286.38	52 124-126	71.30 71.19	7.74 7.71	9.78 9.70	0.64
XVIII	$C_{17}H_{22}N_2O_2$ 286.38	51 163-165	71.30 71.21	7.74 7.70	9.78 9.75	0.82

Table 2. ^{1}H NMR spectral data of compounds **III**-**X**

No.	R	^{1}H NMR δ (ppm)/ $CDCl_3$
III	2-CH_3	1.74-2.22 (8H, m, cyclopentane), 2.34 (3H, s, -CH_3), 2.72 (2H, s, imide), 6.03 (1H, brs, -NH-), 6.47 (1H, d, $H_{arom.}$, *J*=7.95 Hz), 6.87-6.92 (1H, m, $H_{arom.}$), 7.06-7.12 (2H, m, $H_{arom.}$)
IV	3-CH_3	1.73-2.23 (8H, m, cyclopentane), 2.28 (3H, s, -CH_3), 2.71 (2H, s, imide), 6.06 (1H, brs, -NH-), 6.53-6.58 (2H, m, $H_{arom.}$), 6.78-6.80 (1H, m, $H_{arom.}$), 7.12 (1H, t, $H_{arom.}$, *J*=7.69 Hz)
V	4-CH_3	1.71-2.21 (8H, m, cyclopentane), 2.26 (3H, s, -CH_3), 2.69 (2H, s, imide), 6.06 (1H, brs, -NH-), 6.68-6.72 (2H, m, $H_{arom.}$), 7.03-7.06 (2H, m, $H_{arom.}$)
VI	2-CF_3	1.75-2.25 (8H, m, cyclopentane), 2.75 (2H, s, imide), 6.55 (1H, d, $H_{arom.}$, *J*=8.20 Hz), 6.60 (1H, brs, -NH-), 7.03 (1H, t, $H_{arom.}$, *J*=7.18 Hz), 7.39 (1H, t, $H_{arom.}$, *J*=7.18 Hz), 7.56 (1H, d, $H_{arom.}$, *J*=7.95 Hz)
VII	2-CH_3	1.37-1.92 (10H, m, cyclohexane), 2.34 (3H, s, -CH_3), 2.69 (2H, s, imide), 6.01 (1H, brs, -NH-), 6.45 (1H, d, $H_{arom.}$, *J*=7.44 Hz), 6.86-6.91 (1H, m, $H_{arom.}$), 7.08-7.12 (2H, m, $H_{arom.}$)
VIII	3-CH_3	1.36-1.91 (10H, m, cyclohexane), 2.28 (3H, s, -CH_3), 2.67 (2H, s, imide), 6.04 (1H, brs, -NH-), 6.52-6.57 (2H, m, $H_{arom.}$), 6.77-6.80 (1H, m, $H_{arom.}$), 7.11 (1H, t, $H_{arom.}$, *J*=7.82 Hz)
IX	4-CH_3	1.35-1.90 (10H, m, cyclohexane), 2.26 (3H, s, -CH_3), 2.65 (2H, s, imide), 6.04 (1H, brs, -NH-), 6.67-71 (2H, m, $H_{arom.}$), 7.02-7.05 (2H, m, $H_{arom.}$)
X	2-CF_3	1.35-1.92 (10H, m, cyclopentane), 2.71 (2H, s, imide), 6.54 (1H, d, $H_{arom.}$, *J*= 8.20 Hz), 6.59 (1H, brs, -NH-), 7.02 (1H, t, $H_{arom.}$, *J*=7.56 Hz), 7.38 (1H, t, $H_{arom.}$, *J*=7.56 Hz), 7.55 (1H, d, $H_{arom.}$, *J*=7.95 Hz)

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

No.	R	^{1}H NMR δ (ppm)/ $CDCl_3$
XI	H	0.82 (3H, d, -CH_3, *J*=6.67 Hz), 1.00-1.94 (8H, m, cyclohexane), 2.00-2.12 (1H, m, cyclohexane), 2.48 (1H, d, imide, *J*=18.72 Hz), 2.71 (1H, d, imide, *J*=18.72 Hz), 6.11 (1H, brs, -NH-), 6.76-6.79 (2H, m, $H_{arom.}$), 6.98 (1H, t, $H_{arom.}$, *J*=7.43 Hz), 7.21-7.26 (2H, m, $H_{arom.}$)
XII	2-CH_3	0.84 (3H, d, -CH_3, *J*=6.67 Hz), 1.00-1.94 (8H, m, cyclohexane), 2.00-2.16 (1H, m, cyclohexane), 2.35 (3H, s, -CH_3), 2.49 (1H, d, imide, *J*=18.72 Hz), 2.72 (1H, d, imide, *J*=18.72 Hz), 6.04 (1H, brs, -NH-), 6.49 (1H, d, $H_{arom.}$, *J*=7.69 Hz), 6.86-6.91 (1H, m, $H_{arom.}$), 7.07-7.25 (2H, m, $H_{arom.}$)
XIII	3-CH_3	0.82 (3H, d, -CH_3, *J*=6.67 Hz), 1.00-1.93 (8H, cyclohexane), 2.00-2.09 (1H, m, cyclohexane), 2.27 (3H, s, -CH_3) 2.48 (1H, d, imide, *J*=18.46 Hz), 2.70 (1H, d, imide, *J*=18.46 Hz), 6.07 (1H, brs, -NH-), 6.55-6.58 (2H, m, $H_{arom.}$), 6.77-6.80 (1H, m, $H_{arom.}$), 7.11 (1H, t, $H_{arom.}$, *J*=7.56 Hz)
XIV	4-CH_3	0.80 (3H, d, -CH_3, *J*=6.67 Hz), 0.99-1.98 (8H, m, cyclohexane), 2.00-2.09 (1H, m, cyclohexane), 2.26 (3H, s, -CH_3), 2.46 (1H, d, imide, *J*=18.46 Hz), 2.68 (1H, d, imide, *J*=18.46 Hz), 6.05 (1H, brs, -NH-), 6.69-6.72 (2H, m, $H_{arom.}$), 7.02-7.05 (2H, m, $H_{arom.}$)
XV	2-CF_3	0.86 (3H, d, -CH_3), *J*=6.67 Hz), 0.97-1.95 (8H, m, cyclohexane), 2.02-2.14 (1H, m, cyclohexane), 2.52 (1H, d, imide, *J*=18.72 Hz), 2.74 (1H, d, imide, *J*=18.72 Hz), 6.57 (1H, d, $H_{arom.}$, *J*=8.72 Hz), 6.60 (1H, brs, -NH-), 7.02 (1H, t, $H_{arom.}$, 7.31 Hz), 7.36 (1H, t, $H_{arom.}$, *J*=7.82 Hz), 7.55 (1H, d, $H_{arom.}$, *J*=7.95 Hz)

pletely removed, the temperature of the reaction mixture was raised up to 180°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, v/v) as an eluent. After evaporation of the solvents, the oily products were recrystallized from isopropanol. Physicochemical data, yields, elemental analysis and R_f values are present-

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

No.	R	^{1}H NMR δ (ppm)/ $CDCl_3$
XVI	H	1.03-1.78 (10H, m, cyclohexane), 1.97-2.17 (1H, m, cyclohexane), 2.60-2.92 (3H, m, imide), 6.10 (1H, brs,-NH-), 6.75-6.79 (2H, m, $H_{arom.}$), 6.98 (1H, t, $H_{arom.}$, *J*=7.30 Hz), 7.21-7.26 (2H, m, $H_{arom.}$)
XVII	2-CH_3	1.04-1.79 (10H, m, cyclohexane), 1.97-2.06 (1H, m, cyclohexane), 2.34 (3H, s, -CH_3), 2.61-2.93 (3H, m, imide), 6.02 (1H, brs, -NH-), 6.46-6.49 (1H, m, $H_{arom.}$), 6.86-6.91 (1H, m, $H_{arom.}$), 7.08-7.12 (2H, m, $H_{arom.}$)
XVIII	4-CH_3	1.02-1.81 (10H, m, cyclohexane), 1.95-2.17 (1H, m, cyclohexane), 2.26 (3H, s, -CH_3), 2.58-2.91 (3H, m, imide), 6.04 (1H, brs, -NH-), 6.68-6.73 (2H, m, $H_{arom.}$), 7.02-7.05(2H, m, $H_{arom.}$)

Table 5. Anticonvulsant screening project (ASP) phase I test in mice of compounds **III-X**

	Dose	MES[a]		sc.Met[b]		Tox[c]		ASP[d]
No.	mg/kg	0.5h	4h	0.5h	4h	0.5h	4h	Class
III	30	0/1	0/1	0/1	0/1	1/4	0/2	3
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	300	0/1	0/1	0/1	0/1	0/4	0/2	
IV	30	0/1	0/1	0/1	0/1	0/4	0/2	2
	100	0/3	0/1	0/1	0/1	0/8	0/4	
	300	1/1	0/3	0/1	0/1	1/4[1]	0/2	
V	30	0/1	0/1	0/1	0/1	0/4	0/2	2
	100	0/3	0/3	0/1	0/1	1/8	0/4	
	300	0/1	0/1	3/5[2]	0/1	1/4	0/2	
VI	30	0/1	0/1	0/1	0/1	0/4	0/2	2
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	300	0/1	0/1	4/5[2]	0/1	1/4	0/2	
VII	30	0/1	0/1	0/1	0/1	0/4	0/2	3
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	100	0/1	0/1	0/1	0/1	2/4	0/2	
VIII	30	0/1	0/1	0/1	0/1	0/4	0/2	2
	100	0/3	0/3	0/1	0/1	4/8	1/4	
300	1/1	0/1	0/1	5/5[2]	0/1	2/4[1]	0/2	
IX	30	0/1	0/1	1/5[2]	0/1	0/4	0/2	1
	100	0/3	0/3	0/1	0/1	1/8	0/4	
	300	0/1	0/1	0/1	0/1	0/4	0/2	
X	30	0/1	0/1	0/1	0/1	0/4	0/2	3
	100	0/3	0/3	0/1	0/1	2/8	0/4	
	300	0/1	0/1	0/1	0/1	2/4[1]	0/2	

[a)] Maximal electroshock test (number of animals protected/ number of animals tested); [b)] Subcutaneous metrazole test; [c)] Rotorod toxicity (number of animals exhibiting toxicity/ number of animals tested);[d)]The classification are as follows: anticonvulsant activity at doses 100 mg/kg or less (calss 1); anticonvulsant activity at doses greater than 100 mg/kg (class 2); compound inactive at 300mg/kg (class 3).
Response comments: [1] unable to grasp rotorod, [2] myoclonic jerks, [3] tremors

Table 6. Anticonvulsant screening project (ASP) phase I test in mice of compounds **XI-XVIII**

No.	Dose mg/kg	MES[a] 0.5h	MES[a] 4h	sc.Met[b] 0.5h	sc.Met[b] 4h	Tox[c] 0.5h	Tox[c] 4h	ASP[d] Class
XI	30	0/1	0/1	0/1	0/1	0/4	0/2	2
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	300	0/1	0/1	0/1	0/1	3/4[3]	0/2	
XII	30	0/1	0/1	0/1	0/1	0/4	0/2	3
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	300	1/1	0/1	0/1	0/1	0/4	0/2	
XIII	30	0/1	0/1	0/1	0/1	1/4	1/2	3
	100	0/3	0/3	0/1	0/1	4/8	1/4	
	300	0/1	0/1	3/5[2]	0/1	3/4	0/2	
XIV	30	0/1	0/1	1/5[2]	0/1	0/4	0/2	1
	100	0/3	0/3	0/1	0/1	3/8	0/4	
	300	0/1	0/1	1/1[2]	0/1	2/4	1/2	
XV	30	0/1	0/1	0/1	0/1	0/4	0/2	1
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	300	0/1	0/1	0/1	0/1	2/4	2/2	
XVI	30	0/1	0/1	0/1	1/1[2]	0/4	0/2	3
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	300	1/1	0/1	1/1	0/1	0/4	0/2	
XVII	30	0/1	0/1	0/1	0/1	0/4	0/2	2
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	300	0/1	0/1	1/1[2]	0/1	3/4	0/2	
XVIII	30	0/1	0/1	0/1	0/1	0/4	0/2	3
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	300	0/1	0/1	0/1	0/1	3/4	0/2	

[a], [b], [c], [d] and response comments see Table 5.

ed in Table 1. ^{1}H NMR spectral data are shown in Tables 2, 3 and 4.

PHARMACOLOGY

All obtained compounds 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), by use of testing procedures which have been described elsewhere (13, 14). Phase I studies of the investigated compounds involved three tests: maximal electroshock (MES), subcutaneous metrazole (sc. Met) and rotorod test for neurological toxicity (TOX). All the compounds were injected intraperitoneally to mice, as a suspension in 0.5% methylcellulose, at the dose levels of 30, 100, and 300 mg/kg. The compounds were classified into following categories: anticonvulsant activity at 100 mg/kg or less (class 1), anticonvulsant activity at doses greater than 100 mg/kg (class 2) and compounds inactive at 300 mg/kg (class 3). The results are shown in Tables 5 and 6.

RESULTS

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

The compounds investigated revealed diversified anticonvulsant properties (Tables 5 and 6).

Except for compounds **IV** and **XI**, effective only in the MES test at a dose of 300 mg/kg (1/1 animals protected at 0.5 h), all other derivatives showed protection especially in sc. Met screen. The most active N-(3-methylphenyl)-amino-2-azaspiro[4.5] decane-1,3-dione [**VIII**] inhibited both the metrazole (5/5 animals protected at 0.5 h) and electrically

provoked (1/1 animals protected at 0.5 h) seizures at a dose of 300 mg/kg, whereas its 6-methyl analogue [**XIII**] was inactive. The comparable results were obtained for N-(4-methylphenyl)-amino-2-azaspiro[4.4]nonane-1,3-dione [**V**], which protected 3/5 animals at a dose of 300 mg/kg in the sc. Met screen. The change of the size of a cycloalkyl unit to cyclohexane [**IX**], as well as introduction of the methyl group into position-6 [**XIV**] decreased the anticonvulsant activity. Both **IX** and **XIV** revealed only marginal protection at doses of 30 mg/kg (1/5 animals protected at 0.5 h), however, according to the procedures of Anticonvulsant Drug Development Program were ascribed to 1 ASP class. Compound **XIV** was also effective at a dose of 300 mg/kg (1/1 animal protected at 0.5 h).

As had been reported previously (8), the presence of the trifluoromethyl substituents at position-2 of the aryl ring attached to the imide nitrogen atom, directly or through the -CH_2- linker, proved to enhance the anticonvulsant activity. However, in the present study only N-(2-trifluoromethylphenyl)-amino-2-azaspiro[4.4]nonane-1,3-dione [**VI**] showed the anti-sc. Met activity at a dose of 300 mg/kg (4/5 animals protected at 0.5 h). The enlargement of size of the cycloalkyl unit to cyclohexane [**X**] as well as introduction of the methyl group into 6-position [**XV**] yielded an inactive compound **X** and derivative **XV**, that revealed protection only at a dose of 30 mg/kg (1/1 animal protected at 0.5 h), without activity at doses of 100 mg/kg and 300 mg/kg. Surprisingly enough, all the 2-CH_3 analogues [**III**, **VII**, **XII**] were found to be inactive. It was in contrary to earlier experiments indicating that the presence of this substituent at the position-2 of the aryl ring is important for the anticonvulsant activity (6-8, 15).

To evaluate the role of the cycloalkyl fragment attached to the C3 spiro carbon atom in aspect of influence on anticonvulsant activity of spirosuccinimides described above, a series of 3-cyclohexylpyrrolidine-2,5-diones [**XVI-XVIII**] has been synthesized. These molecules were designed as analogues of respective active or inactive spirosuccinimides. The obtained results revealed that the introduction of the cyclohexyl moiety as a flexible fragment at 3-position of the imide ring decreased the anticonvulsant efficacy. Moreover, in opposite to the data observed for azaspiranes, in this series only derivative **XVII** with the methyl group at 2-position of the aryl moiety inhibited the sc. Met seizures at a dose of 300 mg/kg (1/1 animal protected at 0.5 h), whereas 4-CH_3 analogue [**XVIII**] as well as unsubstituted compound [**XVI**] were found to be inactive.

In the neurotoxicity screen only compounds **XII** and **XVI** were devoid of toxicity at the maximum administrated dose (300 mg/kg). The other derivatives were found to be neurotoxic at a dose of 30 mg/kg [**III**, **XIII**], 100 mg/kg [**V**, **VIII**-**X**, **XIII**, **XV**] and 300 mg/kg [**IV**-**VIII**, **X**, **XI**, **XIII**-**XV**, **XVII**, **XVIII**]. The mice were unable to grasp rotorod after administration of compounds **IV**, **VIII** and **X** (300 mg/kg at 0.5 h). All the compounds effective in the sc. Met test induced myoclonic jerks at a dose at which the anticonvulsant activity was demonstrated.

In conclusion, the results obtained, revealed that a number of novel N-phenylamino derivatives of spirosuccinimides were moderately effective especially in sc. Met screen, however, none of them was more potent than unsubstituted N-phenylamino-2-azaspiro[4.4]nonane-1,3-dione [**I**], used as a lead structure. The activity of those compounds 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. In general, the methyl groups in position-3 or -4 increased anticonvulsant activity, whereas the *ortho* isomers were found inactive. The introduction of the trifluoromethyl group, which is known as bioactive structural element (8,16), did not improve anticonvulsant properties significantly. Furthermore, the most active were compounds with cyclopentane unit at the C3 spiro carbon, whereas the enlargement of size of the cycloalkyl system to cyclohexane, as well as the presence of additional methyl group at position-6 decreased anticonvulsant efficacy. The comparison of the 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 the anticonvulsant activity of that type of compounds.

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