Available online at www.sciencedirect.com
SCIENCE DIRECT
Il Farmaco 60 (2005) 529–539
IL FARMACO
http://france.elsevier.com/direct/FARMAC/

Synthesis, physicochemical and anticonvulsant properties of *N*-benzyl and *N*-aminophenyl derivatives of 2-azaspiro[4.4]nonane and [4.5]decane-1,3-dione. Part I

Jolanta Obniska [a,*], Agnieszka Dzierzawska-Majewska [b], Agnieszka Zagorska [a], Pawel Zajdel [a], Janina Karolak-Wojciechowska [b]

[a] *Department of Pharmaceutical Chemistry, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland*
[b] *Institute of General and Ecological Chemistry, Technical University, Zwirki 36, 90-924 Lodz, Poland*

Received 20 July 2004; received in revised form 5 March 2005; accepted 10 May 2005

Available online 13 June 2005

Abstract

To continue our systematic SAR studies, two series of *N*-benzyl- (X = CH_2) and *N*-aminophenyl- (X = NH) derivatives of 2-azaspiro[4.4]nonane (**1a**–**1j**) and 2-azaspiro[4.5]decane-1,3-dione (**2a**–**2j**) were synthesized, and evaluated in maximum electroshock seizure (MES), subcutaneous pentylenetetrazole (sc.MET) and rotorod (TOX) tests for their anticonvulsant activity. Among those derivatives, the most potent *N*-aminophenyl-2-azaspiro[4.4]nonane-1,3-dione **1j** had ED_{50} = 76.27 mg kg^{-1}. X-ray structures for two pairs of derivatives with a different linker were solved. Then 3-D data for the active **1j** versus less active **2j**, both having an imine linker (X = NH), and the respective parent of compounds with a methylene linker (X = CH_2) (**1a** and **2a**) were discussed.

Keywords: 2-Azaspiro[4.4]nonane-1,3-dione; 2-Azaspiro[4.5]decane-1,3-dione; Spirosuccinimides; Pyrrolidine-2,5-diones; Anticonvulsant activity; X-ray structures

1. Introduction

Anticonvulsant activity was found in compounds having a spirocycloalkyl fragment connected with the heterocyclic ring in many types of derivatives, e.g. spirohydantoins [1,2], spirobarbiturates [3], and spirosuccinimides [4–7]. Structure activity relationship studies conducted with a group of spirosuccinimides showed a pivotal role of the cycloalkyl system attached to the C3 spiro carbon atom, as well as the significance of the kind of substituents in the aromatic ring in the aspect of influence of that type of compounds on anticonvulsant activity. Also the influence of a linker joining the endocyclic nitrogen atom and the aromatic ring was investigated [8,9]. A research conducted by Edafiogho et al. [10] led to the synthesis of *N*-benzyl-2-azaspiro[4.4]nonane-1,3-dione **1a**, which was devoid of anticonvulsant activity, later those authors modified the linker between the imide ring and the aryl moiety by changing the methylene group into an oxymethylene one. The resulting *N*-benzyloxy-2-azaspiro[4.4]-nonane-1,3-dione was classified as a potent anticonvulsant compound (ED_{50} = 111 mg kg^{-1}).

Our earlier SAR studies with a group of *N*-pirydyl-3-aryl succinimide derivatives indicated that their anticonvulsant activity was closely connected with the conformational and electronic properties of those molecules. An analysis of the molecular electrostatic potential (MEP) distribution showed that the differences in MEP minima depth in the proximity of both carbonyl oxygen atoms of the imide ring were different for active and inactive compounds [11–14]. On a basis of those facts and our earlier chemical and pharmacological investigations with a group of differently 1,3-substituted pyrrolidine-2,5-diones [15–20], in the present study we designed two series of 2-azaspiro[4.4]nonane- and 2-azaspiro[4.5]decane-1.3-diones with different linkers (methylene, imine) between the imide moiety and the aromatic center. Furthermore, we examined the impact of the aromatic ring substitution mode on anticonvulsant activity. The synthesis and the results of

* Corresponding author.
E-mail address: mfobnisk@cyf-kr.edu.pl (J. Obniska).

0014-827X/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.farmac.2005.05.006

pharmacological investigation are presented below. For two pairs of compounds with the methylene (**1a**, **2a**) or the imine (**1j**, **2j**) linker, crystallographic studies were carried out to correlate the conformation and the electronic properties of those compounds with their anticonvulsant activity.

2. Chemistry

Compounds **1a**[1]**–j** and **2a–j** were synthesized according to Scheme **1**. The starting dicarboxylic acids were prepared as reported previously [7] using a two-step procedure. First, in a reaction of cyclopentanone or cyclohexanone with ethyl cyanoacetate in the presence of acetic acid and ammonium acetate, the corresponding cycloalkylidene cyjanoethyl esters were obtained. Treatment of the intermediate products with potassium cyanide, followed by a hydrolysis, furnished the respective acids. The final N-substituted spirosuccinimides (**1a–j** and **2a–j**) were obtained in a one-pot cyclization reaction of the prepared acids and appropriately substituted benzylamines or phenylhydrazines by heating them at ca. 190–200 °C for 1.5–2 h (Scheme 1).

Compounds **1j**, **2a** and **2j** had been previously described by El-Telbany et al. [21,22] (no ^{1}H-NMR data are available), but none of them were tested for their anticonvulsant activity. Products **1b–i** and **2b–i** are the new ones.

3. Experimental

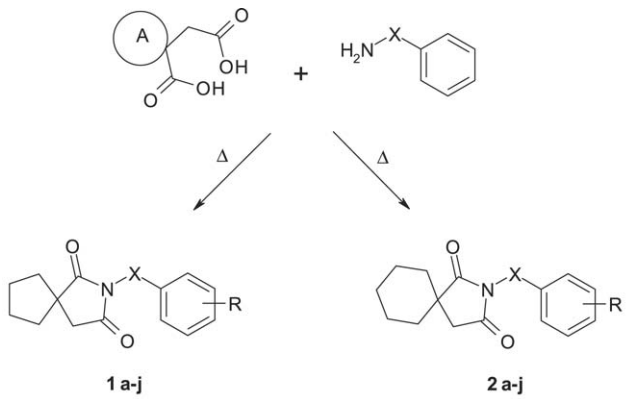
3.1. Chemistry

The chemical structures of the obtained compounds were confirmed by elemental and spectral analyses (^{1}H-NMR, IR). ^{1}H-NMR spectra were determined on a Varian Mercury 300 MHz spectrometer, in $CDCl_3$ with TMS as an internal standard. All chemical shifts are given in δ values. Signal multiplicities are represented by the following abbreviations: s (singlets), m (multiplets). IR spectra were recorded with a Fourier transform spectrometer FT/IR-410 (Jasco Corp., Japan) using KBr discs. Elemental analyses (C, H, N) were within ± 0.4% of the theoretical values.

The purity of all the synthesized compounds was checked by a thin-layer chromatography performed on Merck Silica gel GF_{254} aluminum plates, using the following systems: A. chloroform/acetone (9:1), B. ethyl acetate/n-hexane (3:7). After developing chromatograms, the plates were inspected under UV light (λ = 254 nm), and spots were additionally visualized with 0.05 mol I_2 in a 10% HCl solution. Melting points (°C) are uncorrected.

3.1.1. General procedure for the preparation of N*-benzyl- or* N*-aminophenyl-2-azaspiro[4.4]nonane- (**1a–j**) and 2-aza-spiro[4.5]decane-1,3-dione (**2a–j**) derivatives*

A total of 0.02 mol of the appropriately substituted benzylamine or phenylhydrazine was dissolved in 25 ml of water,



Ring A = cyclopentane, cyclohexane

Comp.	1a*	1b	1c	1d	1e	1f	1g	1h	1i	1j
X	CH_2	CH_2	CH_2	CH_2	CH_2	CH_2	CH_2	CH_2	CH_2	NH
R	H	2-Cl	2,4-Cl	3-Cl	4-Cl	2-CH_3	4-CH_3	2-OCH_3	4-OCH_3	H

*Compound described previously in [10]

Comp.	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j
X	CH_2	CH_2	CH_2	CH_2	CH_2	CH_2	CH_2	CH_2	CH_2	NH
R	H	2-Cl	2,4-Cl	3-Cl	4-Cl	2-CH_3	4-CH_3	2-OCH_3	4-OCH_3	H

Scheme 1. Synthesis and structures of 2-azaspiro[4.4]nonane- (**1a–1j**) and 2-azaspiro[4.5]decane-1,3-dione (**2a–2j**) derivatives.

and 0.02 mol of cyclopentane-1-carboxy-1-acetic acid or cyclohexane-1-carboxy-1-acetic acid was gradually added. The mixture was heated in an oil bath with the simultaneous distillation of water. After the water was completely removed, the temperature of the reaction mixture was raised up to 190–200 °C and maintained for 1.5 h. Crude products were recrystallized from a 96% ethanol.

3.1.1.1. N*-benzyl-2-azaspiro[4.4]nonane-1,3-dione (**1a**)*[2]. (Yield: 65%); m.p. 58–60 °C[3]; R_f = 0.79A, R_f = 0.45B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.58–2.13 (m, 8H, cyclopentane), 2.58 (s, 2H, imide), 4.65 (s, 2H, CH_2), 7.23–7.36 (m, 4H, arom.); IR ν (cm^{-1}) 1773, 1691[4] (C=O), *Anal.* $C_{15}H_{16}O_2N_1$ (C, H, N).

3.1.1.2. N*-(2-chlorobenzyl)-2-azaspiro[4.4]nonane-1,3-dione (**1b**)*. (Yield: 55%); m.p. 72–74 °C; R_f = 0.86A, R_f = 0.43B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.58–2.20 (m, 8H, cyclopentane), 2.65 (s, 2H, imide), 4.79 (s, 2H, CH_2), 7.08–7.37 (m, 4H, arom.); IR ν (cm^{-1}) 1772, 1698 (C=O), *Anal.* $C_{15}H_{16}O_2N_1Cl_1$ (C, H, N).

3.1.1.3. N*-(2,4-dichlorobenzyl)-2-azaspiro[4.4]nonane-1,3-dione (**1c**)*. (Yield: 59%); m.p. 79–81 °C; R_f = 0.91A, R_f = 0.49B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.59–2.21 (m, 8H, cyclopentane), 2.68 (s, 2H, imide), 4.78 (s, 2H, CH_2), 7.18–7.42 (m, 3H, arom.); IR ν (cm^{-1}) 1773, 1709 (C=O), *Anal.* $C_{15}H_{15}O_2N_1Cl_2$ (C, H, N).

[1] Compound **1a** was earlier described in ref. [10].

[2] Reference [10].

[3] 63.5 °C.

[4] 1780 (C=O), 1715 (C=O).

3.1.1.4. N-*(3-chlorobenzyl)-2-azaspiro[4.4]nonane-1,3-dione (**1d**).* (Yield: 65%); m.p. 97–99 °C; R_f = 0.88A, R_f = 0.38B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.61–2.20 (m, 8H, cyclopentane), 2.53 (s, 2H, imide), 4.65 (s, 2H, CH_2), 7.27–7.37 (m, 4H, arom.); IR ν (cm^{-1}) 1772, 1700 (C=O), *Anal.* $C_{15}H_{16}O_2N_1Cl_1$ (C, H, N).

3.1.1.5. N-*(4-chlorobenzyl)-2-azaspiro[4.4]nonane-1,3-dione (**1e**).* (Yield: 65%); m.p. 92–94 °C; R_f = 0.84A, R_f = 0.41B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.57–2.12 (m, 8H, cyclopentane), 2.57 (s, 2H, imide), 4.60 (s, 2H, CH_2), 7.27–7.32 (m, 4H, arom.); IR ν (cm^{-1}) 1772, 1701 (C=O), *Anal.* $C_{15}H_{16}O_2N_1Cl_1$ (C, H, N).

3.1.1.6. N-*(2-methylbenzyl)-2-azaspiro[4.4]nonane-1,3-dione (**1f**).* (Yield: 68%); m.p. 76–78 °C; R_f = 0.88A, R_f = 0.55B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.59–2.19 (m, 8H, cyclopentane), 2.46 (s, 3H, CH_3), 2.66 (s, 2H, imide), 4.71 (s, 2H, CH_2), 7.16–7.20 (m, 4H, arom.); IR ν 1773, 1698 (C=O), *Anal.* $C_{16}H_{19}O_2N_1$ (C, H, N).

3.1.1.7. N-*(4-methylbenzyl)-2-azaspiro[4.4]nonane-1,3-dione (**1g**).* (Yield: 58%); m.p. 78–80 °C; R_f = 0.90A, R_f = 0.54B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.57–2.13 (m, 8H, cyclopentane), 2.30 (s, 3H, CH_3), 2.56 (s, 2H, imide), 4.60 (s, 2H, CH_2), 7.09–7.11 (d, 2H, aromat, J = 7.98 Hz), 7.23–7.26 (d, 2H arom. J = 7.70 Hz); IR ν (cm^{-1}) 1772, 1701 (C=O), *Anal.* $C_{15}H_{19}O_2N_1$ (C, H, N).

*3.1.1.8. 2-(2-Methoxybenzyl)-2-azaspiro[4.4]nonane-1,3-dione (**1h**).* (Yield: 62%); m.p. 58–60 °C; R_f = 0.85A, R_f = 0.47B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.62–2.15 (m, 8H, cyclopentane), 2.60 (s, 2H, imide), 3.82 (s, 3H, OCH_3), 4.71 (s, 2H, CH_2), 6.83–7.22 (m, 4H, arom.); IR ν (cm^{-1}) 1772, 1690 (C=O), *Anal.* $C_{15}H_{19}O_3N_1$ (C, H, N).

3.1.1.9. N-*(4-methoxybenzyl)-2-azaspiro[4.4]nonane-1,3-dione (**1i**).* (Yield: 56%); m.p. 69–71 °C; R_f = 0.81A, R_f = 0.40B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.56–2.12 (m, 8H, cyclopentane), 2.55 (s, 2H, imide), 3.77 (s, 3H, OCH_3), 4.58 (s, 2H, CH_2), 6.79–7.32 (m, 4H, arom.); IR ν (cm^{-1}) 1769, 1706 (C=O), *Anal.* $C_{15}H_{19}O_3N_1$ (C, H, N).

3.1.1.10. N-*aminophenyl-2-azaspiro[4.4]nonane-1,3-dione (**1j**).* (Yield: 68%); m.p. 124–126 °C; R_f = 0.61A, R_f = 0.19B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.71–2.23 (m, 8H, cyclopentane), 2.69 (s, 2H, imide), 6.25 (s, 1H, N*H*), 6.73–7.27 (m, 5H, arom.); IR ν (cm^{-1}) 1791, 1715 (C=O), *Anal.* $C_{14}H_{16}O_2N_2$ (C, H, N).

3.1.1.11. N-*benzyl-2-azaspiro[4.5]decane-1,3-dione (**2a**).* (Yield: 52%), m.p. 49–51 °C, R_f = 0.86A, R_f = 0.53B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.21–1.85 (m, 10H, cyclohexane), 2.54 (s, 2H, imide), 4.63 (s, 2H, CH_2), 7.23–7.33 (m, 5H, arom.); IR ν (cm^{-1}) 1773, 1697 (C=O), *Anal.* $C_{16}H_{19}O_2N_1$ (C, H, N).

3.1.1.12. N-*(2-chlorobenzyl)-2-azaspiro[4.5]decane-1,3-dione (**2b**).* (Yield: 62%); m.p. 100–102 °C; R_f = 0.86A, R_f = 0.50B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.32–1.88 (m, 10H, cyclohexane), 2.62 (s, 2H, imide), 4.77 (s, 2H, CH_2), 7.06–7.36 (m, 4H, arom.); IR ν (cm^{-1}) 1777, 1707 (C=O), *Anal.* $C_{16}H_{18}O_2N_1Cl_1$ (C, H, N).

3.1.1.13. N-*(2,4-dichlorobenzyl)-2-azaspiro[4.5]decane-1,3-dione (**2c**).* (Yield: 63%), m.p. 88–90 °C; R_f = 0.85A, R_f = 0.47B ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.28–1.88 (m, 10H, cyclohexane), 2.60 (s, 2H, imide), 4.72 (s, 2H, CH_2), 7.06–7.37 (m, 3H, aromat); IR ν (cm^{-1}) 1775, 1693 (C=O), *Anal.* $C_{16}H_{17}O_2N_1Cl_2$ (C, H, N).

3.1.1.14. N-*(3-chlorobenzyl)-2-azaspiro[4.5]decane-1,3-dione (**2d**).* (Yield: 69%), m.p. 108–109 °C; R_f = 0.89A, R_f = 0.59B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.25–2.20 (m, 10H, cyclohexane), 2.60 (s, 2H, imide), 4.63 (s, 2H, CH_2), 7.23–7.35 (m, 4H, arom.); IR ν (cm^{-1}) 1772, 1702 (C=O), *Anal.* $C_{16}H_{18}O_2N_1Cl_1$ (C, H, N).

3.1.1.15. N-*(4-chlorobenzyl)-2-azaspiro[4.5]decane-1,3-dione (**2e**).* (Yield: 67%); m.p. 112–114 °C; R_f = 0.84A, R_f = 0.38B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.24–1.83 (m, 10H, cyclohexane), 2.53 (s, 2H, imide), 4.58 (s, 2H, CH_2), 7.23–7.30 (m, 4H, arom.); IR ν (cm^{-1}) 1773, 1689 (C=O), *Anal.* $C_{16}H_{18}O_2N_1Cl_1$ (C, H, N).

3.1.1.16. N-*(2-methylbenzyl)-2-azaspiro[4.5]decane-1,3-dione (**2f**).* (Yield: 62%); m.p. 94–96 °C; R_f = 0.90A, R_f = 0.55B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.32–1.92 (m, 10H, cyclohexane), 2.44 (s, 3H, CH_3) 2.62 (s, 2H, imide), 4.69 (s, 2H, CH_2), 7.13–7.29 (m, 4H, arom.), IR ν (cm^{-1}) 1771, 1696 (C=O), *Anal.* $C_{17}H_{21}O_2N_1$ (C, H, N).

3.1.1.17. N-*(4-methylbenzyl)-2-azaspiro[4.5]decane-1,3-dione (**2g**).* (Yield: 69%); m.p. 72–74 °C; R_f = 0.91A, R_f = 0.54B, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.28–1.84 (m, 10H, cyclohexane), 2.30 (s, 3H, CH_3) 2.53 (s, 2H, imide), 4.58 (s, 2H, CH_2), 7.08–7.23 (m, 4H, arom.); IR ν (cm^{-1}) 1773, 1703 (C=O), *Anal.* $C_{17}H_{21}O_2N_1$ (C, H, N).

*3.1.1.18. 2-(2-Methoxybenzyl)-2-azaspiro[4.5]decane-1,3-dione (**2h**).* (Yield: 65%); m.p. 120–122 °C; R_f = 0.84A, R_f = 0.38B ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.27–1.88 (m, 10H, cyclohexane), 2.57 (s, 2H, imide), 3.81 (s, 3H, OCH_3), 4.68 (s, 2H, CH_2), 6.82–6.89 (dd, 2H, arom. J = 7.80 Hz), 7.01–7.04 (d, 1H, arom. J = 7.42 Hz), 7.19-7.24 (t, 1H, arom. J = 7.0 Hz); IR ν (cm^{-1}) 1773, 1710 (C=O), *Anal.* $C_{17}H_{21}O_3N_1$ (C, H, N).

3.1.1.19. N-*(4-methoxybenzyl)-2-azaspiro[4.5]decane-1,3-dione (**2i**).* (Yield: 53%); m.p. 88–90 °C; R_f = 0.80A, R_f = 0.44B; ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.23–1.82 (m, 10H, cyclohexane), 2.51 (s, 2H, imide), 3.76 (s, 3H, OCH_3), 4.55 (s, 2H, CH_2), 6.79–7.29 (m, 4H, arom.); IR ν (cm^{-1}) 1773, 1689 (C=O), *Anal.* $C_{17}H_{21}O_3N_1$ (C, H, N).

3.1.1.20. N-*aminophenyl-2-azaspiro[4.5]decane-1,3-dione* (***2j***). (Yield: 60%); m.p. 114–116 °C; R_f = 0.65A, R_f = 0.23B; ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.33–1.94 (m, 10H, cyclohexane), 2.64 (s, 2H, imide), 6.290 (s, 1H, N*H*), 6.71–7.23 (m, 5H, arom.); IR ν (cm^{-1}) 1793, 1715 (C=O), *Anal.* $C_{15}H_{18}O_2N_2$ (C, H, N).

3.2. Pharmacology

Compounds **1a–j** and **2a–j** 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 [23,24].

Phase I studies of the investigated compounds involved three tests: maximum electroshock seizure (MES), subcutaneous pentylenetetrazole (sc.MET) and a rotorod test for neurological toxicity (TOX). Male albino mice (CF#1 strain, weighing 18–25 g) and male albino rats (Sprague–Dawley, weighing 100–150 g) were used as experimental animals.

In the MES test, an electrical stimulus of 0.2 s in duration (50 mA) was delivered via corneal electrodes primed with an electrolyte solution containing an anesthetic agent. Mice were tested using the following doses 30, 100 and 300 mg kg^{-1} of investigated compounds. The compounds were injected intraperitoneally as a suspension in a 0.5% methylcellulose/water mixture, in a volume of 0.01 ml g^{-1} body weight for mice and 0.04 ml g^{-1} body weight for rats. In the MES seizure test one animal was used at 30 and 300 mg kg^{-1} and three animals at 100 mg kg^{-1}. Abolition of the hindlimb tonic extensor component indicates the test compound's ability to inhibit MES-induced seizure spread.

The sc.MET test utilizes of pentylenetetrazole (85 mg kg^{-1} in mice and 70 mg kg^{-1} in rats). This produce 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 tested compounds were dissolved in 0.9% saline and injected intraperitoneally at a volume of 0.01 ml g^{-1} body weight in mice and 0.04 ml g^{-1} in rats. Animals were observed over a 30 min period. Absence of clonic seizure in the observed time of period indicated an ability of compounds to abolish the effect of pentylenetetrazole on seizure threshold.

A neurological toxicity test (TOX) induced by a compound was detected in mice using standardized rotorod test. Untreated control mice, when placed on a 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. For these experiments four animals were used at 30 and 300 mg kg^{-1} and eight at 100 mg kg^{-1}.

According to the ADD program activity of the compounds was classed with the following categories: active at doses of 100 mg kg^{-1} or less (class 1), active at doses greater than 100 mg kg^{-1} (class 2), inactive at 300 mg kg^{-1} (class 3). The results of preliminary screening are presented in Tables 1 and 2.

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^{-1} for both the MES and the rotorod toxicity tests. Rats were tested at five times period ranging from one quarter to 4 h post drug administration. The rats classification are as follows: 4/4 of the animals protected (class 4), 3/4 of the animals protected (class 3), 2/4 of the animals protected (class 2), 1/4 of the animals protected (class 1). The results are shown in Table 3.

Compound **1j**, which was active in mice and rats, was quantitatively evaluated in phase II of the ADD program. The quantitative determination of the median effective (ED_{50}) and toxic doses (TD_{50}) were conducted at previously calculated time of peak effect using oral route in rats. Groups of eight rats received various doses of the compound until at least two points were established between the limits of 100% protection or toxicity and 0% protection or minimal toxicity. The dose of the compounds required to produce the desired endpoint (abolition of hindlimb tonic extensor) in 50% of the animals in each test, and 95% confidence interval were calculated by computer program based on the method described previously [25]. The results of quantitative pharmacological studies are presented in Table 4.

3.3. X-ray structure analysis

An X-ray structure analysis was carried out for the selected compounds **1a**, **1j**, **2a** and **2j**. It is noteworthy that the structure of **1a** had been described earlier [28]. In the present paper, that structure was determined a new for the structural unification of results.

All the monocrystals were obtained by slowly evaporating the ethanol solution. The measurements of crystals were performed on a Kuma4CCD κ-axis diffractometer with graphite-monochromated MoKα radiation (λ = 0.71073 Å) [26] at a room temperature. All the crystallographic data and experimental details are presented in Table 5. The structures were solved by a direct method using the SHELXTS [27] program and refined with SHELXTL [28].

An E-map provided positions for all non-H-atoms. The full-matrix least-squares refinement was carried out on $F^{2\prime}$s using anisotropic temperature factors for all non-H-atoms. The H-atoms were located geometrically then the positions of H-atoms were refined in a riding model with isotropic thermal parameters taken as 1.5 times the temperature factors for their parent-atoms. Atomic coordinates are shown in Table 6a,6b.

3.4. Computational procedures

The geometry of molecules of **1a**, **2a**, **1j** and **2j** (starting with the crystallographically obtained geometry) was optimized with the Gaussian98 using rhf/6-311 ++ (d,p) [29].

Table 1
Anticonvulsant screening project (ASP) phase I in mice (**1a–j**)

Compound	Dose	MES [a]		sc.MET [b]		TOX [c]		ASP [d] class
	(mg kg^{-1})	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
1a [e]	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	0/4	0/2	
1b	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	1/1	0/1	2/5	0/1	2/4	0/2	
1c	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	1/1	0/1	2/5	0/1	2/4	0/2	
1d	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	0/4	0/2	
1e	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	0/4	0/2	
1f	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	1/1	0/1	0/1	0/1	0/4	0/2	
1g	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	0/4	0/2	
1h	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	1/1	0/1	5/5	0/1	4/4[14]	0/2	
1i	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	1/4	0/2	
1j	30	0/1	0/1	0/1	1/5	0/4	0/2	1
	100	1/3	0/3	0/1	0/1	0/8	0/4	
	300	1/1	0/1	1/1	0/1	4/4[14]	0/2	

Response comments [14] unable to grasp rotorod.
[a] Maximal electroshock test (number of animals protected/number of animals tested).
[b] Subcutaneous pentylenetetrazole test (number of animals protected/number of animal tested.
[c] Rotorod toxicity (number of animals exhibiting toxicity/number of animals tested).
[d] The classification as follows: 1 – anticonvulsant activity at doses 100 mg kg^{-1} or less; 2 – anticonvulsant activity at doses greater than 100 mg kg^{-1}; 3 – compound inactive at 300 mg kg^{-1}.
[e] Data from Ref. [10].

4. Results and discussion

The synthesized derivatives of *N*-benzyl and *N*-aminophenyl 2-azaspiro[4.4]nonane (**1a–j**) and [4.5]decane-1,3-dione (**2a–j**) showed diversified anticonvulsant properties.

In the first part of our study we investigated the substitution mode in the aryl ring of the 2-azaspiro[4.4]nonane-1,3-dione set as compared to that of the model compound **1a**. Considering our earlier experiments, introduction of a substituent into position-2 should enhance activity. Such an effect was observed for 2-Cl (**1b**), 2-CH_3 (**1f**) and 2-OCH_3 (**1h**) derivatives, which demonstrated activity at a dose of 300 mg kg^{-1} (1/1 of the animals protected at 0.5 h) in the MES test and were more active than the unsubstituted analog **1a**. Additionally, compounds **1b** and **1h** were active at dose of 300 mg kg^{-1} (5/5 of the animals protected at 0.5 h) in the sc.MET test. Derivatives that contained 3-Cl (**1d**), 4-Cl (**1e**), 4-CH_3 (**1g**) and 4-OCH_3 (**1i**) groups were devoid of anticonvulsant activity, probably due to an unfavorable steric interaction.

Next, to have an insight into the physicochemical influence of the size of a spirocycloalkyl unit, we designed a set of 2-azaspiro[4.5]decane analogues with the same substituents in the aryl ring. The unsubstituted compound **2a** and its 2-Cl derivative **2b** were active at doses of 100 mg kg^{-1} (2/3 of the animals protected at 0.5 h) and 300 mg kg^{-1} (1/1 of the animals protected at 4 h) in the MES test. Moreover, compound **2a** exhibited anti-sc.MET activity at doses of 100 and 300 mg kg^{-1} (1/5 and 1/1 of the animals protected at 0.5 h, respectively), and **2b** at a dose of 300 mg kg^{-1} (1/1 of the animals protected at 0.5 and 4 h). The 2,4-dichloro derivative **2c** was effective in the MES procedure at doses of 100 and 300 mg kg^{-1} (3/3 and 1/1 of the animals protected at 4 h). Thus, the introduction of a spirocyclohexane moiety had a positive effect on anticonvulsant activity in the case of unsubstituted (**2a**), 2-Cl substituted (**2b**) and 2,4-dichloro deriva-

Table 2
ASP phase I in mice (**2a–j**)

Compound	Dose (mg kg^{-1})	MES [a]		sc.MET [b]		TOX [c]		ASP [d] class
		0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
2a	30	0/1	0/1	0/1	0/1	0/4	0/2	1
	100	2/3	0/3	1/5	0/1	3/8	0/4	
	300	1/1	1/1	1/1	0/1	4/4	0/2	
2b	30	0/1	0/1	0/1	0/1	0/4	0/2	1
	100	2/3	0/3	0/1	0/1	4/8	0/4	
	300	1/1	1/1	1/1[25]	1/1	4/4	1/2	
2c	30	0/1	0/1	0/1	0/1	0/4	0/2	1
	100	0/3	3/3	0/1	0/1	2/8	0/4	
	300	0/1	1/1	0/1	0/1	2/4	1/2	
2d	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	0/4	0/2	
2e	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	1/1	4/5	0/1	0/4	0/2	
2f	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	0/4	0/2	
2g	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	0/4	0/2	
2h	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	0/4	0/2	
2i	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	0/4	0/2	
2j	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[25]	0/1	3/4	0/2	

Response comments [25]myoclonic yerks.
[a] Maximal electroshock test (number of animals protected/number of animals tested).
[b] Subcutaneous pentylenetetrazole test (number of animals protected/number of animals tested).
[c] Rotorod toxicity (number of animals exhibiting toxicity/number of animals tested).
[d] The classification as follows: 1 – anticonvulsant activity at doses 100 mg kg^{-1} or less; 2 – anticonvulsant activity at doses greater than 100 mg kg^{-1}; 3 – compound inactive at 300 mg kg^{-1}.

Table 3
ASP phase VIa. Test results in rats (p.o. administration at a dose of 30 mg kg^{-1})

Compound	MES [a]					TOX [b]				
	0.25 h	0.5 h	1 h	2 h	4 h	0.25 h	0.5 h	1 h	2 h	4 h
1b	1/4	1/4	1/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4
1f	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
1j	4/4	1/4	1/4	3/4	3/4	0/4	0/4	0/4	0/4	0/4
2a	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
2b	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
2c	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
2e	0/4	1/4	1/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4

[a] Maximal electroshock test, number of animals protected/number of animals tested.
[b] Rotorod test for neurological toxicity, number of animals exhibiting toxicity/number of animals tested.

tives (ASP class 1). In contrast, the activity of 2-CH_3 (**2f**) and 2-OCH_3 (**2h**) derivatives was decreased in comparison with their cyclopentane analogues. Surprisingly, increased anticonvulsant activity was observed in the case of the 4-Cl derivative (**2e**), which was potent at a dose of 300 mg kg^{-1} (1/1 of the animals protected at 0.5 h) in the MES test, and at the same dose in the sc.MET tests (4/5 of the animals protected at 4 h). As was expected, the 4-substituted compounds **2g** and **2i** were inactive.

Finally, we chose model unsubstituted compounds (**1a** and **2a**) and altered the linker between the imide moiety and the aromatic ring. The introduction of an imine group (NH) instead of a methylene joining arm yielded *N*-aminophenyl-2-azaspiro[4.4]nonane-1,3-dione (**1j**). The latter compound

Table 4
Quantitative anticonvulsant data in rats dosed orally [a] (phase II ASP)

Compound	ED_{50} [b] MES (mg kg^{-1})	ED_{50} [b] sc.MET (mg kg^{-1})	TD_{50} [c] (mg kg^{-1})	PI [d] MES (TD_{50}/ED_{50})	TPE [e] MES (h)
1j	72.61 (47.86–100.27)	< 250	> 250	3.44	0.25
Phenytoin [e]	29.8 (21.9–38.9)	> 800	> 3000	> 100	4

[a] Pharmacological values given in this Table are follows.
[b] The ED_{50} dose of drug required to assure anticonvulsant protection in 50% animals.
[c] The TD_{50} dose eliciting minimal neurological toxicity in 50% animals.
[d] The PI, protection index. [e)] The TPE, time to peak effect.
[e] Data on phenytoin are from Ref. H.S. White, J.H. Woodhead, M.R. Franklin, General principles: experimental selection, quantification and evaluation of anticonvulsant drugs, in: R. Levy, Mattson, B.S. Meldrum (Eds.), Antiepileptic Drugs, fourth ed., Raven Press, New York, 1995, pp. 99–110.

Table 5
Crystal data and structure refinement details for **1a**, **1j**, and **2a**, **2j**

	1a	**1j**	**2a**	**2j**
Empirical formula	$C_{15}H_{17}NO_2$	$C_{14}H_{16}N_2O_2$	$C_{16}H_{19}NO_2$	$C_{15}H_{18}N_2O_2$
Formula weight	243.30	244.29	257.32	258.31
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/c$	$P2_1/n$	$C2/c$
a (Å)	13.377(3)	11.161(2)	14.040(3)	24.530(5)
b (Å)	6.599(1)	6.389(1)	6.760(10)	6.152(1)
c (Å)	14.976(3)	17.32 (3)	15.280 (3)	18.071(4)
α (°)	90.00	90.00	90.00	90.00
β (°)	101.00(3)	96.20(3)	102.72(3)	91.90(3)
γ (°)	90.00	90.00	90.00	90.00
Volume $(Å)^3$	1297.5(4)	1226.4(4)	1414.6(5)	2725.6(9)
z	4	4	4	8
Calculated density (g m^{-3})	1.245	1.329	1.208	1.259
Wavelength (Å)	MoK_α 0.07093			
Absorption coefficient (mm^{-1})	0.083	0.090	0.079	0.085
F(000)	520	520	552	1104
Reflections cool./unique	5881/1198	5454/2147	14,330/2481	3582/1719
Data/parameters	1198/164	2147/164	2481/173	1791/173
Theta range data (°)	3.10 ÷ 20.00	3.41 ÷ 24.99	2.97 ÷ 25.00	3.41 ÷ 25.50
Index ranges	$-12 < h < 12$	$-13 < h < 11$	$-16 < h < 16$	$-26 < h < 26$
	$-6 < k < 6$	$-7 < k < 7$	$-7 < k < 8$	$0 < k < 6$
	$-13 < l < 14$	$-20 < l < 20$	$-18 < l < 18$	$0 < l < 19$
S	1.085	0.963	1.059	0.967
R_1	$R_1 = 0.059$	$R_1 = 0.054$	$R_1 = 0.058$	$R_1 = 0.077$
wR_2	w$R_2 = 0.1389$	w$R_2 = 0.095$	w$R_2 = 0.1055$	w$R_2 = 0.130$
Extinction	0.010(2)	0.013(2)	0.026(3)	0.00
Largest difference in peak and hole ($eÅ^{-3}$)	0.118; –0.138	0.168; –0.151	0.117; –0.139	0.212; –0.193

was active at doses of 30 mg kg^{-1} (1/5 of the animals protected at 4 h) and 300 mg kg^{-1} (1/1 of the animals protected at 0.5 h) in the sc.MET test, and also was active at doses of 100 and 300 mg kg^{-1} in the MES test (1/3 and 1/1 of the animals protected at 0.5 h, respectively). Compound **2j** was effective at a dose of 300 mg kg^{-1} (4/5 of the animals protected at 0.5 h), in the sc.MET test, but at the same dose it produced myoclonic jerks. Therefore, the simple modification led to an increase in activity in the case of the 5-membered spirocyclopentane ring (**1j**), but the same procedure did not significantly influence the activity of the respective spirocyclohexane analogue (**2j**).

In a neurological toxicity screening test compounds **1b**, **1c** and **1h–j** showed neurotoxicity at the higher dose (300 mg kg^{-1}) after 0.5 h. The mice were unable to grasp the rotorod after administration of **1h** and **1j** at a dose of 300 mg kg^{-1} at 0.5 h. In a series of 2-aza-spiro[4.5]decane-1,3-diones, compounds **2a–c** showed neurotoxicity at a dose of 100 mg kg^{-1} at 0.5 h.

Further pharmacological investigation was concerned with an oral evaluation of the anti-MES and the neurotoxic activity (phase VIa) of the selected compounds **1b**, **1f**, **1j**, **2a–c** and **2e** in rats. Those compounds were administered per os at a dose of 30 mg kg^{-1}, and their effects were studied after 0.25, 0.5, 1, 2, and 4 h. Compound **1b** protected 25% of the animals at all time period, **2e** also inhibited seizure in 25% of animals at 0.5 and 1 h, and in 50% of animals at 4 h. Compounds **1f**, **2a** and **2b** exhibited only marginal activity in this test. The 2,4-dichloro substituted derivative **2c**, which was active in mice (class 1 ASP) turned out to be inactive in rats. The most active, in this series, compound **1j** protected 100% (4/4) of the animals at 0.25 h, and 75% (3/4) of the animals tested at 2 h and 4 h. When given orally, none of the tested compounds was neurotoxic.

Table 6a
Atomic coordinates (×10^4) and equivalent isotropic displacement parameters (Å^2 × 10^3) for **1a** and **2a**. *U*(eq) is defined as one third of the trace of the orthogonalized Uij tensor

	Compound **1a**					Compound **2a**			
	x	*y*	*z*	*U*(eq)		*x*	*y*	*z*	*U*(eq)
N(1)	1461(3)	2230(5)	5902(2)	41(1)	N(1)	3574(2)	2350(3)	–916(2)	45(1)
C(20)	1009(3)	3640(6)	6462(3)	48(1)	C(20)	006(2)	3736(4)	–451(2)	54(1)
O(1)	2976(2)	1723(4)	6878(2)	62(1)	O(1)	2082(1)	1972(3)	–875(1)	64(1)
O(2)	182(2)	2347(4)	4647(2)	65(1)	O(2)	4858(2)	2299(3)	305(1)	68(1)
C(2)	2410(3)	1359(7)	6162(3)	43(1)	C(2)	2646(2)	1554(4)	–178(2)	45(1)
C(3)	2582(3)	–123(6)	5443(3)	41(1)	C(3)	2477(2)	109(4)	–468(2)	43(1)
C(4)	1728(3)	406(7)	4640(3)	53(1)	C(4)	3402(2)	324(5)	287(2)	59(1)
C(5)	1013(4)	1723(6)	5016(3)	48(1)	C(5)	4049(2)	1735(4)	–67(2)	50(1)
C(11)	3652(3)	–77(7)	5230(3)	61(1)	C(11)	1542(2)	653(5)	–155(2)	60(1)
C(12)	3798(4)	–2151(8)	4812(4)	88(2)	C(12)	1297(2)	–924(6)	472(2)	70(1)
C(13)	3044(4)	–3554(7)	5152(3)	71(2)	C(13)	1158(3)	–2939(6)	13(3)	83(1)
C(14)	2490(3)	–2290(6)	5767(3)	54(1)	C(14)	2094(3)	–3528(5)	–286(2)	82(1)
C(21)	263(3)	2701(7)	6969(3)	40(1)	C(15)	2375(2)	–1961(4)	–903(2)	61(1)
C(22)	394(3)	756(7)	7318(3)	49(1)	C(21)	4726(2)	2768(5)	–910(2)	50(1)
C(23)	–284(4)	–42(8)	7821(3)	65(2)	C(22)	4595(2)	869(5)	–254(2)	60(1)
C(24)	–1090(4)	1122(10)	7988(3)	79(2)	C(23)	5263(3)	33(6)	–697(2)	83(1)
C(25)	–1215(4)	3076(9)	7642(4)	79(2)	C(24)	6066(3)	1110(10)	–787(3)	103(2)
C(26)	–39(4)	3864(7)	7136(3)	61(1)	C(25)	6206(3)	3003(9)	–454(3)	104(2)
					C(26)	5530(3)	3853(6)	–009(2)	78(1)

Table 6b
Atomic coordinates (× 10^4) and equivalent isotropic displacement parameters (Å^2 × 10^3) for **1j** and **2j**. *U*(eq) is defined as one third of the trace of the orthogonalized Uij tensor

	Compound **1j**					Compound **2j**			
	x	*y*	*z*	*U*(eq)		*x*	*y*	*z*	*U*(eq)
N(1)	4672(2)	2888(4)	1126(2)	43(1)	N(1)	2356(2)	3004(7)	1457(3	38(1)
N(2)	5636(2)	4127(4)	973(2)	64(1)	N(2)	1806(2)	3205(9)	1621(3)	59(2)
O(1)	4117(2)	1830(4)	–121(1)	56(1)	O(1)	2645(2)	1807(7)	2589(3)	53(1)
O(2)	4663(2)	3772(4)	2409(2)	72(1)	O(2)	2284(2)	4847(7)	352(2)	67(2)
C(2)	3978(3)	1778(5)	560(2)	39(1)	C(2)	2750(2)	2326(10)	1970(4)	44(2)
C(3)	3051(3)	545(5)	940(2)	33(1)	C(3)	3276(2)	2467(10)	1588(3)	40(2)
C(4)	3138(3)	1441(5)	1767(2)	44(1)	C(4)	3155(2)	3780(10)	908(3)	51(2)
C(5)	4230(3)	2822(5)	1848(2)	45(1)	C(5)	2562(3)	3977(10)	842(4)	38(2)
C(11)	1791(3)	659(5)	493(2)	48(1)	C(11)	3723(3)	3575(11)	2125(3)	58(2)
C(12)	1168(3)	–330(6)	701(3)	81(1)	C(12)	4292(3)	3416(16)	1828(4)	92(3)
C(13)	2126(3)	–861(6)	999(2)	63(1)	C(13)	4443(3)	1128(17)	1657(4)	91(3)
C(14)	3325(3)	–817(5)	927(2)	44(1)	C(14)	4044(3)	–14(11)	1115(4)	72(2)
C(21)	6807(3)	3536(5)	1261(2)	39(1)	C(15)	3463(3)	107(11)	1415(3)	59(2)
C(22)	7086(3)	1581(5)	1552(2)	48(1)	C(21)	1456(3)	1722(12)	1256(3)	49(2)
C(23)	8273(3)	1103(6)	1814(2)	57(1)	C(22)	919(3)	2333(12)	1223(4)	61(2)
C(24)	9167(3)	2560(7)	1784(2)	66(1)	C(23)	527(3)	962(16)	918(4)	84(3)
C(25)	8878(3)	4519(7)	1493(2)	65(1)	C(24)	687(4)	–999(15)	615(4)	77(2)
C(26)	7702(3)	5026(5)	1233(2)	50(1)	C(25)	1222(3)	–602(12)	644(4)	67(2)
					C(26)	1601(2)	–221(10)	956(3)	41(2)

Compound **1j** was chosen for phase II evaluation to quantify its anticonvulsant activity and neurotoxicity. In the sc.MET test **1j** was active at a dose greater than 250 mg kg^{-1}. In the MES test, a median effective dose (ED_{50} = 72.6 mg kg^{-1}) was recorded. The tested compound had its TD_{50} value higher than 250 mg kg^{-1}, and showed a protective index (PI) value of 3.44. The time to peak effect (TPE) was 0.25 h.

In view of structure and activity studies (SAR), it was of special interest to study the 3-D structures of the representative derivatives. To cover the structural diversity, compounds which had a different type of the linker (X = imine or methylene) and the size of a spirocycloalkyl moiety were selected for an X-ray analysis. First of all, the most active compound **1j** and its spirocyclohexane analogue **2j** were chosen. Then, the respective analogues **1a** and **2a** with a methylene linker were selected for crystallographic and molecular structure studies.

The molecules of **1a**, **1j**, **2a** and **2j**, together with the observed sequences of H-bonds in the crystal, are shown in Fig. 1. In the structures of those compounds, the undulated five-member succinimide ring was the crucial fragment. After

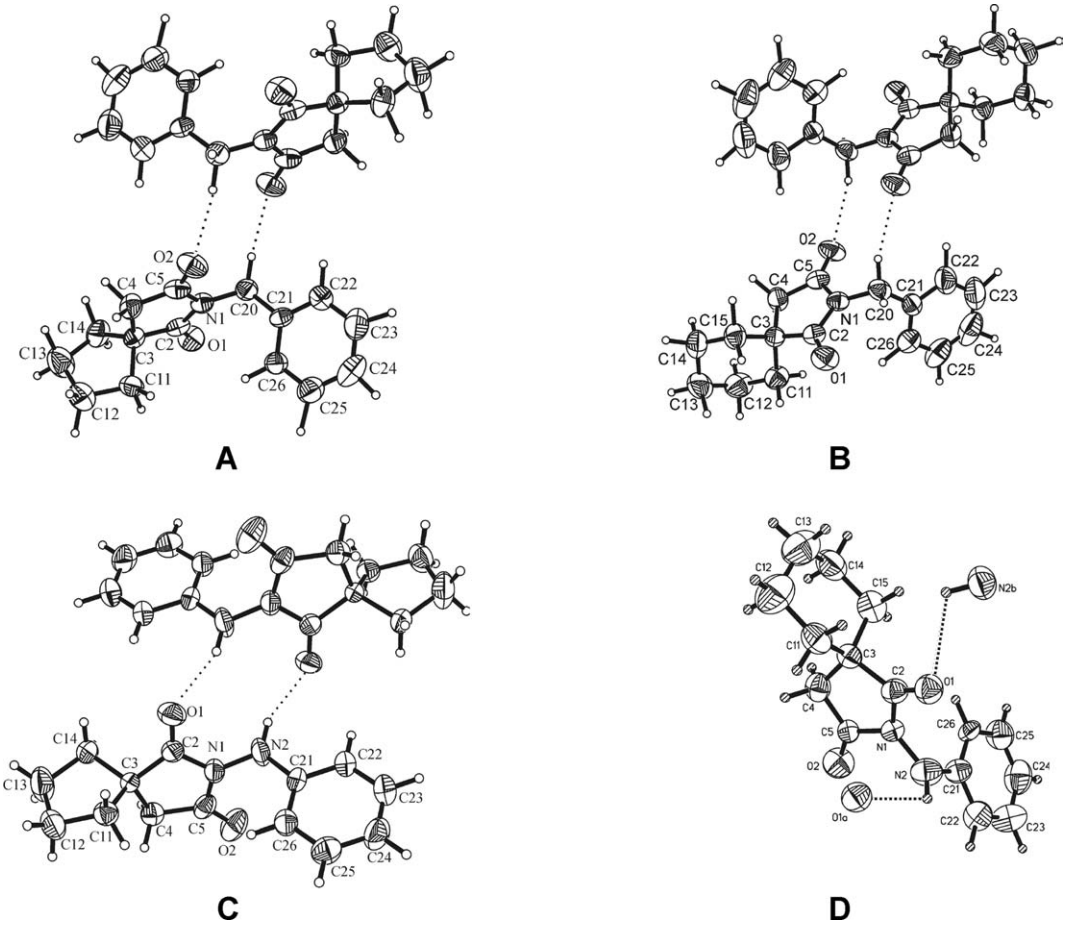


Fig. 1. The molecules of **1a** (a), **2a** (b), **1j** (c) and **2j** (d) together with observed sequences of H-bonds in the crystal.

Table 7
Selected geometrical and electronic data from **1a**, **2a**, **1j** and **2j** molecules

Compound	a) Succinimide ring description					
	Σ angles at N1 (°)	Bond lengths (Å)		Parameter *d* [a] (°)	MEP (eV mol^{-1})	
		N1–X	X–C21		O1	O2
			X = CH_2			
1a	356.9	1.458 (5)	1.498 (5)	0.031 (4)	–22.353	–22.353
2a	360.0	1.461 (2)	1.502 (4)	0.013 (3)	–22.355	–22.351
			X = NH			
1j	359.7	1.384 (3);	1.399 (4)	0.047 (3)	–22.353	–22.347
2j	358.1	1.396 (3)	1.403 (7)	0.111 (6)	–22.349	–22.355

	b) H-bonds in the crystal				
	D–H A (*sym. code*)	D–H (Å)	H··A (Å)	D··A (Å)	D–H··A (°)
		Strong H-bonds			
1j	N2–H2···O1 [$-x+1, -y+1, -z$]	0.90	2.14	2.999(4)	159.6
2j	N2–H2···O1 [$-x+1/2, y+1/2, -z+1/2$]	0.90	2.43	2.938(7)	116.2
		Weak H-bond			
1a	C20–H20B···O2 [$-x, 1-y, 1-z$]	0.96	2.41	3.362(5)	170.2
2a	C20–H20B···O2 [$1-x, 1-y, -z$]	1.00	2.43	3.402(4)	163.7

[a] For definition see Fig. 2.

a careful analysis, it was found that all the important differences in this ring were connected with the X-linker variation. That was easily visualized by two subsequent descriptors: the N1–X bonds length (Table 7) and the value of the d parameter describing the out-of-plane deviation of N1 (Table 7 and Fig. 2). In *N*-aminophenyl-derivatives (**1j**, **2j**), the linking

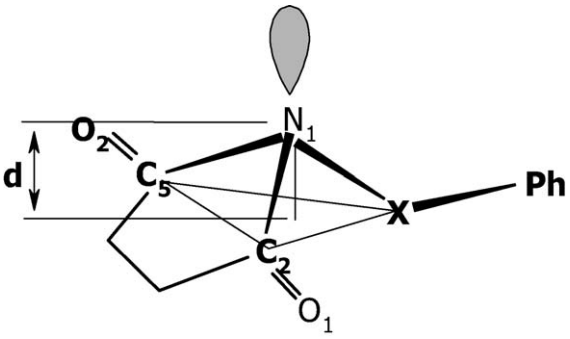


Fig. 2. Parameter *d* defining out-of-plane deviation for N1; X = NH, CH_2.

atom N2 adopted planar configuration, and the free electron pair from the N1 atom in the molecule of **1j** had slightly stronger contribution to the N1–N2 bond conjugation, than that in **2j**. In the case of the *N*-benzyl analogues **1a**, **2a** (X = CH_2), only a typical bond length in the linker was found.

In both crystals of the *N*-aminophenyl derivatives (**1j**, **2j**), the main H-bond motive was always based on N2–H2···O1 strong interactions (Table 7). However, the crystal nets were different. In the crystal of **1j**, dimer formation (Fig. 1c) was a direct result of those interactions, while in the crystal of **2j** molecules were arranged in one chain (Fig. 1d). For details see Table 7. In the crystals of *N*-benzyl derivatives (X = CH_2), the main motive has created dimers with weak H-bonds of C20–H20B···O2 (Table 7 and Fig. 1a, b).

Subsequently, an electrostatic potential (MEP) at the respective oxygen atoms (Table 7) was calculated for all crystallographically studied compounds (ab initio calculations) [29]. It was established that, in the molecule of **1j**, the MEP minimum at O1 was deeper than that at O2. However, in molecule **2j**, the respective minima depths were in inverse relation: the minimum at O2 was deeper than that at O1 (Table 7).

In the case of compounds **1a** and **2a**, the electrostatic potential MEP-values at two oxygen atoms O1 and O2 were almost identical in either molecule (Table 7).

5. Conclusions

The present study showed that the anticonvulsant activity of the investigated compounds depended on the size of the spirocycloalkyl moiety and the kind and position of the substituents in the aromatic ring. Moreover, alteration of the linker (NH instead CH_2) between the imide nitrogen atom and the aromatic fragment increased anticonvulsant activity and the resulting **1j** was classified as potent anticonvulsant agent in this series of compounds. As had been earlier postulated for different classes of succinimides [12,14], it was confirmed that the anticonvulsant activity was connected with the MEP-values in the proximity of O1 and O2. Deeper minima at O1 turned out to be much more convenient for binding processes. The obtained results indicate that further investigation with this group compounds are necessary to validate the role of the linker joining spirosuccinimide and the aromatic center.

Subsequent studies will be published shortly.

Acknowledgements

The authors wish to thank Dr. James Stables for providing them with pharmacological data within the Antiepileptic Drug Development Program (Epilepsy Branch, National Institute of Neurological Disorders and Stroke National Institute of Health Bethesda, MD, USA).

This study was supported by the KBN grand No. 3P05F 024.

Supplementary material

Crystallographic data (excluding structural factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center and allocated the deposition numbers: CCDC 235191-235194 for compounds **1a**, **1j**, **2a** and **2j**. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 1EW, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

References

[1] W. Oldfield, C.H. Cashin, The chemistry and pharmacology of a series of cycloalkanespiro-5-hydantoins, J. Med. Chem. 8 (1965) 239–269.

[2] E. Naydenowa, N. Pencheva, J. Popova, N. Stoyanov, M. Lazarova, B. Aleksiew, Aminoderivatives of cycloalkenespirohydantoins: synthesis and biological activity, Farmaco 57 (2002) 189–194.

[3] E.M. Galati, M.T. Monforte, N. Miceli, E. Raneri, Anticonvulsant and sedative effects of some 5-substituted bromopyrazolinic spirobarbiturates, Farmaco 56 (2001) 459–461.

[4] M.L. Traver, J.M. Nicholson, K.R. Scott, Spirosuccinimides as potential anticonvulsants, J. Pharm. Sci. 74 (1985) 785–787.

[5] M.R. Borenstein, P.H. Doukas, Anticonvulsant activity of indanylospirosuccinimide Mannich bases, J. Pharm. Sci. 76 (4) (1987) 300–302.

[6] K.R. Scott, I.O. Edafiogho, J.A. Moore, J.A. Moore, J.M. Nicholson, Synthesis and anticonvulsant activity of a spirosuccinimide, Pharm. World J. 8 (1991) 44–50.

[7] K.R. Scott, J.A. Moore, T.B. Zalusky, J.M. Nicholson, J.A.M. Lee, Spiro[4.5] and spiro[4.6] carboxylic acids: cyclic analogues of valproic acid. Synthesis and anticonvulsant evaluation, J. Med. Chem. 28 (1985) 413–417.

[8] V.A. Farrar, M. Ciechanowicz-Rutkowska, J. Grochowski, P. Serda, T. Pilati, G. Filippini, C.N. Hinko, A. El-Assadi, J.A. Moore, I.O. Edafiogho, C.W. Andrews, M. Cory, J.M. Nicholson, K.R. Scott, Synthesis and clog P correlation of imidooxy anticonvulsants, J. Med. Chem. 36 (1993) 3517–3525.

[9] M.S. Alexander, J.P. Stables, M. Ciechanowicz-Rutkowska, M.B. Hursthouse, D.E. Hibbs, I.O. Edafiogho, V.A. Moore, K.R. Scott, Spiranes 6. Ring A homologues of *N*-benzyloxy-2-azaspiro [4,4]nonane-1,3-dione. Synthesis, X-ray analysis and anticonvulsant evaluation, Eur. J. Med. Chem. 31 (1996) 787–795.

[10] I.O. Edafiogho, K.R. Scott, J.A. Moore, V.A. Farrar, J.M. Nicholson, Synthesis and anticonvulsant activity of imidooxy derivatives, J. Med. Chem. 34 (1991) 387–392.

[11] J. Karolak-Wojciechowska, W. Kwiatkowski, J. Obniska, A. Zejc, Structures of *N*-pyridyl-2-phenylsuccinimides and structural evidence for substituent effects on anticonvulsant properties, Acta Crystallogr. C 46 (1990) 108–112.

[12] W. Kwiatkowski, J. Karolak-Wojciechowska, Use of molecular electrostatic potentials for analysis of anticonvulsant activities of phenylsuccinimides, SAR QSAR Environ. Res. 1 (1993) 233–243.

[13] J. Karolak-Wojciechowska, W. Kwiatkowski, J. Obniska, A. Zejc, 3-(*m*-Bromophenyl)-1-(3-methyl-2-pyridyl)-pyrrolidine-2,5-dione, Acta Crystallogr. C52 (1996) 1808–1809.

[14] J. Karolak-Wojciechowska, M. Błaszczyk, W. Kwiatkowski, J. Obniska, A. Zejc, Electronic conditions for anticonvulsant activity of diphenylsuccinimides: crystal and molecular structure of 3,3-diphenyl-*N*-(2-pyridyl)-succinimide, J. Chem. Crystallogr. 27 (1997) 297–301.

[15] A. Zejc, J. Obniska, E. Chojnacka-Wójcik, E. Tatarczyńska, B. Wiczyńska, Synthesis and anticonvulsant properties of *N*-methylpyridyl derivatives of *m*- and *p*-bromo-phenylsuccinimides, Pol. J. Pharmacol. Pharm. 39 (1987) 91–95.

[16] A. Zejc, J. Obniska, M. Wilimowski, M. Rutkowska, J. Witkowski, L. Barczyńska, L. Kędzierska-Gożdzik, W. Wojewódzki, K. Orzechowska-Juzwenko, T. Pławiak, E. Duś, J. Gryska, M. Gliniak, Synthesis and anticonvulsant properties of some arylsuccinate methylpyridylimides, Pol. J. Pharmacol. Pharm. 42 (1990) 69–77.

[17] J. Obniska, K. Kulig, A. Zejc, Synthesis and anticonvulsant properties of new *N*-piperazinylalkyl imides of succinic acid, Acta Polon. Pharm-Drug Res. 55 (1998) 223–231.

[18] J. Obniska, A. Zejc, J. Karolak-Wojciechowska, Synthesis and anticonvulsant properties of new *N*-pyridyl derivatives of 3-phenyl- and 3,3-diphenyl-succinimides, Farmaco 54 (1999) 423–429.

[19] J. Obniska, A. Zejc, A. Zagórska, Synthesis and anticonvulsant properties of new 1-phenyl and 1-phenylamino-3-phenylpyrrolidine-2,5-dione derivatives, Acta Polon. Pharm.-, Drug Res. 59 (2002) 209–213.

[20] J. Obniska, A. Zagórska, Synthesis and anticonvulsant properties of new *N*-[(4-phenylpiperazin-1-yl)-methyl] derivatives of 3-phenylpyrrolidine-2,5-dione and 2-aza-spiro[4.4]nonane-1,3-dione, Farmaco 58 (2003) 1227–1234.

[21] F.A. El-Telbany, K.M. Ghoneim, M. Khalifa, Synthesis of certain spiro compounds, part 1, Pharmazie 31 (H) (1976) 442–444.

[22] F.A. El-Telbany, K.M. Ghoneim, M. Khalifa, Synthesis of certain spiro compounds for pharmacological study, part II, J. Pharm. Belg. (1976) 403–410.

[23] R.L. Krall, J.K. Penry, B.G. White, H.J. Kupferberg, E.A. Swinyar, Antiepileptic drug development II. Anticonvulsant drug screening, Epilepsia 19 (1978) 409–428.

[24] H.J. Kupferberg, Antiepileptic drug development program: a cooperative effort of government and industry, Epilepsia 30 (Suppl.) (1989) 51–56.

[25] G.D. Gladding, H.J. Kupferberg, E.A. Swinyard, Handbook of Experimental Pharmacology, Antiepileptic Drugs, Springer, Berlin, Tokyo, 1985.

[26] Oxford Diffraction, CrysAlisCCD and CrysAlisRED (ver. 1.169), 2001.

[27] G.M. Sheldrick, SHELXTL PC^{MT}, Siemens Analytical X-Ray Instruments Inc, Madison, Wisconsin, USA, 1990.

[28] G.M. Sheldrick, SHELXL-97.A FORTRAN-77 Program for the Refinement of Crystal Structures from Diffraction Data, University of Göttingen, Germany, 1997.

[29] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, V.G. Zakrzewski, J.A. Montgomery, R.E. Stratmann, J.C. Burant, S. Dapprich, J.M. Millam, A.D. Daniels, K.N. Kudin, M.C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G.A. Petersson, P.Y. Ayala, Q. Cui, K. Morokuma, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J. Cioslowski, J.V. Ortiz, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P.M.W. Gill, B.G. Johnson, W. Chen, M.W. Wong, J.L. Andres, M. Head-Gordon, E.S. Replogle, J.A. Pople, Gaussian 98 (Revision A.7), Gaussian, Inc, Pittsburgh, PA, 1998.