Full Paper

Synthesis, Physicochemical and Anticonvulsant Properties of New *N*-4-Arylpiperazin-1-yl Amides of (2-Aza-1,3-dioxospiro[4.4]non-2-yl)- and [4.5]dec-2-yl)-propionic Acid

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In continuation of the search of new anticonvulsants, a series of *N*-4-arylpiperazin-1-yl 2-aza-1,3-dioxospiro[4.4]non-2-yl- (**5**–**8**) and [4.5]dec-2-yl- (**9**–**15**) propionamides, structurally related to the previously described *N*-4-arylpiperazin-1-yl amides of 2-aza-1,3-dioxospiro[4.5]dec-2-yl-acetic acid, were synthesized. The designed compounds **5**–**15** were prepared by condensation of the formerly obtained (2-aza-1,3-dioxospiro[4.5]dec-2-yl)- (**3**) and (2-aza-1,3-dioxo[4.4]non-2-yl)-(**4**) propionic acids with the appropriately substituted 4-arylpiperazines, in the presence of the *N,N*-carbonyldiimidazole (CDIM) reagent. All the compounds were tested for their anticonvulsant activity in the maximum electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ) seizure threshold tests. Several compounds **7**–**10**, **13** and **14** revealed protection in the MES screening.

Keywords: Anticonvulsant activity / *N*-4-arylpiperazin-1-yl-2-aza-1,3-dioxospiro[4.4]non-2-yl- and [4.5]dec-2-yl-propionamides / Pyrrolidine-2,5-diones

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Introduction

Numerous compounds are getting synthesized and screened for their anticonvulsant activities each year. To make the discovery of new anticonvulsants more rational, several investigators identify structural fragments that may enhance anticonvulsant properties and permit also for orientating the synthesis of novel compounds in which some of these active fragments can appear [1]. One of the structural features that play a significant role in relation to anti-epileptic activity is an amide function [2, 3]. This moiety may be introduced into a heterocyclic ring, *e.g.* ethosuximide, phenytoin or as an anilide nucleus, *e.g.* ameltolide (Fig. 1.).

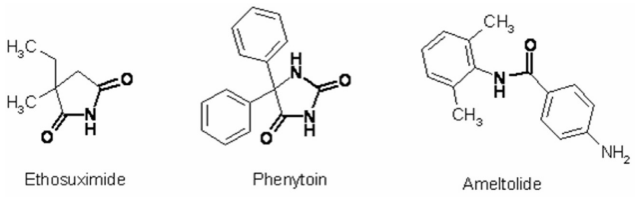

Figure 1. Structure of ethosuximide, phenytoin and ameltolide.

In the course of developing new, potentially anticonvulsant agents, our attention has been focused on the group of pyrrolidine-2,5-dione derivatives variously substituted at the nitrogen atom as well as at the position-3 of the imide ring [4–8]. In this series of compounds the most promising were derivatives containing a 4-arylpiperazine moiety, connected to the imide nitrogen atom by the alkylene spacer [9–11]. Their anticonvulsant properties depended on the kind of substitution mode at position-3 of the pyrrolidine-2,5-dione ring, the length of the alkylene chain joining the endocyclic imide nitrogen atom and the 4-arylpiperazine fragment as well as the nature of the substituents at the aromatic area.

Taking into consideration a vital influence of amide moiety on anticonvulsant activity, we have recently

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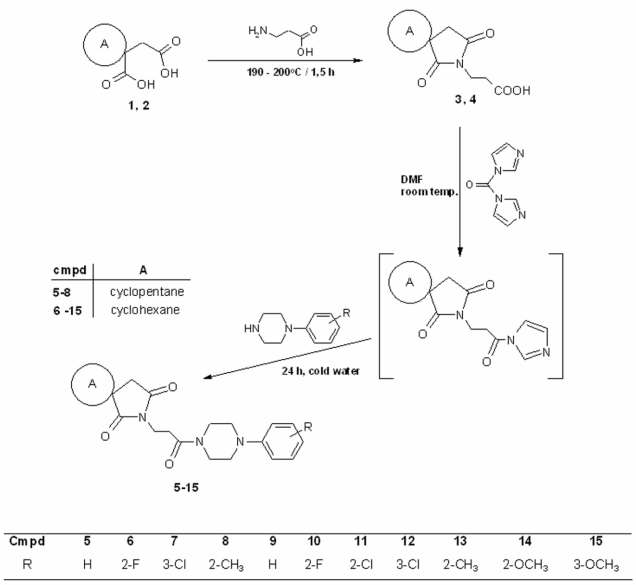

Scheme 1. Synthesis and structures of the obtained compounds.

obtained a series of 2-aza-1,3-dioxospiro[4.5]dec-2-yl-acetamides with the 4-arylpiperazine derivatives as amide function [12]. This molecules may be recognized as analogues of respective *N*-[(4-arylpiperazine)-alkyl]-spirosuccinimides with an additional amide function in the alkyl side chain. Several of those compounds were effective in the maximal electroshock (MES) or subcutaneous metrazole (scPTZ) screenings. On the other hand, the respective *N*-phenyl and *N*-benzyl amides of 3-spirocycloalkylpyrrolidine-2,5-dione acetic acid were devoid of anticonvulsant activity [13]. It proves the unique role of the 4-arylpiperazine fragment as pharmacophoric system of such type of compounds.

In view of these data, in this work, we have designed and synthesized a new series of *N*-4-arylpiperazin-1-yl-2-aza-1,3-dioxospiro[4.4]non-2-yl- (**5**–**8**) and [4.5]dec-2-yl- (**9**–**15**) propionamides. The aim of this study was to evaluate the influence of the introduction of an ethylene spacer between the imide nitrogen atom and the carbonyl amide group on the anticonvulsant activity, in comparison to the methylene analogues described previously.

Results and discussion

Chemistry

Compounds **5**–**15** were synthesized according to Scheme 1. First, the condensation reaction of starting 1-carboxy-1-cyclopentane- (**1**) or 1-carboxy-1-cyclohexane-acetic (**2**) acids, which were obtained according to the procedures described elsewhere [14], with 3-aminopropionic acid yielded the corresponding 3-(1,3-dioxo-2-azaspiro[4.4]non-2-yl)- (**3**) or [4.5]dec-2-yl-propionic(**4**) acids. These products were converted to the respective final 4-arylpiperazine amides **5**–**15**, by use of the amide-bond formation reaction in the presence of the *N*,*N*-carbonyldiimidazole (CDIM) reagent. The structures of the compounds synthesized were confirmed by elemental analysis as well as examination of their ^{1}H-NMR spectra.

Table 1. Anticonvulsant and neurotoxicity screening results of investigated compounds.

Compound	Intraperitoneal injection in mice[a]						ASP[b] class
	MES		scPTZ		TOX[c]		
	0.5 [h]	4 [h]	0.5 [h]	4 [h]	0.5 [h]	4 [h]	
5	–	–	–	–	–	–	3
6	–	–	–	–	–	–	3
7	300	–	–	–	300[14]	–	2
8	300	–	–	–	300[14]	–	2
9	300	–	–	–	300[14]	–	2
10	300	–	–	–	–	–	2
11	–	–	–	–	300	–	3
12	–	–	–	–	–	–	3
13	300	–	–	–	–	–	2
14	300	–	30[25]	–	300	–	2
15	–	–	–	–	–	–	3

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

[b] Toxicity screening: minimum dose of compound whereby toxicity was exhibited. Response comments: [14] unable to grasp rotorod, [25] myoclonic jerks.

[b] The ASP classification is as follows: 1 – anticonvulsant activity at doses of 100 mg/kg or less; 2 – anticonvulsant activity at doses of 100 mg/kg and more; 3 – compound inactive at doses of 300 mg/kg.

Anticonvulsant activity

The maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) tests are claimed to detect compounds affording protection against generalized tonic-clonic seizures and generalized absence seizures, respectively. Thus, the MES and scPTZ screens have become the most widely employed seizure models for early identification of candidate anticonvulsants. The results of anticonvulsant identification studies in mice are shown in Table 1.

The results obtained revealed that, except for compound **14**, which was active in both MES and scPTZ tests, all other derivatives inhibited only electrically provoked seizures (MES-test) **7**–**10**, **13** or were inactive **5**, **6**, **11**, **12**,

15. In the series of 4-arylpiperazin-1-yl-(2-aza-1,3-dioxospiro[4.5]dec-2-yl)-propionamides, the most active were compound **9** and its analogues with fluoro- **10**, methyl- **13** and methoxy- **14** substituents at the position-2 of the 4-arylpiperazine moiety. These molecules protected 100% of the animals tested at a dose of 300 mg/kg at 0.5 h. Additionally, compound **14** exhibited anti-scPTZ activity at a dose of 30 mg/kg at 0.5 h, however, at the same concentration provoked myoclonic jerks.

Change of the size of the cycloalkyl unit from cyclohexane to cyclopentane, caused only a marginal decrease in anticonvulsant activity which was observed in a group of 4-arylpiperazin-1-yl amides of (2-aza-1,3-dioxospiro[4.4]-non-2-yl)-propionic acid **5**–**8**. In this series of compounds, the 3-chloro- **7** and 2-methyl- **8** derivatives were active in the MES test at a dose of 300 mg/kg at 0.5 h.

In the neurological toxicity screening, compounds **7**–**9**, **11** and **14** were found to be toxic at the maximum administrated dose (300 mg/kg). The mice were unable to grasp the rotorod after administration of derivatives **7**, **8** and **9** at a dose of 300 mg/kg.

In conclusion, the introduction of an ethylene spacer between the imide nitrogen atom and the carbonyl amide group decreased the anticonvulsant activity in comparison to 4-arylpiperazin-1-yl amides of (2-aza-1,3-dioxospiro[4.5]dec-2-yl)-acetic acid described previously [12]. As mentioned above, the amide function is one of the structural elements that play a significant role regarding the anticonvulsant activity for several groups of compounds. However, the results of our studies indicate that incorporation of the additional amide function into the alkyl chain joining the nitrogen atom of succinimide and 4-arylpiperazine moiety decreased the anticonvulsant properties in relation to the respective alkylene analogues.

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Experimental

Chemistry

All the chemicals and solvents were purchased from Merck (Darmstadt, Germany) and were used without further purification. Melting points (mp.) 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), using the developing systems A: benzene : ethyl acetate : acetone (10 : 5 : 1) and B: chloroform : acetone (9 : 1). Spots were detected by their absorption under UV light (λ = 254 nm) and by visualization with 0.05 mol iodine in 10% HCl. The chemical structures were confirmed by elemental and spectral analyses (^{1}H-NMR). ^{1}H-NMR spectra were obtained in a Varian Mercury 300 MHz spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$, with TMS as an internal standard. Chemical shifts are reported in δ values (ppm) and *J* values in Hertz (Hz). Signal multiplicities were given by the following abbreviations: s (singlet), br. s (broad singlet), dd (doublet of doublets), t (triplet), dt (doublet of triplets), m (multiplet). Elemental analyses for C, H, N were carried out with an Elementar Vario EL III (Hanau, Germany).

*General procedure for the preparation of (2-aza-1,3-dioxospiro[4.4]non-2-yl)- (**3**) and [4.5]dec-2-yl)-propionic acids (**4**)*

The total of 1-carboxy-1-cyclopentane- (**1**) or 1-carboxy-1-cyclohexane-acetic (**2**) acids (0.04 mol) were dissolved in 20 mL of water and 3-aminopropionic acid (0.04 mol) 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 was raised to 180°C and maintained at that level for approx. 1.5 h. The crude products were recrystallized from ethanol.

*2-Aza-1,3-dioxospiro[4.4]non-2-yl-propionic acid **3****

White solid (yield 82%), mp. 113–115°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.63–2.12 (8H, m, $-C_4H_8-$), 2.58 (2H, s, imide), 2.68 (2H, t, CH_2-CH_2-CO, *J* = 7.18 Hz), 3.82 (2H, t, CH_2-CH_2, *J* = 7.18 Hz), 10.41 (1H, br. s, COOH). Anal. calcd. for $C_{11}H_{15}N_1O_4$: C, 58.66; H, 6.71; N, 6.22. Found: C, 58.40; H, 6.60; N, 6.10.

* Mondon [15] described this compound as by-product; however, no physicochemical and spectral data were available so far.

*2-Aza-1,3-dioxospiro[4.5]dec-2-yl-propionic acid **4***

White solid (yield 78%), mp. 120–122°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.29–1.78 (8H, m, $-C_4H_8-$), 2.54 (2H, s, imide), 2.64 (2H, t, CH_2-CH_2-CO, *J* = 7.18 Hz), 3.78 (2H, t, CH_2-CH_2, *J* = 7.18 Hz), 10.32 (1H, br. s, COOH). Anal. calcd. for $C_{12}H_{17}N_1O_4$: C, 60.24; H, 7.16; N, 5.85. Found: C, 60.0; H, 7.10; N, 6.00.

*General procedure for the preparation of compounds **5-15***

The obtained 2-aza-1,3-dioxospiro[4.4]non-2-yl- (**3**) or [4.5]dec-2-yl-propionic (**4**) acids (0.01 mol) were dissolved in 20 mL of DMF and *N,N*-carbonyl-diimidazole (0.01 mol) was added. The mixture was stirred for 0.5 h at a room temperature. Afterwards, the appropriately substituted 4-arylpiperazine (0.01 mol) was added. After 24 h of stirring at room temperature, the reaction mixture was left in an ice cold bath. The product was precipitated with cold water and was purified by recrystallization from isopropyl alcohol.

*N-(4-Phenylpiperazin-1-yl)amide of 2-aza-1,3-dioxospiro[4.4]non-2-yl-propionic acid **5***

Compound **5** was obtained as white solid (yield 88%), mp. 123–125°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.61–2.17 (8H, m, $-C_4H_8-$), 2.58 (2H, s, imide), 2.69 (2H, t, CH_2-CH_2-CO, *J* = 7.69 Hz), 3.13–3.19 (4H, m, piperazine), 3.61 (2H, br. s, piperazine), 3.75 (2H, br. s, piperazine), 3.84 (2H, t, CH_2-CH_2, *J* = 7.69 Hz), 6.89–

6.94 (2H, m, arom.), 7.24 – 7.31 (3H, m, arom.). Anal. calcd. for $C_{21}H_{27}N_3O_3$: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.20; H, 7.40; N, 11.30.

N-[4-(2-Fluorophenyl)-piperazin-1-yl]amide of 2-aza-1,3-dioxospiro[4.4]non-2-yl-propionic acid ***6***

Compound **6** was obtained as white solid (yield 77%), m.p 134 – 136°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.59 – 2.16 (8H, m, $-C_4H_8-$), 2.57 (2H, s, imide), 2.67 (2H, t, CH_2-CH_2-CO, J = 7.69 Hz), 3.02 – 3.10 (4H, m, piperazine), 3.61 (2H, t, piperazine, J = 5.13 Hz), 3.76 (2H, t, piperazine, J = 5.13 Hz), 3.82 – 3.87 (2H, m, CH_2-CH_2), 6.90 – 7.10 (4H, m, arom.). Anal. calcd. for $C_{21}H_{26}N_3O_3F$: C, 65.18; H, 6.76; N, 10.85. Found: C, 65.0; H, 6.90; N, 10.80.

N-[4-(3-Chlorophenyl)-piperazin-1-yl]amide of 2-aza-1,3-dioxospiro[4.4]non-2-yl-propionic acid ***7***

Compound **7** was obtained as white solid (yield 74%), mp. 99 – 101°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.62 – 2.17 (8H, m, $-C_5H_{10}-$), 2.57 (2H, s, imide), 2.7 (2H, t, CH_2-CH_2-CO, J = 7.69 Hz), 3.17 (4H, dt, piperazine, J = 5.01 Hz), 3.10 (2H, t, piperazine, J = 5.01 Hz), 3.73 (2H, t, piperazine, J = 5.21 Hz), 3.84 (2H, dd, CH_2-CH_2, J = 6.90 Hz), 6.76 – 6.87 (3H, m, arom.), 7.15 – 7.20 (1H, m, arom.). Anal. calcd. for $C_{21}H_{26}N_3O_3Cl$: C, 62.45; H, 6.49; N, 10.40. Found: C, 62.30; H, 6.50; N, 10.40.

N-[4-(2-Methylphenyl)-piperazin-1-yl]amide of 2-aza-1,3-dioxospiro[4.4]non-2-yl-propionic acid ***8***

Compound **8** was obtained as white solid (yield 61%), mp. 102 – 104°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.62 – 2.17 (8H, m, $-C_4H_8-$), 2.32 (3H, s, CH_3), 2.58 (2H, s, imide), 2.68 (2H, t, CH_2-CH_2-CO, J = 7.95 Hz), 2.85 – 2.93 (4H, m, piperazine), 3.60 (2H, t, piperazine, J = 4.86 Hz), 3.74 (2H, t, piperazine, J = 4.87 Hz), 3.83 – 3.88 (2H, m, CH_2-CH_2), 6.90 – 6.99 (2H, m, arom.), 7.00 – 7.20 (2H, m, arom.). Anal. calcd. for $C_{22}H_{29}N_3O_3$: C, 68.99; H, 7.63; N, 10.97. Found: C, 68.71; H, 7.40; N, 10.70.

N-(4-Phenylpiperazin-1-yl)-amide of 2-aza-1,3-dioxospiro[4.5]dec-2-yl-propionic acid ***9***

Compound **9** was obtained as white solid (yield 74%), mp. 139 – 141°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.20 – 1.84 (10H, m, $-C_5H_{10}-$), 2.54 (2H, s, imide), 2.67 (2H, t, CH_2-CH_2-CO, J = 7.69 Hz), 3.12 – 3.19 (4H, m, piperazine), 3.60 (2H, br, s, piperazine), 3.74 (2H, br. s, piperazine), 3.83 (2H, t, CH_2-CH_2, J = 7.55 Hz), 6.89 – 6.93 (2H, m, arom.), 7.28 – 7.31 (3H, d, arom., J = 8.72 Hz). Anal. calcd. for $C_{22}H_{29}N_3O_3$: C, 68.99; H, 7.63; N, 10.97. Found: C, 69.10; H, 7.50; N, 10.80.

N-[4-(2-Fluorophenyl)-piperazin-1-yl]amide of 2-aza-1,3-dioxospiro[4.5]dec-2-yl-propionic acid ***10***

Compound **10** was obtained as white solid (yield 69%), mp. 129 – 131°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.59 – 2.16 (8H, m, $-C_4H_8-$), 2.57 (2H, s, imide), 2.68 (2H, t, CH_2-CH_2-CO, J = 7.69 Hz), 3.02–3.10 (4H, m, piperazine), 3.61 (2H, t, piperazine, J = 5.13 Hz), 3.76 (2H, t, piperazine, J = 5.13 Hz), 3.82 – 3.87 (2H, m, CH_2-CH_2), 6.90 – 7.10 (4H, m, arom.). Anal. calcd. for $C_{22}H_{28}N_3O_3F$: C, 65.90; H, 7.04; N, 10.48. Found: C, 65.70; H, 7.20; N, 10.70.

N-[4-(2-Chlorophenyl)-piperazin-1-yl]amide of 2-aza-1,3-dioxospiro[4.5]dec-2-yl-propionic acid ***11***

Compound **11** was obtained as white solid (yield 63%), mp. 126 – 128°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.19–1.84 (10H, m, $-C_5H_{10}-$), 2.54 (2H, s, imide), 2.66 (2H, t, CH_2-CH_2-CO, J = 7.69 Hz), 2.99–3.07 (4H, m, piperazine), 3.62 (2H, t, piperazine, J = 4.87 Hz), 3.76 (2H, t, piperazine, J = 4.99 Hz), 3.83 (2H, t, CH_2-CH_2, J = 7.56 Hz), 6.98-7.03 (2H, m, arom.), 7.20-7.23 (1H, m, arom.), 7.38 (1H, dd, arom., J = 6.66 Hz). Anal. calcd. for $C_{22}H_{28}N_3O_3Cl$: C, 63.22; H, 6.77; N, 10.08. Found: C, 63.0; H, 6.70; N, 9.85.

N-[4-(3-Chlorophenyl)-piperazin-1-yl]amide of 2-aza-1,3-dioxospiro[4.5]dec-2-yl-propionic acid ***12***

Compound **12** was obtained as white solid (yield 89%), mp. 159 – 161°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.19 – 1.84 (10H, m, $-C_5H_{10}-$), 2.54 (2H, s, imide), 2.66 (2H, t, CH_2-CH_2-CO, J = 7.56 Hz), 3.14 – 3.22 (4H, m, piperazine), 3.60 (2H, t, piperazine, J = 5.12 Hz), 3.71 – 3.74 (2H, t, piperazine, J = 5.13 Hz), 3.82 (2H, t, CH_2-CH_2, J = 7.56 Hz), 6.77 – 6.90 (3H, m, arom.), 7.18 (1H, t, arom. J = 8.08 Hz). Anal. calcd. for $C_{22}H_{28}N_3O_3Cl$: C, 63.22; H, 6.77; N, 10.08. Found: C, 63.40; H, 6.50; N, 9.80.

N-[4-(2-Methylphenyl)-piperazin-1-yl]amide of 2-aza-1,3-dioxospiro[4.5]dec-2-yl-propionic acid ***13***

Compound **13** was obtained as white solid (yield 62%), mp. 120 – 122°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.19 – 1.84 (10H, m, $-C_5H_{10}-$), 2.33 (3H, s, CH_3), 2.54 (2H, s, imide), 2.67 (2H, t, $-CH_2-CH_2-CO$, J = 7.69 Hz), 2.86 (2H, t, piperazine, J = 5.00 Hz), 2.92 (2H, t, piperazine, J = 5 Hz), 3.60 (2H, t, piperazine, J = 4.74 Hz), 3.74 (2H, t, piperazine, J = 4.87 Hz), 3.84 (2H, t, CH_2-CH_2, J = 7.56 Hz), 7.02 (2H, t, J = 8.08 Hz, arom.), 7.15 – 7.20 (2H, m, arom.). Anal. calcd. for $C_{23}H_{31}N_3O_3$: C, 69.59; H, 7.87; N, 10.58. Found: C, 69.40; H, 7.70; N, 10.40.

N-[4-(2-Methoxyphenyl)-piperazin-1-yl]amide of 2-aza-1,3-dioxospiro[4.5]dec-2-yl-propionic acid ***14***

Compound **14** was obtained as white solid (yield 67%), mp. 128 – 129°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.20 – 1.80 (10H, m, $-C_5H_{10}-$), 2.54 (2H, s, imide), 2.66 (2H, t, $-CH_2-CH_2-CO$, J = 7.69 Hz), 3.05 (4H, br. s, piperazine), 3.62 (2H, br. s, piperazine), 3.77 (2H, br. s, piperazine), 3.83 (2H, t, $-CH_2-CH_2-$, J = 7.69 Hz), 3.88 (3H, s, OCH_3), 6.87 – 7.05 (4H, m, arom.). Anal. calcd. for $C_{23}H_{31}N_3O_4$: C, 66.89; H, 7.57; N, 10.17. Found: C, 66.90; H, 7.40; N, 9.88.

N-[4-(3-Methoxyphenyl)-piperazin-1-yl]amide of 2-aza-1,3-dioxospiro[4.5]dec-2-yl-propionic acid ***15***

Compound **15** was obtained as white solid (yield 77%), mp. 125 – 127°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.20 – 1.84 (10H, m, $-C_5H_{10}-$), 2.54 (2H, s, imide), 2.66 (2H, t, $-CH_2-CH_2-CO$, J = 7.56 Hz), 3.13 – 3.19 (4H, m, piperazine), 3.59 (2H, br. s, piperazine), 3.73 (2H, br. s, piperazine), 3.79 (3H, s, OCH_3), 3.83 (2H, t, CH_2-CH_2-, J = 7.44 Hz) 6.45-6.58 (3H, m, arom.), 7.18 (1H, t, arom. J = 8.20 Hz). Anal. calcd. for $C_{23}H_{31}N_3O_4$: C, 66.89; H, 7.57; N, 10.17. Found: C, 66.60; H, 7.60; N, 10.30.

Pharmacology

Compounds **5** – **15** 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 [16, 17].

Phase I studies of the compounds investigated involved three testes: maximal electroshock seizure (MES), subcutaneous pentylenetrazole seizure (scPTZ) 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 anaesthetic agent. Mice were tested using the following doses 30, 100 and 300 mg/kg of the 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 hind limb tonic extensor component indicates the test compound's ability to inhibit MES-induced seizure spread.

The scPTZ test utilizes of pentylenetetrazole (85 mg/kg). This produces clonic seizures lasting for a period of at least five seconds 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 at a volume of 0.01 mL/g body weight in mice. Animals were observed over a 30-minute period. Absence of clonic seizures in the observed time period indicated an ability of the compounds to abolish the effect of pentylenetetrazole on seizure threshold.

Neurological toxicity (TOX) induced by a compound was detected in mice using standardized rotorod 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 of greater than 100 mg/kg (class 2), inactive at 300 mg/kg (class 3). The results of preliminary screening for compounds **5**–**15** are presented in Table 1.

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