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Original article

Synthesis and evaluation of in vivo activity of diphenylhydantoin basic derivatives

Tomasz Dyląg [a,1], Małgorzata Zygmunt [b], Dorota Maciąg [b], Jadwiga Handzlik [a], Marek Bednarski [b], Barbara Filipek [b], Katarzyna Kieć-Kononowicz [a,*]

[a] *Department of Technology and Biotechnology of Drugs, Collegium Medicum of the Jagiellonian University, Medyczna 9, Pl 30-688 Kraków, Poland*
[b] *Laboratory of Pharmacological Screening, Collegium Medicum of the Jagiellonian University, Medyczna 9, Pl 30-688 Kraków, Poland*

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Abstract

During the search for antiarrhythmic agents among amide derivatives of phenytoin, compound **7** {3-ethyl-1-[2-hydroxy-3-(4-phenyl-piperazin-1-yl)-propyl]-2,4-dioxo-5,5-diphenyl-imidazolidine} was selected as it showed antiarrhythmic as well as antihypertensive activity. Treating this compound as a lead, new derivatives **8–19** were synthesised, differing in piperazine phenyl ring substitution (2-, 3-, 4-Cl, 2-CH_3O) as well as in hydantoin N_3 alkyl chain (ethyl, ethyl acetate or ethyl 2-propionate). The obtained compounds in form of hydrochlorides **7a–19a** were examined for prophylactic antiarrhythmic and antihypertensive properties. Compounds containing ethyl 2-propionate moiety **(17a, 18a)** exhibited the highest antihypertensive properties. Water-soluble compounds, containing 2-methoxyphenylpiperazine group **(11a, 19a)**, showed strong antiarrhythmic properties in adrenaline-induced arrhythmia; compound **9a** {1-[3-(4-(3-chloro-phenyl)-piperazin-1-yl)-2-hydroxy-propyl]- 3-ethyl-2,4-dioxo-5,5-diphenyl-imidazolidine hydrochloride} exhibited the highest antiarrhythmic activity in barium chloride arrhythmia model.
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Keywords: Diphenylhydantoin derivatives; Antiarrhythmic activity; Hypotensive activity; Anticonvulsant activity; Microwave

1. Introduction

Phenytoin DPH – 5, 5 – diphenylhydantoin (Fig 1) was the starting structure for all presented compounds. It is frequently used as anticonvulsant drug, however, it is also applied in treatment of heart rhythm disturbances caused by intoxication with *Digitalis* glycosides. Phenytoin is a blocker of inactivated sodium channels and shortens the action potential in heart muscle cells and, therefore – according to Vaughan-Williams classification – it is a member of class Ib antiarrhythmic drugs. Side effects, including CNS – central nervous system complaints (ataxia, nystagmus or mental confusion) [1,2], are considered as the major drawbacks of phenytoin treatment, which have to be faced.

A number of phenytoin structure modifications have been performed to improve its therapeutic properties [3–7]. One direction of research led to the change of phenytoin character from acidic to basic, i.e. **ropitoin**, active in various types of arrhythmia models after oral and intravenous administration [3]. A series of amide phenytoin derivatives were obtained (Fig 1) and examined, including **RH-7**, exhibiting high prophylactic antiarrhythmic activity in chloroform and barium chloride-induced arrhythmia after intravenous injection, being less active in adrenaline-induced arrhythmia. As the action of **RH-7** is similar to that of class Ia antiarrhythmic drugs, introduction of hydroxyethylpiperazine substituent into position 3 of the hydantoin ring was followed by a change in the mechanism of action. **RH-7**, possessing lidocaine-like anaesthetizing activity, is bereft of CNS activity and is less toxic than phenytoin. A lack of the activity after oral administration was its main disadvantage [5]. Among others, KF-2 was a promising one in a group of compounds with 3-amine-2-propanol substituent in position 1 of the hydantoin ring and ethyl or ethyl acetate in position 3. The

* Corresponding author. tel./fax +48-12-6570488.
E-mail address: mfkonono@cyf-kr.edu.pl (K. Kieć-Kononowicz).
[1] Present address: Neurobiochemistry Group, Faculty of Chemistry, Jagiellonian University, Ingardena 3, Pl 30-060 Kraków, Poland

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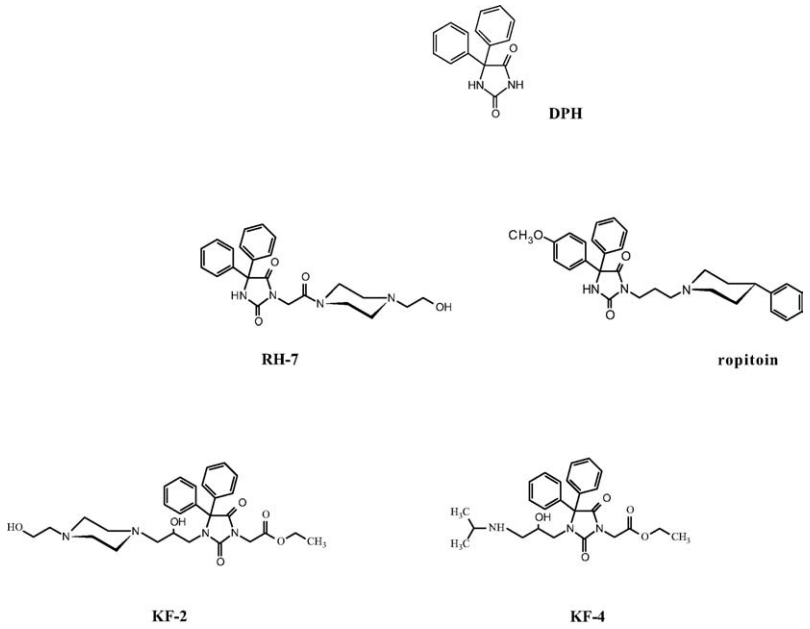
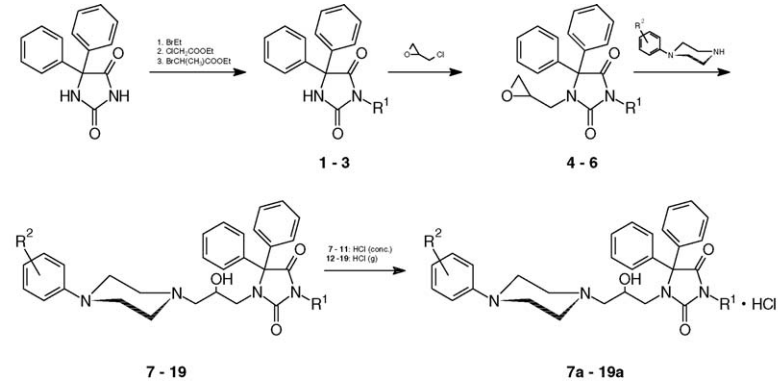


Fig. 1. Structures of some basic derivatives of phenytoin (DPH).

compound prevented symptoms of barium chloride-induced arrhythmia in 66% of animals, although it was less active in adrenaline-induced arrhythmia. In both cases, however, **KF-2** prolonged the survival time of animals. **KF-4** was another active compound [8]. These compounds, possessing a hydroxypropyl fragment characteristic for beta-blockers, display a mechanism of action similar to that of antiarrhythmic Ia group. For the obtained compounds, however, 3-ethyl-5,5-diphenylhydantoin derivative also comprising a phenylpiperazine substituent **7a** (Fig 2) revealed the highest



Compound	R^1
1, 4	CH_3
2, 5	CH_2COOCH_3
3, 6	$CH(CH_3)COOCH_3$

Compound	R^1	R^2
7, 7a	CH_3	H
8, 8a		2-Cl
9, 9a		3-Cl
10, 10a		4-Cl
11, 11a		2-CH_3O
12, 12a	CH_2COOCH_3	H
13, 13a		2-Cl
14, 14a		3-Cl
15, 15a		4-Cl
16, 16a		2-CH_3O
17, 17a	$CH(CH_3)COOCH_3$	H
18, 18a		2-Cl
19, 19a		2-CH_3O

Fig. 2. Synthesis of basic arylpiperazine hydantoin derivatives **7a–19a**.

activity. This compound, which antiarrhythmic activity was confirmed in both barium chloride and adrenaline – induced arrhythmias, was additionally examined for its hypotensive action. It proved to diminish both systolic and diastolic blood pressure in normotensive rats after intraperitoneal administration in a statistically significant manner. In this way, it was confirmed that introduction of arylpiperazine moiety into the molecule endowed it with hypotensive action. Analysis of structure and activity of a series of compounds, used either in therapy or as selective α_1-AR ligands in pharmacology, justifies hypothesis that a likely α_1-adrenergic receptor antagonist was obtained. Compound **7a** comprises also hydroxypropyl side chain, typical for beta-blockers.

Interesting properties of **7a** prompted us to obtain and analyse pharmacological activity of compounds being derivatives of that structure. We decided to estimate influence of the substitution pattern of phenylpiperazine phenyl ring in the compounds on hypotensive action, since this substituent may account for interaction with adrenergic receptors. The presence of hydroxypropyl chain suggested possible β-blocking activity, which should be desirable for antiarrhythmic action.

Keeping that in mind, we designed and synthesized a series of derivatives of **7a** differing in the substitution of piperazine phenyl ring as well as in hydantoin N_3 alkyl chain. The structurally modified compounds **8a–19a** have been examined for their influence on normal ECG and antiarrhythmic and antihypertensive action. In addition, anticonvulsant activity and neurological toxicity for some compounds were evaluated.

2. Chemistry

The preparation of basic diphenylhydantoin derivatives was accomplished with the route depicted in Fig 2.

The first step of synthetic work was based on an introduction of substituents into position 3 of hydantoin ring, which was performed by heating of respective bromo- or chloroalkyl derivatives with DPH. This process was performed at elevated temperature in two phase-catalytic conditions in the presence of sodium or potassium carbonate and benzyltriethylammonium chloride (TEBA) as phase-transfer catalyst and afforded compounds **1–3**.

The aim of the second step was to introduce reactive 2,3-epoxypropyl moiety into the 1 position of hydantoin. This step involved phase-transfer catalysed reactions of compounds **1–3** with epichlorhydrine used in 20% excess. The reaction was performed at room temperature with the use of TEBA and sodium carbonate and led to the formation of compounds **4–6**.

In the following step, the epoxy bridge was cleaved with secondary amines: phenylpiperazine or its 2-, 3-, 4-chloro and 2-methoxy derivatives to obtain compounds **7–19**. The first technique to achieve this goal included heating of equimolar quantities of substrates in toulene for 6.5–15.5 hours (method A). Time consumption as well as the necessity to use a solvent that impedes isolation of product from the reaction liquid are the major drawbacks of this method. In the other method, mixed substrates were irradiated in a standard domestic microwave oven (method B) previously melting of some reagents (method B*). In this method, without having to use a solvent for conducting the synthesis, simple crystallisation of the reaction mixture from ethanol afforded pure product. Rapidity and efficiency are the supreme benefits of the method shorter than ten-minute irradiation suffices to obtain products with yields substantially higher than in the same reactions performed in the toluene environment. Also the purification was much easier than in traditional method [9–11]. To improve the solubility of free bases **7–19**, which was desirable for pharmacological screening purposes, compounds were converted into corresponding hydrochlorides **7a–19a**. Compounds **7–11** were turned into salts by addition of concentrated hydrochloric acid and heating until its excess evaporated. The presence of easily hydrolysing ester bonds in compounds **12–19** prevented the use of liquid acid and, therefore, they were solved in dry ethanol and saturated with gaseous hydrogen chloride. In spite of converting the bases into hydrochlorides, the solubility of the compounds (with the exception of compounds **11a** and **19a**) was not high enough to allow intravenous administration. As indicated by elemental analyses, in all cases, monohydrochloride salts were obtained. For the pharmacological screening purposes, all compounds were used as mixtures of enantiomers.

3. Pharmacology

Obtained compounds were examined in vivo. The investigation included effects on normal electrocardiogram, influence on blood pressure in normotensive rats and prophylactic antiarrhythmic action in animal models of heart rhythm disturbances caused by intravenous injection of either barium chloride or adrenaline. The obtained compounds were tested as hydrochlorides, which possessed different water solubility. Thus, the two ways of administrations were used. Most of the compounds were examined after i.p. administration because their water solubility was low. Only two compounds were soluble enough for more profitable intravenous administration.

Protection against rhythm disturbances caused by injection of arrhythmogens (barium chloride or adrenaline) depicts the ability of a compound to act as a potential antiarrhythmic agent. The two arrhythmogenes act via different mechanisms. Barium chloride takes effect by decrease of the outward potassium currents, whereas adrenaline stimulates β-adrenergic receptors.

Improvement of antiarrhythmic and antihypertensive properties of phenytoin derivatives can lead to a decrease in their CNS interactions. Treating anticonvulsant activity as a CNS interaction symptom, three of the obtained compounds

Table 1
Effects of an intravenous injection of the investigated compounds on heart rate and ECG intervals in anaesthetised male Wistar rats

Compound	Dose (mg/kg)	Parameters	Time of observation (min) 0	1	5	15
11a	50	Beats per minute	341.1 ± 11.4	303.1 ± 14.3*	326.8 ± 16.0	327.8 ± 16.8
	50	P-Q	49.4 ± 2.3	59.1 ± 4.2*	57.7 ± 3.2	59.4 ± 2.4*
	50	Q-T	75.7 ± 2.4	76.9 ± 3.0	77.1 ± 3.0	74.3 ± 3.7
	50	QRS	18.6 ± 0.7	19.1 ± 1.1	19.1 ± 0.7	20.0 ± 1.0
19a	50	Beats per minute	324.0 ± 9.4	260.0 ± 15.4****	271.0 ± 15.4***	284.0 ± 7.5*
	50	P-Q	60.4 ± 3.6	66.8 ± 2.8	66.4 ± 3.5	63.4 ± 2.3
	50	Q-T	70.2 ± 2.6	85.4 ± 6.0*	77.8 ± 3.6	72.4 ± 2.7
	50	QRS	15.4 ± 0.2	16.8 ± 0.9	15.8 ± 0.7	15.4 ± 0.5

The data are the means of six experiments ± S.E.M. Statistical analyses were performed using a one-way ANOVA test
* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$

were tested by the Antiepileptic Drug Development (ADD) Program. The investigation containing Phase I of the evaluation involved three tests: maximal electroshock (MES), subcutaneous penthylenetetrazole (scMet), and neurologic toxicity [12].

4. Results

The influence of compounds **11a** and **19a** on normal rats ECG was tested after i.v. administration (Table 1). Compound **11a** slightly decreased the number of cardiac beats in 1st minute and prolonged PQ interval in 1st and 15th minutes of the test. Compound **19a** showed significant decrease of cardiac beats number for the duration of observation and slightly prolonged QT interval in first minute after injection.

Some compounds tested intraperitoneally (Table 2), especially **9a** and **10a**, did not significantly affect the normal ECG but other compounds had selective effects inducing decreased rate of cardiac beats in 60th **(8a)**, prolonged PQ intervals in 15th **(18a)**, 30th and 60th minutes **(17a, 18a)**, prolonged QT interval in 15th **(12a, 16a)**, 30th **(16a)** and

Table 2
Effects of an intraperitoneal injection of the investigated compounds on heart rate and ECG intervals in anaesthetised male Wistar rats

Compound	Dose (mg/kg)	Parameters	Time of observation (min) 0	15	30	60
8a	50	Beats per minute	420.0 ± 14.9	397.3 ± 16.8	382.0 ± 15.3	368.2 ± 15.6*
	50	P-Q	49.2 ± 2.0	51.2 ± 3.0	49.6 ± 1.9	50.8 ± 3.1
	50	Q-T	58.8 ± 2.3	67.6 ± 6.1	68.0 ± 3.7	74.0 ± 5.4*
	50	QRS	17.2 ± 1.2	17.6 ± 1.0	17.2 ± 1.2	17.2 ± 1.2
9a	50	Beats per minute	367.2 ± 32.0	330.9 ± 29.8	320.8 ± 33.1	318.4 ± 39.9
	50	P-Q	53.0 ± 2.4	56.3 ± 3.0	56.3 ± 3.0	57.7 ± 3.9
	50	Q-T	75.0 ± 4.3	79.3 ± 2.6	81.6 ± 4.0	87.3 ± 6.8
	50	QRS	19.3 ± 0.4	19.7 ± 0.6	19.7 ± 1.0	20.7 ± 0.7
10a	50	Beats per minute	332.7 ± 29.5	334.8 ± 34.4	331.4 ± 36.2	325.4 ± 36.01
	50	P-Q	52.5 ± 1.5	52.5 ± 1.0	55.0 ± 0.6	56.0 ± 1.4
	50	Q-T	73.5 ± 2.4	68.5 ± 3.0	70.0 ± 2.4	74.0 ± 4.8
	50	QRS	22.0 ± 2.9	21.5 ± 3.0	20.5 ± 1.9	22.0 ± 2.8
12a	50	Beats per minute	308.9 ± 17.1	298.9 ± 6.3	305.5 ± 4.4	320.1 ± 13.9
	50	P-Q	56.5 ± 4.7	56.5 ± 2.9	54.5 ± 2.6	58.5 ± 4.3
	50	Q-T	72.5 ± 2.5	81.5 ± 3.0**	76.5 ± 2.4	79.0 ± 1.0
	50	QRS	17.5 ± 0.0	16.5 ± 0.5	16.0 ± 0.1**	16.1 ± 0.1**
16a	50	Beats per minute	330.7 ± 17.3	313.1 ± 15.0	302.4 ± 18.9	293.8 ± 24.8
	50	P-Q	51.2 ± 2.9	53.6 ± 2.1	55.6 ± 2.0	59.2 ± 2.0*
	50	Q-T	67.5 ± 4.8	77.0 ± 1.9*	77.5 ± 1.0**	80.5 ± 0.5***
	50	QRS	19.6 ± 0.7	20.0 ± 0.6	20.8 ± 0.8	21.6 ± 0.7
17a	50	Beats per minute	344.7 ± 9.1	345.5 ± 6.8	350.4 ± 13.4	372.6 ± 15.0
	50	P-Q	53.0 ± 1.8	55.3 ± 0.7	56.3 ± 1.0*	58.3 ± 1.0***
	50	Q-T	81.7 ± 1.7	81.7 ± 1.7	76.7 ± 2.1	76.7 ± 2.1
	50	QRS	17.3 ± 0.8	17.3 ± 0.8	17.0 ± 0.9	17.7 ± 1.0
18a	50	Beats per minute	381.3 ± 18.2	344.1 ± 20.1	334.3 ± 16.0	333.2 ± 12.8
	50	P-Q	40.8 ± 0.8	46.4 ± 1.0***	46.8 ± 1.4***	50.0 ± 1.7****
	50	Q-T	76.0 ± 6.8	88.4 ± 8.5	86.0 ± 6.8	88.8 ± ± 3.4
	50	QRS	20.0 ± 0.6	20.8 ± 0.8	20.8 ± 0.8	20.8 ± 0.8

The data are the means of six experiments ± S.E.M. Statistical analyses were performed using a one-way ANOVA test
* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$

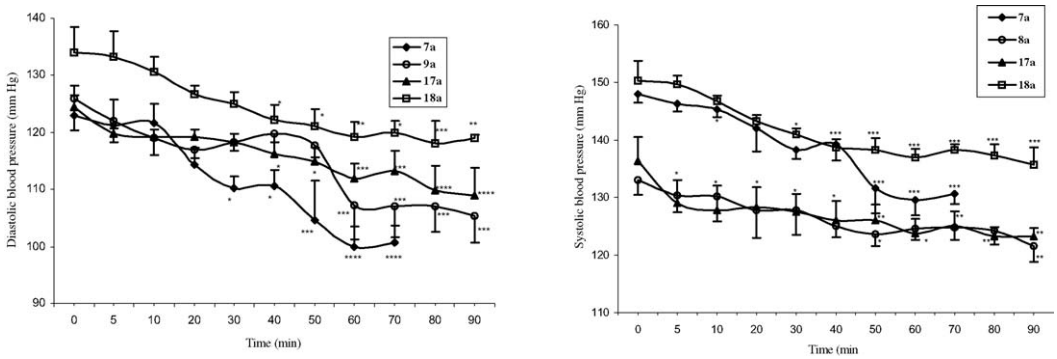


Fig. 3. Hypotensive activity of compounds tested after i. p. administration in anaesthetised normotensive rats.

60th **(8a, 16a)** minutes of observation. Only compound **12a** demonstrated statistically significant prolongation of QRS complex (in 30th and 60th minutes after i.p. administration).

The obtained compounds were evaluated for antihypertensive properties after intraperitoneal or intravenous administration in anaesthetised Wistar rats. Compounds of low water solubility **(7a–10a, 12a, 16a–18a)** were tested for 90 (70 in case of **7a**) minutes after i.p. administration. The lead compound **7a** significantly decreased both systolic (max. 12%) and diastolic (max 8.7%) blood pressure at 30th minute of the measurement. Furthermore, three of i.p. tested compounds **(8a, 17a, 18a)** showed statistically significant decrease of rat systolic blood pressure prolonged to the end of observation. Especially, compound **17a** significantly decreased systolic pressure (5.7–9.8%) from 5th till 90th minutes of the observation. The compounds **9a, 17a, 18a** depressed diastolic blood pressure (7.2–16.4%) lasting for 40–60 minutes of the test (Fig 3).

Influence of water soluble compounds **11a** and **19a** on rat blood pressure was assessed for 60 minutes after i.v. injection in dose of 2.5 mg/kg. In addition, compound **19a** was tested in dose of 10 mg/kg (used also in antiarrhythmic activity test). Both compounds affected the normal blood pressure of animals only for 1–3 minutes after administration. During this time, compound **19a** in higher dose (10 mg/kg) showed undesirable sudden decrease of both systolic and diastolic pressure causing death of 33% of investigated animals. Compounds **11a** and **19a** (2.5 mg/kg) did not possess hypotensive property demonstrating just one weak statistically significant decrease of rat blood pressure (systolic and diastolic) in 2nd minute of the measured time (Fig 4).

Elementary antiarrhythmic activity of obtained compounds was evaluated for anesthetized rats, using the adrenaline and barium chloride models of arrhythmia.

For examined rats, intravenous injections of high dose of barium chloride (32 mg/kg) caused sinus bradycardia and a rapid ventricular extrasystoles, ventricular tachycardia and ventricular fibrillation (100%), which led to the death of all animals within 3-5 minutes (Fig 5). The lead compound **7a** i.p. administrated showed similar activity in both barium chloride and adrenaline-induced arrhythmia tests. In barium chloride induced arrhythmia models, compound **7a** inhibited premature ventricular beats in 50% of tested animals, prevented occurrence of ventricular fibrillation in 75% and also diminished arrhythmogen caused mortality to 25%. In adrenaline arrhythmia model (Fig 6), compound **7a** decreased the number of sinal extrasystoles (44%) and protected against death all of the tested rat population. The results are shown in Fig 7 and Fig 8.

Among obtained compounds only one **(9a)** was more effective in $BaCl_2$-induced arrhythmia test than **7a**. This compound **9a** decreased number of extrasystoles (50%), totally reduced incidence of ventricular fibrillation and protected against death 100% of tested animals. Significant

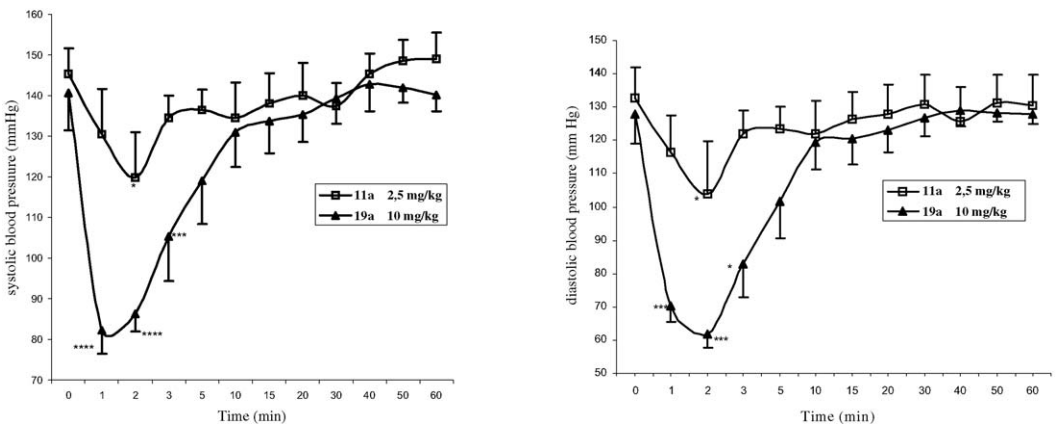


Fig. 4. Hypotensive activity of compounds tested after i. v. administration in anaesthetised normotensive rats.

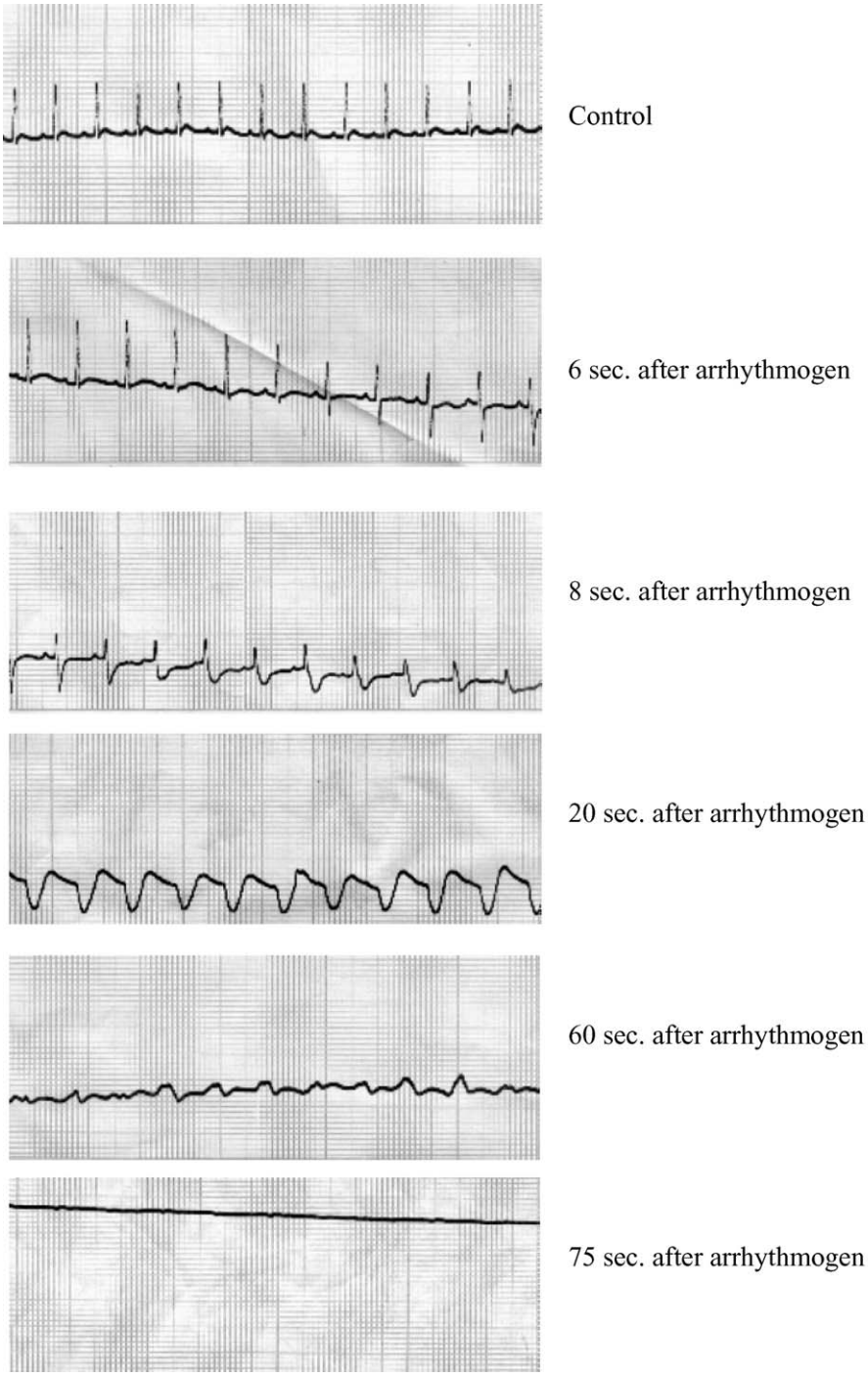


Fig. 5. Heart rhythm disturbances caused by intravenous injection of high dose of barium chloride (32 mg/kg i.v.). The surface ECG lead II with paper speed 50 mm/s and display amplification 1 mV/cm are shown.

activity was shown by i.v. injected compound **11a**, preventing the appearance of barium chloride arrhythmia in 50% of animals which also diminished their mortality to 25%. Compounds **12a** and **16a** in dose of 50 mg/kg i.p. caused the return of sinal rhythm and prolonged the survival time for more than 60% of tested rats. Compounds **8a, 10a, 17a, 18a** possessed weak antiarrhythmic properties after i.p. administration, slightly reducing number of barium chloride induced rhythm disturbances and mortality. The lowest activity was demonstrated by compound **19a**, which was not active at dose of 10 mg/kg i.v. in 100% of tested animals following their death within 2–4 minutes after $BaCl_2$ administration (Fig 7).

Rapid intravenous injection of adrenaline at dose of 20 μg/kg caused anesthetized reflex bradycardia (100%), atrioventricular disturbances, ventricular and supraventricular extrasystoles (94%), bigeminy (50%) which led to death of approx. 50% of animals within 10±5 minutes.

The compound **19a** (10 mg/kg i.v.) was the most active in adrenaline test, preventing the appearance of adrenaline-

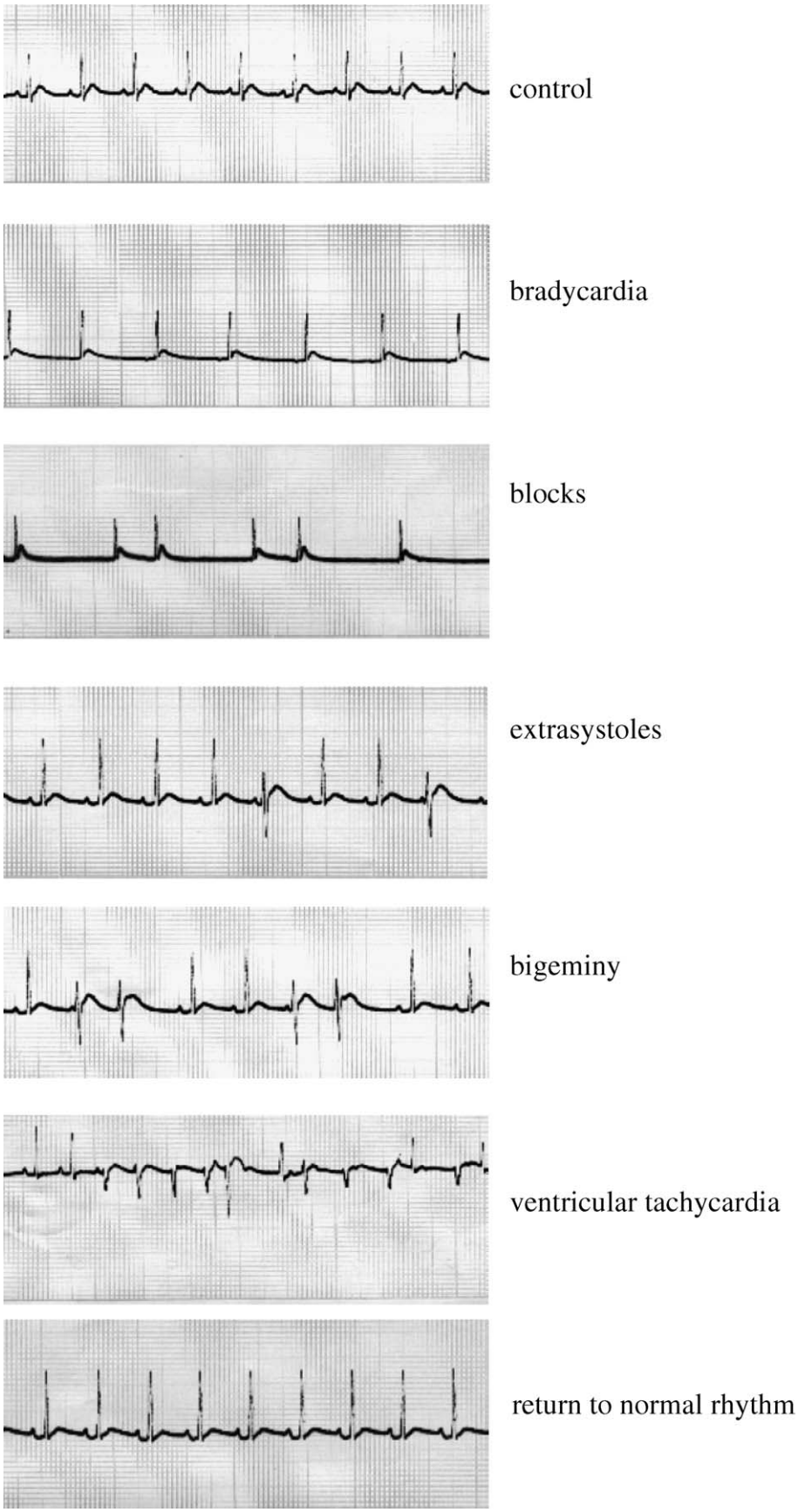


Fig. 6. Heart rhythm disturbances caused by rapid intravenous injection of adrenaline (20 μg/kg i.v.). The surface ECG lead II with paper speed 50 mm/s and display amplification 1 mV/cm are shown.

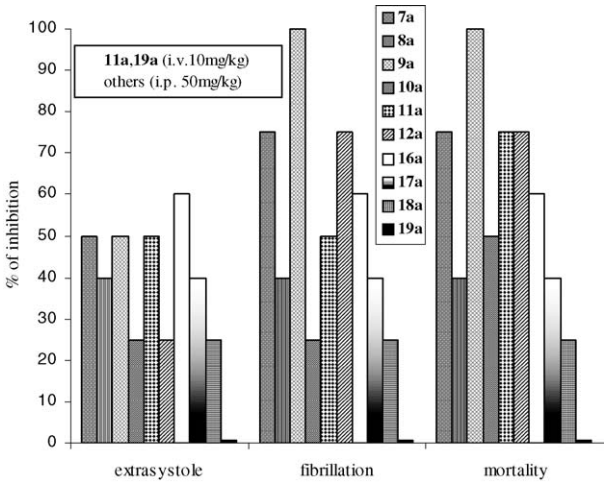


Fig. 7. Prophylactic antiarrhythmic activity in barium-induced arrhythmia.

induced arrhythmia symptoms in 100% of tested rats. The other active compound, namely **11a**, decreased number of adrenaline induced cardiac rhythm disturbances and mortality to 25%. Among compounds administrated intraperitoneally, two of them diminished adrenaline induced premature ventricular beats **(9a, 10a)** for more than 50% of investigated rats. The lowest activity in adrenaline induced model of arrhythmia was shown by compound **12a** (Fig 8).

Antiepileptic properties of compounds **9a, 10a** and **16a** were tested in Phase I of ADD Program. Compounds **9a** and **16a** were devoid of anticonvulsant activity in the used tests and did not show neurological toxicity. Compound **10a** was active in ScMet test in dose of 100 mg/kg after 0.5 hour and demonstrated neurological toxicity in dose of 100 mg/kg and 300 mg/kg after 0.5 hour and 4 hours, respectively.

5. Discussion

The introduction of new moieties into 1- and 3- position of hydantoin ring changed pharmacological properties of phenytoin which belongs to class Ib of antiarrhythmic agents.

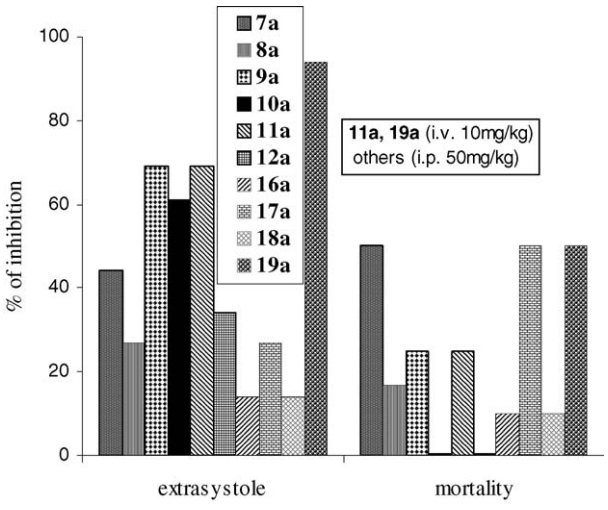


Fig. 8. Prophylactic antiarrhythmic activity in adrenaline-induced arrhythmia.

The conducted preliminary studies showed that some of the compounds slightly decreased the heart rate, lengthened the time of duration of P-Q and Q-T intervals. The strongest effects were produced by compounds **8a, 11a, 12a, 16a, 17a, 18a** and **19a**, which reduced significantly the heart rate **(8a, 11a, 19a)**, prolonged the P-Q **(11a, 16a, 17a, 18a)** and Q-T **(8a, 12a, 16a, 19a)** intervals, without affecting the QRS complex; only compound **12a** shortened the QRS complex. Compounds of class Ib reveal little effect on phase 0 (normal tissues) and have negligible or shortening effect on repolarization. Thus, class Ib antiarrhythmic drugs markes no significant influence on P-Q and QRS and tends to decrease Q-T interval [13-15]. The presented group of new compounds rather prolonged Q-T interval (Table I, Table 2), being similar to class Ia [8,13-15]. In contrast to class Ia, these compounds did not significantly prolong and sometimes even shorten **(12a)** QRS complex. In case of compounds **8a, 16a, 19a**, the prolongation of Q-T and lack of influence on QRS complex can indicate some similarities to drugs of class III of Vaughan-Williams classification.[1,13-15].

Other compounds **(9a, 10a)** marked no significant effect on P-Q, QRS and Q-T intervals. Especially, the electrocardiographic changes, observed after administration of **10a**, were similar to those observed for phenytoin, which usually exerts no significant effect on P-Q and QRS duration, whereas the Q-T is unaltered or slightly shortened [15].

An analysis of the results shows that the type and position of substituent in phenylpiperazine phenyl ring as well as the substituent in 3 position of hydantoin moiety play an important role in the pharmacological activity of the investigated compounds.

For three isomers **8a, 9a, 10a**, *ortho* **(8a)**, *meta* **(9a)** and *para* **(10a)** chlorophenylopiperazine derivatives of lead compound **7a**, a significant influence of the chloride position on expected pharmacological properties was observed . The *para* isomer demonstrated weak antiarrhythmic activity, lack of hypotensive property and significant influence on normal rat ECG. Results of Phase I of anticonvulsant assay ascribe this *para*-chloro isomer to I class antiepileptic drugs, accentuating its clear neurological toxicity. This finding suggests that *para* position of substituent in phenylpiperazine ring may cause undesirable interaction with CNS and does not enhance expected antiarrhythmic and hypotensive efficacy. Pharmacological test results for *ortho* and *meta* isomers are in agreement with this hypothesis. The *meta*-chloro derivative **(9a)** shows neither anticonvulsant activity nor neurological toxicity in the Phase I anticonvulsant assay but displays the highest antiarrhythmic properties among considered isomers. Especially, in whole series of described compounds, the **9a** is the most potent in barium chloride-induced arrhythmia tests. This may suggest that *meta* substituent in phenylpiperazine phenyl moiety is profitable for antiarrhythmic action in barium chloride arrhythmia model.

The ventricular tachyarrhythmias, produced by barium chloride, are most severe and hard to influence. The mechanism by which barium chloride exerts its arrhythmogenic

action is complex and not fully understood. It was postulated that – in Purkinje fibers of the sheep – the changes caused by barium ions are mainly brought about by an increase in sodium inward current [16]. Barium ions decrease the outward potassium currents, blocking the ultra-rapid delayed rectifier K^+ channels and inwardly rectifying K^+ channels (Kir), pacemaker K^+ channels (I_K), background K^+ channels (I_{K1}), ACh-induced K^+ current ($I_{K,ACh}$), calcium-activated K^+ channels (I_{Ca}) and ATP-sensitive K^+ channels ($I_{K,ATP}$), fatty acids-activated K^+ channels (I_{AA}) [17,18]. Blocking the potassium channels by barium ions causes rapid diastolic depolarization and initiation of spontaneous repetitive activity by mechanisms of early afterdepolarizations and spontaneous phase 4 depolarization (abnormal automaticity). As a consequence of its K^+ chanel-blocking action, barium prolongs Q-T interval (Fig 5) [19]. It is known that local anesthetic antiarrhythmic drugs (lidocaine, phenytoin), blocking Na^+ channel, reduce the Na^+ current and abolish early- and delayed after depolarization (EADs and DADs).

Some of the obtained compounds (especially **7a, 9a, 12a, 16a**) delayed the onset time of this arrhythmia, reduced the incidence of ventricular tachycardia and ventricular fibrillation, prolonged the survival time and prevented or diminished mortality caused by $BaCl_2$.

Compounds **19a, 11a, 9a, 10a** diminished or prevented the appearance of adrenaline-induced arrhythmia symptoms. Adrenaline is an agonist of β-adrenergic receptors (β-AR), which are located also in heart muscle tissue. β-adrenergic stimulation is one of the most important regulatory mechanisms of ions channel function. In the heart, the stimulation of β-AR increases the magnitude of $I_{Ca\text{-}L}$, I_f, I_{Cl} , I_{Kur}, I_{KATP}, I_{st} and these actions are mediated by cAMP-dependent protein kinase [17,18]. β-adrenergic stimulation, via voltage-dependent Ca^+, I_{Kur} and also Na^+ channels, increases the probability of a variety of supraventricular and ventricular arrhythmias. Based on our results, we hypothesize that the antiarrhythmic effects of these tested compounds are probably related to the arrest of the intraflow of Na^+ and/or Ca^{2+} in the cardiac cells and the depression of their cardiac autoarrhythmicity and conductivity. As a consequence of overdose of adrenaline in rats, prolongation of P-Q, QRS and Q-T intervals with concomitant decrease of heart rate was observed (Fig 6) [20].

In case of compound **11a**, the introduction of methoxy group in *ortho* position of lead phenyl ring significantly improves its antiarrhythmic property in adrenaline model but deprives the compound of antihypertensive potency. This change of pharmacological properties may be evoked by increase of water solubility. The similar trend is seen for compounds **17a** and **19a**, possessing ethyl propionate moiety in 3 position of hydantoin ring, and phenylpiperazine **(17a)** or *ortho*-methoxyphenylpiperazine **(19a)** at the end of hydroxypropyl chain. The water soluble compound **19a** demonstrates the strongest antiarrhythmic efficacy against adrenaline arrhythmogen among all the presented compounds and is an obstacle to expected antihypertensive activity. On contrary, the water insoluble compounds **17a** as well as its *ortho*-chloro analogue **18a**, are the most potent hypotensive agents among all tested series but display weak antiarrhythmic activity. The third pair of analogue compounds, namely phenylpiperazine **(12a)** and 2-methoxyphenylpiperazine **(16a)** derivatives of 3-hydantoin ethyl acetate, is insoluble in water. Both compounds are almost equiactive in the tests showing very low antiarrhythmic action, and are bereft of hypotensive activity. Considering a behaviour of three pairs of phenylpiperazine and 2-methoxyphenylpiperazine analogues, it appears that not simply presence of *ortho*-methoxy group but rather increased water solubility caused by this group enhances antiarrhythmic and decreases hypotensive properties of the compounds. Although the increase of antiarrhythmic property of soluble *ortho*-methoxyphenylpiperazine derivatives is in agreement with other works [21], the fall in hypotensive properties is a strange inverse.

According to the in vivo results, a substituent in 3 position of hydantoin ring is also related to the pharmacological efficacy. Replacement of lead ethyl group **(7a)** with ethyl 2-propionate **(17a)** improves its antihypertensive activity but introduction of ethyl acetate **(12a)** enhances neither antihypertensive nor antiarrhythmic properties of the compound. The similar trend is seen for 2-chlorophenylpiperazine derivatives **(8a, 18a)**. Derivatives of 2-methoxyphenylpiperazine **(11a, 16a, 19a)** demonstrated similar behaviour in case of adrenaline induced arrhythmia test. The water insoluble ethyl acetate derivative **16a** is significantly less active than the ethyl one **(11a)**, which in turn is slightly less active than the compound **19a**, possessing ethyl 2-propionate moiety.

Searching for effective antiarrhythmic drugs, it is needful to assess a protection against arrhythmogens as well as an influence of investigated compounds on ECG components. Especially, an influence of a compound on Q-T interval is an important criterion/issue. Lengthening of the Q-T interval causes no symptoms but both the congenital and the aquired form of the long Q-T syndrom (LQTS) can precipitate life-threatening polymorphic ventricular tachycardia known as torsade de pointes. Thus, three obtained compounds **9a, 11a** and **19a** are particularly promising in the search for novel antiarrhythmic agents. Those compounds displayed the strongest antiarrhythmic activity in barium chloride- **(9a)** or adrenaline **(11a, 19a)** induced arrhythmia models and they hardly influenced the ECG components. Compound **19a** displayed weak statistically significant prolongation of Q-T interval only in the very first minute after administration. Compound **11a** did not cause significant lengthening of Q-T interval, while the **9a** one caused no statistically significant change of any ECG component. It can suggest lack of proarrhytmic properties of these compounds.

The presented work is an introduction into the new family of phenylpiperazine derivatives of 5,5-diphenylhydantoin. The performed, on preliminary level, pharmacological tests, divided the derivatives of lead **7a** into five groups of different activities: hypotensive **(17a, 18a)**, antiarrhythmic in barium

chloride arrhythmia model **(9a)**, antiarrhythmic in adrenaline model **(19a, 11a)**, anticonvulsant **(10a)** and compounds of low activity in presented tests **(8a, 12a, 16a)**.

6. Experimental

^{1}H-NMR and H,H-COSY spectra were recorded on Varian Mercury VX 300 MHz PFG instrument in $CDCl_3$ at ambient temperature. For H,H-COSY experiment of **12**, 4 repetitions of 256 increments were performed. Chemical shifts are given in parts per million relative to tetramethylsilane, coupling constants are given in Hz. IR spectra were recorded on a Jasco FT/IR-410 apparatus using KBr pellets and are reported in cm^{-1}. For column chromatography Merck silica gel 60 (Merck) of seed diameter 0.063–0.200 mm [mesh 70–230 ASTM] was used. Thin-layer chromatography was performed on pre-coated Merck silica gel 60 F_{254} aluminium plates, the used solvent systems were: (A) toluene/acetone/methanol 5:5:1; (B) chloroform/ethyl acetate 1:1. Melting points are uncorrected. Analyses indicated by the symbols of the elements or functions were within ± 0.4% of the theoretical values unless stated otherwise.

IR spectra were recorded for hydrochloride salts, NMR spectra were recorded for free bases unless stated otherwise.

6.1. Chemistry

6.1.1. Synthesis of N_3-substituted 5,5-diphenylhydantoin derivatives (1–3)

Compounds **1–2** were prepared according to the procedure described earlier [7,8].

6.1.1.1. 2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)-propionic acid ethyl ester (3). A mixture of DPH (25.2 g, 0.1 mol), TEBA (3 g, 0.013 mol), potassium carbonate (40 g) and acetone (500 ml) was refluxed for 1 hour. Next, a solution of ethyl 2-bromopropionate (18.0 g, 0.1 mol) in 50 ml acetone was added dropwise and the whole was refluxed for the next 3 h. The solid residue was removed from the reaction mixture, the filtrate was condensed and the oily residue was recrystallized from EtOH to give **3** at 81.7% yield, m.p. 92–94 ºC, R_f(B) = 0.82.

6.1.2. Synthesis of N_3- and N_1-substituted 5,5-diphenylhydantoin derivatives (4–6)

Compounds **4–5** were prepared according to the procedure described earlier [7,8].

6.1.2.1. 2-[3-(2,3-Epoxypropyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl]-propionic acid ethyl ester (6). A suspension of **3** (14.8 g, 42 mmol), TEBA (1.26 g, 5.5 mmol) and potassium carbonate (17 g) in acetone (120 ml) was stirred at room temperature for 30 minutes, then, a solution of freshly distilled epichlorohydrin (4.65 g, 50 mmol) in acetone (25 ml) was added dropwise. After being stirred for the next 7.5 h, the precipitate was removed by filtration and the filtrate was condensed to yield oily residue which was purified by means of column chromatography (mobile phase: $CHCl_3$). Fractions containing product were concentrated in vacuo to afford **6** in the form of oil, which was used for further reactions.

6.1.3. Synthesis of N_1-phenylpiperazinealkyl-N_3-alkyl-5,5-diphenylhydantoin derivatives (7a–19a)

Commercially available monohydrochloride salts of N-2- and N-3-chlorophenylpiperazine as well as N-4-chlorophenylpiperazine dihydrochloride were converted into free bases according to the method described by Pawłowski et al. [22].

6.1.3.1. 3-Ethyl-1-[2-hydroxy-3-(4-phenylpiperazin-1-yl)-propyl]-2,4-dioxo-5,5-diphenylimidazolidine hydrochloride (7a). Method B: A mixture of **4** (1.68 g, 5 mmol) and N-phenylpiperazine (0.81 g, 5 mmol) was prepared in a flat-bottomed 50 ml flask covered with a small inverted funnel. The flask along with a water-filled beaker were placed in a standard household microwave oven and irradiated (300 W) for 3 minutes altogether. The presence of the product was observed on TLC plates after increasing the power and so irradiation was continued for the next 2 minutes at 450 W. Longer heating did not cause any increase of the product spot (TLC) intensity. The reaction mixture was crystallized from EtOH to afford 1.42 g of pure **7**. M.p. 136–138 ºC; yield 57%, TLC: R_f(A) = 0.75; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm) of base **7**: 1.27 (t, J = 7.14, 3H, CH_2–CH_3), 2.05 (dd def., 1H, N–C$H_{2(a)}$–CHOH), 2.20–2.27 (m, 1H, N–C$H_{2(b)}$–CHOH), 2.28–2.48 (m, 4H, 2 x (CH_2)–N–CH_2–CHOH), 3.06 (t, J = 4.94, 4H, 2 x Ph–N–(CH_2)–), 3.00–3.18 (m, 1H, C*H*OH), 3.40–3.59 (m, 2H, CHOH–CH_2–N), 3.60–3.75 (m, 3H, CH_2–CH_3, CHO*H*), 6.80–6.92 (m, 3H, Ar–H_2, Ar–H_4, Ar–H_6 Ph-piper), 7.20–7.34 (m, 6H, 2 x Ar–H_2, 2 x Ar–H_6 DPH; Ar–H_3, Ar–H_5 Ph-piper), 7.36–7.46 (m, 6H, 2 x Ar–H_3, 2 x Ar–H_4, 2 x Ar–H_5 DPH). Concentrated hydrochloric acid was added to solid 7 and the mixture was evaporated to dryness. The residue was recrystallized from EtOH to quantitatively afford pure hydrochloride salt **7a**, m.p. 245–248 ºC. IR: 3467 (OH), 3339 (OH), 2973 (CH), 2160, 1766 (C_2=O), 1710 (C_4=O), 1597 (Ar).

6.1.3.2. 1-[3-(4-(2-Chlorophenyl)-piperazin-1-yl)-2-hydroxypropyl]-3-ethyl-2,4-dioxo-5,5-diphenylimidazolidine hydrochloride (8a). Method A: A mixture of **4** (1.68 g, 5 mmol) and N-2-chlorophenylpiperazine (0.98 g, 5 mmol) in toluene (15 ml) was refluxed for 10 h under TLC control. The solvent was evaporated. The precipitate was washed with EtOH and crystallized from the same solvent to obtain 1.44 g of **8**; m.p. 131–133 ºC; yield 54%; TLC: R_f(A) = 0.75; ^{1}H-NMR of base ($CDCl_3$, TMS) δ (ppm): 1.27 (t, J = 7.14, 3H, CH_2–CH_3), 2.06 (dd def., 1H, N–C$H_{2(a)}$–CHOH), 2.23 (dd def., 1H,

N–C$H_{2(b)}$–CHOH, 2.17–2.28 (m, 4H, 2 x (CH_2)–N–CH_2–CHOH), 2.93 (br.s, 4H, 2 x Ph–N–(CH_2)), 3.02–3.16 (m, 1H, C*H*OH), 3.38–3.58 (m, 2H, CHOH–CH_2–N), 3.65 (m, 3H, CH_2–CH_3, CHO*H*), 6.90–7.00 (m, 2H, Ar–H_4, Ar–H_6 Ph-piper), 7.15–7.24 (m, 2H, Ar–H_3, Ar–H_5 Ph-piper), 7.24–7.37 (m, 4H, 2 x Ar–H_2, Ar–H_6 DPH), 7.37–7.47 (m, 6H, 2 x Ar–H_3, Ar–H_4, Ar–H_5 DPH).

8a was obtained by the same method as **7a**; m.p. 255–258 ºC; IR: 3413, 3243 (OH), 2958, 2919, 2839 (CH), 2552 (NH^+), 1769 (C_2=O), 1713 (C_4=O), 1589 (Ar); ^{1}H-NMR of hydrochloride ($CDCl_3$, TMS) δ (ppm): 1.25 (t, *J* = 7.14, 3H, CH_2–CH_3), 2.54–2.96 (m, 3H, N–CH_2–CHOH, C*H*OH), 2.96–3.16 (m, 2H, CHOH–CH_2–N), 3.18–3.38 (m, 2H, 2 x Ph–N–(C$H_{2(a)}$)–), 3.40–3.60 (t def, 4H, 2 x Ph–N–(C$H_{2(b)}$), 2 x C$H_{2(a)}$–NH^+–CH_2–CHOH), 3.60–3.78 (m, 4H, 2 x C$H_{2(b)}$–NH^+–CH_2–CHOH, CH_2–CH_3), 5.27 (br s, 1H, OH), 6.95–7.04 (t, 2H, *J* = 7.42, Ar–H_4, Ar–H_6 Ph-piper), 7.20–7.28 (m, 1H, Ar–H_5 Ph-piper), 7.36 (d, *J* = 7.97, 1H, Ar–H_3 Ph-piper), 7.10–7.50 (m, 10H, Ar–H DPH), 11.78 (br s, 1H, NH^+).

*6.1.3.3. 1-[3-(4-(3-Chlorophenyl)-piperazin-1-yl)-2-hydroxypropyl]-3-ethyl-2,4-dioxo-5,5-diphenylimidazolidine hydrochloride **(9a)**. Method A*: The reaction followed the route used to obtain **8**, with the use of **4** (1.55 g, 4.6 mmol) and equimolar quantity of N-3-chlorophenylpiperazine (0.90 g). After a part of the solvent was evaporated, the reaction mixture was separated by column chromatography ($CHCl_3$/AcOEt 1:1). Fractions containing product were pooled and condensed to obtain the oily residue. Its recrystallization from 99.8% EtOH furnished 0.80 g of **9**; yield 33%.

*Method B**: A mixture of **4** (2.05 g, 6.2 mmol) and N-3-chlorophenylpiperazine (1.20 g, 6.2 mmol) was prepared in a flat-bottomed flask covered with a small inverted funnel. The whole was irradiated (450 W) to melt into a homogenous liquid and then irradiation was continued for 2 minutes (300 W). The reaction progress was controlled with TLC. The glasslike mass was crystallized from EtOH to obtain 2.14 g of **9**; yield 65%; m.p. 139–141 ºC; TLC: R_f(A) = 0.78; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 1.27 (t, *J* = 6.87, 3H, CH_2–CH_3), 2.03 (dd, J_1 = 12.36, J_2 = 4.94, 2H, N–C$H_{2(a)}$–CHOH), 2.16–2.44 (m, 5H, N–C$H_{2(b)}$–CHOH, 2 x (CH_2)–N–CH_2–CHOH), 3.06 (t, *J* = 4,94, 4H, 2 x Ph–N–(CH_2)–), 3.01–3.16 (m, 1H, C*H*OH), 3.40–3.58 (m, 2H, CHOH–CH_2–N), 3.58–3.75 (m, 3H, CH_2–CH_3, CHO*H*), 6.68–6.83 (m, 3H, Ar–H_2, Ar–H_4, Ar–H_6 Ph-piper), 7.14 (t, *J* = 7.97, 1H, Ar–H_5 Ph-piper), 7.22–7.34 (m, 4H, 2 x Ar–H_2, 2 x Ar–H_6 DPH), 7.36–7.45 (m, 6H, 2 x Ar–H_3, 2 x Ar–H_4, 2 x Ar–H_5 DPH).

9a was obtained by the same method as **7a**; m.p. 230–232 ºC; IR: 3323, 3244 (OH), 2918 (CH), 2549, 2457 (NH^+), 1770 (C_2=O), 1711 (C_4=O), 1595 (Ar).

*6.1.3.4. 1-[3-(4-(4-Chlorophenyl)-piperazin-1-yl)-2-hydroxypropyl]-3-ethyl-2,4-dioxo-5,5-diphenylimidazolidine hydrochloride **(10a)**. Method A*: Synthesis was analogous to that of **9**, but with the use of **4** (1.55 g, 4.6 mmol) and N-4-chlorophenylpiperazine (0.90 g, 4.6 mmol) as substrates. Column chromatography ($CHCl_3$:AcOEt 1:1) and crystallization form EtOH afforded 0.60 g of **10**; yield 24%.

*Method B**: Compound **4** (2.67 g, 7.9 mmol) and N-4-chlorophenylpiperazine (1.56 g, 7.9 mmol) were placed in a flat-bottomed flask covered with an inverted funnel and melted by irradiating (450 W) twice for 1 minute. The whole was then irradiated for the next 50 s (300 W). A product was precipitated by 96% EtOH from the glassy residue and recrystallized from the same solvent to give 2.17 g of **10**; m.p. 139–141 ºC; yield 52%; TLC R_f(A) = 0.70; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 1,26 (t, *J* = 7.14, 3H, CH_2–CH_3), 2.03 (dd, J_1 = 12.36, J_2 = 4.94, 1H, N–C$H_{2(a)}$–CHOH), 2.22 (dd, J_1 = 8.79, J_2 = 1.64, 1H, N–C$H_{2(b)}$–CHOH), 2.24–2.46 (m, 4H, 2 x (CH_2)–N–CH_2–CHOH), 3.03 (t def., 4H, 2 x Ph–N–(CH_2)–), 3.03–3.16 (m, 1H, C*H*OH), 3.38–3.60 (m, 2H, CHOH–CH_2–N), 3.62 (m, 1H, CHO*H*), 3.67 (q, *J* = 7.14, 2H, CH_2–CH_3), 6.76–6.80 (m, 2H, Ar–H_2, Ar–H_6 Ph-piper), 7.14–7.22 (m, 2H, Ar–H_3, Ar–H_5 Ph-piper), 7.24–7.34 (m, 4H, 2 x Ar–H_2, 2 x Ar–H_6 DPH), 7.39–7.42 (m, 6H, 2 x Ar–H_3, 2 x Ar–H_4, 2 x Ar–H_5 DPH).

10a was obtained by the same method as **7a**; m.p. 260–262 ºC; IR: 3561, 3388, 3245 (OH), 2937, 2829 (CH), 2576, 2461 (NH^+), 1772 (C_2=O), 1712 (C_4=O), 1599 (Ar).

*6.1.3.5. 3-Ethyl-1-[2-hydroxy-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-propyl]-2,4-dioxo-5,5-diphenylimidazolidine hydrochloride **(11a)**. Method A*: A mixture of **4** (1.68 g, 5 mmol) and N-2-methoxyphenylpiperazine (0.96 g, 5 mmol) in toluene (15 ml) was stirred at reflux for 6.5 h. The solvent was evaporated. Washing with diethyl ether and recrystallization from ethanol did not afford crystals. Concentrated hydrochlorid acid was added to the oily residue and the mixture was evaporated to dryness. The residue was recrystallized from EtOH to afford hydrochloride **11a**; m.p. 212–216 ºC; yield 38.2 %; TLC: R_f(A) = 0.67; IR: 3527, 3394, 3275 (OH), 2962 (CH), 2832, 2662, 2583, 2457 (NH^+), 1769 (C_2=O), 1713 (C_4=O), 1594 (Ar); ^{1}H-NMR of hydrochloride: ($CDCl_3$, TMS) δ (ppm): 1.21–1.28 (m, 5H, CH_2–CH_3 and 1/2 CH_3–CH_2–OH), 2.52–2.86 (m, 3H, NH^+–CH_2–CHOH, C*H*OH), 2.98–3.12 (d, *J* = 10.71, 2H, CHOH–CH_2–N), 3.25–3.58 (m, 6H, H piper), 3.59–3.76 (m, 5H, H piper, CH_2–CH_3, 1/2 CH_3–CH_2–OH), 3.87 (s, 3H, OCH_3), 5.3 (s, 1H, O*H*), 6.84–6.96 (m, 3H, Ar–H_3, Ar–H_5, Ar–H_6 Ph-piper), 7.02–7.11 (m, 1H, Ar–H_4 Ph-piper), 7.18–7.32 (m, 4H, 2 x Ar–H_2, 2 x Ar–H_6 DPH), 7.38–7.50 (m, 6H, 2 x Ar–H_3, 2 x Ar–H_4, 2 x Ar–H_5 DPH), 11.61 (br s, 1H, NH^+).

*6.1.3.6. {3-[2-Hydroxy-3-(4-phenylpiperazin-1-yl)-propyl]-2,5-dioxo-4,4-diphenylimidazolidin-1-yl}-acetic acid ethyl ester hydrochloride**(12a)**. Method A*: A mixture of **5** (1.97 g, 5 mmol) and N-phenylpiperazine (0.81 g, 5 mmol) in toluene (15 ml) was refluxed for 9.5 h. After solvent evaporation, double recrystallization from ethanol afforded 2.13 g of analytically pure **12**; m.p. 95–99 ºC; yield 64%; TLC R_f(A) = 0.73; R_f(B) = 0.27; ^{1}H-NMR ($CDCl_3$, TMS) δ

(ppm): 1.22 (t, J = 7.14, 3H, CH_2–CH_3), 1.29 (t, J = 7.14, 3H, CH_3–CH_2–OH), 2.03–2.14 (dd def, 1H, N–C$H_{2(a)}$–CHOH), 2.19–2.26 (m, 1H, N–C$H_{2(b)}$–CHOH), 2.27–2.50 (m, 4H, 2 x (CH_2)–N–CH_2–CHOH), 3.05 (t, J = 4.94, 4H, 2 x Ph–N–(CH_2)–), 3.00–3.10 (m, 1H, CHOH), 3.52 (d, J = 6.32, 2H, CHOH–CH_2–N), 3.69 (q def., 2H, CH_3–CH_2–OH), 4.24 (q, J = 7.14, 2H, CH_2–CH_3), 4.32 (s, 2H, N–CH_2–COO), 6.80–6.90 (m, 3H, Ar–H_2, Ar–H_4, Ar–H_6 Ph-piper), 7.20–7.28 (m, 2H, Ar–H_3, Ar–H_5 Ph-piper), 7.32–7.50 (m, 10H, Ar–H DPH). Ascription of ^{1}H-NMR signals to particular groups of protons was facilitated by H,H-COSY spectrum.

Dry ethanol (20 ml) was added to a 1 g of **12**. The suspension was saturated with dried gaseous hydrogen chloride until acidic pH was obtained and, then, the solid was separated by filtration to quantitatively obtain hydrochloride **12a**; m.p. 236–238 ºC; yield 64%; IR: 3256 (OH), 2979 (CH), 2500, 2441 (NH^+), 1776 (C_2=O), 1748 (C=O ester), 1725 (C_4=O), 1597 (Ar).

*6.1.3.7. {3-[3-(4-(2-Chlorophenyl)-piperazin-1-yl)-2-hydroxypropyl]-2,5-dioxo-4,4-diphenylimidazolidin-1-yl}-acetic acid ethyl ester hydrochloride **(13a)**. Method B*: In a flat-bottomed flask **5** (3.28 g, 8.32 mmol) and N-2-chlorophenylpiperazine (1.64 g, 8.32 mmol) were mixed and melted on a heating jacket. The flask was then covered with an inverted funnel and irradiated in a domestic microwave oven (450 W) for consecutive 5 and 4 minutes; additionally, a beaker with water was present in the oven to avoid overheating of the system. The crude product was isolated by column chromatography ($CHCl_3$/AcOEt 1:1) and recrystallized from EtOH to furnish 1.68 g of **13**; m.p. 106–109 ºC; yield 34%; TLC: R_f(A) = 0.80; R_f(B) = 0.21; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 1.30 (t, J = 7.14, 3H, CH_2–CH_3), 2.11 (dd def., 1H, N–C$H_{2(a)}$–CHOH), 2.19–2.26 (m, 1H, N–C$H_{2(b)}$–CHOH), 2.29–2.52 (m, 4H, 2 x (CH_2)–N–CH_2–CHOH), 2.93 (br s, 4H, 2 x Ph–N–(CH_2)–), 3.00–3.12 (m, 1H, CHOH), 3.42–3.60 (m, 2H, CHOH–CH_2–N), 4.26 (q, J = 7.14, 2H, CH_2–CH_3), 4.32 (s, 2H, N–CH_2–COO), 6.92–6.99 (m, 2H, Ar–H_4, Ar–H_6 Ph-piper), 7.16–7.23 (m, 1H, Ar–H_5 Ph-piper), 7.31–7.36 (dd, J_1 = 7.79, J_2 = 1.65, 1H, Ar-H_3 Ph-piper), 7.37–7.45 (m, 10H, Ar–H DPH).

13a was obtained by the same method as **12a**; m.p. 198–210 ºC; IR: 3587, 3437, 3218 (OH), 2986, 2961, 2841 (CH), 2522, 2443 (NH^+), 1776 (C_2=O), 1748 (C=O ester), 1719 (C_4=O), 1589 (Ar).

*6.1.3.8. {3-[3-(4-(3-Chlorophenyl)-piperazin-1-yl)-2-hydroxypropyl]-2,5-dioxo-4,4-diphenylimidazolidin-1-yl}-acetic acid ethyl ester hydrochloride **(14a)**. Method B*: Compound **5** (1.62 g, 4.1 mmol) and N-3-chlorophenylpiperazine (0.81 g, 4.1 mmol) were melted on a heating jacket to obtain oily mass and, then, they were irradiated twice for 5 minutes in a domestic microwave oven (450 W, under cover of an inverted funnel). The oily reaction mixture was crystallized from EtOH to obtain 1.50 g of **14**; m.p. 80–83 ºC; yield 62%; TLC: R_f(A) = 0.84; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 1.20–1.33 (m, 4H, CH_2–CH_3 + CH_3–CH_2–OH), 2.08 (dd def., 1H, N–C$H_{2(a)}$–CHOH), 2.16–2.24 (m, 1H, N–C$H_{2(b)}$–CHOH), 2.24–2.45 (m, 4H, 2 x (CH_2)–N–CH_2–CHOH), 3.03–3.06 (m, 5H, 2 x Ph–N–(CH_2), CHOH), 3.50 (d, J = 6.32, 1H, CHOH–CH_2–N), 3.72 (q, J = 7.14, 1H, CH_3–CH_2–OH), 4.25 (q, J = 7.14, 2H, CH_2–CH_3), 4.32 (s, 2H, N–CH_2–COO), 6.68–6.83 (m, 3H, Ar–H_2, Ar–H_4, Ar–H_6 Ph-piper), 7.14 (t, J = 7.97, 1H, Ar–H_5 Ph-piper), 7.38–7.45 (m, 10H, Ar–H DPH).

14a was obtained by the same method as **12a**; m.p. 235–238 ºC; IR: 3420, 3254 (OH), 2979, 2927, 2851 (CH), 2544, 2445, 2364 (NH^+), 1775 (C_2=O), 1745 (C=O ester), 1719 (C_4=O), 1596 (Ar).

*6.1.3.9. {3-[3-(4-(4-Chlorophenyl)-piperazin-1-yl)-2-hydroxypropyl]-2,5-dioxo-4,4-diphenylimidazolidin-1-yl}-acetic acid ethyl ester hydrochloride **(15a)**. Method B*: The synthesis followed the method used to obtain **14**. As substrates **5** (1.65 g, 4.2 mmol) and N-4-chlorophenylpiperazine (0.82 g, 4.2 mmol) were used to furnish 1.35 g of **15**; m.p. 115–118 ºC; yield 54%; TLC: R_f(A) = 0.77; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 1.29 (t, J = 7.14, 3H, CH_2–CH_3), 2.08 (dd def., 2H, N–C$H_{2(a)}$–CHOH), 2.21 (dd def., 2H, N–C$H_{2(b)}$–CHOH), 2.27–2.45 (m, 4H, 2 x (CH_2)–N–CH_2–CHOH), 2.97–3.02 (m, 4H, 2 x Ph–N–(CH_2)–), 2.96–3.12 (m, 1H, CHOH), 3.51 (d, J = 6.32, 2H, CHOH–CH_2–N), 4.25 (q, J = 7.14, 2H, CH_2–CH_3), 4.32 (s, 2H, N–CH_2–COO), 6.78 (d, J = 9.06, 2H, Ar–H_2, Ar–H_6 Ph-piper), 7.18 (d, J = 8.79, 2H, Ar–H_3, Ar–H_5 Ph-piper), 7.36–7.44 (m, 10H, 2 x Ar–H DPH).

15a was obtained by the same method as **12a**; m.p. 223–225 ºC; IR: 3431, 3210 (OH), 2985, 2937, 2843 (CH), 2522, 2442 (NH^+), 1775 (C_2=O), 1740 (C=O ester), 1718 (C_4=O), 1598 (Ar).

*6.1.3.10. {3-[2-Hydroxy-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-propyl]-2,5-dioxo-4,4-diphenylimidazolidin-1-yl}-acetic acid ethyl ester hydrochloride **(16a)**. Method A*: A mixture of **5** (1.97 g, 5 mmol) and 2-methoxyphenylpiperazine (0.96 g, 5 mmol) in toluene (15 ml) was refluxed for 10 hours altogether. The solvent was evaporated from the reaction mixture and the residue was crystallized twice from EtOH to give 0.71 g of analytically pure **16**; yield 55%.

*Method B**: The synthesis followed, in general, the method used to obtain **10**. The substrates **5** (3.94 g, 10 mmol) and N-2-methoxyphenylpiperazine (1.92 g, 10 mmol) were melted by three-minute irradiation (450 W) and were further irradiated (300 W) for 1 minute. Crystallization from EtOH furnished 4.45 g of **16**; m.p. 137–138 ºC; yield 76%, TLC: R_f(A) = 0.62; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 1.29 (t, J = 7.14, 3H, CH_2–CH_3), 2.09 (dd def., 2H, N–C$H_{2(a)}$–CHOH), 2.17–2.24 (m, 2H, N–C$H_{2(b)}$–CHOH), 2.30–2.55 (m, 4H, 2 x (CH_2)–N–CH_2–CHOH), 2.94 (br s, 4H, 2 x Ph–N–(CH_2)–), 3.02–3.12 (m, 1H, CHOH), 3.51 (t def., 2H, CHOH–CH_2–N), 3.61 (br s, 1H, CHOH), 3.85 (s, 3H, OCH_3), 4.25 (q, J = 7.14, 2H, CH_2–CH_3), 4.32 (s, 2H,

N–CH_2–COO), 6.82–6.93 (m, 3H, Ar–H_3, Ar–H_5, Ar–H_6 Ph-piper), 6.95–7.02 (m, 1H, Ar–H_4 Ph-piper), 7.39–7.45 (m, 10H, 2 x Ar–H DPH).

16a was obtained by the same method as **12a**; m.p. 240–242 ºC, IR: 3195 (OH), 2957, 2829 (CH), 2451 (NH^+), 1775 (C_2=O), 1751 (C=O ester), 1721 (C_4=O), 1593 (Ar).

*6.1.3.11. 2-{3-[2-Hydroxy-3-(4-phenylpiperazin-1-yl)-propyl]-2,5-dioxo-4,4-diphenylimidazolidin-1-yl}-propionic acid ethyl ester hydrochloride **(17a)**. Method A*: A suspension of **6** (3.40 g, 8.3 mmol) and N-phenylpiperazine (1.21 g, 7.5 mmol) in toluene (16.5 ml) was heated at reflux for 8 h. After the solvent evaporation, the residue was purified by column chromatography ($CHCl_3$/AcOEt 1:1) to obtain 1.94 g of **17**; m.p. 99–102 ºC; yield 45%; TLC: R_f(A) = 0.77; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 1.23 (t, *J* = 7.14, 3H, CH_2–CH_3), 1.65 (d, *J* = 7.42, 3H, CH–CH_3), 2.02–2.12 (m, 2H, N–C$H_{2(a)}$–CHOH), 2.16–2.25 (m, 2H, N–C$H_{2(b)}$–CHOH), 2.25–2.41 (m, 4H, 2 x (CH_2)–N–CH_2–CHOH), 3.01–3.07 (m, 5H, 2 x Ph–N–(CH_2), C*H*OH), 3.46–3.60 (m, 2H, CHOH–CH_2–N), 3.58 (s, 1H, CH_2–CHO*H*–CH_2), 4.14–4.28 (m, 2H, CH_2–CH_3), 4.85 (q, *J* = 7.42, 1H, C*H*–CH_3), 6.80–6.90 (m, 3H, Ar–H_2, Ar–H_4, Ar–H_6 Ph-piper), 7.21–7.28 (m, 2H, Ar–H_3, Ar–H_5 Ph-piper), 7.30–7.46 (m, 10H, Ar–H DPH).

17a was obtained by the same method as **12a**; m.p. 187–192 ºC; IR: 3255 (OH), 2993, 2942 (CH), 2428 (NH^+), 1771 (C_2=O), 1744 (C=O ester), 1713 (C_4=O), 1597 (Ar).

*6.1.3.12. 2-{3-[3-(4-(2-Chlorophenyl)-piperazin-1-yl)-2-hydroxypropyl]-2,5-dioxo-4,4-diphenylimidazolidin-1-yl}-propionic acid ethyl ester hydrochloride **(18a)**. Method A*: A suspension of **6** (2.83 g, 6.93 mmol) and N-2-chlorophenylpiperazine (1.21 g, 6.15 mmol) in toluene (15 ml) was refluxed for 15.5 h. After evaporation of the solvent, the reaction mixture was purified by column chromatography ($CHCl_3$/AcOEt 1:1) to give 1.48 g of **18** in the form of oil. A trial of recrystallization form EtOH did not afford crystals. To the oil dry ethanol (25 ml) was added and the mixture was saturated with dried gaseous hydrogen chloride until precipitation in acidic pH, then the solid was removed by filtration to obtain hydrochloride **18a**; m.p. 215–218 ºC; yield 32%; TLC: R_f(A) = 0.74; IR: 3231 (OH), 2924 (CH), 2442 (NH^+), 1772 (C_2=O), 1717 (C_4=O), 1588 (Ar). ^{1}H-NMR of hydrochloride ($CDCl_3$, TMS) δ (ppm): 1.26–1.32 (m, 3H, CH_2–CH_3), 1.64–1.80 (m, 6H CH–CH_3), 2.58–2.90 (m, 3H, NH^+–CH_2–CHOH, C*H*OH), 2.90–3.20 (m, 2H, CHOH–CH_2–N), 3.20–3.81 (m, 8H, H-piper), 4.20–4.29 (m, 2H, CH_2–CH_3), 4.80–4.91 (m, 1H, C*H*–CH_3), 5.32 (d, *J* = 4.12, 1H, OH), 7.01–7.08 (m, 2H, Ar–H_4, Ar–H_6 Ph-piper), 7.20–7.50 (m, 12H, Ar–H DPH, Ar–H_3, Ar–H_5 Ph-piper), 11.57 (br s, 1H, NH^+).

*6.1.3.13. 2-{3-[2-Hydroxy-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-propyl]-2,5-dioxo-4,4-diphenylimidazolidin-1-yl}-propionic acid ethyl ester hydrochloride **(19a)**. Method B*: The reaction followed the method used to obtain **14** with substrates **6** (2.05 g, 5 mmol) and N-2-methoxyphenylpiperazine (0.96 g, 5 mmol). The reaction mixture was purified by column chromatography ($CHCl_3$/AcOEt 1:1) to give **19** as oil, to which dry ethanol (25 ml) was added. The mixture was saturated with dried gaseous hydrogen chloride until precipitation in acidic pH took place and, then, the solid was separated by filtration to obtain hydrochloride **19a**; m.p. 192–196 ºC; yield 24%; TLC: R_f(A) = 0.75; IR: 3611, 3434, 3263 (OH), 2948, 2835 (CH), 2662, 2591, 2450 (NH^+), 1773 (C_2=O), 1740 (C=O ester), 1720 (C_4=O), 1593 (Ar); ^{1}H-NMR of hydrochloride ($CDCl_3$, TMS) δ (ppm): 1.27 (q, *J* = 7.15, 3H, CH_2–CH_3), 1.62–1.70 (m, 3H, CH–CH_3), 2.55–2.90 (m, 3H, NH^+–CH_2–CHOH, C*H*OH), 2.95–3.15 (m, 2H, CHOH–CH_2–N), 3.27–3.79 (m, 8H, H-piper), 3.87 (s, 3H, OCH_3), 4.20–4.28 (m, 2H, CH_2–CH_3), 4.79–4.89 (m, 1H, C*H*–CH_3), 5.37 (br s, 1H, OH), 6.85–6.96 (m, 3H, Ar–H_3, Ar–H_5, Ar–H_6 Ph-piper), 7.01–7.11 (m, 1H, Ar–H_4 Ph-piper), 7.25–7.37 (m, 4H, 2 x Ar–H_2, 2 x Ar–H_6 DPH), 7.38–7.51 (m, 6H, 2 x Ar–H_3, 2 x Ar–H_4, 2 x Ar–H_5 DPH), 11.49 (br s, 1H, NH^+).

6.2. Pharmacology

6.2.1. Chemicals

Adrenaline (Adrenalinum hydrochloride, Polfa), barium chloride (Barium chloratum, POCh), sodium chloride (Natrium chloratum, POCh), heparin (Heparinum natrium, Polfa), methylcellulose (Methylcellulose, Fluka), thiopental (Thiopentone, Biochemie GmbH).

6.2.2. Animals

Experiments were carried out on male Wistar rats (130–380 g). Animals were housed in standard cages, under a 12 h light-dark cycle at the temperature of 20–24 ºC and relative humidity of 60%. The animals had free access to standard pellet food and water.

6.2.3. Statistical analysis

The data are expressed as means ± SEM. The statistical significance was calculated using a one-way ANOVA test. Differences were considered significant when $p < 0.05$.

6.2.4. Influence on normal electrocardiogram in vivo

Physiological ECG recordings were taken from rats anaesthetised with thiopental (60 mg/kg i.p.) and, afterwards, an examined compound was injected. Compounds **8a–10a, 12a** and **16a–18a** were suspended in 0.5% methylcellulose and administered intraperitoneally at the dose of 50 mg/kg. ECG parameters were collected after 15, 30 and 60 minutes of administration. Compounds **11a** and **19a** were dissolved in physiological saline and injected intravenously at the dose of 10 mg/kg, the ECG recordings were taken after 1, 5 and 10 minutes after administration of **11a**.

6.2.5. Antiarrhythmic activity

6.2.5.1. Barium chloride – induced arrhythmia[23]. The arrhythmia was evoked in rats anaesthetised with thiopental (60 mg/kg i.p.) by i.v. injection of barium chloride (32 mg/kg, in a volume of 1 ml/kg). Compounds **8a–10a, 12a** and **16a–18a** were suspended in 0.5% methylcellulose and administered i.p. at the constant dose of 50 mg/kg, 60 minutes prior to arrhythmogen administration. Compounds **11a** and **19a** were injected i.v. as saline physiological solution at the dose of 10 mg/kg, 15 minutes prior to arrhythmogen.

Overdose of $BaCl_2$ induced progressively increasing disturbances of cardiac rhythm, associated with premature ventricular beats, ventricular tachycardia and ventricular fibrillation, leading to death within 2-5 minutes (Fig 5).

Attenuation or lack of disturbances and the decreased mortality after $BaCl_2$ administration were accepted as a criterion of the antiarrhythmic effect of the compound.

6.2.5.2. Adrenaline – induced arrhythmia. Cardiac arrhythmia was induced according to Szekeres [23] by i.v. administration of adrenaline (20μg/kg in a volume of 1 ml/kg) to anaesthetised rats (thiopenthal 60 mg/kg i.p.) The routes of administration and doses of the analyzed compounds were identical to those in case of barium chloride – induced arrhythmia experiment.

Rapid intravenous injection of adrenaline induced reflex bradycardia, blocks, premature ventricular beats (VBs), ventricular tachycardia with ventricular and supraventricular ectopic (4 or more successive VBs) for 100% of the animals, and frequently, even ventricular fibrillation and death for 50 % of the animals of the control group (Fig 6). Arrhythmias were analysed according to the Lambeth Convention [24].

The lack of extrasystoles and of heart rhythm disturbances, in comparison with the control group, were accepted as criteria of the antiarrhythmic activity.

6.2.6. Influence on blood pressure

Influence on blood pressure was analysed in normotensive rats under thiopental anaesthesia (60 mg/kg i.p.). The right carotid artery was cannulated with polyethylene tub filled with heparin in saline to facilitate pressure measurements using a Datamax apparatus (Columbus Instruments). Both systolic and diastolic pressure values were measured before and after administration of compounds. Compounds **8a–10a, 12a** and **16a–18a** were suspended in 0.5% methylcellulose and administered intraperitoneally (50 mg/kg) and, thereafter, blood pressure measurement was continued for 90 minutes. Compounds **11a** and **19a** were dissolved in physiological saline and injected intravenously at the dose of 2.5 mg/kg **(11a, 19a)** and 10 mg/kg **(19a)**. Administration of **11a** and **19a** was followed by 60 minutes blood pressure measurement.

6.2.7. Anticonvulsant activity

Anticonvulsant activity of **9a, 10a** and **16a** was estimated in vivo in a maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (scMet) tests in mice within the framework of the Anticonvulsant Screening Project at the National Institute of Health, Bethesda, MD, USA. The test, performed in male Carworth Farm No. 1 (CF 1) mice, used small groups of animals (1–8). Compounds were suspended in 30% polyethylene glycol 400 for intraperitoneal administration and tested at 30 minutes and 4 hours following doses 30, 100 and 300 mg/kg.

In the MES test, an electrical stimulus of 0.2 s in duration (50 mA) delivered via coroneal electrodes primed with an electrolite solution containing an anaesthetic agent.

The ScMet utilised a dose of penthylenetetrazol (85 mg/kg) administrated subcutaneously at the anticipated time of testing.

In the minimal neurotoxicity test, toxicity induced by the compounds was detected using the standardised rotorod test. Untreated control mice, when placed on a 6 r.p.m. rotation rod could maintain their equilibrium for a prolonged period of time. Neurological impairment can be demonstrated by the inability of a mouse to maintain equilibrium for one minute in each of three succesive trials [25,26].

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