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Short communication

Synthesis, anticonvulsant activity and 5-HT_{1A}, 5-HT_{2A} receptor affinity of new *N*-[(4-arylpiperazin-1-yl)-alkyl] derivatives of 2-azaspiro[4.4]nonane and [4.5]decane-1,3-dione

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Abstract

The synthesis, physicochemical and pharmacological properties of new *N*-[(4-arylpiperazin-1-yl)-alkyl]-2-azaspiro[4.4]nonane- (**8a–c, 10a–d**) and [4.5]decane-1,3-dione (**9a–c, 11a–d**) derivatives were described. The antiepileptic effects of those compounds were examined by a maximal electroshock (MES) and a pentylenetetrazole (*sc*. PTZ) tests, and their neurotoxicity was determined using a rota-rod test. Compounds **8c, 9c, 10c, d, 11c, d** with a CF_3 group at the 3-position of the 4-arylpiperazine fragment exhibited anti-seizure properties in the MES model; in contrast, their 2-CH_3 and 2-OCH_3 analogues were inactive in both the tests used. Moreover, since the investigated compounds belong to the class of long-chain arylpiperazines, their serotonin 5-HT_{1A} and 5-HT_{2A} receptor affinity was determined. The relationship between the length of alkylene spacer and 5-HT_{1A}/5-HT_{2A} receptor activity was observed. Compounds with an ethylene and a propylene bridge (**10a–d** and **11a–d**) were 3–80-fold more potent (K_i ranged from 3.1 to 94 nM for 5-HT_{1A} and 32–465 nM for 5-HT_{2A}) than their methylene analogues (**8a–c** and **9a–c**; K_i ranged from 81 to 370 nM for 5-HT_{1A} and 126–1370 nM for 5-HT_{2A}). The highest 5-HT_{1A} receptor affinity was displayed by 2-OCH_3 and 3-CF_3 phenyl derivatives (**10b**, **11b**: $K_i = 6.8$ and 5.7 nM, respectively, and **10c, 11c**: $K_i = 6.0$ and 3.1 nM, respectively), while in the case of 5-HT_{2A} receptor the highest affinity was observed for the 3-CF_3 phenyl derivatives **10c, d, 11c, d** (K_i ranged from 32 to 86 nM).

Keywords: 2-Azaspiro[4.4]nonane- and [4.5]decane-1,3-diones; 4-Arylpiperazine derivatives; Anticonvulsant activity; 5-HT_{1A}, 5-HT_{2A} receptor affinity

1. Introduction

Epilepsy is one of the most frequent neurological disorders characterized by spontaneous recurrent seizures arising from excessive electrical activity in some portion of the brain. Uncontrolled electrical activity in the central nervous system may occur via either a reduction in inhibitory neurotransmission or an increase in excitatory transmission. Changes in ionic conductance through neuronal membranes may underlie the above-mentioned abnormalities [1].

Additionally, there has been a growing evidence that serotoninergic neurotransmission modulates a wide variety of experimentally induced seizures and is involved in the enhanced seizure susceptibility observed in some genetically prone rats [2–5]. Furthermore, the experimental data obtained with animals show that 5-HT_{1A} receptors are predominantly located in limbic areas and they suggest that serotonin mediates the anticonvulsant effect via these receptors [6–8]. On the other hand, following the findings of Stean et al. [9], the mixed 5-$HT_{1A, 1B, 1D}$ receptor agonists SFK 99101 and RU 24969 produce marked increases in the seizure threshold. It has also been found that stimulation of 5-HT_2 receptors is linked to the anticonvulsant action of trifluoromethylphenylpiperazine (5-HT_{2A}/5-HT_{2C}) and *m*-chlorophenylpiperazine (5-HT_{2A}/5-HT_{2C}) in an animal maximal electroshock test [10,11].

In the course of developing new potential anticonvulsant agents and 5-HT_{1A}/5-HT_{2A} receptor ligands we focused our attention on a group of 1,3-substituted pyrrolidine-2,5-diones (succinimides) with a piperazin-1-yl-alkyl fragment at the imide nitrogen atom. In that series of derivatives, anticonvulsant activity was observed for compounds with an aromatic

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ring at the 3-position of pyrrolidine-2,5-dione and 4-aryl or 4-methyl-piperazin-1-yl alkyl moiety at the imide nitrogen atom [12,13]. Prompted by the fact that the series of pyrrolidine-2,5-diones containing at the 3-position cycloalkyl moiety connected by a spiro carbon atom showed potent anticonvulsant activity [14–17], we replaced the 3-aryl ring with a spirocycloalkyl fragment thus obtaining a series of *N*-[(4-arylpiperazin-1-yl)-alkyl]-2-azaspiro[4.4]nonane- and [4.5]decane-1,3-dione derivatives. In that series of azaspiranes connected to the arylpiperazine moiety with a propylene chain, we identified several compounds displaying activity in an *sc*. PTZ test [18]. As shown in Fig. 1, some of them exhibited high 5-HT_{2A} receptor affinity and were also found to be fairly potent 5-HT_{1A} receptor ligands [18,19]. Introduction of a spirocycloalkyl fragment into the 3-position of the pyrrolidine-2,5-dione ring significantly enhanced 5-HT_{1A}/5-HT_{2A} receptor affinity, in contrast to 3-phenyl analogues which were only slightly active (K_i > 200 nM for both receptor subtypes) [20].

In line with the above findings, in the present study we obtained two series of *N*-[(4-arylpiperazin-1-yl)-alkyl]-2-azaspiro [4.4]nonane- and [4.5]decane-1,3-dione derivatives with different length of alkyl spacer and two kinds of substituent: the electron-attracting CF_3 and the electron–donating OCH_3 and CH_3 at the 4-arylpiperazine moiety. All the above-mentioned compounds were tested in vivo for their anticonvulsant activity through the Anticonvulsant Screening Program (ASP), and in vitro for their affinity towards 5-HT_{1A} and 5-HT_{2A} receptors.

2. Chemistry

The synthesis of compounds **8a–c**, **9a–c**, **10a–d** and **11a–d** is shown in Scheme 1. The starting 1-carboxy-1-cyclopentane- (**4**) and 1-carboxy-1-cyclohexane-acetic acids (**5**) were prepared from related cycloalkyl ketones according to the previously described procedure [14]. The synthesis of 2-azaspiro [4.4]nonane- (**6**) and 2-azaspiro[4.5]decane-1,3-dione (**7**) (spirosuccinimide) had been described in a separate publication [21]. Compounds **8a–c** and **9a–c** were prepared by the Mannich-type reaction from the appropriately 3-substituted spirosuccinimide (**6, 7**), formaldehyde and the corresponding 4-arylpiperazine to obtain the designed derivatives. The reaction was carried out in ethanol at a room temperature for ca. 6–12 h and was eventually refluxed for 30 min. The synthesis of compounds **10a–d** and **11a–d** were prepared using a one-pot cyclization reaction of the obtained acids **4** or **5** and appropriately substituted 1-amino-alkyl-4-arylpiperazine.

All compounds **8a–c, 9a–c, 10a–d** and **11a–d** were isolated as the hydrochloride salts and were recrystallized from anhydrous ethanol. Their molecular formulas were established on the basis of elemental (C, H, N) analyses (data not shown). The structures of **8a–c, 9a–c, 10a–d** and **11a–d** as well as their physicochemical data are presented in Table 1.

The structures of the investigated compounds were confirmed by the examination of their ^{1}H NMR and MS spectra (Tables 2–4).

The ^{1}H NMR spectra of the investigated compounds revealed characteristic chemical shifts agreed with their proposed structures. In all derivatives (**8a, c, 9a, c, 10a–d** and **11a–d**) the signal due to pyrrolidine-2,5-dione ring CH_2 protons appeared at about δ 2.57–2.88 ppm, as singlets. The chemical shifts of the cyclopentane and cyclohexane rings were observed as multiplets within the range of δ 1.66–2.23 ppm (**8a, c, 10a–d**), and δ 1.21–1.93 ppm (**9a, c, 11a–d**). Appearance of a sharp singlet at about δ 4.55–4.81 ppm, due to the two protons of methylene group confirms the formation of compounds **8a, c** and **9a, c**. The piperazine protons of compounds **8a, c** and **9a, c** were observed as two multiplets in the range of δ 2.77–2.80 ppm and δ 2.90–3.24 ppm (**8a, c**), and δ 2.75–3.46 ppm and δ 3.20–3.73 ppm (**9a, c**).

The ^{1}H NMR spectra of compounds **10a–d** in general were almost similar to compounds **11a–b**. The difference was only in chemical shift of two piperazine protons, for compounds **10b, 11b,** which were shifted considerably down field and were observed as a triplets at δ 5.09 ppm (*J* ca.12 Hz). The

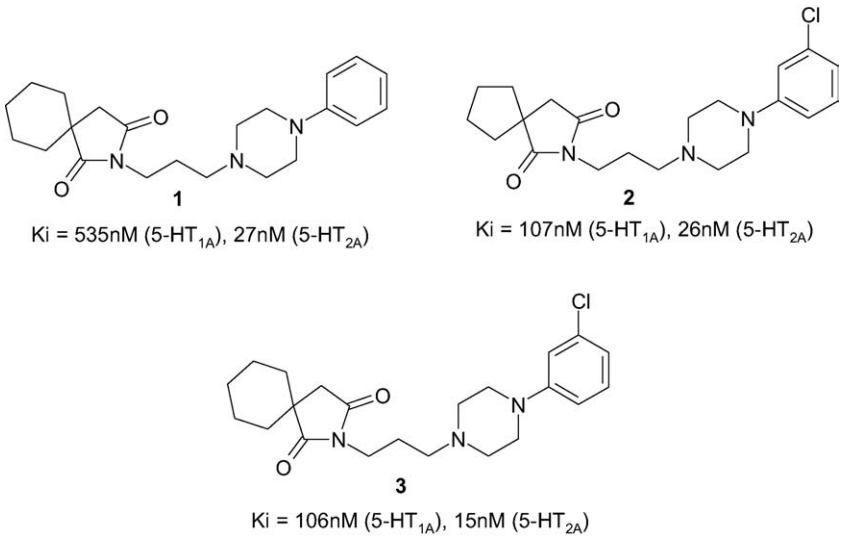
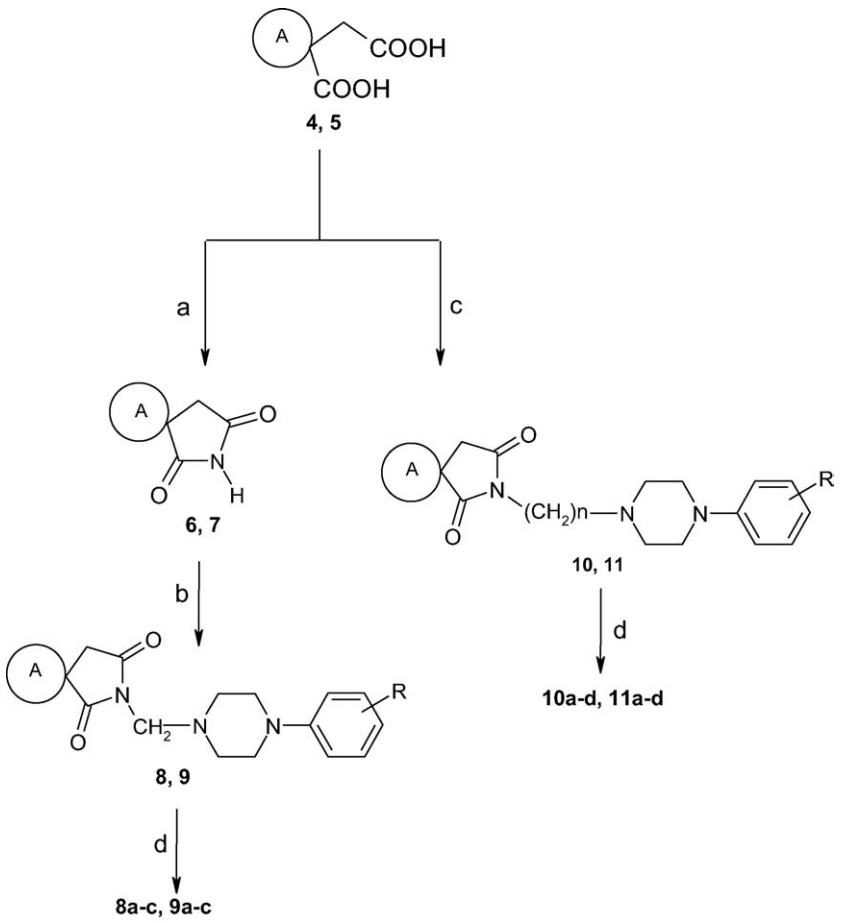
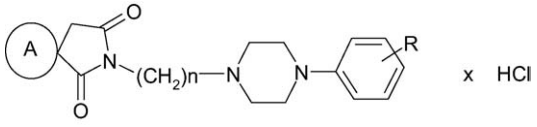


Fig. 1. Chemical structure of compounds **1**–**3**.



Scheme 1. Synthesis of pathways of the investigated compounds **8a–c, 9a–c, 10a–d** and **11a–d** Reagents conditions: (a) 25% NH_4OH, reflux 2 h, (b) 4-arylpiperazine derivatives, formaldehyde, 96% ethyl alcohol, reflux for 0.5 h, ca. 6–12 h room temp. (c) 1-aminoalkyl-4-arylpiperazine, cyclocondensation, 2 h, 180–200°C, (d) ethanol HCl solution.

Table 1
Physicochemical properties of the hydrochlorides of *N*-[(4-arylpiperazin-1-yl)-alkyl]-2-azaspiro[4.4]nonane- (**8a, c, 10a–d**) and [4.5]decane-1,3-dione (**9a, c, 11a–d**) derivatives



Compound	Ring A	R	*N*	Yield (%)	m.p. (°C)	Formula[a]	Molecular weight
8a	Cyclopentane	2-CH_3	1	72	183–185	$C_{20}H_{27}O_2N_3 \cdot HCl$	377.92
8c	Cyclopentane	3-CF_3	1	77	179–181	$C_{20}H_{24}O_2N_3F_3 \cdot HCl$	431.89
9a	Cyclohexane	2-CH_3	1	69	205–207	$C_{21}H_{29}O_2N_3 \cdot HCl$	391.94
9c	Cyclohexane	3-CF_3	1	65	190–192	$C_{21}H_{26}O_2N_3F_3 \cdot HCl$	445.92
10a	Cyclopentane	2-CH_3	2	70	235–237	$C_{21}H_{29}O_2N_3 \cdot HCl$	391.94
10b	Cyclopentane	2-OCH_3	2	65	211–213	$C_{21}H_{29}O_3N_3 \cdot HCl$	407.94
10c	Cyclopentane	3-CF_3	2	62	215–217	$C_{21}H_{26}O_2N_3F_3 \cdot HCl$	445.92
10d	Cyclopentane	3-CF_3	3	60	255–257	$C_{22}H_{28}O_2N_3F_3 \cdot HCl$	459.94
11a	Cyclohexane	2-CH_3	2	68	246–248	$C_{22}H_{31}O_2N_3 \cdot HCl$	405.97
11b	Cyclohexane	2-OCH_3	2	70	219–221	$C_{22}H_{31}O_3N_3 \cdot HCl$	421.97
11c	Cyclohexane	3-CF_3	2	63	223–225	$C_{22}H_{28}O_2N_3F_3 \cdot HCl$	459.94
11d	Cyclohexane	3-CF_3	3	64	227–229	$C_{23}H_{30}O_2N_3F_3 \cdot HCl$	473.97

[a] The values obtained by elemental analysis were within ± 0.4% of theoretical values.

Table 2
^{1}H NMR spectral data of compounds **8a, c, 9a, c, 10a–d**

Compound	δ ppm in $CDCl_3$ (200 Hz)
8a	1.70–2.25 (m, 8H, C_4H_8), 2.29 (s, 3H, *CH_3*), 2.68 (s, 2H, imide), 2.77–2.79 (m, 4H, piperazine), 2.90–2.94 (m, 4H, piperazine), 4.56 (s, 2H, *CH_2*), 6.97–7.20 (m, 4H, arom.), 10.20 (br. s, 1H, *H*Cl).
8c	1.67–2.21 (m, 8H, C_4H_8), 2.66 (s, 2H, imide), 2.77–2.80 (m, 4H, piperazine), 3.21–3.24 (m, 4H, piperazine), 4.57 (s, 2H, *CH_2*), 7.04–7.11 (m, 3H, arom.), 7.36 (t, *J* = 7.84 Hz 1H, arom.), 10.70 (br. s, 1H, *H*Cl).
9a	1.21–1.91 (m, 10H, C_5H_{10}), 2.34 (s, 3H, CH_3), 2.78 (s, 2H, imide), 3.28–3.46 (m, 4H, piperazine), 3.64–3.73 (m, 4H, piperazine), 4.81 (s, 2H, *CH_2*), 7.04–7.23 (m, 4H, arom.), 9.94 (br. s, 1H, *H*Cl).
9c	1.27–1.93 (m, 10H, C_5H_{10}), 2.62 (s, 2H, imide), 2.75–2.79 (m, 4H, piperazine), 3.20–3.23 (m, 4H, piperazine), 4.55 (s, 2H, *CH_2*), 7.03–7.11 (m, 3H, arom.), 7.36 (t, *J* = 7.97 Hz, 1H, arom.), 10.15 (br. s, 1H, *H*Cl)
10a	1.67–2.23 (m, 8H, C_4H_8), 2.30 (s, 3H, *CH_3*), 2.87 (s, 2H, imide), 3.02 (t, *J* = 11.82 Hz, 2H, CH_2–*CH_2*), 3.14 (d, *J* = 13.40 Hz, 2H, piperazine), 3.33–3.38 (m, 2H, piperazine), 3.67 (t, *J* = 11.55 Hz, 2H, *CH_2*–CH_2), 3.93–3.99 (m, 4H, piperazine), 7.05–7.23 (m, 4H, arom.), 12.87 (br. s, 1H, *H*Cl)
10b	1.74–2.21 (m, 8H, C_4H_8), 2.88 (s, 2H imide), 3.48 (br. s, 2H, piperazine), 3.60 (t, *J* = 12.60 Hz, 2H, CH_2-*CH_2*), 3.93–4.00 (m, 4H, 2H, piperazine, 2H, *CH_2*–CH_2), 4.07 (s, 3H, O*CH_3*), 4.42 (br. s, 2H, piperazine), 5.09 (t, *J* = 12.24 Hz, 2H, piperazine), 7.05–7.10 (m, 2H, arom.), 7.49 (t, *J* = 7.30 Hz, 1H, arom.), 8.19 (d, *J* = 8.2 Hz, 1H, arom.), 13.70 (br. s, 1H, *H*Cl)
10c	1.66–2.20 (m, 8H, C_4H_8), 2.86 (s, 2H imide), 2.96 (t, *J* = 12.60 Hz, 2H, CH_2–*CH_2*), 3.34 (br. s, 2H, piperazine), 3.67–3.80 (m, 4H, piperazine), 3.95–3.98 (m, 4H, 2H, piperazine, 2H, *CH_2*–CH_2), 7.09–7.23 (m, 3H, arom.), 7.42 (t, *J* = 7.80 Hz, 1H, arom.), 13.09 (br. s, H, *H*Cl)
10d	1.68–2.22 (m, 10H, 8H, C_4H_8, 2H, CH_2–*CH_2*–CH_2), 2.30 (t, *J* = 8.20 Hz, 2H, CH_2–CH_2–*CH_2*), 2.62 (s, 2H, imide), 2.92–3.10 (m, 4H, piperazine), 3.62–3.80 (m, 6H, 4H, piperazine, 2H, *CH_2*–CH_2–CH_2), 7.06–7.22 (m, 3H, arom.), 7.39 (t, *J* = 7.90 Hz, 1H, arom.), 13.20 (br. s, 1H, *H*Cl)

Table 3
^{1}H NMR spectral data of compounds **11a–d**

Comp.	δ ppm in $CDCl_3$ (200 Hz)
11a	1.27–1.83 (m, 10H, C_5H_{10}), 2.29 (s, 2H, *CH_3*), 2.83 (s, 2H, imide), 3.0 (t, *J* = 11.35 Hz, 2H, CH_2–*CH_2*), 3.14 (d, *J* = 12.93 Hz, 2H, piperazine), 3.34 (br. s, 2H, piperazine), 3.65 (t, *J* = 12.24 Hz, 2H, *CH_2*-CH_2), 3.95 (t, *J* = 5.5 Hz, 4H, piperazine), 7.04–7.22 (m, 4H, arom.), 12.82 (br. s, 1H, *H*Cl).
11b	1.27–1.90 (m, 10H, C_5H_{10}), 2.86 (s, 2H, imide), 3.47 (br. s, 2H, piperazine), 3.61 (t, *J* = 12.2 Hz, 2H, CH_2–*CH_2*), 3.91–3.99 (m, 4H, 2H, piperazine, 2H, *CH_2*-CH_2), 4.07 (s, 3H, *OCH_3*), 4.41 (br., s, 2H, piperazine), 5.14 (t, *J* = 11.50 Hz, 2H, piperazine), 7.05–7.11 (m, 2H, arom.), 7.49 (t, *J* = 6.7 Hz 1H, arom.), 8.21 (d, *J* = 8.2 Hz, 1H, arom.), 13.78 (br. s, 1H, *H*Cl).
11c	1.28–1.89 (m, 10H, C_5H_{10}), 2.57 (s, 2H, imide), 2.62–2.67 (m, 6H, 4H, piperazine, 2H, CH_2–*CH_2*), 3.16–319 (m, 4H, piperazine), 3.69 (t, *J* = 12.38 Hz, 2H, *CH_2*–CH_2), 7.05–7.11 (m, 3H, arom.), 7.36 (t, *J* = 8.00 Hz, 1H, arom.), 13.58 (br. s, 1H, *H*Cl).
11d	1.24–1.80 (m, 10H, C_5H_{10}), 2.21–2.30 (m, 2H, CH_2-*CH_2*–CH_2), 2.58 (s, 2H, imide), 2.90–3.08 (m, 4H, piperazine), 3.62 (t, *J* = 12.5 Hz, 2H, *CH_2*–CH_2–CH_2), 3.67–3.79 (m, 6H, 4H, piperazine, 2H, CH_2–CH_2-*CH_2*), 7.05–7.12 (m, 2H, arom.), 7.20 (d, *J* = 7.4 Hz, 1H, arom.), 7.38 (t, *J* = 7.9 Hz, 1H, arom.), 13.24 (br. s, 1H, *H*Cl).

other piperazine protons were observed as multiplets (**10a–d, 11c, d**), doublets with coupling constant ca. 13 Hz (**10a, 11a**) and broad singlets (**10b, 11a, b**). The two methylene protons neighboring with piperazine nitrogen atom appear as triplets with coupling constants about 12 Hz or as multiplets.

In derivatives **10d** and **11d,** the signal due to (–CH_2–) propylene spacer appeared about δ 2.20 ppm as multiplets. All the aromatic protons were well separated and observed at expected region. Results are presented in Tables 2 and 3.

The structures of selected compounds were further verified by EIMS spectra (70eV) where the *m/z* values of molecular ion peaks were in complete agreement with calculated molecular weight for individual compounds. The compounds having ethylene (**10a, c, 11a**) or propylene (**10d**) spacer had relatively small molecular ions, whereas the ions derived from the methylene analogues (**8a, c, 9a, c**) were the most intensive (100%). In case of compounds **10a, c, d** and **11a**, the major fragments at *m/z* 189 and *m/z* 243 (100%) appeared by the cleavage of alkylene chain. The fragmentation of the 2-azaspiro[4.4]nonane (**8a, c, 10a, c, d**) and 2-azaspiro[4.5]decane-1,3-dione (**9a, c, 11a**) rings was confirmed by the characteristic ions at *m/z* 70, *m/z* 81 and *m/z* 95, which were within the range of 2–25% intensity. The other ions m/z 146 and m/z 200 arising from the fragmentation of 4-arypiperazine moiety. For the details see Table 4.

3. Pharmacological results

3.1. In vivo test

3.1.1. Anticonvulsant activity

Anticonvulsant assays were performed for all obtained compounds **8a–c, 9a–c, 10a–d** and **11a–d**. The compounds were injected intraperitoneally into mice and evaluated in the maximal electroshock (MES), subcutaneous pentylenetetrazole (*sc.* PTZ) and neurotoxicity screens, using doses of 30, 100 and 300 mg/kg at two different time intervals (0.5 and 4 h). The maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc.* PTZ) tests are claimed to detect compounds affording protection against generalized tonic-clonic seizures and generalized absence seizures, respectively. Thus the MES and *sc.* PTZ screens have become the most widely employed seizure models for early identification of candidate anticonvulsants.

In all investigated series of compounds, derivatives with 3-CF_3 group (**8c, 9c, 10c, d** and **11c, d**) showed anticonvulsant

Table 4
MS spectral data of selected *N*-[(4-arylpiperazin-1-yl)-alkyl]-2-azaspiro[4.4]nonane- (**8a, c, 10a, c, d**) and [4.5]decane-1,3-dione (**9a, c, 11a**) derivatives

Compound	Formula[a] m.w.	EIMS *m/z* (% intensity)
8a	$C_{20}H_{27}O_2N_3$ 341.46	[M^+] 341 (100), 194 (20), 189 (42), 175 (25), 166 (12), 146 (72), 91 (10), 81 (6), 70 (9)
8c	$C_{20}H_{24}O_2N_3F_3$ 395.43	[M^+] 395 (100), 243 (43), 229 (41), 200, (58), 195 (22), 166 (22), 145 (15), 81 (9), 70(11).
9a	$C_{21}H_{29}O_2N_3$ 355.48	[M^+] 355 (100), 208 (20), 189 (51), 180 (12), 176 (40), 167 (6), 146 (88), 95 (7), 91 (15), 70 (15),
9c	$C_{21}H_{26}O_2N_3F_3$ 409.46	[M^+] 409 (100), 243 (69), 229 (48), 209 (28), 200 (43), 180 (26), 166 (2), 95 (10), 70 (14).
10a	$C_{21}H_{29}O_2N_3$ 355.48	[M^+] 355 (11), 189 (100), 174 (6), 146 (15), 91 (4), 81 (2), 70 (25).
10c	$C_{21}H_{26}O_2N_3F_3$ 409.46	[M^+] 409 (12), 243 (100), 228 (4), 200(10), 180 (2), 145 (2), 81 (1), 70 (10)
10d	$C_{22}H_{28}O_2N_3F_3$ 423.48	[M^+] 423 (26), 257 (2), 243 (100), 229 (3), 223 (12), 200 (11), 194 (9), 166 (2), 145 (3), 81 (2), 70 (13)
11a	$C_{22}H_{31}O_2N_3$ 369.51	[M^+] 369 (13), 223 (1), 189 (100), 146 (14), 95 (2), 91 (3) 70 (23).

[a] Calculated for free basis.

Table 5
Anticonvulsant screening project (ASP): phase I test results in mice

Compound	Intraperitoneal injection in mice						ASP[d] class
	MES[a]		sc. PTZ[b]		TOX[c]		
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
8c	300	100	–	–	300	300	1
9c	–	30	–	–	300	> 300	1
10c	100	100	–	–	100	300	1
10d	100	30	–	–	30[1, 22]	100	4
11c	–	100	–	–	> 300	> 300	1
11d	–	100	–	–	100	300	1

Response comments: [1] death, [22] continuous seizures activity.
[a] Maximal electroshock: doses of 30, 100 and 300 mg/kg were administrated intraperitoneally in mice; the figures in the table indicate the minimum dose whereby anticonvulsant activity was demonstrated in 100% of the animals.
[b] Subcutaneous pentylenetetrazole test: doses of 30, 100 and 300 mg/kg.
[c] Neurotoxicity screen: dose of compound whereby neurotoxicity was exhibited in half or more of the animals. The dash indicates an absence of activity at maximum dose administrated (300 mg/kg).
[d] The ASP classification is as follows: 1: anticonvulsant activity at a doses 100 mg/kg or less; 4: compound active but toxic at a dose of 30 mg/kg or only toxic at the same dose.

Table 6
Anticonvulsant screening project (ASP) phase VIa

Compound	MES oral administration to rats (at a dose of 30 mg/kg)[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
9c	1	0	2	2	1
11c	1	2	0	1	3
Phenytoin[b]	1	4	3	3	3

[a] The figures indicate the number of rats out of four, which were protected.
[b] Data from [30].

activity in the MES test but none of them was active in the *sc*. PTZ screen. The results are shown in Table 5.

Some selected compounds (**9c, 11c**), active in mice were evaluated orally in rats in the MES test at a dose of 30 mg/kg, at several time points (Table 6).

3.2. In vitro test

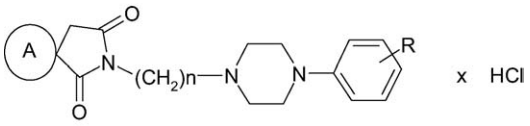
3.2.1. 5-HT_{1A} and 5-HT_{2A} binding assays

The results of in vitro binding studies of the newly synthesized compounds **8a–c, 9a–c, 10a–d** and **11a–d** are shown in Table 7. The investigated compounds showed diversified affinity for both 5-HT_{1A} and 5-HT_{2A} receptors and it's depended on length of aliphatic chain between two nitrogen atoms. All the ethylene analogues (**10a–c** and **11a–c**) displayed high affinity for 5-HT_{1A} receptor sites (K_i = 3.1–45 nM); moreover, they showed moderate affinity for 5-HT_{2A} receptors (K_i = 32–465 nM). In contrast, compounds with the methylene spacer (**8a–c** and **9a–c**) were found to displayed moderate to low affinity for both 5-HT_{1A} (K_i = 81–370 nM) and 5-HT_{2A} receptors (K_i = 126–1370 nM). The two propylene derivatives **10d** and **11d** demonstrated comparable, moderate affinity for 5-HT_{1A} (K_i = 82–94 nM) and for 5-HT_{2A} (K_i = 36–82 nM).

4. Discussion

The 2-azaspiro[4.4]nonane- and [4.5]decane-1,3-dione derivatives containing a 4-aryl-piperazine moiety connected with the nitrogen imide atom by an alkylene spacer were synthesized and tested for their anticonvulsant activity. Two kind of groups: electron-donating (–CH_3, –OCH_3) and electron-attracting (–CF_3) were chosen as substituents at the phenyl ring (either being often present in compounds showing anticonvulsant activity). Anticon-

Table 7
Chemical structure and the 5-HT_{1A} and 5-HT_{2A} receptor affinities of the investigated compounds (**8a–c, 9a–c, 10a–d** and **11a–d)**



Compound	Ring A	*N*	R	K_i ± S.E.M. (nM)	
				5-HT_{1A}	5-HT_{2A}
8a	Cyclopentane	1	2-CH_3	370 ± 42	1210 ± 90
8b	Cyclopentane	1	2-OCH_3	230 ± 18	1370 ± 55
8c	Cyclopentane	1	3-CF_3	300 ± 35	150 ± 9
9a	Cyclohexane	1	2-CH_3	326 ± 29	870 ± 37
9b	Cyclohexane	1	2-OCH_3	81 ± 7	1190 ± 2
9c	Cyclohexane	1	3-CF_3	260 ± 9	126 ± 9
10a	Cyclopentane	2	2-CH_3	45 ± 5	171 ± 12
10b	Cyclopentane	2	2-OCH_3	6.8 ± 1	465 ± 34
10c	Cyclopentane	2	3-CF_3	6.0 ± 1.3	47 ± 6
10d	Cyclopentane	3	3-CF_3	94 ± 8	82 ± 6
11a	Cyclohexane	2	2-CH_3	6.7 ± 0.5	43 ± 5
11b	Cyclohexane	2	2-OCH_3	5.7 ± 0.8	254 ± 12
11c	Cyclohexane	2	3-CF_3	3.1 ± 0.3	32 ± 2
11d	Cyclohexane	3	3-CF_3	82 ± 12	36 ± 2
Ketanserin				1933 ± 219	1.5 ± 0.2

vulsant activity was observed for compounds with a trifluoromethyl substituent (**8c, 9c, 10c, d, 11c**, **d**) in the MES test. Among those compounds, *N*-[{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-propyl]-2-azaspiro[4.4]-nonane-1,3-dione **10d** and *N*-[{4-(3-trifluoromethylphenyl)-piperazin-1-yl)-methyl]-2-azaspiro [4.5]decane-1,3-dione **9c** were the most active, displaying seizure protection at a dose of 30 mg/kg after 4 h. Since compound **10d** was neurotoxic at a dose of 30 mg/kg irrespective of its activity, following the ASP rules, it was ascribed to the 4 ASP class. All the other active compounds (**8c, 9c, 10c, 11c**, **d**) were assigned to the 1 ASP class (Table 5). Compounds **9c** and **11c**—being relatively the least toxic—were selected for the oral evaluation of anti-MES and neurotoxic activity in rats (phase VIa). They were administered in a dose of 30 mg/kg, and their effects were studied after 0.25, 0.5, 1, 2 and 4 hours. When given orally, none of those compounds was found to be neurotoxic. The most active **11c** protected 50% of the animals (2/4) after 0.5 h, and 75% (3/4) after 4 h. The least active **9c** protected 50% of the animals (2/4) after 1 and 2 h (Table 6).

It is noteworthy that introduction of the 2-methyl (**8a, 9a, 10a, 11a**) or the 2-methoxy (**8b, 9b, 10b**, **11b**) group into the 4-arylpiperazine fragment made all the compounds inactive in both tests used.

The highest anticonvulsant activity shown by the derivatives with a 3-CF_3 group with respect to the inactive 2-CH_3 and 2-OCH_3 analogues indicated that introduction of this bioactive [22,23], electron-attracting substituent is important to anticonvulsant activity.

In line with our previous findings [18,19], all the tested compounds displayed significant affinity for serotonin $5HT_{1A}$ and 5-HT_{2A} receptors, which ranged from moderate (K_i = 370 nM) to very high (K_i = 3.1 nM) for $5HT_{1A}$, and from low (K_i = 1370 nM) to high (K_i = 32 nM) for 5-HT_{2A} sites. Differences resulted mainly from the changes in the alkyl spacer length and the phenyl ring substitution pattern, whereas cycloalkyl portion hardly influenced serotonin receptor affinity (cyclohexane derivatives being slightly more active than cyclopentane ones). In all the cases ethylene spacer analogues (**10a–c, 11a–c**) possessed higher affinity for both receptor subtypes, than methylene ones (**8a–c, 9a–c**), whereas introduction of a longer propylene spacer for two 3-CF_3 analogues (**10d, 11d**) significantly decreased 5-HT_{1A} and 5-HT_{2A} receptor affinity than **10c** and **11c.** The above, described effect was contrary to that observed previously for a series of structurally related hydantoin derivatives. The influence of substitution at the phenyl ring was in line with the general knowledge about the structure-affinity relationships for arylpiperazine derivatives, especially in the case of methylene spacer analogues [24]. Derivatives with 2-OCH_3 group (**8b, 9b**) showed the highest affinity for $5HT_{1A}$ receptors, and the lowest for 5-HT_{2A} sites, while the highest 5-HT_{2A} receptor affinity was displayed by compounds **8c, 9c** with a 3-CF_3 phenyl moiety.

Summing up, compounds **10c**, **11a** and **11c** may be regarded as dual $5HT_{1A}$ and 5-HT_{2A} receptor ligands, whereas compounds **10b** and **11b** are $5HT_{1A}$ receptor ligands, being 68- and 44-fold more selective for 5-HT_{2A} sites, respectively.

In conclusion, in this study, we described several azaspiranes containing a 4-aryl-piperazin-1-yl-alkyl moiety, examined for their anticonvulsant activity in the MES test, as well as for their 5-HT_{1A} and 5-HT_{2A} receptor affinity. Among this series of derivatives we found anticonvulsant agents and some interesting serotonin receptors ligands, especially of the 5-HT_{1A} subtype. Although no direct correlation between anticonvulsant activity and receptor affinity was found, the active 3-CF_3 derivatives **10c**, **11c** with an ethylene spacer exhibited both anticonvulsant activity (1 class ASP) and high 5-HT_{1A} (K_i = 6 and 3 nM, respectively) and 5-HT_{2A} receptors affinity (K_i = 47 and 32 nM, respectively). The obtained results indicate that further

studies on involvement of 5-HT_{1A} and 5-HT_{2A} receptors on anticonvulsant activity are required.

5. Experimental protocols

5.1. Chemistry

All the chemicals and solvents were purchased from Sigma-Aldrich. Melting points (m.p.) were determined with electrothermal digital melting point apparatus and are left uncorrected. All the compounds used (**8a–c, 9a–c, 10a–d** and **11a–d**) were subjected to a quantitative elemental analysis (C, H, N) by a micro method using the elemental Vario EI III Elemental analyzer, and gave results for the elements stated with ± 0.4% of the theoretical values (data not shown).

The purity of the compounds was checked by a thin-layer chromatography (TLC) performed on Merck silica gel GF_{254} aluminum sheets, using the developing solvent: benzene/ethyl acetate/acetone (10:5:1). Spots were detected by their absorption under UV light and visualization was carried out with 0.05 mol I_2 in a 10% HCl.

^{1}H NMR spectra (in $CDCl_3$) were recorded on a Varian Gemini 200 (200 Hz) spectrometer. Chemical shifts were given in ppm (δ) from tetramethylsilane (TMS) as an internal standard.

Mass spectra (EI) were recorded on an AMD-604 mass spectrometer operating at 70 eV.

The preparation of 1-(2-aminoethyl)- and 1-(3-aminopropyl)-4-arylpiperazine was previously reported [25]. The synthesis and physicochemical data of compounds **8b** and **9b** has been published [12,21].

5.1.1. Synthesis of the N-[(4-arylpiperazin-1-yl)-methyl]-2-azaspiro[4.4]nonane- (**8a, c**) and [4.5]decane-1,3-dione derivatives (**9a, c**)

5.1.1.1. General procedure. The mixture of 2-azaspiro[4.4] nonane- (1.53 g, 0.01 mol), or [4.5]decane-1,3-dione (1.67 g, 0.01 mol), 35% formaldehyde solution (0.9 ml, 0.01 mol) and corresponding 4-arylpiperazine (0.01 mol) in 96% ethanol (40 ml) was refluxed for 0.5 h and then left for ca. 6–12 h at room temperature and refrigerated ca. –10 °C for 24 h. The products were washed with cold ethanol and crystallized from a 96% ethanol. The solid products were separated by filtration. Free bases were converted into hydrochloride salts in anhydrous ethanol saturated with HCl gas, and were crystallized from anhydrous ethanol (Scheme 1).

5.1.2. Synthesis of the N-[(4-arylpiperazin-1-yl)-alkyl]-2-azaspiro[4,4]nonane- (**10a–d**) and [4,5]decane-1,3-dione (**11a–d**) derivatives

5.1.2.1. General procedure. The obtained 1-cyclopentane-1-carboxy-1-acetic acid (1.72 g, 0.01 mol) or 1-cyclohexane-1-carboxy-1-acetic acid (1.86 g, 0.01 mol) was dissolved in water (10 ml) and appropriately substituted 1-(2-aminoethyl)- or 1-(3-aminopropyl)-4-arylpiperazine (0.01 mol) was added. The mixture was heated in oil bath with the simultaneous distillation of water. After the water was completely removed, the temperature of reaction was raised up to 190–200 °C and maintained for 1.5 h. The precipitated crude products were recrystallized from 96% ethanol. Free bases were converted into hydrochloride salts in anhydrous ethanol saturated with HCl gas, and were crystallized from anhydrous ethanol (Scheme 1).

5.2. Anticonvulsant assay

The compounds (**8a–c, 9a–c, 10a–d** and **11a–d**) 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 [26,27].

Phase I studies of the investigated compounds involved three testes: maximal electroshock (MES), subcutaneous pentylenetetrazole (*sc*. PTZ) and rota-rod test for neurological toxicity (TOX). All the compounds were injected intraperitoneally, as a suspension in a 0.5% methylcellulose, at the dose levels of 30, 100, and 300 mg/kg at 0.5 and 4 hours time periods. These data are presented in Table 5.

Promising compounds from phase I underwent phase VIa. The compounds were administrated orally into rats using four animals at a fixed dose of 30 mg/kg for both the MES and the rota-rod toxicity tests. Rats were tested at five time periods ranging from one quarter to 4 h post drug administration. The results are shown in Table 6.

5.3. In vitro experiments

5.3.1. 5-HT_{1A} and 5-HT_{2A} binding assays

The affinity of the investigated compounds for 5-HT_{1A} and 5-HT_{2A} receptors in vitro was assessed on the basis of their ability to displace [^{3}H]-8-OH-DPAT (170 Ci/mmol, NEN Chemicals, USA) and [^{3}H]-ketanserin (88 Ci/mmol, NEN Chemicals, USA), respectively. Radioligand binding experiments were carried out on rat brain using tissues from the hippocampus for 5-HT_{1A} receptors, and from the cortex for 5-HT_{2A} receptors, according to the previously published procedures [28]. K_i values were determined from at least three competition binding experiments in which 10–14 sample concentrations, run in triplicate, were used. The Cheng and Prusoff [29] equation was used for K_i calculations.

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