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Orginal article

Synthesis, structure and antiarrhythmic properties evaluation of new basic derivatives of 5,5-diphenylhydantoin

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Abstract

The synthesis of 1-*N* and 3-*N* aminoalkyl derivatives of 5,5-diphenylhydantoin is described. Structural elucidation based on X-ray analysis was performed for two representative compounds **4a** and **9b**. The effect of tested compounds on the electrocardiogram was examined in vitro in the non-working heart perfusion test and antiarrhythmic activity in the rat coronary artery ligation–reperfusion model. The most active 1-*N* derivatives **4a** and **7b** have shown properties of the compounds belonging to class Ia, according to the Vaughan Williams classification. The spatial organisation of the chosen compounds necessary for their pharmacological activity was discussed.

Keywords: Antiarrhythmic activity; Diphenylhydantoin derivatives; X-ray structure determination; anticonvulsant properties

1. Introduction

In 1938 Merrit and Putnam have found that 5,5-diphenylhydantoin (phenytoin) shows anticonvulsant properties. Twelve years later it was stated that apart from antiepileptic activity phenytoin possesses antiarrhythmic activity. It belongs to class Ib according to Vaughan Williams classification of antiarrhythmic agents [1] modulating voltage-gated sodium channels conductance [2]. Limited clinical effectiveness and undesired central side-effects of phenytoin, like nystagmus, ataxia, drowsiness, stupor, and coma [3,4] encouraged several authors to synthesise numerous hydantoin derivatives which proved to have antiarrhythmic properties [5–8]. In our research program, some basic derivatives of 5,5-diphenylhydantoin have been already investigated [9–12]. The aim of the present derivatives designing was to change the acidic properties of the starting phenytoin molecule to basic one and to obtain more hydrophilic compounds in order to enhance the antiarrhythmic activity and reduce unwanted central side-effects. Recently series of amide derivatives was examined in which 5,5-diphenyl-3-{2-oxo-2-[4-(2-hydroxyethyl)-1-piperazinyl]ethyl}hydantoin was the most active one [12]. In our current study we have designed the synthesis of aminoalkyl derivatives of phenytoin, which have a typical for β-blockers propanolo-2 element of pharmacophore, situated either at N1 or at N3 of hydantoin ring. Their structure and physical, chemical and pharmacological properties are discussed and compared.

2. Results

2.1. Chemistry

As starting material 5,5-diphenylhydantoin (**1**) Fig. 1 was used which upon alkylation with 2,3-epichlorhydrin to product **8** underwent nucleophillic attack with

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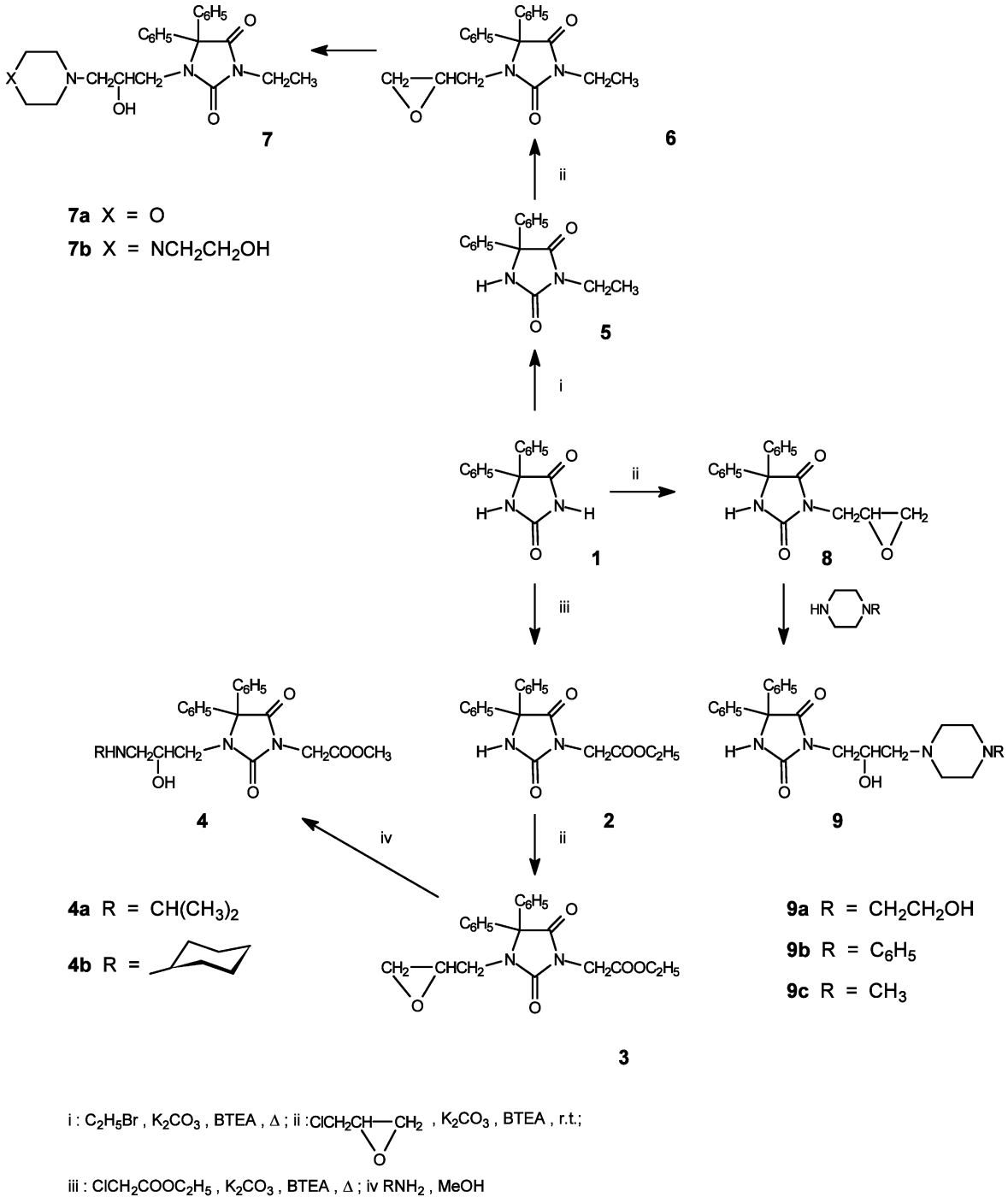

Fig. 1. Procedure for the preparation of basic 1-*N* (**4a**, **4b**, **7a**, **7b**) and 3-*N* (**9a**–**9c**) substituted 5,5-diphenylhydantoin derivatives.

piperazine derivatives yielding 3-*N*-substituted derivatives of **1** (**9a**–**9c**). Phenytoin alkylated, under phase transfer catalysis conditions (acetone, K_2CO_3, BTEA-catalyst, reflux), with ethyl bromide or methyl chloroacetate formed intermediates (**5** and **2**—selective 3-*N* alkylation, yields $\approx$85%) which reacted with 2,3-epichlorhydrin (acetone, K_2CO_3, BTEA-catalyst, room temperature) to **6** and **3**, respectively (1-*N* alkylation, yields 80–85%). The epoxide bonds were cleaved with secondary (**7a**, **7b**) or primary amines (**4a**, **4b**) giving 1-*N* aminoalkyl substituted derivatives. The obtained aminoalkyl derivatives were transformed into compounds better soluble in water. The homogenity of the compounds was checked by TLC, their structure was confirmed by elemental and spectral (^{1}H-NMR, IR) analyses.

2.2. Crystal structure determination

Molecular structures of **4a** and **9b** as found from X-ray analysis are shown in Fig. 2. The parameters of their molecular geometry (bond lengths, bond angles and torsion angles) are given in Table 1. The hydantoin moiety of the both compounds is compared with the structure of 5,5-diphenylhydantoin itself (**1**) [13]. The observed elongation of N(3)–C(4) bond length in **4a**, followed by the shortening of C(4)–O(4), is due to a stronger interaction of N(3) electron lone pair with the ester substituent than with the chain containing HO–C–C–N(amine) moiety in **9b**. In **4a** N(1)–C(2) is much shorter than in **9b** and **1**, because of the influence of N(1)-substituent. C(2)–O(2) of **4a** is elongated as well. The geometry of HO–C–C–N(amine) pharmacophore showing the synclinal conformation is similar in **4a** and **9b** (56.3 and 65.8°, respectively). A difference between geometry of these compounds, seen in the close vicinity

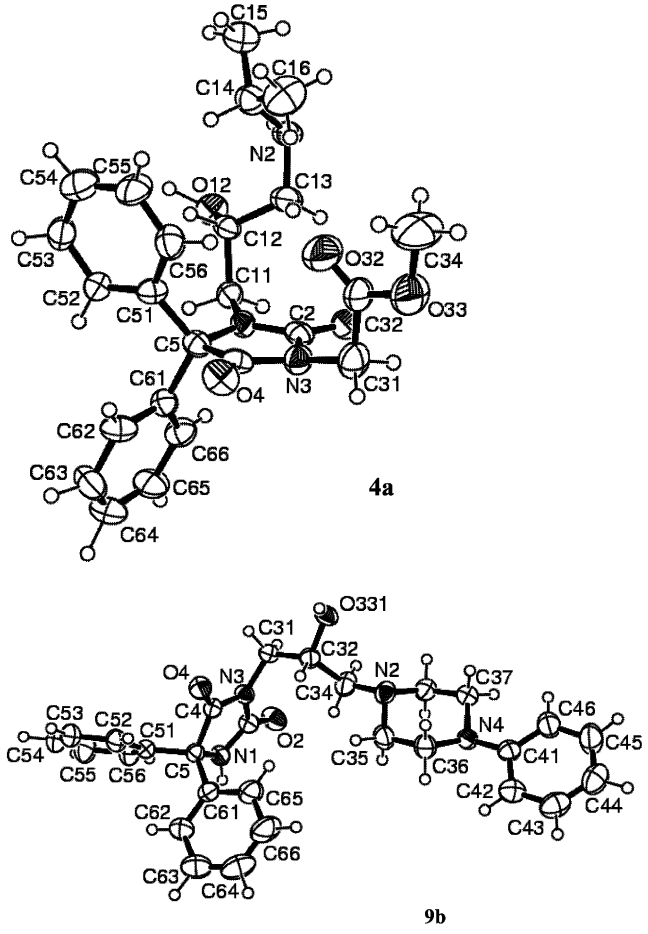

Fig. 2. Conformation of the molecules **4a** and **9b** in the crystalline state. The atom-numbering scheme is given. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as spheres of arbitrary radii. Here **4a** and **9b** are presented in the same S-configuration at C(12) and C(32), respectively. Notice that for **4a** the absolute structure could not be determined reliably and **9b** formed the racemate in the crystalline state (space group $P\bar{1}$).

Table 1
Selected bond lengths (Å) and angles (°) for **4a** and **9b**

Bond lengths			
	4a	**9b**	**1**
Hydantoin			
N(1)–C(2)	1.323(8)	1.346(2)	1.341(2)
C(2)–N(3)	1.408(8)	1.410(2)	1.391(2)
N(3)–C(4)	1.376(9)	1.366(1)	1.361(2)
C(4)–C(5)	1.531(9)	1.552(2)	1.536(2)
C(5)–N(1)	1.459(8)	1.460(1)	1.463(1)
C(2)–O(2)	1.227(8)	1.213(1)	1.220(1)
C(4)–O(4)	1.198(8)	1.211(1)	1.211(2)
C(5)–C(51)	1.526(9)	1.528(2)	1.535(2)
C(5)–C(61)	1.555(8)	1.534(2)	1.537(3)
N(1)-substituent			
N(1)–C(11)	1.485(8)		
C(11)–C(12)	1.545(9)		
C(12)–O(12)	1.399(8) [a]		
C(12)–C(13)	1.527(9) [a]		
C(13)–N(2)	1.488(8) [a]		
N(2)–C(14)	1.490(9)		
C(14)–C(15)	1.521(12)		
C(14)–C(16)	1.529(11)		
N(3)-substituent			
N(3)–C(31)	1.445(9)	1.459(2)	
C(31)–C(32)		1.530(2)	
C(32)–O(331) [b]		1.395(2) [a]	
C(32)–C(34)		1.519(2) [a]	
C(34)–N(2)		1.478(2) [a]	
N(2)–C(38)		1.462(2)	
N(2)–C(35)		1.466(2)	
C(35)–C(36)		1.517(2)	
C(36)–N(4)		1.474(2)	
C(37)–N(4)		1.469(2)	
C(37)–C(38)		1.514(2)	
N(4)–C(41)		1.428(2)	
Interbonding angles			
Hydantoin			
N(1)–C(2)–N(3)	108.1(6)	107.3(1)	107.4(1)
C(2)–N(3)–C(4)	110.5(5)	111.61(9)	111.8(1)
N(3)–C(4)–C(5)	106.5(5)	107.03(9)	107.3(1)
C(4)–C(5)–N(1)	100.8(5)	100.38(8)	100.1(1)
C(5)–N(1)–C(2)	113.0(5)	113.5(1)	113.2(2)
N(1)–C(2)–O(2)	128.9(6)	127.9(1)	128.4
N(3)–C(4)–O(4)	125.5(6)	125.4(1)	127.0
N(1)–C(5)–C(51)	110.7(5)	112.62(9)	112.4
N(1)–C(5)–C(61)	112.5(5)	109.36(9)	111.2
C(4)–C(5)–C(51)	112.1(5)	108.79(9)	113.4
C(4)–C(5)–C(61)	107.0(5)	110.91(9)	107.3
N(1)-substituent			
C(2)–N(1)–C(11)	122.1(6)		
C(5)–N(1)–C(11)	124.8(5)		
N(1)–C(11)–C(12)	114.7(5)		
C(11)–C(12)–O(12)	107.9(5)		
O(12)–C(12)–C(13)	109.0(5)		
C(12)–C(13)–N(2)	114.3(6)		
C(13)–N(2)–C(14)	115.3(6)		
N(2)–C(14)–C(15)	108.0(7)		
N(2)–C(14)–C(16)	109.8(7)		
N(3)-substituent			
C(2)–N(3)–C(31)	121.7(6)	124.7(1)	

Table 1 (*Continued*)

	4a	9b	1
Bond lengths			
C(4)–N(3)–C(31)	123.2(6)	123.7(1)	
N(3)–C(31)–C(32)		112.6(1)	
C(31)–C(32)–O(331) [b]		108.57(1)	
O(331)–C(32)–C(34) [b]		108.57(1)	
C(32)–C(34)–N(2)		112.08(1)	
C(34)–N(2)–C(35)		112.09(1)	
C(34)–N(2)–C(38)		110.35(1)	
Torsion angles			
Hydantoin			
N(1)–C(2)–N(3)–C(4)	−5.5(7)	0.9(1)	4.7
C(2)–N(3)–C(4)–C(5)	10.0(6)	1.8(1)	−2.1
N(3)–C(4)–C(5)–N(1)	−10.1(6)	−3.6(1)	−1.0
C(4)–C(5)–N(1)–C(2)	7.3(6)	4.5(1)	4.1
C(5)–N(1)–C(2)–N(3)	−1.8(7)	−3.6(1)	−5.5
C(5)–N(1)–C(2)–O(2)	177.5(6)	175.8(1)	175.1
C(4)–N(3)–C(2)–O(2)	175.2(6)	−178.5(1)	−175.9
C(2)–N(3)–C(4)–O(4)	−174.4(6)	1.6(2)	−178.7
N(1)–C(5)–C(4)–O(4)	174.4(6)	175.5(1)	175.6
N(1)-substituent			
O(2)–C(2)–N(1)–C(11)	−5.0(10)		
C(2)–N(1)–C(11)–C(12)	−87.3(8)		
N(1)–C(11)–C(12)–O(12)	−176.7(6)		
N(1)–C(11)–C(12)–C(13)	63.4(8)		
C(11)–C(12)–C(13)–N(2)	175.7(6)		
O(12)–C(12)–C(13)–N(2)	56.3(8) [a]		
C(12)–C(13)–N(2)–C(14)	67.8(9)		
C(13)–N(2)–C(14)–C(15)	−162.6(7)		
C(13)–N(2)–C(14)–C(16)	77.8(8)		
N(3)-substituent			
O(2)–C(2)–N(3)–C(31)	18.7(10)	2.6(2)	
C(2)–N(3)–C(31)–C(32)		−97.5(1)	
N(3)–C(31)–C(32)–O(331) [b]		−166.9(1)	
N(3)–C(31)–C(32)–C(34)		73.7(1)	
C(31)–C(32)–C(34)–N(2)		−174.9(1)	
O(331)–C(32)–C(34)–N(2) [b]		65.8(2) [a]	
C(32)–C(34)–N(2)–C(35)		96.6(1)	
C(32)–C(34)–N(2)–C(38)		−143.3(1)	

The geometry of hydantoin fragment is compared with that of **1** [13].
[a] Geometry of the HO–C–C–N(amine) pharmacophore indication.
[b] Geometry of a disordered part of **9b** molecule (keton: 13.6%):

C(32)–O(332): 1.269(10)
C(31)–C(32)–O(332): 121.0(5)
O(332)–C(32)–C(34): 121.0(5)
N(3)–C(31)–C(32)–O(332): −70.9(7)
O(332)–C(32)–C(34)–N(2): −32.1(7)

of N(2) atom, could be related to its secondary (**4a**) or tertiary (**9b**) character. The hydroxyl groups of both **4a** and **9b** are able to form strong intermolecular hydrogen bonds. The geometrical parameters of the hydrogen bonds observed in the crystalline state are given in Table 2.

Table 2
Hydrogen-bonds for **4a** and **9b** (Å, °)

D–H···A	d(D–H)	d(H···A)	d(D···A)	<(DHA)
4a				
O(12)–H(12O)···N(2) #1	1.11(8)	1.70(8)	2.798(7)	168(6)
N(2)–H(1N2)···O(12)	1.05(7)	2.51(6)	2.887(7)	100(4)
9b				
N(1)–H(1)···N(4) #2	0.92(2)	2.04(2)	2.950(1)	171(1)
O(331)–H(331)···N(2) #3	0.86(3)	2.05(3)	2.875(2)	160(3)

Symmetry transformations used to generate equivalent atoms: #1 $-x+1, y-1/2, -z+1$; #2 $-x+1, -y+1, -z+1$. #3 $-x, -y+2, -z+1$.

2.3. Pharmacology

2.3.1. Non-working heart perfusion

All the novel 5,5-diphenylhydantoin derivatives (**4a**, **4b**, **7a**, **7b**, **9a**, **9b**, **9c**) decreased the number of cardiac beats per minute (Fig. 3), prolonged $P-Q$ and $Q-T$ intervals, and QRS complex (Fig. 4). The strongest cardiodepressive activity was found for the compound **9a**, which in dose of 10^{-8} M significantly decreased the number of cardiac beats per minute (by 29%), prolonged intervals $P-Q$ (by 167%) and $Q-T$ (by 54%), and QRS complex (by 30%). It reduced the coronary flow (by 22%). Given in dose of 10^{-7} M, **9a** produced arrhythmia and cardiac arrest. The compound **9b**, used in concentration 10^{-9} to 10^{-6} M, decreased the number of cardiac beats per minute (by 7–38%), prolonged $P-Q$ (by 23–105%) and $Q-T$ (by 15–70%) intervals, and QRS complex (by 44%). Application of higher concentration was impossible due to low solubility of the compound. Compounds **4a** and **4b**, used in concentration 10^{-9} to 10^{-6} M, decreased the number of cardiac beats per minute (by 3–24%), prolonged $P-Q$ (by 12–155%) and $Q-T$ (by 11–42%) intervals, and QRS complex (by 1.5–41%). **4a** and **4b** reduced the coronary flow by (6–28%). Compounds **7a**, **7b** and **9c**, used in concentration 10^{-9} to 10^{-5} M, reduced heart rate (by 5–37%), prolonged $P-Q$ (by 16–166%) and $Q-T$ (by 17–73%) intervals, and QRS complex (by 1.5–87%). Compounds **7b** and **9c** diminished the coronary flow (by

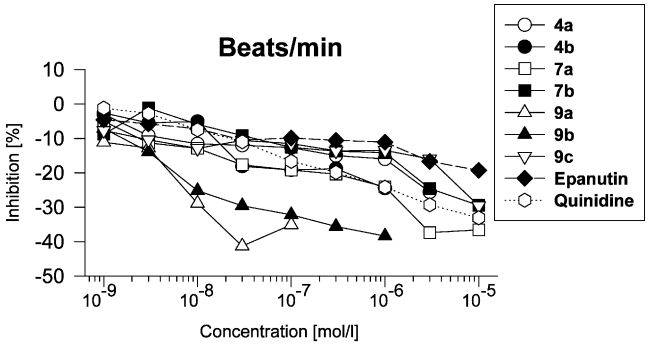

Fig. 3. Influence of tested compounds on heart rate.

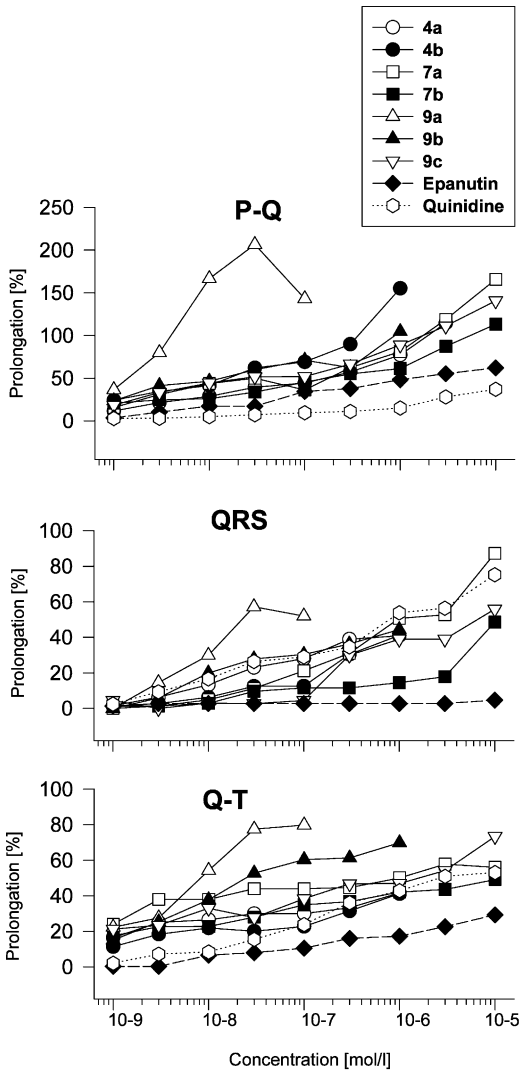

Fig. 4. Influence of tested compounds on ECG parameters.

8–26%). The electrocardiographic changes observed after administration of the tested compounds were identical to those observed for quinidine, whereas epanutin, which prolonged $P–Q$ and $Q–T$ interval, did not change the QRS complex (Fig. 4). The obtained results suggest that the new 5,5-diphenylhydantoin derivatives possess quinidine-like properties.

2.3.2. Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the non-working isolated perfused rat heart

During the 30 min period of coronary artery reperfusion, all hearts in the control group developed ventricular premature beats (VBs). The incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) was 62.5 and 37.5%, respectively (Table 3 and Fig. 5). Perfusion with compound **4a** and **7b**, in concentration of 10^{-8} M, did not reduce the incidence of VBs, VT and VF. At concentration of 10^{-7} M they also did not reduce significantly the incidence of VBs, but decreased the number of VT and VF. The incidence of VT was reduced from 62.5 in untreated hearts to 0 (**4a**) and 25% (**7b**), while the incidence of VF was reduced from 37.5 to 12.5 and 0%, respectively. The arrhythmias severity-index values are presented in Fig. 5 and Table 3. Application of higher concentration (10^{-6} M) led to the weakness of antiarrhythmic activity (Table 3). Remaining compounds, used in concentrations of 10^{-9} to 10^{-7} M, did not show antiarrhythmic activity in this test.

2.3.3. Anticonvulsant assays

Chosen compounds (**4a**, **4b**, **7b** and **9b**) were evaluated for anticonvulsant properties by the Antiepileptic Drug Development (ADD) Program. The tests of Phase I and the threshold tonic extension (TTE) were carried out. Phase I of the evaluation consisted of three tests: maximal electroshock (MES), subcutaneous pentylenetetrazol (ScMet) and the rotorod test for neurological toxicity (Tox).

3. Discussion

3.1. Physicochemical properties

The values of log P, log D and pK_a of basic and acidic groups of compounds **4a**, **4b**, **7a**, **7b**, **9a**–**9c**, phenytoin (**1**) and quinidine, **Qdn**, were calculated with PALLAS program (version 1.2) [15], using prediction modules: prolog P 5.1, prolog D 2.0 and pKalc 3.1. The obtained results are presented in Table 4. The most expressed hydrophilic properties possess compounds **9a**, **7b** and **9c** (negative values of log P) with alkyl piperazine substituents. Cycloalkyl (**4b**), phenyl (**9b**) and alkyl amine (**4a**) derivatives have more lipophilic properties possessing positive log P values similar to that of phenytoin (**1**) and quinidine. The obtained compounds have basic character which is stronger for secondary than for tertiary amines (pK_a values). According to the basic character the compounds are protonated under physiological conditions. To assess the degree of protonation their distribution coefficients (log D) for typical physiological pH values (pH 7.0 and pH 7.4) were calculated. These coefficients allow to predict the distribution of molecules between organic and water phase, considering the degree of ionisation of the investigated molecules. The highest negative distribution factors were observed for **4a**, **9a** and **9c**. The degree of protonation of secondary amine derivatives **4a**, **4b** was higher than that of tertiary amines e.g. **7a**, **7b** (indicated by the difference between log P and log D at pH 7.0 or pH 7.4). Compound **9b** with phenylpiperazine substituent remained the most lipophilic one in physiological conditions. As it could be expected log P and log D values for acidic phenytoin were almost equal, while

Table 3
Effect of tested compounds on reperfusion-induced arrhythmia

Compound	Concentration (M)	VBs incidence (%)	VT incidence (%)	VF incidence (%)	Arrhythmia severity index [13]
Control		100	62.5	37.5	5.25±1.09
4a	10^{-8}	100	50	12.5	3.62±0.48
	10^{-7}	100	0	12.5	1.75±0.36****
4b	10^{-8}	100	87.5	12.5	4.87±0.69
7a	10^{-8}	100	100	0	5.0±0
	10^{-7}	100	62.5	25	4.37±0.37
7b	10^{-8}	100	75	25	4.5±0.28
	10^{-7}	100	25.0	0	2.12±0.44***
	10^{-6}	100	28.6	0	2.85±0.55*
9c	10^{-8}	100	100	0	5.0±0
	10^{-7}	100	50	50	5.25±0.25
Epanutin	10^{-6}	100	100	0	4.33±0.32
	10^{-5}	100	25	0	2.6±0.4**
Control		100	62.5	12.5	4.8±0.86
Quinidine	10^{-6}	83.3	50	0	2.3±0.76*
	5×10^{-6}	16.7	33.3	0	1.2±0.60****

Each value was obtained from 6–8 hearts. Significantly different to control: *$P < 0.05$, **$P < 0.02$, ***$P < 0.01$, ****$P < 0.001$.

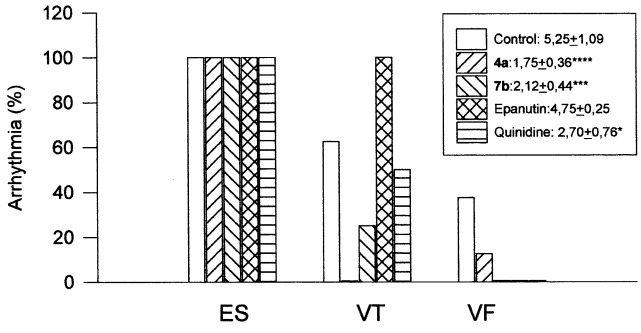

Fig. 5. Effect of compounds **4a** and **7b** on reperfusion induced arrhythmia.

lipophilic quinidine ($\log P = 2.65$) was protonated in symulated physiological conditions—$\log P$ (pH 7) = −0.66 and $\log P$ (pH 7.4) = −0.56.

3.2. Pharmacological properties

3.2.1. Effect on electrocardiogram

For the group of antiarrhythmic agents, ECG analysis is of special interest in the characterisation not only of the side-effects, but also for the analysis of the mode of action [4]. It is known that class Ia drugs (quinidine, procainamide, disopyramide) prolong $P-Q$ interval (a surrogate measure of sodium channel activity), widen the QRS complex (indication of conduction velocity), and increase $Q-T$ interval (effect of prolonged repolarisation). Contrary to that class Ib (lidocaine, phenytoin, mexiletine, tocainide) decrease $Q-T$ interval with no change in the QRS or $P-Q$ interval and class Ic (flecainide, propafenone) increase $P-Q$ interval and QRS complex, causing no change or prolongation of $Q-T$ interval. As showed results CAST [16,17], antiarrhythmic drugs lengthening $Q-T$ interval can cause cardiac arrhythmias. Analysing the previously mentioned influence of the class I antiarrhythmic drugs on the ECG, only those from class Ib do not cause $Q-T$ prolongation, having least pro-arrhythmic activity. Taking the above into the consideration modifications of phenytoin structure were undertaken.

To study the effects of tested compounds on the electrocardiogram (ECG) the Langendorff-non-working rat heart has been used. The preliminary studies showed that the novel **1** derivatives reduced the heart rate, lengthened the time of duration of the intervals $P-Q$ and $Q-T$, and QRS complex; and diminished the coronary flow. This last effect probably related to reduction of the heart rate. The most strong prolongation of the time of duration of the $Q-T$ interval was observed after application of the compounds **9a** and **9b**. They significantly prolonged the $Q-T$ interval at concentration 10–100 times lower than that of other tested compounds. Moreover, compound **9a** and **9b** showed a significant negative chronotropic property with a concomitant prolongation of the QRS complex. The strong prolongation of the $Q-T$ interval lead to arrhythmia, as it was earlier suggested by many authors (e.g. [18]). On the basis of all above results, compounds **9a** and **9b** were excluded from further investigations. The electrocardiographic changes observed after administration of compounds **4a**, **4b**, **7a**, **7b** and **9c** were comparable to that reported for quinidine, but not for epanutin, which prolonged $P-Q$ and $Q-T$ intervals, but not QRS complex. These results unfortunately

Table 4
Prediction [15] of physicochemical properties of basic compounds **4a**, **4b**, **7a**, **7b**, **9a**–**9c** compared to those of phenytoin (1) and quinidine (**Qdn**)

Cpd	Log *P*	pK_a	pK_a	Log *D*	
		Basic group	Acidic group	pH 7.0	pH 7.4
4a	1.33	10.19	14.23	−1.40	−1.22
4b	2.85	10.16	14.23	0.01	0.24
7a	0.51	7.03	13.86	0.20	0.36
7b	−0.34	7.52 2.41 −4.30	13.77 15.03	−0.96	−0.70
9a	−1.15	7.52 2.41 −4.30	14.00 15.03	−1.77	−1.51
9b	1.55	7.36	14.00	1.04	1.27
9c	−0.34	8.14 2.41	14.00	−1.51	−1.15
1	1.65	–	8.06	1.62	1.57
Qdn	2.65	11.05 2.30	14.15	−0.66	−0.56

indicate that the novel derivatives of **1** could be assigned to class Ia of antiarrhythmic drugs possessing quinidine-like properties.

3.2.2. Antiarrhythmic properties

The numerous and varied methods could be used in screening of new antiarrhythmic agents [19,20]. A large number of literature reports suggest that the rat coronary artery ligation–reperfusion model in vitro can be recommended as a screen for new antiarrhythmic drugs of any Vaughan Williams class [1,18,21–24].

Following this suggestion the antiarrhythmic activity of compounds **4a**, **4b**, **7a**, **7b** and **9c** was examined in arrhythmia associated with coronary artery occlusion and reperfusion rat models. Re-opening of the occluded left coronary artery led to arrhythmias which began almost immediately after the restoration of coronary flow. The products of hypoxic metabolism and reperfusion of ischemic myocardium play the important role in the genesis of ventricular arrhythmias associated with coronary artery occlusion and reperfusion. These products include locally released catecholamines, potassium, thromboxane and oxygen-derived free radicals [25,26]. Compared with control hearts, compound **4a** and **7b** statistically diminished the incidence of VT and VF associated with coronary artery occlusion and reperfusion in the isolated rat heart in a concentration unrelated-fashion, but did not protect the heart from single VBs. It was interesting to observe that the compounds showed activity inferior to that of quinidine, which decreased the incidence of all ventricular arrhythmias in a concentration-related fashion, but superior to that of epanutin which diminished the incidence of VT and VF, but at concentration about 10–100 times higher (Table 3). These results were confirmed by the analysis of arrhythmic severity-index calculated according to Bernauer [14] for the studied compounds as well as for epanutin and quinidine. It turned out that **4a** and **7b** have the lowest severity—index of 1.75 ($\pm$0.36) and 2.12 ($\pm$0.44) significantly different to control ($P < 0.001$) and ($P < 0.01$), respectively. Their effectiveness is better than that determined for quinidine and epanutin in the same experimental procedure but in higher concentration. The effect of **4a** and **7b** on reperfusion-induced arrhythmia is shown in Fig. 5. On the basis of the obtained results, compounds **4a** and **7b** were selected for further pharmacological evaluation.

3.2.3. Anticonvulsant properties

For anticonvulsant assays compounds **4b** and **9b** with the most lipophilic properties (log $P = 2.85$, 1.55) were chosen, since it is known that an optimal lipophilicity for penetration through the blood–brain barrier appears to exist at about log $P = 2$ [27]. Additionally the most active in reperfusion-induced arrhythmia compounds **4a** and **7b** were evaluated, too. In doses up to 300 mg kg^{-1} these derivatives have shown no neurotoxicity and were devoid of anticonvulsant activity in the used tests. The obtained results indicate that examined compounds are lacking of CNS activity characteristic for the starting phenytoin.

3.2.4. Spatial properties

Compounds **4a** and **9b** were chosen for X-ray analysis as typical representative of the reaction path (ii) and (iii). We were not able to grow crystals of **7b** suitable for X-ray analysis. **4a** has the pharmacophore typical of class Ia antiarrhythmics in the chain substituted at

hydantoin N1 atom. **9b**, which was eliminated because of disadvantageous prolongation of $Q-T$ interval, has its substituent at hydantoin N3 atom. In general, the conformation of the spacer substituted at N(1) in **4a** and at N(3) in **9b** does not differ significantly (Table 1). Hence, for optimal pharmacological activity, the position of the side-chain against hydrophobic moiety (i.e. one of the benzene rings of **1**) is getting essential. In the crystalline state the conformation of **9b** is extended, probably due to intermolecular hydrogen bonds, whereas that of **4a** is folded and stabilised by an intramolecular hydrogen bond of N–H···O type (Table 2). The folded conformation seems to be a convenient form of class Ia antiarrhythmics to interact with a receptor site as it was reported earlier [12].

Fig. 6 presents the geometrical relation of the pharmacophore, included in the side-chain, to each of the benzene rings of **4a**. It can be clearly seen from the figure that nitrogen amine atom N(2) is situated close to the Ph_1 plane (5.049 Å) with its hydrogen atom directed towards the opposite site. N(2) is also close to the best plane of Ph_2, which could be considered as having possibility to interact with the hydrophobic pocket of a receptor. To emphasise relationships between the molecular structure, observed in the crystalline state, and the pharmacological activity, the values of chosen descriptors for **4a** and **9b** are compared in Table 5 with those calculated for quinidine (**Qdn**) [28], the recognised representative of class Ia antiarrhythmics. The shape of N–C–C–OH pharmacophore seems to be similar for the compared compounds. The distance of amine nitrogen atom from Ph_1 benzene ring is the same for **4a** and **Qdn**. From the point of view of placing the amine nitrogen atom against the best plane of the benzene ring (Ph_2), the best compatibility is found once more between **4a** and **Qdn**.

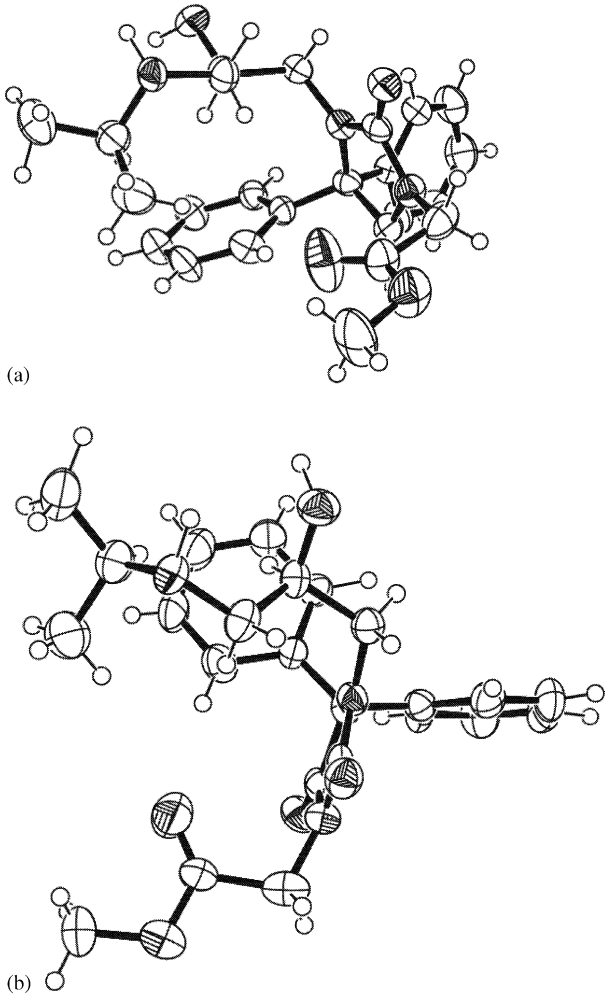

Fig. 6. (a) View of **4a** molecule along C(13)–C(12) bond of the pharmacophore N(amine)–C–C–OH. The position of N(amine) atom in relation to C(51)–C(56) benzene ring (Ph_1) is seen. (b) Projection of the pharmacophore of **4a** onto C(51)–C(56) plane. Ph_2 benzene ring, C(61)–C(66), is free to interact with a receptor pocket.

In conclusion, among the new studied derivatives **4a** and **7b** show the quinidine-like antiarrhythmic properties. The analysis of the structure-activity relationship indicates that physicochemical properties of all considered compounds (described by log P and log D) correlate in limited range with their pharmacological properties. These properties were explained by the 3D structure analysis.

4. Experimental protocols

4.1. Chemistry

4.1.1. General remarks

Melting points are uncorrected and were recorded on a Mel.-Temp. II (LD Inc., USA) apparatus. The TLC was performed on Merck silica gel GF_{254} precoated TLC Al sheets; the used solvent systems were: A toluene:acetone:methanol (1:1:1); B toluene:acetone:-methanol (5:5:1). IR spectra were measured with FT IR 410 spectrometer (Jasco) in KBr pellets.

The ^{1}H-NMR spectra were recorded on a Bruker AC-200F spectrometer in $CDCl_3$ using TMS as an internal standard (chemical shifts are reported in δ units). The

Table 5
The values of chosen geometrical descriptors (Å, °) of the conformation of β-aminoethanol moiety and the receptor-pocket model

	4a	9b	Qdn
N–C–C–OH	56.3 [a]	65.8	75.9
$N_{amine}\cdots O_{hydroxyl}$	2.887 [a]	2.912	3.093
$O_{hydroxyl}\cdots Ph_1$	4.516	7.089	3.722
$N_{amine}\cdots Ph_1$	5.049	6.351	5.110
$N_{amine}\cdots\pi$ (Ph_2)	−1.751	−0.761	−1.724 [b]

Data for quinidine (**Qdn**) are taken from Ref. [28].
[a] Intramolecular H-bond (Table 2).
[b] For **Qdn**: $Ph_2 = Ph_1 = C(5)C(6)-C(10)$.

elemental analyses were performed at the Department of Pharmaceutical Chemistry of the Jagiellonian University, Kraków (Poland) and were within ±0.4% of the theoretical values.

The starting alkylated 5,5-diphenylhydantoin derivatives **2**, **3**, **5**, **6** and **8** were obtained as previously described [9,29,30].

4.1.2. Preparation of basic 5,5-diphenylhydantoin derivatives

*4.1.2.1. 1-[3-(2-propylo)amino-2-propanolo]-3-carbmethoxymethyl-5,5-diphenylhydantoin (**4a**).* A mixture of 1-(2,3-epoxypropyl)-3-carbmethoxymethyl-5,5-diphenylhydantoin (**3**) (3.94 g, 0.010 mol) and 2-propylamine (0.2 g, 0.033 mol) in 25 mL of methanol was refluxed for 3 h. After cooling the solid was separated by suction and purified by crystallisation from ethanol, m.p.: 172–173 °C; yield: 57%; TLC: R_f (A) 0.23; ^{1}H-NMR (200 MHz) δ: 0.92 (d, $J = 3.9$ Hz, 3H, CH_3CH), 0.95 (d, $J = 3.9$ Hz, 3H, CH_3CH), 2.18–2.39 (m, 2H, NHCH_2), 2.57 (sept, 1H, CH(CH$_3$)$_2$) 2.84 (q, 1H, CHOH), 3.44 (dd, $J = 1.7$ Hz, $J = 6.4$ Hz, 2H, NCH_2CHOH), 3.79 (s, 3H, OCH$_3$), 4.33 (s, 2H, NCH$_2$CO), 7.35–7.44 (m, 10H, Ar–H); IR ν: 2961 (CH$_2$), 1771 (C$_4$=O), 1733 (COOCH$_3$), 1718 (C$_2$=O), 1447, 1235, 773, 700 cm^{-1}. Anal. (C$_{24}$H$_{29}$N$_3$O$_5$) C, H, N.

*4.1.2.2. Hydrochloride of **4a**.* **4a** was suspended in dry ethanol cooled with water bath, and saturated with gaseous HCl. The solid dissolved and precipitated a new one, m.p.: 198–200 °C (from ethanol). Anal. (C$_{24}$H$_{29}$N$_3$O$_5$ HCl) C, H, N.

*4.1.2.3. 1-[3-cyclohexylamino-2-propanolo)-3-carbmethoxymethyl-5,5-diphenylhydantoin (**4b**).* Prepared analogous to **4a**, m.p.: 170–172 °C (ethanol, yield: 40%; TLC: R_f (A) 0.29; ^{1}H-NMR (200 MHz) δ: 0.81–1.90 (m, 10H, cyclohexane), 2.00–2.43 (m, 3H, CHNHCH_2), 2.73–2.84 (m, 1H, CHOH), 3.42–3.47 (m, 2H, NCH_2CHOH), 3.78 (s, 1H, OCH$_3$), 4.33 (s, 2H, NCH$_2$CO), 7.34–7.42 (m, 10H, Ar–H); IR ν: 2931, 2854 (CH$_2$), 1776 (C$_2$=O), 1749 (COOCH$_3$), 1720 (C$_4$=O), 1449, 1219, 699 cm^{-1}. Anal. (C$_{27}$H$_{33}$N$_3$O$_5$) C, H, N.

*4.1.2.4. 1-(3-N-morpholino-2-propanolo)-3-ethyl-5,5-diphenylhydantoin (**7a**).* Prepared according to the procedure described earlier [9].

^{1}H-NMR (200 MHz) δ: 1.25 (t, $J = 7.2$, 3H, CH$_3$), 1.92–2.30 (m, 6H, CH$_2$N(CH$_2$)$_2$), 2.99–3.11 (m, 1H, CHOH), 3.36–3.72 (m, 9H, O(CH$_2$)$_2$, NCH_2CHOH, NCH_2CH$_3$, OH), 7.24–7.31 (m, 4H, Ar–H), 7.36–7.43 (m, 6H, Ar–H).

*4.1.2.5. Dihydrochloride of 1-[4-(2-hydroxyethyl)-1-piperazine-2-propanolo]-3-ethyl-5,5-diphenylhydantoin (**7b**).* Prepared according to the procedure described earlier [9].

*4.1.2.6. 3-[4-(2-hydroxyethyl)-1-piperazine-2-propanolo]-5,5-diphenylhydantoin (**9a**).* A mixture of 3-(2,3-epoxypropyl)-5,5-diphenylhydantoin (**8a**) (3.08 g, 0.010 mol) 1-(2-hydroxyethyl)-piperazine (1.3 g, 0.010 mol) in toluene (20 mL) was refluxed for 5 h, the solvent was evaporated in vacuo, the oily residue was recrystallised from acetone, m.p.: 106–109 °C, yield: 62%; TLC: R_f (A) 0.17; R_f (B) 0.05; ^{1}H-NMR (200 MHz) δ: 2.32–2.51 (m, 12H, CH$_2$pipCH$_2$), 3.51–3.72 (m, 4H, NCH_2CHOH, CH_2OH), 4.00 (q, $J = 6.3$ Hz, 1H, CHOH), 7.18–7.42 (m, 11H, Ar–H, N$_1$–H); IR ν: 3137 (NH), 2947, 2825 (CH$_2$), 1772 (C$_2$=O), 1713 (C$_4$=O), 1444, 1419, 1316, 1147, 757, 702. Anal. (C$_{24}$H$_{30}$N$_4$O$_4$) C, H, N. Dihydrochloride of **9a** was obtained as dihydrochloride of **4a**, m.p.: 237–240 °C. Anal. (C$_{24}$H$_{30}$N$_4$O$_4$ 2HCl) C, H, N.

*4.1.2.7. 3-[(4-phenyl-1-piperazine)-2-propanolo]-5,5-diphenylhydantoin (**9b**).* Prepared analogous to **9a**, m.p.: 178–179 °C (from ethanol), yield: 69; TLC: R_f (A) 0.84; R_f (B) 0.66.

^{1}H-NMR (200 MHz) δ: 2.41 (d, $J = 6.7$ Hz, 2H, CH$_2$N), 2.47–2.57 (m, 2H, NCH$_2$), 2.66–2.76 (m, 2H, NCH$_2$), 3.14 (t, $J = 4.9$ Hz, 4H, (CH$_2$)$_2$NPh), 3.50 (br s, 1H, OH), 3.56–3.76 (m, 2H, NCH_2CHOH), 4.07 (q, 1H, CHOH), 6.82–6.91 (m, 3H, H-3′, H-4′, H-5′), 7.02 (s, 1H, N$_1$–H), 7.21–7.26 (m, 2H, H-2′, H-6′), 7.29–7.42 (m, 10H, Ar–H); IR ν: 3056 (NH), 2821 (CH$_2$), 1775 (C$_2$=O), 1714 (C$_4$=O), 1446, 1414, 1230, 765, 291 cm^{-1}. Anal. (C$_{28}$H$_{30}$N$_4$O$_3$) C, H, N. Hydrochloride of **9b** was obtained as dihydrochloride of **9c**, m.p.: 192–194 °C (from acetone). Anal. (C$_{28}$H$_{30}$N$_4$O$_3$ HCl) C, H, N.

*4.1.2.8. 3-[(4-methyl-1-piperazine)-2-propanolo]-5,5-diphenylhydantoin (**9c**).* A mixture of 3-(2,3-epoxypropyl)-5,5-diphenylhydantoin (**8**) (3.08 g, 0.010 mol), 1-methylpiperazine (1.0 g, 0.010 mol) in propanol (10 mL) was refluxed for 10 h. The solvent was evaporated in vacuo, the oily residue was recrystallised from ethanol, m.p.: 168–170 °C, yield: 35%, TLC: R_f (A) 0.05; R_f (B) 0.17.

^{1}H-NMR (200 MHz) δ: 2.22 (s, 5H, CH_2, CH_3), 2.31–2.60 (m, 8H, pip) 3.58–3.66 (m, 2H, NCH_2-CHOH), 3.94–4.03 (m, 1H, CH–OH), 7.31–7.39 (m, 11H, Ar–H, N$_1$–H); IR ν: 2941, 2805 (CH$_2$), 1771 (C$_2$=O) 1715 (C$_4$=O), 1433, 1409, 1148, 1008, 760, 698 cm^{-1}. Anal. (C$_{23}$H$_{28}$N$_4$O$_3$) C, H, N. Dioxalate of **9c**: m.p.: 171–173 °C (from ethanol). Anal. (C$_{23}$H$_{28}$N$_4$O$_3$ 2C$_2$H$_2$O$_4$) C, H, N.

Dihydrochloride of **9c**: to the solid of **9a** concentrated hydrochloric acid was added and evaporated to dryness. The obtained oil was recrystallised from ethanol, m.p.: 239–241 °C. Anal. (C$_{23}$H$_{28}$N$_4$O$_3$ 2HCl) C, H, N.

4.2. X-ray diffraction analysis of 1-[3-(2-propylo)amino-2-propanolo]-3-carbmethoxymethyl-5,5-diphenylhydantoin (**4a**) and 3-[(4-phenyl-1-piperazine)-2-propanolo]-5,5-diphenylhydantoin (**9b**)

Crystals of **4a** and **9b**, suitable for X-ray analysis, were obtained by slow recrystallisation from ethanol at room temperature.

4.2.1. Crystal data for **4a**

$C_{24}H_{29}N_3O_5$, MW = 439.50, monoclinic, $P12_11$, a = 8.0652(6), b = 8.7724(6), c = 16.2481(17) Å, β = 99.004(3), V = 1135.41(17) Å^3, Z = 2, D = 1.286 Mg m^{-3}, λ(Mo Kα) = 0.71073 Å, μ = 0.091 mm^{-1}, $F(0\,0\,0)$ = 468, T = 293(2) K. The lack of a heavy atom unabled us to determine the absolute structure reliably.

4.2.2. Crystal data for **9b**

$C_{28}H_{29.75}N_4O_3$, MW = 470.31, triclinic, $P\bar{1}$, a = 8.7791(2), b = 10.3175(2), c = 15.5652(4) Å, α = 71.762(1), β = 84.682(1), γ = 68.098(1)°, V = 1241.93(5) Å^3, Z = 2, D = 1.258 Mg m^{-3}, λ(Mo Kα) = 0.71073 Å, μ = 0.083 mm^{-1}, $F(0\,0\,0)$ = 500, T = 293(2) K.

The final fractional atomic coordinates with thermal displacement parameters for the crystal structures and other numerical data are deposited as supplementary material [31].

Intensity data were collected on a Nonius KappaCCD diffractometer using graphite monochromated Mo Kα radiation. Programs COLLECT [32] and DENZO-SMN [33] were applied for the data collection, cell refinement and data reduction. The structures were solved by direct methods using SHELXS-97 [34] and refined by full-matrix least-squares on F^2 using SHELXL-97 [34]. Weighting scheme $w = [\sigma^2(F_o^2) + ((AP)^2 + BP)]^{-1}$, where $P = (F_o^2 + 2F_c^2)/3$ was used. The final R-indices and goodness of fit parameter S obtained for **4a** and **9b** were: R_1 = 0.089, wR_2 = 0.187, S = 1.577 for 3243 unique reflections and R_1 = 0.052, wR_2 = 0.132, S = 1.021, for 8540 unique reflections, respectively.

The non-hydrogen atoms positions were refined together with anisotropic thermal displacements. Hydrogen atoms were localised on difference Fourier map and refined isotropically. The figures were drawn with ORTEP-3 [35].

In the crystal structure of **9b** beside the alcohol (86.4%) a keton, in amount of 13.6(4)%, was observed probably due to an oxidation of the starting compound in the crystalline state under X-ray exposure (NMR studies of **9b** in DMSO-d_6 solution carried out before X-ray exposure did not show any instability).

4.3. Pharmacological methods

4.3.1. Materials and methods

4.3.1.1. Compounds. Diphenylhydantoin sodium salt (Epanutin, Parke–Davis), quinidine sulphate (Polfa), sodium heparin (Polfa), thiopental sodium (HEFA-Frenon Arzneim.).

4.3.1.2. Animals. The experiments were carried out on male Wistar rats (180–250 g). Animals were housed in wire mesh cages in room at 20±2° with natural light–dark cycles. The animals had free access to standard pellet diet and water, and used after a minimum of 3 days acclimation to the housing conditions. Control and experimental group consisted of 8–10 animals each.

4.3.1.3. Reference compound. Diphenylhydantoin sodium salt and quinidine were used as a reference compounds.

4.3.1.4. Statistical analysis. The data are expressed as means±S.E.M. The results were statistically analysed by the one-way ANOVA or Student's t-test. Differences were considered significant when $P < 0.05$.

4.3.2. Non-working heart perfusion

Hearts from thiopental-anaesthetised (45–60 mg kg^{-1}, i.p.) rats were perfused according to the Langendorff technique [36] at constant pressure of 70 cm H_2O (6.87 kPa) with Chenoweth–Koelle solution continuously gassed with 95% O_2 plus 5% CO_2 of the following composition (mmol l^{-1}): NaCl (120.0), KCl (5.6), $MgCl_2$ (2.2), $NaHCO_3$ (19.0), $CaCl_2$ (2.4) and glucose (10.0).

The effect of tested compounds, in concentration of 10^{-8} to 10^{-5} M, on coronary flow (cardiac effluent), ECG (obtained by two stainless steel electrodes, one inserted into the muscle of the ventricular wall and another attached to the metal aortic cannula) were assessed after 15–20 min of initial stabilisation.

4.3.3. Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the non-working isolated perfused rat heart [37]

Non-working isolated hearts were mounted as described above for recording coronary flow and ECG. After a 15-min stabilisation period, acute regional myocardial ischaemia was produced for 15 min by installing a clip on the left coronary artery close to its origin (ischaemic period). The clip was then reopened, and changes during reperfusion were monitored for 30 min (reperfusion period). Occlusion and reperfusion was verified by measuring coronary flow before occlusion, after occlusion and after reperfusion.

Ligation of the coronary artery resulted in 24–28% reduction in coronary flow. Reperfusion was followed by a return of the coronary flow. Reperfusion induced arrhythmias, manifested by VBs, VT and VF.

ECGs were analysed according to the guidelines of the Lambeth Conventions [24] for VBs, bigeminy, salvos (<4 successive VBs), VT (4 or more successive VBs) and VF.

In order to obtain a measure for the intensity of the arrhythmias, an arrhythmia severity-index was calculated for each heart according to Bernauer [14] the occurrence of up to 10 ventricular extrasystoles during the first 30 min of reperfusion was attributed the value 1, more than 10 the value 2, VT or ventricular flutter the value 3, and VF the value 4. Bigeminy and salvos were not quantified separately but included with VBs. Agents were added to the perfusion medium 15 min before coronary artery ligation and the concentration was maintained for the rest of the perfusion period.

4.3.4. Anticonvulsant assays

Antiepileptic activity and neurological toxicity assays were carried out by the ADD Program, Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institute of Health, Bethesda, MD. Compound was injected intraperitoneally into mice as suspensions in 0.5% methylcellulose at three dosage levels (30, 100 and 300 mg kg^{-1}). Phase I of the evaluation was a qualitative assay which used small groups of animals (1–8) and included three tests: MES seizure, subcutaneous pentylenetetrazol (ScMet), and rotorod test for neurological toxicity (Tox) with anticonvulsant activity and neurotoxicity noted 30 min and 4 h after administration. The TTE test was similar to the MES screen but used a lower level of electrical current. The details of these procedures have been published [38,39].

5. Supplementary material

Crystallografic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications number: **4a** 209329, **9b** 209330. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; (fax: +44-1223-336-033, e-mail: deposit@ccdc.cam.ac.uk).

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