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Impact of aromatic substitution on the anticonvulsant activity of new N-(4-arylpiperazin-1-yl)-alkyl-2-azaspiro[4.5]decane-1,3-dione derivatives

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Abstract:
A series of N-[(4-arylpiperazin-1-yl)-alkyl]-2-azaspiro[4.5]decane-1,3-dione derivatives were synthesized and evaluated for their anticonvulsant and neurotoxic properties. The main modifications to that series of compounds consisted in the introduction of an aromatic area to the cyclohexane ring as a flexible fragment with conformational freedom (**1a–h**), or as a rigidified skeleton (**2a–h**). Except for N-[3-(4-phenylpiperazin-1-yl)-propyl]-8-phenyl-2-aza-spiro[4.5]decane-1,3-dione derivative (**1e**), all the other compounds displayed anticonvulsant activity in the MES test, but some of them (**1c, 2f** and **2g**) were found to be neurotoxic at a dose of 30 mg/kg, irrespective of their activity. The most potent and relatively weakly neurotoxic analogues of that series, i.e. N-[2-{4-(3-chlorophenyl)-piperazin-1-yl}-ethyl]-[7,8-f]benzo-2-aza-spiro[4.5]decane-1,3-dione (**2c**) and N-[3-{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-propyl]-[7,8-f] benzo-2-aza-spiro[4.5]decane-1,3-dione (**2h**) had ED_{50} values of 205 mg/kg (**2c**) and 23 mg/kg (**2h**) respectively, in the MES-test in mice, and showed higher protection than magnesium valproate (ED_{50} = 211 mg/kg), used as a standard substance.

Key words:
anticonvulsant activity, 2-azaspiro[4.5]decane-1,3-diones, pyrrolidine-2,5-diones, spirosuccinimides

Introduction

Recently a large number of active anticonvulsant compounds with diverse chemical structures have been synthesized [5, 6, 16, 22, 24]. The comparison of the structural characteristics of above molecules have identified a common pattern, defined as a 5- or a 6-membered heterocyclic ring, one or two carbonyl groups as well as an aromatic system [23].

Following these findings, in the course of developing new, potentially anticonvulsant compounds, our attention was focused on a group of 3-substituted pyrrolidine-2,5-diones with different substituents at the nitrogen atom [14, 15]. Recently we demonstrated that a great number of 3-arylpyrrolidine-2,5-dione derivatives with a 4-aryl-piperazin-1-yl-alkyl moiety at the imide nitrogen atom exhibited anticonvulsant activity, especially in the maximal electroshock (MES) test (e.g. N-[4-(3-chlorophenyl)-piperazin-1--yl-methyl]- 3-(2-chlorophenyl)-pyrrolidine-2,5-dione; ED_{50} = 14.20 mg/kg) [13]. On the other hand, studies with spirosuccinimides indicated that the introduction of a spiro nucleus into the 3-position of a pyrrolidine-2,5-

dione ring enhanced anticonvulsant activity [1, 3, 17, 18, 21].

On the basis of the above findings, we previously replaced the 3-aryl ring with a cycloalkyl fragment connected to pyrrolidine-2,5-dione by a spiro carbon atom, yielding N-[(4-aryl-piperazin-1-yl)-alkyl]-2-azaspiro[4.4]nonane and [4.5]decane-1,3-dione derivatives. However, none of those compounds was more potent than the derivatives with an aromatic ring at the same position [10, 11]. Thus, in an attempt to obtain new spiro compounds with enhanced activity, in the present study we decided to introduce an aromatic structure to the cyclohexane ring as a flexible fragment (**1a–h**), or as a rigid skeleton (**2a**–**h**) in order to determine whether such modifications would enhance anticonvulsant activity. Furthermore, we examined the impact of the kind of substituents at the 4-arylpiperazine moiety, especially the bioactive -CF_3 group, on its anticonvulsant efficacy [16, 19].

Materials and Methods

CHEMICAL PART

All the chemicals and solvents were purchased from Sigma-Aldrich. Melting points (m.p.) were determined in electrothermal digital melting point apparatus and are presented uncorrected. ^{1}H-NMR spectra were obtained in a Varian Mercury spectrometer operating at 300 MHz. Chemical shifts were given in ppm from tetramethylsilane (TMS) as an internal standard. Elemental analyses for C, H, N were carried out using a Perkin-Elmer model 240c analyser, and were within ± 0.4% of the theoretical values. Purity and homogeneity were checked by TLC, performed on Merck silica gel GF_{254} aluminium plates.

The starting 4-phenylcyclohexane-1-carboxy-1-acetic acid was synthesized according to the procedures described previously [3], 3,4-dihydro-1(*2H*) naphthalene-2-carboxy-2-acetic acid was prepared by the method reported by Faust [4].

The preparation of 1-(2-aminoethyl)- and 1-(3-aminopropyl)-4-arylpiperazine was previously reported [2]. The synthesis and physicochemical data of compounds **2e** and **2g** have been described recently [12].

General procedure for the preparation of the N-[(4-arylpiperazin-1-yl)-alkyl]-derivatives of 8-phenyl- and [7,8-f]benzo-2-aza-spiro[4.5]decane-1,3-dione (1a-h and 2a–h)

The obtained 4-phenylcyclohexane-1-carboxy-1-acetic acid (**1**) or 3,4-dihydro-1*(2H)*naphthalene-2-carboxy-2-acetic acid (**2**) (10 mmol) was dissolved in water, and the appropriately substituted 1-(2-aminoethyl)- or 1-(3-aminopropyl)-4-arylpiperazine (10 mmol) was added. The mixture was heated in an oil bath and the water was simultaneously distilled. After the water was completely removed, the temperature of reaction was raised up to 190–200°C and maintained at that temperature for 1.5 h. The precipitated crude products were crystallized from 96% ethanol. Free bases were converted into hydrochloride salts in anhydrous ethanol saturated with the HCl gas, and were crystallized from anhydrous ethanol (Fig. 1).

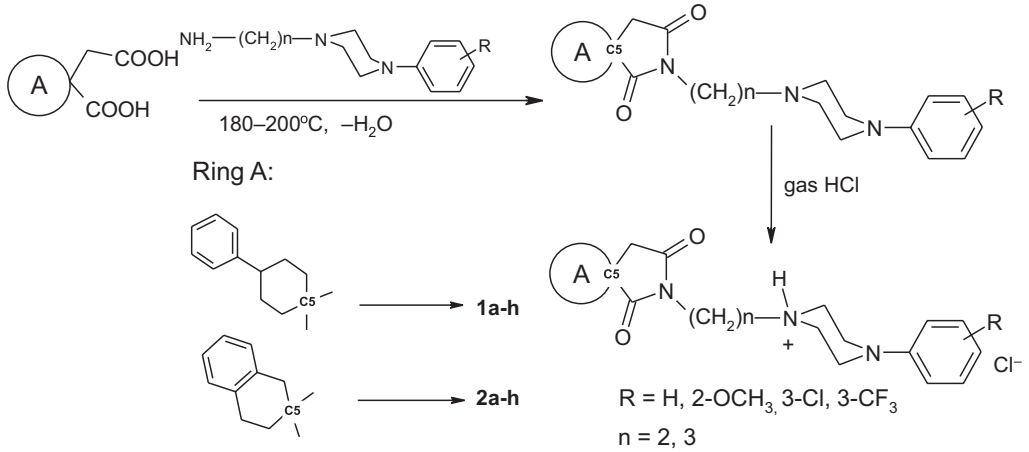
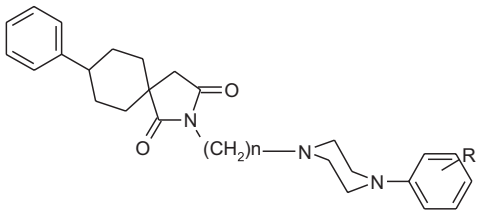
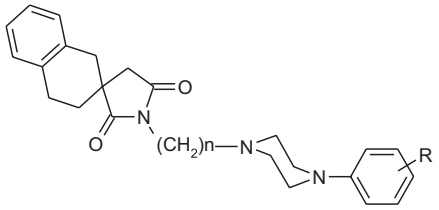


Fig. 1. Synthetic protocol of the target compounds

Tab. 1. Structures and physicochemical data of the synthesized compounds



1a-h



2a-h

Compound	n	R	Yield (%)	Mp (C)	Molecular Formula[a]	R_f[b]
1a	2	H	67	216–218	$C_{27}H_{33}O_2N_3$ x HCl	0.69
1b	2	2-OCH_3	57	230–232	$C_{28}H_{35}O_3N_3$ x HCl	0.72
1c	2	3-Cl	62	243–245	$C_{27}H_{32}O_2N_3Cl_1$ x HCl	0.74
1d	2	3-CF_3	55	203–205	$C_{28}H_{32}O_2N_3F_3$ x HCl	0.77
1e	3	H	72	237–239	$C_{28}H_{35}O_2N_3$ x HCl	0.72
1f	3	2-OCH_3	59	235–237	$C_{29}H_{37}O_3N_3$ x HCl	0.72
1g	3	3-Cl	63	228–230	$C_{28}H_{34}O_2N_3Cl_1$ x HCl	0.75
1h	3	3-CF_3	52	211–213	$C_{29}H_{34}O_2N_3F_3$ x HCl	0.70
2a	2	H	73	231–233	$C_{25}H_{29}O_2N_3$ x HCl	0.72
2b	2	2-OCH_3	78	241–243	$C_{26}H_{31}O_3N_3$ x HCl	075
2c	2	3-Cl	65	237–239	$C_{25}H_{28}O_2N_3Cl_1$ x HCl	0.84
2d	2	3-CF_3	67	232–234	$C_{26}H_{28}O_2N_3F_3$ x HCl	0.80
2e[c]	3	H	59	212–215	$C_{26}H_{31}O_2N_3$ x HCl	0.73
2f	3	2-OCH_3	71	237–239	$C_{27}H_{33}O_3N_3$ x HCl	0.68
2g[c]	3	3-Cl	59	230–233	$C_{26}H_{30}O_2N_3Cl_1$ x HCl	0.79
2h	3	3-CF_3	68	218–220	$C_{27}H_{30}O_2N_3F_3$ x HCl	0.77

[a] Elemental analyses for C, H, N were within ± 0.4 of the theoretical values. [b] Solvent: butanol : acetic acid : water (5:4:1). [c] Physicochemical data for **2e** and **2g** were taken from [12]

The molecular formulas, yields, melting points and R_f values are listed in Table 1.

Monohydrochloride N-[2-(4-phenylpiperazin-1-yl)-ethyl]-8-phenyl-2-aza-spiro[4,5]decane-1,3-dione (1a): ^{1}H-NMR ($CDCl_3$) δ: 1.44–2.30 (m, 8H, -C_4H_8-), 2.40–2.52 (m, 2H, -CH_2-$\underline{CH_2}$-) 2.59 (t, 1H, *J* = 12.4 Hz, Ph-H), 2.82 (s, 2H, imide), 2.92 (d, 2H, *J* = 10.7 Hz, piperazine), 3.31 (d, 2H, *J* = 4.8 Hz, -$\underline{CH_2}$-CH_2-), 3.60–3.80 (m, 2H, piperazine), 3.92 (t, 4H, *J* = 6.2 Hz, piperazine), 6.7–6.93 (m, 3H, Ar), 7.17–7.30 (m, 7H, Ar), 13.01 (br., s, 1H, HCl).

Monohydrochloride N-[2-{4-(2-methoxyphenyl)-piperazin-1-yl}-ethyl]-8-phenyl-2-aza-spiro[4,5]decane-1,3-dione (1b): ^{1}H-NMR ($CDCl_3$) δ: 1.43–2.40 (m, 8H, -C_4H_8-), 2.37 (d, 2H, *J* = 13.8 Hz, -CH_2-$\underline{CH_2}$-), 2.55 (t, 1H, *J* = 12.6 Hz, Ph-H), 2.82 (s, 2H, imide), 2.91 (d, 2H, *J* = 10.2 Hz, piperazine), 3.37–3.55 (m, 2H, -$\underline{CH_2}$-CH_2-), 3.66–3.82 (m, 2H, piperazine), 3.89–3.95 (m, 4H, piperazine), 4.12 (s, 3H, OCH_3), 6.90–6.99 (m, 2H, Ar), 7.17–7.30 (m, 7H, Ar), 13.03 (br., s, 1H, HCl).

Monohydrochloride N-[2-{4-(3-chlorophenyl)-piperazin-1-yl}-ethyl]-8-phenyl-2-aza-spiro[4,5]decane-1,3-dione (1c): ^{1}H-NMR ($CDCl_3$) δ: 1.57–2.38 (m, 8H, -C_4H_8-), 2.37 (d, 2H, *J* = 13.3 Hz, -CH_2-$\underline{CH_2}$-), 2.51–2.59 (m, 1H, Ph-H), 2,82 (s, 2H, imide), 2.90 (d, 2H, *J* = 10.0 Hz, piperazine), 3.30 (d, 2H, *J* = 4.9 Hz, -$\underline{CH_2}$-CH_2-), 3.60–3.78 (m, 2H, piperazine), 3.91–3.96 (m, 4H, piperazine), 6.76–6.92 (m, 3H, Ar), 7.16–7.30 (m, 6H, Ar), 13.00 (br., s, 1H, HCl).

Monohydrochloride N-[2-{4-(3-trifluoromethyl-phenyl)-piperazin-1-yl}-ethyl]-8-phenyl-2-aza-spiro [4,5]decane-1,3-dione (1d): ^{1}H-NMR ($CDCl_3$) δ:

1.47–2.08 (m, 8H, -C_4H_8-), 2.42–2.53 (m, 2H, -CH_2-$\underline{CH_2}$-), 2.60 (t, 1H, *J* = 12.2 Hz, Ph-H), 2.91 (s, 2H, imide), 3.33 (d, 2H, *J* = 4.9 Hz, -$\underline{CH_2}$-CH_2-), 3.64–3.82 (m, 4H, piperazine), 3.92–4.0 (m, 4H, piperazine) 7.08–7.42 (m, 9H, Ar), 13.08 (br., s, 1H, HCl).

MonohydrochlorideN-[3-(4-phenylpiperazin-1-yl)-propyl]-8-phenyl-2-aza-spiro[4,5]decane-1,3-dione (1e): ^{1}H-NMR ($CDCl_3$): δ 1.21–2.18 (m, 10H, 8H -C_4H_8-, 2H -CH_2-$\underline{CH_2}$-CH_2-), 2.25 (d, 2H, *J* = 9.6 Hz, -CH_2-CH_2-$\underline{CH_2}$-), 2.48–2.52 (m, 1H, Ph-H), 2.58 (s, 2H, imide), 2.99–3.03 (m, 2H, -$\underline{CH_2}$-CH_2-CH_2-), 3.62 (br., s, 4H, piperazine), 3.70–3.83 (m, 4H, piperazine), 6.95 (t, 2H, *J* = 8.2 Hz, Ar), 7.00–7.32 (m, 8H, Ar), 13.02 (br., s, 1H, HCl).

Monohydrochloride N-[3-{4-(2-methoxyphenyl)-piperazin-1-yl}-propyl]-8-phenyl-2-aza-spiro[4,5]decane-1,3-dione (1f): ^{1}H-NMR ($CDCl_3$) δ: 1.21–2.17 (m, 10H, 8H -C_4H_8-, 2H -CH_2-$\underline{CH_2}$-CH_2-), 2.28–2.30 (m, 2H, CH_2-CH_2-$\underline{CH_2}$-), 2.54 (t, 1H, *J* = 12.1 Hz, Ph-H), 2.59 (s, 2H, imide), 3.05–3.17 (m, 2H, -$\underline{CH_2}$-CH_2-CH_2-), 3.57–3.73 (m, 4H, piperazine), 4.06 (s, 3H, OCH_3), 4.34 (br., s, 2H, piperazine), 5.03 (br., s, 2H, piperazine), 7.05 (t, 2H, *J* = 8.1 Hz, Ar), 7.17–7.33 (m, 5H, Ar), 7.45 (t, 1H, *J* = 7.8 Hz, Ar), 8.17 (d, 1H, *J* = 7.4 Hz, Ar), 13.82 (br., s, 1H, HCl).

Monohydrochloride N-[3-{4-(3-chlorophenyl)-piperazin-1-yl}-propyl]-8-phenyl-2-aza-spiro[4,5]decane-1,3-dione (1g): ^{1}H-NMR ($CDCl_3$) δ: 1.21–2.18 (m, 10H, 8H -C_4H_8-, 2H -CH_2-$\underline{CH_2}$-CH_2-), 2.23–2.30 (m, 2H, CH_2-CH_2-$\underline{CH_2}$-), 2.52 (t, 1H, *J* = 11.7 Hz, Ph-H), 2.57 (s, 2H, imide), 2.92–3.03 (m, 2H, -$\underline{CH_2}$-CH_2-CH_2-), 3.59–3.73 (m, 8H, piperazine), 6.76–6.92 (m, 3H, Ar), 7.05 (t, 2H, *J* = 8.1 Hz, Ar), 7.17–7.33 (m, 4H, Ar), 13.00 (br., s, 1H, HCl).

Monohydrochloride N-[3-{4-(3-trifluoromethyl-phenyl)-piperazin-1-yl}-propyl]-8-phenyl-2-aza-spiro [4,5]decane-1,3-dione (1h): ^{1}H-NMR ($CDCl_3$) δ: 1.21-2.19 (m, 10H, 8H -C_4H_8-, 2H -CH_2-$\underline{CH_2}$-CH_2-), 2.22–2.30 (m, 2H, CH_2-CH_2-$\underline{CH_2}$-), 2.53 (t, 1H, *J* = 12.6 Hz, Ph-H), 2.58 (s, 2H, imide), 2.95–3.07 (m, 2H, -$\underline{CH_2}$-CH_2-CH_2-), 3.61–3.80 (m, 8H, piperazine), 7.06–7.2.39 (m, 9H, Ar) 13.22 (br., s, 1H, HCl).

Monohydrochloride N-[2-(4-phenylpiperazin-1-yl)-ethyl]-[7,8-f]benzo-2-aza-spiro[4,5]decane-1,3-dione (2a): ^{1}H-NMR ($CDCl_3$): δ 1.23 (t, 1H, *J* = 7.0 Hz, H-10), 1.64 (br., s, 1H, H-10), 2.22 (d, 1H, *J* = 13.6 Hz, imide), 2.69 (d, 1H, *J* = 17.9 Hz, imide), 2.82 (d, 2H, *J* = 6.4 Hz, H-9), 2.90 (t, 2H, *J* = 9.5 Hz, H-6), 2.97 (d, 2H, *J* = 8.5 Hz, -CH_2-$\underline{CH_2}$-), 3.03–3.25 (m, 2H, piperazine) 3.33–3.38 (m, 2H piperazine), 3.62 (d, 2H, *J* = 12.6 Hz, -$\underline{CH_2}$-CH_2-), 3.95–3.97 (m, 4H, piperazine), 6.90–7.33 (m, 9H, Ar), 12,98 (br., s, 1H, HCl).

Monohydrochloride N-[2-{4-(2-methoxyphenyl)-piperazin-1-yl}-ethyl]-[7,8-f]benzo-2-aza-spiro[4,5]decane-1,3-dione (2b): ^{1}H-NMR ($CDCl_3$): δ 1.17–2.29 (m, 3H, 1H, imide, 2H, H-10), 2.70 (d, 1H, *J* = 18.2 Hz, imide), 2.80–2.96 (m, 4H, 2H H-9, 2H H-6), 2.98 (d, 2H, *J* = 8.2 Hz, -CH_2-$\underline{CH_2}$-), 3.01–3.48 (m, 4H, piperazine), 3.58 (d, 2H, *J* = 13.0 Hz, -$\underline{CH_2}$-CH_2-), 3.95 (t, 4H, *J* = 6.7 Hz, piperazine), 4.02 (s, 3H, OCH_3), 7.01–7.43 (m, 8H, Ar), 11.32 (br., s, 1H, HCl).

Monohydrochloride N-[2-{4-(3-chlorophenyl)-piperazin-1-yl}-ethyl]-[7,8-f]benzo-2-aza-spiro[4,5] decane-1,3-dione (2c): ^{1}H-NMR ($CDCl_3$) δ: 1.27 (t 1H, *J* = 7.15 Hz, H-10), 1.67 (br., s, 1H, H-10), 2.25 (d, 1H, *J* = 9.9 Hz, imide), 2.73 (d, 1H, *J* = 17.9 Hz, imide), 2.85 (d, 2H, *J* = 6.6 Hz, H-9), 2.90–3.06 (m, 6H, 2H H-6, 2H piperazine, 2H -CH_2-$\underline{CH_2}$-), 3.28–3.38 (m, 2H, piperazine), 3.75 (d, 2H, *J* =11.8 Hz, -$\underline{CH_2}$-CH_2-), 3.98 (t, 4H, *J* = 5.8 Hz, piperazine), 6.79–6.97 (m, 3H, Ar), 7.09–7.32 (m, 5H, Ar), 13.07 (br., s, 1H, HCl).

Monohydrochloride N-[2-{4-(3-trifluoromethyl-phenyl)-piperazin-1-yl}-ethyl]-[7,8-f]benzo-2-aza-spiro [4,5]decane-1,3-dione (2d): ^{1}H-NMR ($CDCl_3$) δ: 1.27 (t, 1H, *J* = 7.1 Hz, H-10), 1.70 (br., s, 1H, H-10), 2.32 (d, 1H, *J* = 9.0 Hz, 1H, imide), 2.73 (d, 1H, *J* = 17.9 Hz, imide), 2.86 (t, 2H, *J* = 5.6 Hz, H-9), 2.90–3.07 (m, 6H, 2H H-6, 2H piperazine, 2H -CH_2-$\underline{CH_2}$-), 3.28–3.42 (m, 2H, piperazine), 3.70 (d, 2H, *J* = 12.6 Hz, -$\underline{CH_2}$-CH_2-), 3.99 (t, 4H, *J* = 5.5 Hz, piperazine), 7.09–7.45 (m, 8H, Ar), 13.12 (br., s, 1H, HCl).

Monohydrochloride N-[3-{4-(2-methoxyphenyl)-piperazin-1-yl}-propyl]-[7,8-f]benzo-2-aza-spiro[4,5]decane-1,3-dione (2f): ^{1}H-NMR ($CDCl_3$): δ 1.23 (t, 1H, *J* = 7.0 Hz, H-10), 1.86–2.23 (m, 3H, 2H -CH_2-$\underline{CH_2}$-CH_2-, 1H H-10), 2.26 (d, 1H, *J* = 7.9 Hz, imide), 2.60 (d, 1H, *J* = 18.2 Hz, imide), 2.86 (d, 2H, *J* = 5.9 Hz, H-9), 2.90–3.05 (m, 6H, 2H H-6, 2H piperazine, 2H -CH_2-CH_2-$\underline{CH_2}$-), 3.29 (d, 2H, *J* = 13.1 Hz piperazine), 3.48–3.62 (m, 2H, -$\underline{CH_2}$-CH_2- CH_2-), 3.70 (t, 4H, *J* = 6.1 Hz, piperazine), 3.96 (s, 3H, -OCH_3), 6.96–7.23 (m, 8H, Ar), 13.30 (br., s, 1H, HCl).

Monohydrochloride N-[3-{4-(3-trifluoromethyl-phenyl)-piperazin-1-yl}-propyl]-[7,8-f]benzo-2-aza-spiro[4,5]decane-1,3-dione (2h): ^{1}H-NMR ($CDCl_3$) δ: 1.64 (s, 2H, -CH_2-$\underline{CH_2}$-CH_2-), 1.86–1.96 (m, 1H,

H-10), 2.14–2.22 (m, 1H, H-10), 2.25–2.35 (m, 3H, 1H imide, 2H -CH_2-CH_2-$\underline{CH_2}$-), 2.49 (d, 1H, J = 18.4 Hz, imide), 2.86 (d, 2H, J = 6.3 Hz, H-9), 2.90–3.04 (m, 4H, 2H H-6, 2H piperazine) 3.25 (t, 2H, J = 16.2 Hz, piperazine), 3.64–3.78 (m 6H, 4H piperazine, 2H -$\underline{CH_2}$-CH_2-CH_2-), 7.05–7.21 (m, 7H, Ar), 7.39 (t, 1H, J = 8.0 Hz, Ar), 13.18 (br., s, 1H, HCl).

PHARMACOLOGICAL PART

Preliminary anticonvulsant assays

All compounds (**1a**–**h** and **2a**–**h**) 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 [7, 8].

Phase I studies of the investigated compounds involved three testes: maximal electroshock (MES), subcutaneous metrazole (sc. MET) and rota-rod test for neurological toxicity (TOX). All the compounds were injected intraperitoneally, as a suspension in 0.5% methylcellulose, at the dose levels of 30, 100 and 300 mg/kg at 0.5 and 4 hours time periods. The compounds were classified into the following categories: active at 100 mg/kg or less (class 1), active at dose greater than 100 mg/kg (class 2), inactive (class 3) and active but toxic at a dose of 30 mg/kg or toxic at the same dose (class 4). These data are presented in Table 2.

The compounds **2c**, **2d**, **2e** and **2h** were advanced to phase VIa and were administered orally into rats at a dose of 30 mg/kg. The results of that test are shown in Table 3.

Quantitative anticonvulsant assays

The experimental procedures used were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Male Albino-Swiss mice (25–28 g) were used throughout the experiments after at least one-week acclimatization. The animals were housed under standard laboratory conditions (an ambient temperature of 20 ± 1°C, natural light-dark cycle). Tap water and chow pellets were freely available before the experiment. All the experiments were conducted between

Tab. 2. Anticonvulsant screening project (ASP) phase I test in mice

Compound	Intraperitoneal injection in mice				ASP[c] class
	MES[a]		TOX[b]		
	0.5 h	4 h	0.5 h	4 h	
1a	–	100	300	–	1
1b	300	–	100	300[d]	2
1c	–	300	30	–	4
1d	–	300	100	100	2
1e	–	–	300	–	3
1f	300	–	100	300[d]	2
1g	–	300	100	100	2
1h	–	100	–	–	1
2a	300	300	100	300	2
2b	300	–	100[e]	300[d]	2
2c	–	30	300	–	1
2d	100	100	300	300	1
2e	–	100	300	100	1
2f	100	–	30	–	4
2g	100	30	30	30	4
2h	100	30	300	300	1

[a] Maximal electroshock: doses of 30, 100 and 300 mg/kg were administrated intraperitoneally in mice; the values in the Table indicate the minimum dose whereby anticonvulsant activity was demonstrated in 100% of the animals. The dash indicates an absence of activity at maximum dose administrated (300 mg/kg). [b] Neurotoxicity screen: dose of compound whereby neurotoxicity was exhibited in half or more of the animals. [c] The ASP classification is as follows: 1 – anticonvulsant activity at a doses 100 mg/kg or less; 2 – anticonvulsant activity at doses of 300 mg/kg; 3 – compound inactive at dose of 300 mg/kg; 4 – compound active but toxic at a dose of 30 mg/kg. Response comments: [d] unable to grasp rota-rod, [e] muscule spasms

Tab. 3. Anticonvulsant screening project (ASP) phase VIa

Compound	MES oral administration to rats (dose 30 mg/kg)[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
2c	–	–	–	1	1
2d	–	–	1	1	–
2e	–	–	–	1	2
2h	–	1	4	4	2
Phenytoin[b]	1	4	3	3	3

[a] The values indicate the number of rats out of four, which were protected. [b] Data from [24]

10.00 a.m. and 2.00 p.m. (December–January). Each experimental group consisted of 10 animals.

The tested compounds and valproate magnesium (ICN Polfa SA, Rzeszów, Poland) were administrated intraperitoneally (*ip*) as a suspension in a 1% solution of Tween 80 (Sigma, St. Luis, MO, USA) in a volume of 10 ml/kg. Control groups received appropriate volumes of the solvent.

Maximal electroshock seizure (MES) test

Seizures were evoked with an electric current (50 Hz, 50 mA, the duration of impulse 0.2 s, ear-clips electrodes) in mice according to the method of Swinyard [20], at 60 min after administration of the investigated compounds. The number of mice reacting with the tonic extention of hind limbs was recorded. The ED_{50} values, i.e. the doses protecting 50% of mice against MES, were determined from the effect of at least four doses for each compound. ED_{50} values with 95% confidence limits were calculated according to the log-probit method of Litchfield and Wilcoxon [9].

Rota-rod test

Pre-selected mice (holding on to the rotating rod for 2 minute) were placed on a rotating rod (1 cm in diameter, 6 r.p.m) and observed during 2 min. The number of animals falling off the rod was recorded 60 min after administration of the investigated compounds. The TD_{50} values i.e. the median doses preventing 50% of the animals holding of the rod for 2 min were determined from the effect of at least four doses for each compound.

Results and Discussion

Aromatic substitution in the investigated series of compounds was observed to influence anticonvulsant activity. The obtained results revealed that except compound **1e**, all the other derivatives inhibited electrically provoked seizures (MES test).

In the series of N-[(4-arylpiperazin-1-yl)-alkyl]-8-phenyl-2-aza-spiro[4,5]decane-1,3-diones (**1a**–**h**), the compound **1a** (without a substituent at the 4-aryl-piperazine moiety) exhibited activity at a dose of 100 mg/kg at 4 h, whereas its propylene analogue (**1e**) was inactive. Derivatives with 2-methoxy group (**1b**, **1f**) inhibited seizures at a dose of 300 mg/kg 4 h after the treatment but at the same dose mice were unable to grasp the rota-rod. In the case of 3-chloro (**1c**, **1g**) and 3-trifluoromethyl (**1d**, **1h**) derivatives, only compound **1h** at a dose of 100 mg/kg protected the animals after 4 h. The other compounds (**1c**, **1d**, **1g**) at a dose of 300 mg/kg inhibited seizures during the same period of time. In the neurotoxicity screening, the 3-chloro derivative **1c** revealed toxicity at a dose of 30 mg/kg, and irrespective of its activity, was classified into the ASP-4 class following the ASP rules.

The restricted flexibility of phenyl ring connected with the 2-aza-spiro[4,5]decane-1,3-dione system, resulting in rigidization of molecules, was observed for [7,8-f]benzo-2-aza-spiro[4,5]decane-1,3-dione derivatives (**2a**–**h**). In that series of compounds, the highest activity was observed for 3-chloro (**2c**, **2g**) and 3-trifluoromethyl (**2d**, **2h**) derivatives. The anti-MES protection was detected at a dose of 30 mg/kg at 4 h (**2c**, **2g**, **2h)** as well as at a dose of 100 mg/kg at 0.5 h (**2d**, **2g**, **2h)** and at 4 h (**2d**). Despite its activity, the compound **2g** at a dose of 30 mg/kg, exhibited neurological toxicity and was included into the ASP-4 class. The activity of the unsubstituted derivatives (**2a**, **2e)** and their 2-methoxy analogues (**2b**, **2f**) was dependent on the length of alkylene chain, its extension from ethylene (**2a**, **2b**) to propylene (**2e**, **2f**) increased anti-MES protective effect but also, in case of **2f**, increased neurotoxicity (Tab. 2).

Derivatives **2c**, **2d**, **2e** and **2h** were selected for the oral evaluation of anti-MES and neurotoxic activity in rats (phase VIa). Those compounds were administered at a dose of 30 mg/kg *per os* and their effect was studied after 0.25, 0.5, 1, 2 and 4 h. When given orally, none of the compounds was neurotoxic. The most potent 3-trifluoromethyl derivative (**2h**) protected 100% of the animals at 1 and 2 h, and 50% of the animals at 4 h, and its activity is comparable to phenytoin (Tab. 3). Similarly, to **2h**, a 50% protection at the time of 4 h was observed for compound **2e**. Derivatives **2c** and **2d** were less active in this test and inhibited seizures only in 25% of the animals at various time point (1, 2 or 4 h).

On the basis of a preliminary anticonvulsant screening for the most active and relatively weakly neurotoxic 3-chloro (**2c**) and 3-trifluoromethyl (**2h**) derivatives, we decided to carry out quantitative evaluation of pharmacological parameters, i.e. the median effective dose (ED_{50} – a dose protecting 50% of animals against electrically provoked seizures) and

the median neurotoxic dose (TD_{50} – a dose preventing 50% of animals from holding of the rod for 2 minute). The above data enabled us to assess the accurate profile of anticonvulsant activity.

Tab. 4. Anticonvulsant activity and neurotoxic effects in mice

Compound	MES[a]	Rota-rod test[c]
	ED_{50} mg/kg	TD_{50} mg/kg
2c	205 (169.4–248.1)[b]	> 400
2h	23 (20.4–26.0)	60 (53.1–67.8)
Valproate magnesium	211 (168.8–263.8)	342 (285.0–410.4)

[a] The tested compounds were administered *ip* 60 min before the tests. [b] 95% confidence limits given in parentheses. [c] TD_{50} – dose preventing 50% of animals from holding the rod for 2 min

As shown in Table 4, the examined 3-chloro (**2c**) and 3-trifluoromethyl (**2h**) derivatives inhibited electrically provoked seizures, with ED_{50} values of 205 mg/kg (**2c**) and 23 mg/kg (**2h**) respectively, and turned out to be more potent than valproate magnesium (ED_{50} = 211mg/kg) used as a standard antiepileptic drug.

The obtained results show that in series of compounds under study, the introduction of an aromatic system as a flexible phenyl ring (**1a**–**h**) or as a rigidified fragment (**2a**–**h**) is essential for anti-MES activity. It is noteworthy that the rigidified structures of the **2a**–**h** series are preferable to activity. Structural characterization also involves the kind and the position of substituents attached to the phenyl ring of the 4-arylpiperazine moiety, in general, the presence of electron-attracting substituents in positon-3 (3-CF_3, 3-Cl) enhances the anticonvulsant properties.

In conclusion, the insertion of an additional aromatic system into the cyclohexane ring of the 2-azaspiro[4.5]decane-1,3-dione moiety seems to be the most important factor that influences anticonvulsant activity. Maybe the change in shape, as well as the increase in overall size of molecules could strengthen the hydrophobic and π electron interactions with the target site. On the basis of the present study, further research in this area is carried out and will be reported shortly.

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