SYNTHESIS AND ANTICONVULSANT PROPERTIES OF SOME N-ARYL AND N-ARYLAMINOMETHYL DERIVATIVES OF 3-P-ISOPROPOXYPHENYLPYRROLIDINE-2,5-DIONE

SERGEY L. KOCHAROV[1], HENRY PANOSYAN[1], JAROSŁAW CHMIELEWSKI[2],
BARBARA GWOREK[3] and JAROGNIEW J. ŁUSZCZKI[4,5]*

[1]Scientific Technological Centre for Organic and Pharmaceutical Chemistry,
National Academy of Sciences, Yerevan, Armenia
[2]Institute of Environmental Protection, National Research Institute, Warsaw, Poland
[3]Warsaw University of Life Sciences-SGGW, Warsaw, Poland
[4]Department of Pathophysiology, Medical University of Lublin, Lublin, Poland
[5]Isobolographic Analysis Laboratory, Institute of Rural Health, Lublin, Poland

Abstract: Anticonvulsant properties of newly synthesized compounds and potential antiepileptic drugs are usually assessed in screen tests in experimental animals. One of the most commonly used screen tests in mice is the maximal electroshock-induced seizure test that reflects tonic-clonic seizures in humans. A series of 3-p-isopropoxyphenylpyrrolidine-2,5-dione derivatives, including N-aryl and N-arylaminomethyl analogs, were characterized for their anticonvulsant properties in the maximal electroshock-induced seizure test in mice. Electroconvulsions (tonic-clonic seizures) were evoked in adult Albino Swiss mice by a current (sine-wave, 25 mA, 50 Hz, 500 V, 0.2 s stimulus duration) delivered via auricular electrodes. N-aryl derivatives did not show any anticonvulsant activity, whereas some representatives of N-arylaminomethyl derivatives, i.e. N-Mannich bases, exhibited a distinct protective action against maximal electroshock-induced (MES) convulsions in mice. Several N-arylaminomethyl derivatives of 3-p-isopropoxyphenylpyrrolidine-2,5-dione may become in future new antiepileptic drugs, or they could serve as valuable supporting materials for obtaining new derivatives with stronger anticonvulsant activities than their maternal compounds.

Keywords: 3-p-Isopropoxyphenylpyrrolidine-2,5-dione; N-aryl and N-arylaminomethyl derivatives; maximal electroshock-induced seizures

Treatment of epilepsy is still a huge problem for epileptologists and clinicians today because of an insufficient range of highly efficacious antiepileptic drugs accompanied by no adverse side-effects. There is no doubt that the search for newer and better antiepileptic drugs is still much needed (1). To date, several classes of various chemical compounds are used in the treatment of epilepsy (2). However, the design and synthesis of new structures with the required pharmacological activity are useful for studying the structure-activity relationship between the studied compounds, which therefore may assist in elucidating the mechanisms of action.

In spite of the structural diversity of the antiepileptic drugs, some common chemical structures responsible for their therapeutic anticonvulsant action have been denoted. Such chemical elements consist of a nitrogenous heteroatomic system, one phenyl ring, and either additional phenyl nucleus or an alkyl substituent attached to the heterocycle (3, 4). Of note, these elements comprise the well-established antiepileptic drug – ethosuximide (3-ethyl-3-methyl-pyrrolidine-2,5-dione) that belongs to the succinimide class. A review of the literature revealed that this class of chemical compounds can become a source of new drugs with anticonvulsant activity (5-10). For instance, a series of 3-p-alkoxyphenylsuccinimides was synthesized in Mnjoyan's Institute of Fine Organic Chemistry in Yerevan, Armenia (11), and an anticonvulsant compound [3-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione; 3-p-isopropoxyphenylsuccinimide] was licensed in the former Soviet Union as an efficacious

antiepileptic drug (Pufemid®) (12). Two different structural modifications of 3-p-alkoxyphenylsuccin-imides led to alterations in activity levels (13). However, when an additional an methyl group was introduced in position 3 of the same imides, the pattern of resulting activities was found to be dependent on some experimental seizure model. The most effective was the 1,3-dimethyl derivative of 3-p-iso-propoxyphenylsuccinimide (14). To carry out the study of the structure-activity relationship, two molecules of 3-p-isopropoxyphenylsuccinimide were coupled by their N atoms directly (N-N-bond), and through linear alkylene chains (C_1-C_{10}) (15).

Bearing in mind all the above, it was appropriate to extend the search for the influence of additional chemical modifications (i.e., additional aryl residue in the molecule of 3-p-isopropoxyphenyl-succinimide) on its anticonvulsant properties. With this purpose in mind, a series of its N-aryl and N-arylaminomethyl analogs, as well as joining the starting imide through the benzene ring using 1,2-phenylenediamine, were synthesized. Relatively recently, some p-isopropoxyphenylsuccinimide derivatives were tested in the maximal electroshock-induced seizure threshold (MEST) test and their anticonvulsant properties described (16).

In this study, we described the synthesis of a series of N-aryl and N-arylaminomethyl analogs of 3-p-isopropoxyphenylsuccinimide.

EXPERIMENTAL

Chemistry

All chemicals used were of analytical or reagent grade. Melting points were determined on a Boetius PHMK 76/0904 hot stage microscope (GDR) and were uncorrected. Infrared spectra were obtained in Nujol on a spectrometer. ^{1}H NMR spectra were recorded on a Varian Mercury-300 spectrometer, operating at 300 MHz; chemical shifts were reported in ä values (ppm) relative to tetramethylsilane as the internal standard. Coupling constants (J values) were given in Hertz (Hz). The solvents mixture was DMSO-d_6/CCl$_4$, 1 : 3; signals reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), p (pentet), sp (septet), m (multiplet), br. (broad). Mass spectra experiments were performed on an MX-1321A mass spectrometer using electrospray ionization in positive ion mode. Analytical TLC was used to check the purity of the products and performed on Silufol silica gel sheets ('Kavalier', Votice, Czech Republic) developed in chloroform-hexane-ethanol, 2 : 2 : 0.1 (system A), benzene-AcOH-H$_2$O, 7 : 3 : 1
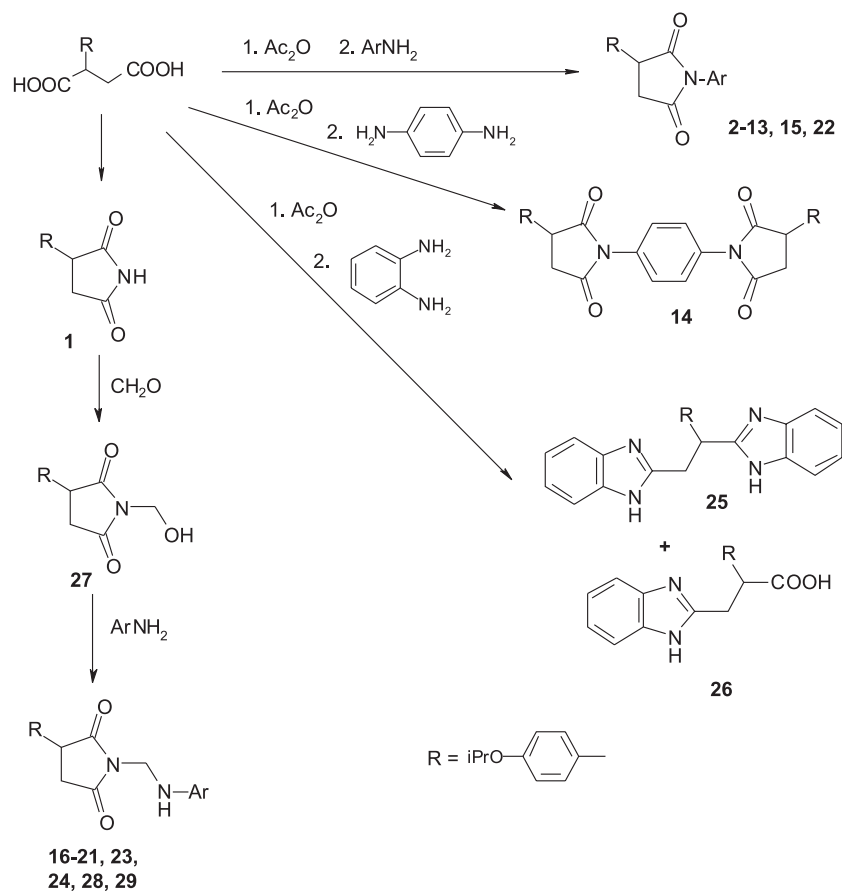
(upper layer) (system B), n-butanol saturated with ammonia water (system C), chloroform-ethanol, 5.5 : 2.5 (system D), chloroform-acetonitrile (3 : 1) (system E). Compounds were detected stained with I$_2$ (iodine), spraying with a 5% solution of phosphomolybdic acid in ethanol, followed by heating at 80-90°C during 5 min; if in the free carboxyl group there was a molecule, Bromocresol Purple indicator (as water solution spray) was also used. Semi-preparative column chromatography for compounds **30** and **31**, separation was performed using silica gel L (100/160, 'Chemapol', Prague, Czech Republic), eluent – system E for TLC. Initial p-isopropoxy-phenylsuccinic acid and compound **1** were prepared as previously reported (11). Only their melting points, ^{1}H-NMR and MS of **1** are given here. All amino compounds used in this study are available commercially and recrystallized before use, except for 2-amino-3-cyano-cycloheptano[b]-thiophene, which was kindly supplied by Dr. G.A. Gevorgyan at the Scientific Technological Centre for Organic and Pharmaceutical Chemistry, National Academy of Sciences of Armenia (NAS RA).

General procedure for syntheses of compounds 2-15, 22

The mixture of p-isopropoxyphenylsuccinic acid (0.012 mol) and acetic anhydride (10 mL) was heated for 6 h at 100°C and the excess of acetic anhydride and acetic acid formed were removed at reduced pressure. To the residue, primary amine (0.012 mol) and glacial acetic acid (7 mL) were added and the mixture refluxed for 2-3 h. On cooling, the product precipitated was filtered, washed by a small amount of acetic acid, water, dried and recrystallized. If there was no precipitate on cooling of the reaction mixture, the latter was concentrated until a solid appeared, or evaporated to dryness in a rotary evaporator under reduced pressure. The residue was re-crystallized with the addition of activated charcoal (Norit). Compound **14** was prepared in the same manner with the difference that a double mole amount of initial substituted succinic acid was used in the reaction with 1,4-phenylenediamine.

General procedures for syntheses of compounds 27, 16-21, 23, 24, 28, 29

To a suspension of **1** (23.33 g, 0.1 mol) in ethanol (100 mL) 26 mL of 35-37% formaldehyde solution (9.91 g, 0.33 mol) was added, the mixture refluxed in a water bath for 1 h and allowed to stand overnight at room temperature. The crystals of **27** precipitated were collected and recrystallized from the benzene-ethanol, 1 : 2 mixture. On concentrating
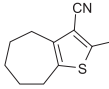
Figure 1. Synthetic pathways of the compounds **1-29**

Compound	Ar
2, 16	Ph
3	p-CH$_3$O-Ph
4, 18	p-CH$_3$CO-Ph
5	p-(CH$_3$)$_2$N-Ph
6, 17	m-Br-Ph
7, 24	m-(CH=CH-COOH)-Ph
8, 19	o-(COOH)-Ph
9, 20	m-(COOH)-Ph
10, 21	p-(COOH)-Ph
11	m-(COOH)-p-(OH)-Ph
12	2, 4-NO$_2$-Ph-NH
13	
15, 23	p-(o-F-Ph-CONH)-Ph
22, 29	p-(COOC$_2$H$_5$)-Ph
28	(CH$_2$)$_2$O(CH$_2$)$_2$

the mother liquid, an additional portion of the product was isolated. A mixture of equimolar amounts of **27** and appropriate amine (usually 0.015 mol of each) in ethanol (25-30 mL) was refluxed 50-60 min. On cooling, the precipitated product was isolated and recrystallized.

Procedure for preparation of compounds 25 and 26

The mixture of anhydride obtained from p-isopropoxyphenylsuccinic acid (0.025 mol), o-phenylenediamine (0.050 mol) and o-xylene (35 mL) was refluxed with a Dean-Stark trap for 4 h. After cooling, the solid obtained was filtered, washed with benzene and dried. A saturated soda solution was added and after vigorous stirring of the suspension undissolved material was filtered, washed with water until neutral pH was obtained, dried and re-crystallized from dioxane to give **25**. From the combined soda solution filtrate and water washings, compound **26** was isolated on acidification. Another way of separating the products may also be used which relies on their different solubilities. Thus, if the obtained solid is subjected to re-crystallization from isopropanol, **25** remains in the solvent, whereas **26** settles on cooling. Moreover, **25** is easily dissolvable in cold glacial acetic acid, **26** may be dissolved rather on heating.

Drug chemistry

The starting substance for obtaining all compounds was p-isopropoxyphenylsuccinic acid (Fig. 1). This was converted into appropriate imide **1** by procedures outlined previously (11). N-aryl substituted derivatives of imide **1** (compounds **2-13, 15** and **22**) may be synthesized by the interaction of initial acid or its anhydride with primary aromatic amines. The way via anhydride was preferred in the presented study. N-Mannich bases derivatives of **1** (compounds **16-21**, **23**, **24**, **28** and **29**) were prepared by the indirect method, i.e. by heating preliminary obtained N-hydroxymethyl analog of **1** (**27**) with corresponding primary aromatic amines in ethanol medium. The well-known hydroxymethylation reaction was used for synthesis of **27**.

The homogeneity of all final substances was checked by thin layer chromatography (TLC), purity by elemental analysis and their structure confirmed based on data from IR and ¹H NMR spectroscopy. If additional support was necessary, mass spectra were recorded. Each sample tested by TLC was detected on chromatograms as a single spot. The analytical samples gave combustion values for carbon, hydrogen and nitrogen, and when necessary,

for halogen within ± 0.4% of the theoretical values (Table 1). IR spectra contain all characteristic absorption bands attributable to succinimide (17-19), benzene rings and other fragments. ¹H NMR spectra of all compounds were consistent with the assigned structures.

The interaction of 1,2-phenylenediamine and dicarboxylic acids or anhydrides has been repeatedly discussed in the literature since 1893 (20). It was assumed that the first derived adduct is 1,2-phenylenediamide which, on being subjected to treatment with hydrochloric acid, converted into benzimidazol-2-yl alkane carboxylic acid. Several variations and subsequent modifications have been proposed (21-23) and different structures have been suggested as the most probable products, but among the few various potential intermediates, benzimidazolyl substituted carboxylic acid and bis-benzimidazolyl alkane are the major products of this reaction. Unsubstituted and symmetrically substituted dicarboxylic acids or anhydrides have been used in the cited techniques. In the presented study, both analogous to those products, i.e. containing benzimidazole ring (**25** and **26**) were detected, isolated and identified in each applied reaction conditions. Besides heating reagents in o-xylene (see experimental protocol) the reaction was carried out in glacial acetic acid and polyphosphoric acid (not shown). An attempt to carry out this reaction in hydrochloric acid failed because of the insolubility of the initial dicarboxylic acid it contained. It should be stressed that both compounds (**25** and **26**) were simultaneous reaction products at each diamine/ anhydride ratio (2 : 1 and 1 : 1) in all variants tested. There was no influence of substituent in initial substituted succinic acid on the structure of the desired product **25**, but theoretically, there is a possibility for the structural isomer of **26** to appear. In accordance with the rule of Anschütz (24), an opening of asymmetrically substituted succinic anhydride ring by amine treatment produces mainly a stronger amidoacid (α-acid-β-amide) with a free carboxyl group at the carbon atom. Hence, the substituted benzimidazolyl propionic acid obtained in the reaction 1,2-phenylenediamine with anhydride (Fig. 1) has the structure **26**. To confirm whether this formula is correct or not, an aliquot part of acid **26** was methylated with dimethyl sulphate by a generally accepted method. Two products of methylation were detected by TLC, separated by column chromatography and identified as N-methyl analog of **26** and its methyl ester (**30** and **31**, respectively) (Fig. 2). The position of the substituted phenyl ring in a molecule of **31** was determined by the method

Table 1. Analytical data for the compounds 1-29.

Compound	Calculated %				Found %			
	C	H	N	Hal	C	H	N	Hal
3-(4-Isopropoxyphenyl)-pyrrolidine-2,5-dione monohydrate (1)	62.13	6.82	5.57		61.90	7.01	5.51	
3-(4-Isopropoxyphenyl)-1-phenyl-pyrrolidine-2,5-dione (2)	73.77	6.19	4.53		73.54	6.33	4.79	
3-(4-Isopropoxyphenyl)-1-(4-methoxyphenyl)-pyrrolidine-2,5-dione (3)	70.78	6.24	4.13		71.05	6.02	4.44	
1-(4-Acetylphenyl)-3-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione (4)	71.78	6.03	3.99		71.61	6.40	4.12	
1-(4-Dimethylaminophenyl)-3-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione (5)	71.57	6.86	7.95		71.44	6.54	7.71	
1-(3-Bromophenyl)-3-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione (6)	58.77	4.67	3.61	20.58	58.49	5.00	3.70	20.35
(E)-3-4-[3-(4-Isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolyl]phenyl-2-propenoic acid (7)	69.64	5.58	3.69		70.01	5.72	3.81	
2-[3-(4-Isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolyl]benzoic acid (8)	67.98	5.42	3.96		67.79	5.55	3.73	
3-[3-(4-Isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolyl]benzoic acid (9)	67.98	5.42	3.96		67.67	5.28	4.01	
4-[3-(4-Isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolyl]benzoic acid (10)	67.98	5.42	3.96		68.09	5.64	3.69	
1-(3-Carboxy-4-hydroxyphenyl)-3-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione (11)	65.03	5.18	3.79		64.92	5.27	3.65	
1-(2,4-Dinitroanilino)-3-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione (12)	55.07	4.38	13.52		54.85	4.19	13.29	
3-Cyano-2-[3-(4-isopropoxyphenyl)-2,5-dioxotetrahydro-1H-pyrrolyl-1]-cyclo-heptano[b]thiophene (13)	67.62	5.92	6.86		67.30	6.04	7.02	
1,4-Bis[3-(4-isopropoxyphenyl)-2,5-dioxotetrahydro-1H-pyrrolyl-1]-benzene (14)	71.09	5.97	5.18		70.87	5.92	5.41	
N-{4-[3-(4-Isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolyl]phenyl}-2-fluoro-benzamide (15)	69.94	5.19	6.27	4.26	69.76	5.21	6.22	
1-Anilinomethyl-3-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione (16)	70.98	6.55	8.28		71.11	6.39	7.99	
1-(3-Bromoanilinomethyl)-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione (17)	57.56	5.07	6.71	19.15	57.29	5.28	6.66	18.98
1-(4-Acetylanilinomethyl)-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione (18)	69.45	6.36	7.36		69.49	6.15	7.50	
2-[3-(4-Isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolylmethylamino]benzoic acid (19)	65.95	5.80	7.33		65.70	5.85	7.19	
3-[3-(4-Isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolylmethylamino]benzoic acid (20)	65.95	5.80	7.33		65.62	5.61	7.35	
4-[3-(4-Isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolylmethylamino]benzoic acid (21)	65.95	5.80	7.33		70.09	5.97	7.41	
Ethyl 4-[3-(4-isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolylamino]benzoate (22)	69.28	6.08	3.67		68.97	6.34	3.71	
N-{4-[3-(4-Isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolylmethylamino]phenyl}-2-fluoro-benzamide (23)	68.20	5.51	8.84	4.00	68.43	5.47	9.00	4.12
(E)-3-4-[3-(4-isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolylmethylamino]-phenyl-2-propenoic acid (24)	67.63	5.92	6.86		67.27	6.00	6.59	
1,2-(Bis-benzimidazolyl-2)-1-(4-isopropoxyphenyl)-ethane (25)	75.73	6.10	14.13		75.60	5.79	13.87	
3-(Benzimidazolyl-2)-2-(4-isopropoxyphenyl)-propionic acid (26)	70.35	6.22	8.64		70.40	5.99	8.51	
1-Hydroxymethyl-3-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione (27)	63.86	6.51	5.32		64.09	6.64	5.17	
3-(4-Isopropoxyphenyl)-1-morpholinomethyl-pyrrolidine-2,5-dione (28)	65.04	7.28	8.43		64.96	7.01	8.49	
Ethyl 4-[3-(4-isopropoxyphenyl)-2,5-dioxotetrahydro-1H-1-pyrrolylmethylamino]-benzoate (29)	69.28	6.08	3.67		69.08	6.33	3.65	

of NMR 2D-NOESY. The presence of intensive NOE between the methyl group at nitrogen atom and methylene group in 2D-NOESY spectrum of **31** indicates that the relative position of these groups to each other is as close as possible.

Animals

Experiments were performed on adult male albino Swiss mice (weighing 22 – 26 g) that were kept in colony cages with free access to food and tap water, under standardized housing conditions. After 7 days of acclimatization to laboratory conditions, the animals were randomly assigned to experimental groups – each comprised of 8 mice. Each mouse was used only once and all tests were performed between 08.00 – 15.00. Procedures involving animals and their care were conducted in accordance with current European Community and Polish legislation on animal experimentation. Additionally, the experimental protocols and procedures described in this study were approved by the First Local Ethics Committee at the Medical University of Lublin (License No.: 18/2006), the Second Local Ethics Committee at the University of Life Sciences in Lublin (License Nos.: 79/2009 and 15/2012), and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

All studied compounds were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered intraperi-toneally (i.p.) as a single injection, in a volume of 5 mL/kg body weight. Fresh drug solutions were prepared on each day of experimentation and administered at 15, 30, 60 and 120 min before the initiation of maximal electroconvulsions. These pre-treatment times before testing of the investigated compounds and the route of i.p. administration were based on information from the Anticonvulsant Screening Programme (25-27).

Maximal electroshock-induced seizure (MES) test

Electroconvulsions were produced by a current (sine-wave, 0.2 s stimulus duration; 500 V, 50 Hz, fixed current intensity of 25 mA) delivered *via* ear clip electrodes by a Rodent Shocker generator (constant-current stimulator Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hind limb extension. The animals were administered with a constant dose of 300 mg/kg of each of the examined compounds and subjected to MES-induced seizures. This experimental procedure has been described in more detail elsewhere (28).

RESULTS

The results clearly indicate that some succinimide derivatives (i.e., **1**, **16**, **19**, **20**, **21**, **27** and **28**) have anticonvulsant properties in the mouse MES-

Table 2. Time-course of anticonvulsant effects of 3-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione and numerous its derivatives (administered i.p. at a constant dose of 300 mg/kg) in the maximal electroshock-induced seizure test (MES) in mice.

Pretreatment time (min)				
Compound*	15	30	60	120
1	7/8	7/8	6/8	4/8
2	0/8	1/8	0/8	0/8
7	0/8	0/8	1/8	0/8
10	1/8	0/8	0/8	0/8
16	6/8	7/8	8/8	6/8
19	5/8	6/8	7/8	5/8
20	7/8	6/8	6/8	5/8
21	1/8	2/8	2/8	1/8
24	0/8	0/8	1/8	0/8
27	7/8	7/8	3/8	2/8
28	7/8	8/8	6/8	1/8

Results are presented as the number of animals protected out of the total number of animals in the experimental group subjected to MES-induced seizures in mice. *Compounds not presented here were practically ineffective in this seizure model
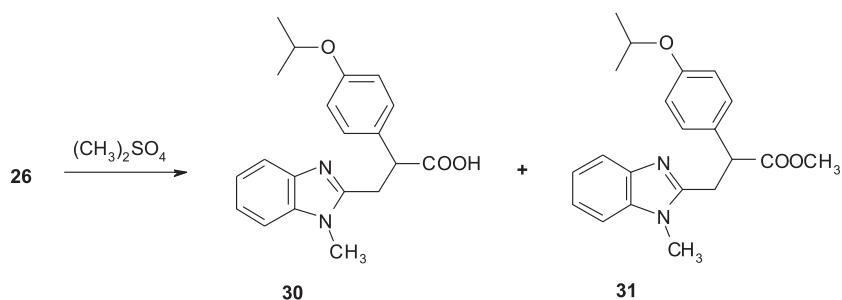
Figure 2. Methylation products of **26**

induced seizure model (Table 2), considered as a basic preclinical screening test allowing for the selection of drugs with anticonvulsant action (27).

The time to peak of maximum anticonvulsant effects ranged between 15 min (for the compound **20**) and 60 min (compounds **16**, **19** and **21**). In contrast, none of the active compounds had the time to peak of anticonvulsant action higher than 120 min after i.p. administration. In such a situation, it can be indirectly ascertained that the studied compounds relatively quickly penetrate through the blood-brain barrier and produce (dose-dependently) protection against MES-induced tonic seizures.

DISCUSSION

According to the bioscreening results (Table 2), it may be concluded that replacement of the hydrogen atom of NH group in succinimide **1** with aryl residue, including both unsubstituted and substituted phenyl moiety, resulted in a complete loss of activity in almost all cases, regardless of the type and position of substituent in the benzene ring; the same inactivating action was observed when condensed thiophene residue was used as imidic N-substituent (**13**) – our only example of inclusion aryl substituent of non-benzene nature – and when a coupling of substituted phenyl to imidic N atom through NH group was carried out (**12**). The last example does not seem to be consistent with data obtained in (9), according to which imine linker joining the endocyclic N atom and aromatic moiety was essential for anticonvulsant activity. Also, the binding of two molecules of **1** by N-atoms through a benzene ring (**14**) gave a negative effect. Compounds **25** and **26**, containing benzimidazolyl fragments are devoid at all of the activity in question, although information

about the anticonvulsant activity of benzimidazole derivatives has been known (29, 30). Converting **1** to its N-hydroxymethyl analog (**27**) was followed by retention of activity, at least during short pretreatment time (15-30 min). Five of ten N-Mannich bases, obtained from **27** and primary aromatic amines, namely, compounds **16**, **19-21**, **28,** were active at testing. On the basis of these studies it is becoming evident that binding phenyl or substituted phenyl radical with succinimide N-atom through CH$_2$-NH-bridge is preferable to direct binding in terms of a higher level of anticonvulsant activity (compare **8**, **9**, **10** with **19**, **20**, **21**, respectively). Previously, 3-(m-bromophenyl) succinimide was shown to be exceptional in activity among a group of succinimides containing m-substituted phenyl residue at position 3 (31). To examine whether m-bromophenyl radical increases the activity of imide **1,** as a substituent at position 1, derivative **6** was obtained. Instead of the potentially expected effect, a complete lack of activity was observed. The use of m-bromoaniline as the amine component in the Mannich reaction also resulted in a complete drop in activity. It is noteworthy that analogous conversion of known anticonvulsants ethosuximide (see Introduction), 3-phenyl- and 3-(4-chlorophenyl)-pyrrolidine-2,5-diones to their N-phenyl- and N-(substituted phenyl)-aminomethyl analogs was carried out long ago (32), but no results of pharmacological screening are available.

There is a suggestion that due to the facile decomposition of N-Mannich bases in water media, the bioactivity data obtained may be unrealistic (33). Lapszewicz et al. (34) recognize pK$_a$ of the parent NH-acidic compound, pK$_a$ and steric properties of the amine component as factors responsible for biotesting results. In their opinion, the former on this

list is the only information required in the case of morpholine Mannich bases. The authors of the presented study do not believe that the potential high hydrolability of N-Mannich bases misrepresents their real activity values in the current experiments because of the considerable variation in the activities within this group of tested compounds. Since all those N-Mannich bases which have shown activity (Table 2) on hydrolysis, may revert to their common starting material (**27**) if not to mother imide **1**, they rather should be of equal activity, similar to that of **27,** especially at a longer pretreatment time. If fast hydrolysis continues until **1** formation, then how can the difference between indices of **27** and **1** at 60 and 120 min pretreatment be explained? Hence, there is no support for this assumption in the presented study.

Other researchers emphasize the importance of the contribution of substituents in imidic C-linked aromatic residue to total lipophilicity for anticonvulsant activity (35). Regarding imidic N-substituent, these authors consider that the duration of effect depends on this, increasing with its hydrophilicity. Electronic and steric factors are believed to be less significant. Based on these considerations, it is difficult to interpret the appreciable difference in activity values obtained for structural isomers **19-21** insofar as they have only steric peculiarities. However, esterification of the carboxyl group of **21** led to the lack of activity for **29**, which indeed may be a consequence of lowering N-substituent's hydrophilicity.

The presented results from testing, at least to some extent, refute the assertion of other authors (35) that in general the more active compounds of succinimides class are those containing either a hydrogen atom or a methyl group at an imidic nitrogen atom. A more detailed comparative analysis of these results and data available in the literature on this subject is beyond the scope of the current study.

CONCLUSIONS

Two types of new derivatives of 3-(4-isopropoxyphenyl)-pyrrolidine-2,5-dione, already known to possess anticonvulsant properties, have been prepared and evaluated for the same bioactivity to compare with that of the parent compound. Phenyl, and substituted at different positions phenyl residues, were chosen for introduction at position 1 of the imide ring, directly or through the aminomethyl bridged group (N-Mannich bases). As bioscreening has shown, replacement of the hydrogen atom in the imidic group with aryl moiety results in the complete disappearance of tested activity in almost all cases. There were a few compounds

in the series of N-Mannich bases which revealed the activity of a degree not less than that of the initial succinimide.

The obtained results were discussed with consideration of varied information on this subject available in the literature (i.e. attempts to understand what special features in the succinimide structure are dominant in the presence of anticonvulsant properties). Since there is no single version to explain the anticonvulsant mechanism of action, there may not be one specific approach to the creation of definite structures possessing predicted values of bioactivity. This is the reason why compounds of already established activity may serve as valuable supporting materials for obtaining new derivatives.

Disclosure of conflicts of interest

The authors have no disclosures to declare.

REFERENCES

1. Lason W., Dudra-Jastrzebska M., Rejdak K., Czuczwar S.J.: Pharmacol. Rep. 63, 271 (2011).
2. Czapinski P., Blaszczyk B., Czuczwar S.J.: Curr. Top. Med. Chem. 5, 3 (2005).
3. Chimirri A., De Sarro A., De Sarro G., Grasso S., Trimarchi G.R. et al.: J. Med. Chem. 32, 93 (1989).
4. Wong M.G., Defina J.A., Andrews P.R.: J. Med. Chem. 29, 562 (1986).
5. Chmielewska B.: Pharmazie 38, 872 (1983).
6. Chmielewska B.: Pharmazie 39, 259 (1984).
7. Edafiogho I.O., Scott K.R., Moore J.A., Farrar V.A., Nicholson J.M.: J. Med. Chem. 34, 387 (1991).
8. Kaminski K., Obniska J.: Bioorg. Med. Chem. 16, 4921 (2008).
9. Kaminski K., Obniska J., Dybala M.: Eur. J. Med. Chem. 43, 53 (2008).
10. Owoyale J.A., Lahan G.D., Osuide G., Edafiogho I.O.: J. Pharm. Sci. 70, 963 (1981).
11. Avetisyan S.A., Mndzhoyan O.L.: Arm. Khim. Zh. 23, 354 (1970).
12. Mndzhoyan O.L., Avetisyan S.A., Akopyan N.E., Gerasimyan D.A., Dzhagatspanyan I.A. et al.: Chem. J. 17, 452 (1983).

13. Avetisyan S.A., Mndzhoyan O.L.: Arm. Khim. Zh. 24, 137 (1971).

14. Mndzhoyan O.L., Avetisyan S.A., Azaryan L.V., Akopyan N.E., Gerasimyan D.A.: Arm. Khim. Zh. 27, 1056 (1974).

15. Avetisyan S.A., Nesunts N.S., Buyukyan N.S., Mndzhoyan O.L., Dzhagatspanyan I.A. et al.: Pharm. Chem. J. 22, 309 (1988).

16. Kocharov S.L., Panosyan H.A., Marzęda P., Wróblewska-Łuczka P., Kochman E. et al.: Health Probl. Civil. 11, 195 (2017).

17. Buczkowski Z., Lange J., Urbanski T.: Roczniki Chem., Ann. Soc. Chim. Polonorum. 39, 231 (1965).

18. Kutlu H.: J. Fac. Pharm. Istanbul. 13, 125 (1977).

19. Matsuo T.: Bull. Chem. Soc. Japan. 37, 1844 (1964).

20. Anderlini F.: Berichte der Deutschen Chemischen Gesellschaft. 26, Ref 600 (1893).

21. Hein D.W., Alheim R.J., Leavitt J.J.: J. Amer. Chem. Soc. 79, 427 (1957).

22. Lombardino J.G., Ewing F.E.: J. Heterocycl. Chem. 19, 923 (1982).

23. Shriner R.L., Upson R.W.: J. Amer. Chem. Soc. 63, 2277 (1941).

24. Anschütz R.: Justus Liebigs Ann. Chem. 354, 117 (1907).

25. Kupferberg H.: Epilepsia 4, 7 (2001).

26. Kupferberg H.J.: Epilepsia 30, S64 (1989).

27. Stables J.P., Kupferberg H.J.: The NIH Anticonvulsant Drug Development (ADD) Program: Preclinical Anticonvulsant Screening Project. John Libbey, London 1997.

28. Luszczki J.J., Wojda E., Andres-Mach M., Cisowski W., Glensk M. et al.: Epilepsy Res. 85, 293 (2009).

29. Chimirri A., De Sarro A., De Sarro G., Gitto R., Zappala M.: Farmaco. 56, 821 (2001).

30. Saxena A.K., Saxena M.: Prog. Drug Res. 44, 185 (1995).

31. Lange J., Rump S., Galecka E., Ilczuk I., Lechowska-Postek M. et al.: Pharmazie 32, 82 (1977).

32. Zejc A., Gross Z.: Dissert. Pharm. Pharmacol. 22, 305 (1970).

33. Bundgaard H., Johansen M.: Int. J. Pharm. 9, 7 (1981).

34. Lapszewicz J., Lange J., Rump S., Walczyna K.: Eur. J. Med. Chem. – Chem. Ther. 13, 465 (1978).

35. Miller C.A., Long L.M.: J. Amer. Chem. Soc. 75, 373 (1953).