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# Antiarrhythmic properties of phenylpiperazine derivatives of phenytoin with $\alpha_1$-adrenoceptor affinities

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ABSTRACT

An association between $\alpha_1$-adrenoceptor affinities, hERG $K^+$-antagonistic properties and antiarrhythmic activities for a series of phenylpiperazine derivatives of hydantoin (**2a–21a**) was investigated. New compounds were synthesized and tested for their affinity for $\alpha_1$-adrenoceptors in radioligand binding assay using [$^3$H]-prazosin as a selective radioligand. Antiarrhythmic activities in adrenaline- and barium chloride-induced arrhythmia models, an influence of the phenylpiperazine derivatives on the ECG-components and blood pressure were tested in vivo in normotensive rats. The hERG $K^+$-antagonistic properties of the most potent antiarrhythmic agents were investigated in silico by the use of program QikProp. The highest $\alpha_1$-adrenoceptor affinity ($K_i$ = 4.7 nM) and the strongest antiarrhythmic activity in adrenaline induced arrhythmia ($ED_{50}$ = 0.1 mg/kg) was found for 1-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione hydrochloride (**19a**). The results indicated a significant correlation between $\alpha_1$-AR affinities ($pK_i$) and antiarrhythmic activity ($ED_{50}$) in adrenaline model ($R^2$ = 0.92, $p$ <0.005). Influence of the examined phenylpiperazine hydantoin derivatives on hERG $K^+$ channel, predicted by means of in silico methods, suggested their hERG $K^+$-blocking properties.



## 1. Introduction

Cardiac arrhythmia is one of the main reasons of sudden death in cardiovascular diseases. Therefore a search for new antiarrhythmic drug targets is a current therapeutic challenge. The first classification of antiarrhythmic agents, introduced by Singh and Vaughan Williams in 1970[1,2] has been revised and modified by newer approaches[3–5] giving the current version, Vaughan Williams Classification—2001, which divides antiarrhythmic agents into four main groups in accordance to their blocking properties for $Na^+$ channels, β-adrenergic receptors, $K^+$ and $Ca^+$ channels, respectively.[6] Although $\alpha_1$-adrenergic agents are not considered in the Vaughan Williams classification, recent studies indicate their increasing role in arrhythmia mechanisms, especially, in the case of ischemic arrhythmia.[7,8] Currently available pharmacological studies on the effect of $\alpha_1$-adrenoceptor stimulation and arrhythmias are not consistent. Some of them describe cardioprotective effect of selective $\alpha_1$-AR agonists against arrhythmias in isolated hearts,[9,10] whereas the others clearly show that stimulation of these receptors promotes arrhythmias.[11,12] Several lines of evidence suggest that prazosin and other $\alpha_1$-adrenoceptor antagonists possess antiarrhythmic properties.[13,14] The $\alpha_1$-adrenergic antagonistic activity of prazosin distinctly decreases the incidence of malignant ventricular arrhythmias associated with either myocardial ischemia or subsequent reperfusion.[15]

The human hERG $K^+$ is another point of interest in search for antiarrhythmic agents.[16–21] Potassium hERG channels regulate heart function because they are responsible for $K^+$ current ($I_{Kr}$), which plays a very important role in the repolarization phase.[17] Blockade of the $K^+$ current however leads to the prolongation of the QT interval and it may be associated with a life-threatening arrhythmia called torsades de pointes.[18] Due to their function, hERG channels can be treated as antitarget or target, respectively.[19] Some drugs such as cisapride, terfenadine or sertindole are able to block hERG channels and they can cause serious heart rate disturbances. It is very important to design new non-cardiologic drugs which are deprived of the ability to block the potassium channels.[20] On the other hand, it is well known that class III of antiarrhythmic drugs acts by blocking potassium channels. In the

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0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
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literature various channel mutations have been described. Among them channelopathy of hERG can be found. It is the base of the newly described disorder called congenital short QT syndrome (SQTS).[21] In this case hERG blockers could serve as useful therapeutic agents. Thus, the hERG channels can be treated as antitarget or target, depending on the proposed therapeutic indication. It should be also pointed out that not only blockers are used. There are also known hERG activators (e.g., mallatoxin), which are very interesting in the treatment of long QT syndrome.[22]

Our previous studies were focused on the search for new antiarrhythmic agents among derivatives of phenytoin[23,24] and as a result several active compounds have been characterized. The compounds displayed cardioprotective properties against reperfusion induced arrhythmia in vitro[24] or against barium chloride- and adrenaline induced arrhythmia in anesthetized rats.[25] Especially, phenylpiperazine derivatives of phenytoin, synthesized as the I-st generation of chemical modifications of compound **1** (**AZ-99**, Fig. 1), have been an interesting chemical group that showed high structural similarity to known $\alpha_1$-adrenoceptor antagonists.[26,27] The previously tested methoxyphenylpiperazine derivatives[25] showed antiarrhythmic activity in the adrenaline-induced model of arrhythmia but they were totally inactive in the barium chloride-induced arrhythmia. We suggested that antiarrhythmic activity of these compounds could be relative to their potential antagonistic properties for $\alpha_1$-adrenoceptors. Our further investigations, including binding tests and functional bioassays,[28,29] confirmed that the compounds displayed significant affinity for $\alpha_1$-adrenoceptor with antagonistic properties. In the same investigation, new phenylpiperazine derivatives of phenytoin (**2–9**) were synthesized and tested in radioligand binding assays giving a series of active compounds (generation II, Fig. 1) with affinities for $\alpha_1$-adrenoceptors in the submicromolar range (Table 1).

In the present work, further modifications are described as the III-rd and IV-th generation of **AZ-99** (Fig. 1). The main goal of the studies was to assess antiarrhythmic activity of the phenylpiperazine derivatives (**2a–21a**). Antiarrhythmic activity of the compounds and an influence on the rat heart rhythm as well as an influence on the blood pressure were investigated in vivo. The association between $\alpha_1$-adrenoceptor affinity and antiarrhythmic properties for a series of $\alpha_1$-adrenoceptor agents (Table 1, Table 2) was evaluated. Theoretical studies to predict hERG-affinities for the most promising antiarrhythmic agents were carried out.

## 2. Results and discussion

### 2.1. Synthesis

Synthesis of the first generation (**2**–**11**) of lead **AZ-99** was described earlier.[25,28] Synthesis of compounds **12–21** was performed according to Scheme 1. Phenylpiperazine hydantoin derivatives with 2-hydroxypropyl linker **12–17** were obtained within three-step synthesis, starting from 5,5-diphenylhydantoin (**22**). Two steps of N-alkylation of hydantoin, giving compounds **23–25** and **26, 27**, were performed using methods described previously.[25,28] In the last step derivatives with oxirane ring (**26** and **27**) were used as alkylating agents to react with corresponding phenylpiperazines **28–30** under microwave irradiation in solvent free conditions.[28] A structure-yield relationship was observed as all N3-methyl derivatives (**12–14**) were obtained with satisfying yields (60–84%) while N3-ester derivatives (**15–17**) with significantly lower one (35–49%).

As starting materials for synthesis of compounds **18–21**, N3-methyl-(**23**) or N3-ester derivatives (**24**, **25**) were used. Compounds **23–25** were alkylated at N1-position of hydantoin within two-phase alkylation using suitable dibromoalkanes, 1,3-dibromopropane and 1,4-dibromobutane, respectively, to give N1-bromoalkylhydantoin derivatives **31**–**34**. The reaction was carried out using two methods, standard (A) or microwave-aided method (B). The standard method was performed at room temperature using long-term stirring (64–92 h) in acetone with $K_2CO_3$ and TEBA. An excess (33%) of dibromolkanes was used to decrease the risk of an appearance of bis-substituted side-products. Nevertheless, TLC-control indicated a presence of a small amount of some side products during the long-term stirring without heating. Primary tests to elaborate the process conditions indicated that the number and an amount of side products increased if the temperature of the process was turned up. A main reason was a nucleophilic substitution within bromide-alkyl fragments in the alkaline environment to give some alcohols that was easy to go in the basic condition. If the temperature of the process was close to 20 °C the appearance of side alcohols was imperceptible, easy to eliminate by crystallization with alcohol. Long-term stirring method gave compounds **31** and **33** with satisfying yields (74–75%). In the case of bromobutyl derivative **32** the yield was slightly lower (57%) and the synthesis was repeated under microwave irradiation using

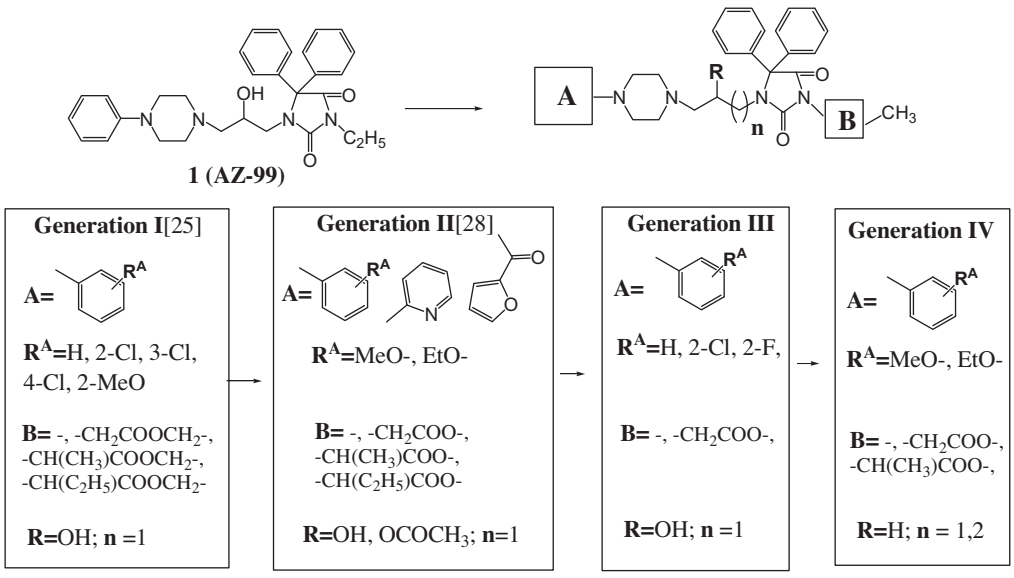
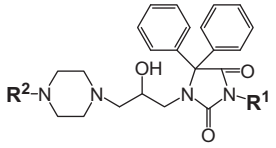



**Figure 1.** Chemical modifications of **AZ-99**: previous modifications (generation I-II), present modifications (generation III-IV).

**Table 1**
Structure and $\alpha_1$-adrenoceptor affinity for compounds **2a**–**11a** obtained previously[25,28]



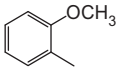
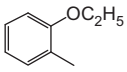
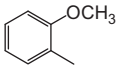
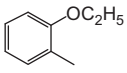
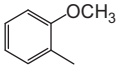
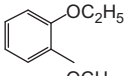
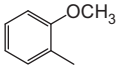
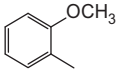
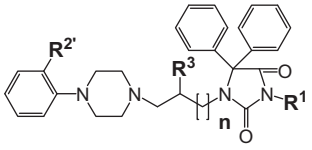

| Compd | $R^1$ | $R^2$ | $\alpha_1$-AR $K_i$ (nM) | $\alpha_1$-AR affinity p$K_i$ |
|---|---|---|---|---|
| **2a** | $-CH_3$ |  | 160.7 ± 13.6 | 6.79 |
| **3a** | $-CH_3$ |  | 121.6 ± 14.9 | 6.92 |
| **4a** | $-CH_3$ |  | >100 000 | — |
| **5a** | $-CH_3$ |  | 691.9 ± 16.5 | 6.16 |
| **6a** | $-CH_2COOCH_3$ |  | 197.8 ± 25.4 | 6.70 |
| **7a** | $-CH_2COOCH_3$ |  | 251.6 ± 3.8 | 6.60 |
| **8a** | $-CH(CH_3)COOCH_3$ |  | 103.9 ± 4.2 | 6.98 |
| **9a** | $-CH(CH_3)COOCH_3$ |  | 167.7 ± 8 | 6.78 |
| **10a** | $-CH(CH_3)COOC_2H_5$ |  | 135.7 ± 31.3 | 6.87 |
| **11a** | $-CH(C_2H_3)COOCH_3$ |  | 3100 ± 200 | 5.51 |

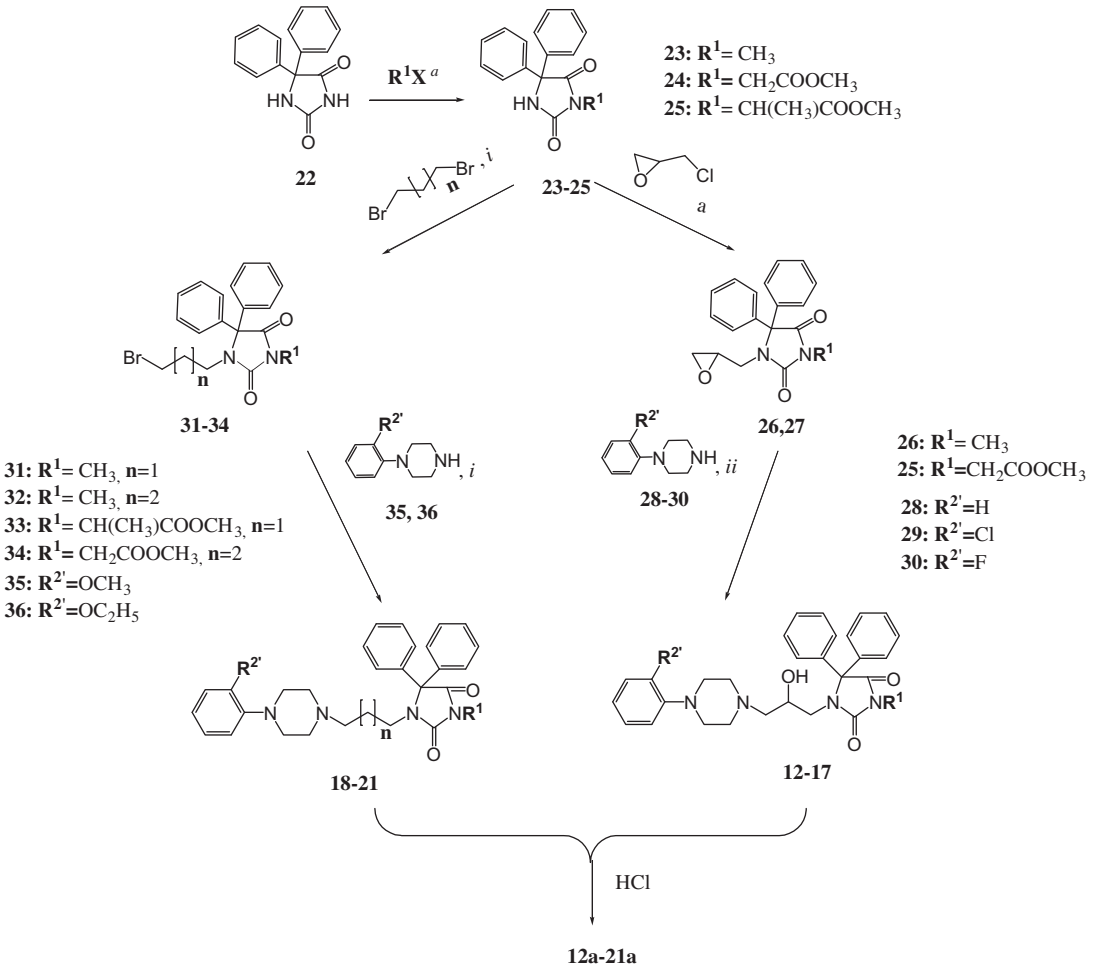
**Table 2**
Structure and $\alpha_1$-adrenoceptor affinity for new compounds (**12a**–**21a**)




| Compd | $R^1$ | $R^{2'}$ | $R^3$ | n | $\alpha_1$-AR $K_i$ (nM) | $\alpha_1$-AR affinity p$K_i$ |
|---|---|---|---|---|---|---|
| **12a** | $-CH_3$ | H | OH | 1 | 542.3 ± 19.4 | 6.27 |
| **13a** | $-CH_3$ | Cl | OH | 1 | 483.0 ± 29.4 | 6.32 |
| **14a** | $-CH_3$ | F | OH | 1 | 564.6 ± 80.5 | 6.25 |
| **15a** | $-CH_2COOCH_3$ | H | OH | 1 | 413.1 ± 75.8 | 6.39 |
| **16a** | $-CH_2COOCH_3$ | Cl | OH | 1 | 354.7 ± 21.6 | 6.45 |
| **17a** | $-CH_2COOCH_3$ | F | OH | 1 | 3300 ± 200 | 5.48 |
| **18a** | $-CH_3$ | $OCH_3$ | H | 1 | 412.9 ± 21 | 6.38 |
| **19a** | $-CH_3$ | $OCH_3$ | H | 2 | 4.7 ± 1.5 | 8.33 |
| **20a** | $-CH(CH_3)COOCH_3$ | $OC_2H_5$ | H | 1 | 401.2 ± 25.9 | 6.40 |
| **21a** | $-CH_2COOCH_3$ | $OCH_3$ | H | 2 | 25.0 ± 2,4 | 7.60 |

method B. The reactants were irradiated under reflux in chemical microwave oven 'Plazmatronika' in temperature 40–50 °C for 2.5 h. The method B allowed to shorten the time of reaction giving compound **32** with a bit higher yield (67%).

Final products **18–21** were obtained by two-phase alkylation of appropriate alkoxyphenylpiperazines (**35, 36**) using an excess (10%) of alkylating agents, bromoalkyl derivatives **31–34**. The process was performed in acetone in similar conditions to those described for compounds **31–34**. A progress of reaction was observed for 0–90 h. The further prolongation of stirring did not improve a yield. In all cases (**18–21**), a small amount of unreacted starting materials was still observed. Especially, a presence of arylpiperazines complicated purification of final products. To eliminate the rest of unreacted arylpiperazines, four-steps extraction with




**Scheme 1.** Synthesis of phenylpiperazine derivatives **12a–21a**; *a*-synthesis described earlier[25,28]; *i*-acetone, TEBA, $K_2CO_3$; rt (met. A) or mv-irradiation (met. B); *ii*-mv-irradiation.

diluted HCl (2%) was used and a residue from organic phase was crystallized with methanol. The method gave pure crystals of compounds **20** and **21** with different yields (32–69%). In the case of compounds **18** and **19**, no crystal was obtained. Methanol solutions of the purified compounds were saturated with gaseous HCl giving pure crystals of corresponding hydrochlorides **18a** and **19a**. Additionally, all compounds obtained in basic form were converted into corresponding hydrochlorides to give compounds **12a**–**17a**, **20a** and **21a**, useful in pharmacological assays. Compounds **2a–17a**, possessing chirality centers were synthesized and tested as racemates.

## 2.2. Pharmacology

### 2.2.1. Route of pharmacological screening

The main goal of all branches of medicinal science is life protection. Although the current pharmacology and medicinal chemistry introduced various bloodless in vitro- or in silico methods helpful in primary screening, they still cannot fully substitute tests in vivo which are necessary before clinical trials. In order to minimize the number of animals used in the study, we designed and applied a new route of primary pharmacological screening (Fig. 2) which allowed us to obtain desirable information about antiarrhythmic properties and structure–activity relationship in the group of phenylpiperazine hydantoin derivatives (**2a–21a**). The classical Szekeres' method was modified.[30,31] The main objective was to reduce undesirable death of animals by elimination of preliminary acute toxicity tests and rational selection of compounds for tests in vivo based on their activity in vitro. The route was divided into two levels (Fig. 2) based on the results of previous tests in vivo[25] and radioligand binding assays.[28] The whole group of compounds (**2a–21a**) was tested in the level I, which included four steps (S1–S4). Based on the testing of level I the most promising antiarrhythmic agents were selected for further tests within level II. In the first step (S1), $\alpha_1$-adrenoceptor affinities for all new phenylpiperazine derivatives **12a–21a** were tested in radioligand binding studies. Then, primary tests in vivo (S2) were performed for compounds of the II-nd and III-rd generations (**2a–9a**, **11a–17a**). The compounds were tested at starting doses of 5–10 mg/kg iv in two models of arrhythmia (adrenaline and $BaCl_2$-induced) and for their influence on both, the ECG-components and the blood pressure. Selection of compounds of IV-th generation for the primary tests in vivo was performed based on their affinity for $\alpha_1$-AR (S3). Results for generations I-III indicated that most of highly active compounds (**2a**, **3a**, **6a**–**10a**), totally protected animals from arrhythmia syndrome at starting doses and had affinities for $\alpha_1$-AR with the p$K_i$ values >6.5. Thus, the p$K_i$ >6.5 was established as a criterion for selection of compounds of IV-th generation for adrenaline induced arrhythmia test in vivo. Only two compounds from this generation, **19a** and **21a** were selected (S3) for primary tests in vivo at starting doses (S4). The most active compounds from this and previous[25,28] studies (**2a, 3a, 6a–10a, 14a, 19a** and

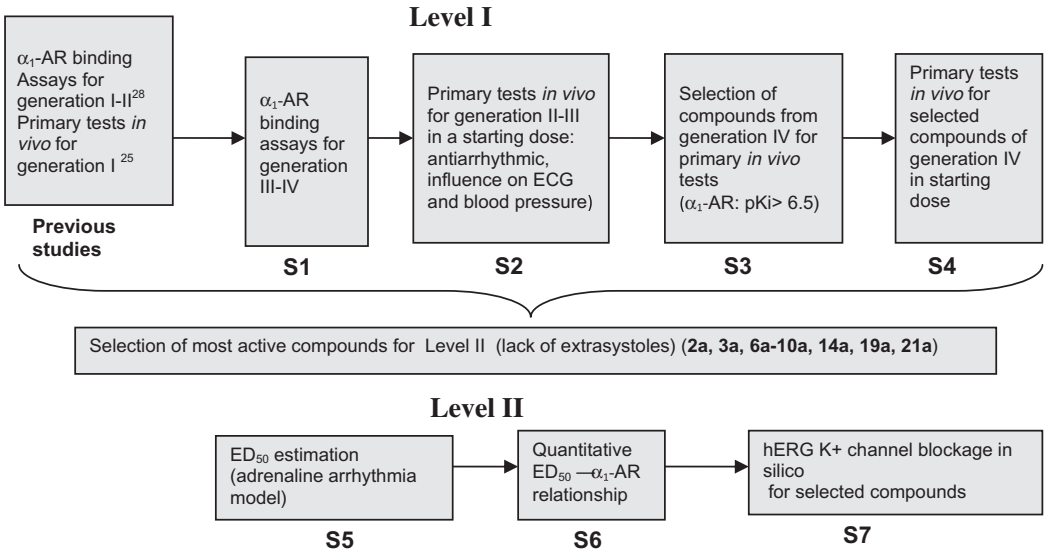



**Figure 2.** Route of pharmacological screening—levels I and II.

**21a**), which totally decreased an occurrence of the arrhythmogens-induced ventricular extrasystoles at their starting doses, were selected for the level 2 testing. The level 2 included an estimation of effective doses ($ED_{50}$) in full antiarrhythmic assays (S5), the quantitative analysis of the $ED_{50}$–p$K_i$ relationship (S6) and studies in silico to predict the hERG-inhibitory properties (S7).

### 2.2.2. Radioligand binding assays

Affinities of compounds **2a–11a** for $\alpha_1$-adrenoceptors evaluated previously[28] are presented in Table 1. Compounds **12a–21a** were tested for their in vitro affinity for $\alpha_1$-adrenoceptors in rat cerebral cortex by the radioligand binding assays using [$^3$H]-prazosin as a specific radioligand. The affinities, described by $K_i$ values (nM) and their dimensionless value p$K_i$, are shown in Table 2. The new synthesized compounds showed different affinity at $\alpha_1$-AR in nano- or micromolar range. A structure–activity relationship can be observed. The most active compounds **19a** ($K_i$ = 4.7 nM) and **21a** possess both 2-methoxyphenylpiperazine fragment and tetramethylene linker (Table 2). Especially, a role of the linker seems to be crucial as butyl derivatives (**19a, 21a**) were much more potent than corresponding hydroxypropyl- (**2a, 6a**) or propyl derivatives (**18a, 20a**). In the case of the most active compounds (**19a, 21a**), a profitable influence of methyl substituent at N3-hydantoin is observed that increased (~5.5-fold) $K_i$-value comparing to the methyl acetate N3-terminal fragment (**21a**). This relationship for N3-substituents is also shown in case of following hydroxypropyl derivatives: 2-methoxy- (**2a, 6a**), 2-ethoxy- (**3a, 7a**) and 2-fluorophenylpiperazine derivatives (**14a, 17a**). Additionally, the results indicated an influence of substituents at phenylpiperazine phenyl ring on the receptor affinity. It is shown that replacement of 2-alkoxy- substituent (**2a, 3a, 6a, 7a**) with 2-halogen (**13a, 14a, 16a, 17a**) or free hydrogen (**12a, 15a**) decreased the affinity at $\alpha_1$-AR. The binding range for halogen substituted- and free phenylpiperazine derivatives is similar. It is contrary to the pharmacophore model of $\alpha_1$-adrenoceptor antagonists which underlines the role of presence of lipophilic substituent (HY-2) at phenylpiperazine.[27]

### 2.2.3. Tests in vivo

All tests in vivo were performed in male Wistar rats using intravenous (iv) administration of tested compounds **2a–9a, 11a–17a, 19a** and **21a** as their water solutions at starting dose of 10 mg/kg. The lower starting doses of 5 m/kg and 2.5 mg/kg were used for weakly soluble compounds (**14a–16a**) and for the most potent $\alpha_1$-AR antagonist (**19a**), respectively.

**2.2.3.1. Influence on the ECG-components.** The effect of a compound on the ECG-components is the key factor for its classification as an antiarrhythmic agent. The effect on the ECG intervals and heart rate was determined for the compounds **2a–9a, 11a–17a**, **19a**, **21a** during the 15-minutes observation period immediately following compound administration. Results are shown in Tables 3 and 4 . All of the compounds, excluding **8a**, tended to slow down the heart rhythm. In particular, compounds **4a**, **7a**, **9a**, **11a–17a** and **21a** significantly decreased the number of cardiac beats per minute. The highest impact was observed for halogen- or unsubstituted phenylpiperazine derivatives **12a–15a**. In the case of the most active $\alpha_1$-AR agents **19a** and **21a**, a statistically significant decrease ($p$ <0.05) was observed only for ester derivative **21a**.

Most of the tested compounds prolonged the P–Q intervals (**2a**, **5a**, **12a**, **14a**, **15a 17a**, **21a**). The strongest effect was observed for compounds with N3-ester terminated fragment, **2a, 15a** and **17a** (9–18%, $p$ <0.01). In the group of ten compounds with higher affinities for $\alpha_1$-AR (p$K_i$ >6.5), six compounds ( **3a, 7a, 8a, 9a, 10a** and **19a**) did not significantly influence the P–Q intervals. Considering the Q–T interval, a tendency towards the prolongation was observed for most of the tested arylpiperazine derivatives. The highest, statistically significant, Q–T prolongation was observed for N3-methylhydantoin derivatives (**12a**, **14a**) and N3-ester (**15a**) whereas active $\alpha_1$-adrenergic receptor antagonists **3a, 7a, 8a, 9a** and **19a** practically did not affect the Q–T intervals at the tested doses. Most of the tested compounds did not affect the QRS complex. Slight, statistically significant, widening was observed only for compounds **15a** and **13a**. The most active $\alpha_1$-AR antagonist **19a** did not significantly influence the ECG-components at its starting dose.

**2.2.3.2. Antiarrhythmic activities in two models of arrhythmia.** Arylpiperazine hydantoin derivatives were tested for their antiarrhythmic properties in two models of arrhythmia, barium chloride- and adrenaline-induced. Results of our previous studies indicated that various hydantoin derivatives have distinct antiarrhythmic activity in barium chloride induced arrhythmia, and an additional introduction of phenylpiperazine moiety is responsible for prophylactic antiarrhythmic activity in adrenaline induced

**Table 3**
Effects of an iv injection of the investigated compounds on the heart rate and ECG intervals in anesthetized male Wistar rats (60 mg thiopental/kg ip)

| Compd | Parameters | Time of observation (min) | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 5 | 15 |
| **2a** | Beats/min | 298 ± 12.5 | 270 ± 11.1 | 274 ± 12.8 | 268 ± 12.6 |
| | P–Q [ms] | 57 ± 1.5 | 64 ± 2.6*** | 60 ± 1.1 | 59 ± 0.4 |
| | Q–T [ms] | 72 ± 3.5 | 104.4 ± 9.2*** | 79 ± 5.9 | 77 ± 2.7 |
| | QRS [ms] | 17 ± 1.4 | 18 ± 2.4 | 17 ± 2.2 | 16 ± 2 |
| **3a** | Beats/min | 285 ± 17.7 | 246 ± 12 | 241 ± 12 | 242 ± 14.2 |
| | P–Q [ms] | 68 ± 6.1 | 75 ± 5.3 | 79 ± 1.8 | 79 ± 0.7 |
| | Q–T [ms] | 103 ± 9 | 109 ± 15 | 114 ± 13.3 | 118 ± 14.4 |
| | QRS [ms] | 15 ± 1.8 | 18 ± 0.6 | 16 ± 1.2 | 17 ± 0.7 |
| **4a** | Beats/min | 329 ± 10.5 | 284 ± 12** | 285 ± 9** | 287 ± 15* |
| | P–Q [ms] | 61 ± 1.2 | 60 ± 2.5 | 61 ± 0.8 | 64 ± 1.7 |
| | Q–T [ms] | 86 ± 4.2 | 86 ± 5.1 | 80 ± 2.3 | 85 ± 7.8 |
| | QRS [ms] | 26 ± 1.9 | 26 ± 2.9 | 24 ± 3.1 | 26 ± 2 |
| **5a** | Beats/min | 320 ± 31.7 | 255 ± 23.1 | 267 ± 19.3 | 290 ± 27.1 |
| | P–Q [ms] | 57 ± 1.5 | 62 ± 1.2* | 59 ± 0.7 | 58 ± 1.2 |
| | Q–T [ms] | 77 ± 3.5 | 87 ± 4.7 | 90 ± 1.1* | 83 ± 2.9 |
| | QRS [ms] | 17 ± 0.7 | 17 ± 0.3 | 17 ± 0 | 17 ± 0.7 |
| **6a** | Beats/min | 292 ± 17.7 | 268 ± 6.2 | 262 ± 29.1 | 261 ± 19.6 |
| | P–Q [ms] | 62 ± 2 | 64 ± 2.3 | 63 ± 0.9* | 63 ± 2 |
| | Q–T [ms] | 72 ± 4 | 98 ± 8.9*** | 76 ± 6 | 74 ± 4.3 |
| | QRS [ms] | 16 ± 2 | 20 ± 2.6 | 18 ± 2.4 | 17 ± 1.4 |
| **7a** | Beats/min | 325 ± 7.9 | 293 ± 10.3 | 281 ± 19.1* | 274 ± 8** |
| | P–Q [ms] | 57 ± 0.6 | 60 ± 2 | 62 ± 3.2 | 60 ± 0.3 |
| | Q–T [ms] | 97 ± 10.1 | 101 ± 10 | 98 ± 12 | 98 ± 11.1 |
| | QRS [ms] | 15 ± 1.3 | 14 ± 0.3 | 15 ± 0.7 | 15 ± 0.3 |
| **8a** | Beats/min | 360 ± 15 | 350 ± 12.8 | 363 ± 7.6 | 362 ± 12.7 |
| | P–Q [ms] | 55 ± 1.3 | 57 ± 1 | 55 ± 1 | 57 ± 1.3 |
| | Q–T [ms] | 82 ± 1.1 | 82 ± 1.4 | 81 ± 0.7 | 82 ± 2 |
| | QRS [ms] | 17 ± 0.7 | 16 ± 0.3 | 15 ± 0.3 | 16 ± 0.3 |
| **9a** | Beats/min | 376 ± 12.6 | 326 ± 16.9** | 316 ± 9.7* | 325 ± 12.1 |
| | P–Q [ms] | 55 ± 1.5 | 59 ± 2.9 | 58 ± 2.1 | 59 ± 3.1 |
| | Q–T [ms] | 75 ± 2.9 | 78 ± 2.6 | 80 ± 2.0 | 78 ± 1 |
| | QRS [ms] | 17 ± 0.8 | 17 ± 1 | 17 ± 0.7 | 16 ± 1 |
| **11a** | Beats/min | 337 ± 10.6 | 261 ± 15.9*** | 294 ± 12.1** | 291 ± 7.6** |
| | P–Q [ms] | 56 ± 1 | 56 ± 2.4 | 57 ± 3.4 | 56 ± 2.1 |
| | Q–T [ms] | 75 ± 4.2 | 82 ± 8.9** | 78 ± 4.7 | 71 ± 1.8 |
| | QRS [ms] | 23 ± 3.7 | 25 ± 2.2 | 23 ± 3.9 | 25 ± 2.5 |

Data represent the mean of 6 experiments ± SEM. Statistical analysis was performed using one-way ANOVA test $^{*}p$ <0.05, $^{**}p$ <0.02, $^{***}p$ <0.01.

model of heart disturbances.[23–25] Therefore these two models of arrhythmia were chosen for primary screening within the present studies. Mechanism of barium chloride-arrhythmia is not fully understood. Barium chloride is considered as the most selective blocker of inward rectifier potassium current $I_{K1}$ (Kir2). $I_{KI}$ blockers prolong atrioventricular (AV) node and ventricular action potential duration (APD) and are effective against various types of ventricular reentrant tachycardias. Moreover, $I_{KI}$ blockers produce membrane depolarization (an effect that slows conduction velocity due to a voltage-dependent inactivation of $Na^+$ channels) and prolongs the QT interval, both actions being proarrhythmic. Furthermore, barium is known to interfere with calcium-mediated inactivation of L-type calcium channels and thus to prolong the open time of the channel, which could contribute to APD and QT interval prolongation. Several lines of evidence[25,32] indicated that ions $Ba^{2+}$ cause an increase of the sodium inward current in Purkinje fibers leading to the heart rhythm disturbances whereas adrenergic receptors are involved in adrenaline-arrhythmia mechanism.[25]

Compounds of generation II and III ( **2a, 3a, 6a–8a, 11a–17a**) were tested for their prophylactic activity in barium induced model of arrhythmia in rat. This drastic arrhythmogen causes progressively increasing disturbances of cardiac rhythm, associated with premature ventricular beats, ventricular tachycardia and ventricular fibrillation, leading to death in 100% of investigated rats in 2–5 min at the absence of any protecting compounds. Most of the tested arylpiperazine derivatives (**2a**, **3a**, **6a**–**8a**, **11a**, **12a** and **15a**–**17a**) of hydantoin were not active in barium chloride induced-arrhythmia assay (Fig. 3). Particularly, all compounds possessing 2-alkoxyphenylpiperazine fragment ( **2a, 3a, 6a, 8a** and **11a**) were totally inactive. Only two compounds, halogenphenylpiperazine derivatives with N3-methyl substituent (**13a** and **14a**), displayed antiarrhythmic properties in barium chloride-model, protecting more than 50% of tested animals against $BaCl_2$-induced heart disturbances and death.

Compounds (**2a–17a**, **19a**, **21a**) were tested for their prophylactic antiarrhythmic activity in adrenaline-induced arrhythmia model in rat.[30,31] Intravenous (iv) injections of adrenaline (20 μg/kg) caused sinus bradycardia (100%), atrioventricular disturbances, ventricular and supraventricular extrasystoles (96%), which led to death of approximately 66% of animals within 10 ± 5 min. Compounds injected 15 min before adrenaline administration, decreased the occurrence of extrasystoles, bigeminy and reduced mortality (Fig. 4). The most active compounds (**2a**, **3a**, **6a–10a, 14a, 19a** and **21a**) were given at starting doses of 2.5–10 mg/kg. They totally protected the animals from the death and all above disturbances. The $ED_{50}$ values were assessed for the most promising compounds (Fig. 4). Most of the compounds in this selected group (**2a, 3a, 6a–10a** and **19a**) displayed $ED_{50}$ values lower than those of tolazoline and propranolol (Fig. 4). The highest activity in this model of arrhythmia was displayed by compound **19a**, which had an $ED_{50}$ value of 0.1 mg/kg. This compound (**19a**), containing 2-methoxyphenylpiperazine moiety attached to N3-methyl hydantoin with butyl linker, had the highest affinity for $\alpha_1$-adrenoceptors (Table 2) among the tested compounds (**2a–18a, 21a**). It was in accordance with our previous observation that affinities for $\alpha_1$-AR are closely associated with antiarrhythmic activities in adrenaline model. To confirm this thesis, quantitative $pK_i$-$ED_{50}$ relationship was calculated using linear regression method (Fig. 5). Results obtained for eight out of ten tested compounds indicated almost a linear correlation ($R^2 = 0.92$, $p$ <0.005). Two compounds (**19a** and **21a**) with the highest affinities (the outliers) were excluded from the correlation analysis.

**2.2.3.3. Influence on the blood pressure.** The hypotensive activity of compounds (**2a–9a**, **12a**, **14a–17a**, **19a**, **21a**) was determined following iv administration at a dose of 2.5–10 mg/kg in normotensive rats. An observation was carried out during the 60 minutes. The results are presented in Figures 6 and 7. Most of the arylpiperazine derivatives of phenytoin (**2a, 6a–9a**, **12a**, **14a–17a**, and **21a**) showed similar behavior during the test, causing sudden decrease of both systolic and diastolic blood pressure in the first minutes after their administration. Then, the blood pressure returned to the baseline level and remained unchanged until the end of the assay. Compounds **3a** (Fig. 6) and **19a** (Fig. 7), 2-methoxyphenylpiperazine derivatives of N3-methylhydantoin differing in the area of alkyl linker, showed a decrease of blood pressure until the end of the assay. Particularly, compound **3a** displayed significant hypotensive properties causing reduction of both, systolic and diastolic pressure during the whole test. Furoylpiperazine derivative **4a** which did not bind to $\alpha_1$-adrenoceptors as well as pyridylpiperazine derivative **5a** did not affect the blood pressure in normotensive rats.

### 2.2.4. hERG-affinity in silico

For the most active compounds (**2a**, **3a**, **6a–10a, 14a, 19a** and **21a**) inhibiting properties at hERG $K^+$ channel were estimated in silico. Affinities for hERG $K^+$ channel were expressed as $logIC_{50}$ values (Table 5). For compounds with chirality centers (**2a**, **3a**, **6a–10a, 14a**), all possible absolute configurations were considered, and mean values of $logIC_{50}$ were calculated as the compounds were synthesized and tested in their racemic forms. Results of the prediction show that all tested phenylpiperazine derivatives (**2a**, **3a**, **6a–10a, 14a, 19a** and **21a**) are likely to possess

**Table 4**
Effects of an iv injection of the investigated compounds on the heart rate and ECG intervals in anesthetized male Wistar rats (60 mg thiopental/kg ip)

| Compd | Parameters | Time of observation (min) | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 5 | 15 |
| **12a** | Beats/min | 322 ± 11.1 | 270 ± 7.5**** | 260 ± 5.3**** | 263 ± 9**** |
| | P–Q [ms] | 57 ± 1.4 | 66 ± 4.9* | 59 ± 1.2 | 59 ± 3.3 |
| | Q–T [ms] | 63 ± 1.5 | 109 ± 7.9**** | 79 ± 5.8* | 74 ± 2.8 |
| | QRS [ms] | 18 ± 1.5 | 23 ± 2.4 | 20 ± 1.5 | 18 ± 0.5 |
| **13a** | Beats/min | 343 ± 23.4 | 279 ± 5.6**** | 278 ± 9**** | 303 ± 12.7** |
| | P–Q [ms] | 57 ± 1.4 | 60 ± 0.8 | 60 ± 1.3 | 60 ± 1.4 |
| | Q–T [ms] | 68 ± 2.8 | 83 ± 5.7 | 76 ± 5.5 | 72 ± 5.7 |
| | QRS [ms] | 15 ± 0.4 | 17 ± 0.3* | 16 ± 0.6 | 16 ± 0.7 |
| **14a** | Beats/min | 322 ± 4.9 | 272 ± 8.9*** | 251 ± 13.7*** | 258 ± 8.6**** |
| | P–Q [ms] | 54 ± 3.1 | 64 ± 3.4 | 62 ± 2.9* | 59 ± 2.9 |
| | Q–T [ms] | 62 ± 1.6 | 81 ± 5.3**** | 74 ± 3.3** | 68 ± 1.9 |
| | QRS [ms] | 15 ± 0.6 | 20 ± 2.4* | 17 ± 0.6 | 16 ± 1.1 |
| | QRS [ms] | 15 ± 1.8 | 18 ± 0.6 | 16 ± 1.2 | 17 ± 0.7 |
| **15a** | Beats/min | 332 ± 10.4 | 246 ± 20.9**** | 261 ± 8.7**** | 272 ± 8.6**** |
| | P–Q [ms] | 57 ± 1 | 72 ± 3.4*** | 65.2 ± 3* | 64 ± 2.6 |
| | Q–T [ms] | 68 ± 4.8 | 97 ± 10.9*** | 81 ± 1.7 | 74 ± 1.6 |
| | QRS [ms] | 21 ± 1.7 | 33 ± 2.4** | 26 ± 3.7 | 22 ± 3.5 |
| **16a** | Beats/min | 315 ± 15.3 | 290 ± 17.5 | 256 ± 21.1* | 268 ± 18.5 |
| | P–Q [ms] | 56 ± 2.3 | 62 ± 4.8 | 63 ± 2.9 | 59 ± 2.6 |
| | Q–T [ms] | 68 ± 3.9 | 76 ± 4.8 | 76 ± 6.2 | 69 ± 3.7 |
| | QRS [ms] | 15 ± 1 | 16 ± 1 | 15 ± 0.5 | 17 ± 0.6 |
| **17a** | Beats/min | 328 ± 1.8 | 284 ± 6.4** | 256 ± 10.2** | 281 ± 13.9*** |
| | P–Q [ms] | 56 ± 2.6 | 66 ± 3.1*** | 61 ± 1.3 | 58 ± 1.5 |
| | Q–T [ms] | 68 ± 3.7 | 86 ± 4.7** | 82 ± 6.3 | 71 ± 3.6 |
| | QRS [ms] | 15.4 ± 0.7 | 17 ± 0.9 | 17 ± 1 | 15 ± 0.5 |
| **19a** | Beats/min | 353 ± 14.4 | 338 ± 15.6 | 333 ± 18.6 | 346 ± 21.2 |
| | P–Q [ms] | 50 ± 3.1 | 50 ± 3.8 | 51 ± 2.9 | 49 ± 3.6 |
| | Q–T [ms] | 84 ± 5.1 | 83 ± 8.6 | 81 ± 6.9 | 86 ± 8.6 |
| | QRS [ms] | 22 ± 1.5 | 22 ± 0.4 | 22 ± 0.4 | 22 ± 1.1 |
| **21a** | Beats/min | 396,7 ± 31,7 | 355,8 ± 23,8* | 354,3 ± 21,0* | 357,8 ± 19,2* |
| | P–Q [ms] | 48,3 ± 5,6 | 62,6 ± 7,8* | 63,3 ± 4,6* | 62,8 ± 9,3* |
| | Q–T [ms] | 62,3 ± 6,9 | 74,6 ± 8,6* | 73,3 ± 9,3* | 75,6 ± 4,8* |
| | QRS [ms] | 19,0 ± 2,8 | 19,6 ± 2,6 | 19,3 ± 1,3 | 19,5 ± 2,5 |

Data represent the mean of 6 experiments ± SEM. Statistical analysis was performed using one-way ANOVA test *$p$ <0.05, **$p$ <0.02, ***$p$ <0.01, ****$p$ <0.001.

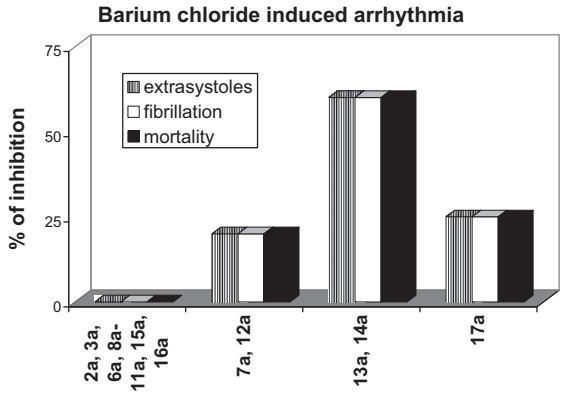
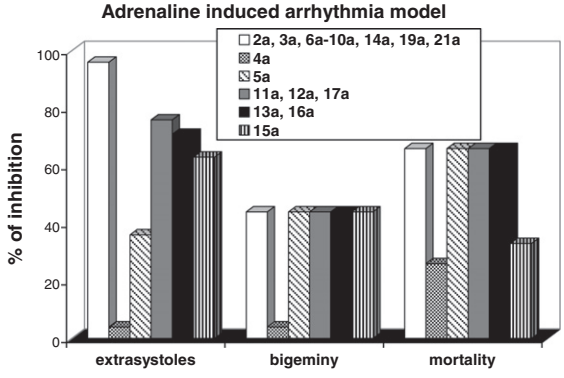



**Figure 3.** Prophylactic antiarrhythmic activity in the barium-induced arrhythmia.




| Cpd | $ED_{50}$ (mg/kg i.v.) | Cpd | $ED_{50}$ (mg/kg i.v.) |
|---|---|---|---|
| **2a** | 0.51 (0.23-1.1) | **10a** | 0.41 (0.21-0.7) |
| **3a** | 0.33 (0.1-1.1) | **14a** | 1.92 (1.09-3.4) |
| **6a** | 0.69 (0.43-1.34) | **19a** | 0.1 (0.01-0.13) |
| **7a** | 0.88 (0.55-1.4) | **21a** | 1.8 (1.55-2.3) |
| **8a** | 0.36 (0.13-1.0) | **Tolazoline** | 3.4 (2.6-3.4) |
| **9a** | 0.34 (0.1-1.15) | **Propranolol** | 1.05 (0.64-1.73) |

**Figure 4.** Prophylactic antiarrhythmic activity in the adrenaline-induced arrhythmia. Each value was obtained from 3 experimental groups. Each group consisted of 6 animals. The $ED_{50}$ values and their confidence limits were calculated according to the methods of Litchfield and Wilcoxon.[30]

hERG $K^+$- blocking potency with significant $logIC_{50}$ values of −8.40 to (−7.72). In our reasoning, two values of $logIC_{50}$ were considered as criteria for potent hERG $K^+$-blocking properties: −6 and −8. Compounds with $logIC_{50}$ <−8 were classified as highly active blockers, whereas those with $logIC_{50}$ >−6 were considered as compounds deprived of hERG $K^+$-blocking abilities. Compounds with $logIC_{50}$ values in the range between −8 to (−6) were considered as weak or moderate hERG-inhibitors. Taking these criteria into account, it seems that all compounds except **14a, 2a** and **3a** could strongly block hERG channels and prolong the QT interval. Structure–activity relationship indicated that the highest hERG-blocking properties were found for 2-methoxyphenylpiperazine derivatives of hydantoin with butyl linker (**21a** > **19a**). These compounds were also among the most active as $\alpha_1$-AR- and antiarrhythmic agents (Table 2, Fig. 4). In the group of compounds with 2-

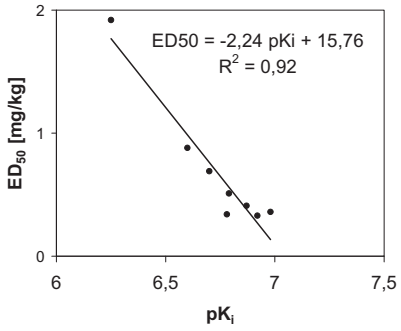



**Figure 5.** Association between $\alpha_1$-adrenoceptor affinity ($pK_i$) and antiarrhythmic properties ($ED_{50}$) in the adrenaline model for compounds **2a**, **3a**, **6a**–**10a** and **14a**. Two compounds with the highest affinities (**19a** and **21a**) were excluded. Quantitative relationship is expressed by linear equation. The correlation was calculated using Pearson worksheet function ($R^2 = 0.92$; $p < 0.005$).

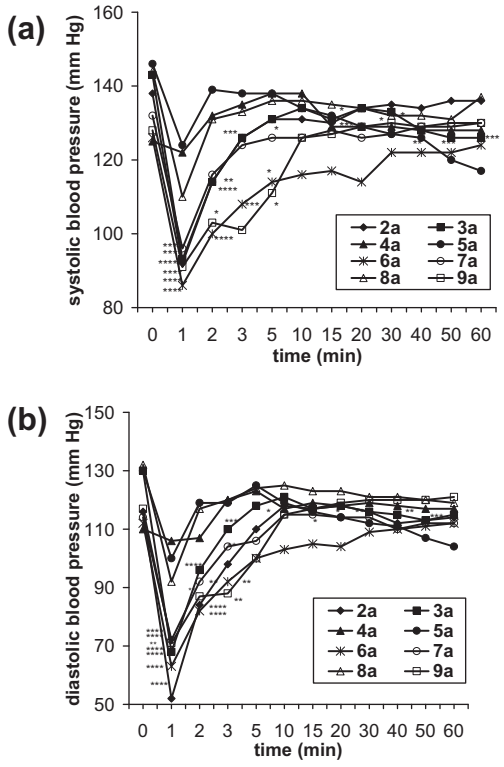



**Figure 6.** Influence on the blood pressure for compounds **2a**–**9a**. Data represent the mean of 6 experiments ± SEM. Statistical analysis was performed using a one-way ANOVA test: $^{*}p < 0.05$, $^{**}p < 0.02$, $^{***}p < 0.01$, $^{****}p < 0.001$.

hydroxypropyl linker (**2a**, **3a**, **6a–10a, 14a**), compounds with branched ester terminate fragment at N3-hydantoin (**10a** > **9a** > **8a**) had higher affinities than those with N3-methyl acetate (**7a > 6a**). Compounds with N3-methyl terminate fragment (**3a > 2a > 14a**) were less potent than the rest of evaluated compounds but their predicted hERG-blocking properties were stronger than theoretical properties of known hERG-antagonist, sertindole ($logIC_{50} = -7.17$) and ibutilide ($logIC_{50} = -6.85$), calculated with the same method. Some influence of the phenylpiperazine phenyl ring substitution was also observed. Thus, the presence of alkoxyl (**2a** and **3a**) seems to induce stronger predicted hERG-blocking effect than that of fluorine (**14a**).

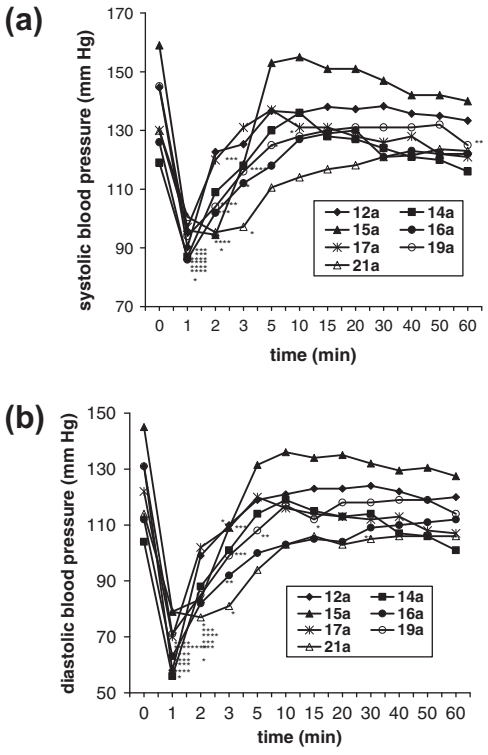



**Figure 7.** Influence on the blood pressure for compounds **12a**–**21a**. Data represent the mean of 6 experiments ± SEM. Statistical analysis was performed using a one-way ANOVA test: $^{*}p < 0.05$, $^{**}p < 0.02$, $^{***}p < 0.01$, $^{****}p < 0.001$.

### 2.3. Estimation of antiarrhythmic properties and SAR-studies

In the tested phenylpiperazine derivatives of phenytoin **2a–21a**, a number of compounds with antiarrhythmic properties were found. Chemical modifications of the lead compound **1** changed pharmacological profile of phenytoin, a member of class Ib of antiarrhythmic drugs. Furthermore, an evolution of pharmacological profile of lead **1** can be observed, as the most active new compounds (**2a**, **3a**, **6a–10a, 19a** or **21a**) displayed more selective activities toward the adrenaline induced arrhythmia, whereas they were totally inactive in the barium chloride one. This could be explained by their stronger $\alpha_1$-adrenolytic properties ($pK_i = 6.60$–$8.33$) in comparison with that of lead **1** ($pK_i = 6.28$).

According to our previous conclusions,[25] phenylpiperazine derivatives of hydantoin displayed properties similar to those of class Ia and III of antiarrhythmic drugs in Vaughan Williams classification.[3–7] The most active compounds slightly decreased the number of cardiac beats per minute. This effect was similar to that of quinidine (class Ia) and it can also correspond to the effect caused by procainamide (Ia) and bretylium (III). A lack of influence or a weak prolongation of the PQ interval caused by phenylpiperazine hydantoin derivatives **2a–21a** was similar to that of bretylium (III), amiodarone (III), sotalol (III) or procainamide (Ia). A tendency of the compounds to prolong time of repolarization (QT interval) was weaker than that of class Ia members (quinidine, procainamide) and some members of class III (amiodarone, sotalol). This property was comparable with that of bretylium (class III). No influence on the QRS complex was observed by most of the tested hydantoin derivatives (**2a–21a**) in contrast to the members of class Ia. Most of the antiarrhythmic drugs belonging to the class Ib and II–IV of Vaughan Wiliams classification do not influence the QRS complex as well.[3]

**Table 5**
Prediction of hERG $K^+$ channel blockage properties in silico

| Compd | Config at CHOH | Config at side chain | hERG $K^+$ Channel Blockage: $\log IC_{50}$ | |
|---|---|---|---|---|
| **2a** | R | — | −7.77 | −7.72 |
| | S | — | −7.66 | |
| **3a** | R | — | −7.88 | −7.76 |
| | S | — | −7.64 | |
| **6a** | R | — | −8.19 | −8.04 |
| | S | — | −7.90 | |
| **7a** | R | — | −8.17 | −8.05 |
| | S | — | −7.94 | |
| **8a** | R | S | −8.14 | −8.08 |
| | S | S | −8.03 | |
| | R | R | −8.04 | |
| | S | R | −8.09 | |
| **9a** | R | S | −8.24 | −8.11 |
| | S | S | −8.13 | |
| | R | R | −8.13 | |
| | S | R | −7.94 | |
| **10a** | R | S | −8.21 | −8.21 |
| | S | S | −8.11 | |
| | R | R | −8.24 | |
| | S | R | −8.28 | |
| **14a** | R | — | −7.66 | −7.59 |
| | S | — | −7.52 | |
| **19a** | — | — | −8.33 | |
| **21a** | — | — | −8.40 | |
| **Ibutilide** | R/S | | −6.85 (−7.55)* | |
| **Sertindole** | — | — | −7.17 (−7.85)* | |
| **Tolazoline** | — | — | −4.21 | |

* Evaluated experimentally.[43,44]

Adrenergic receptors (ARs) play many roles in the myocardium ranging from positive inotropic and chronotropic effects, cardiac preconditioning, arrhythmogenesis and cardiac hyperthrophy. The mechanism of the positive inotropic effect induced by $\alpha_1$-adrenoceptor stimulation is still a matter of debate: the $\alpha_1$-adrenoceptor stimulation-induced activation of phospholipase C (PLC) in the cell membrane, and increased 1,4,5-triphosphate-formation (IP3), which might mediate the release of $Ca^{2+}$ from intracellular stores, and diacylglycerol, which activates protein kinase C (PKC). In addition, sustained $\alpha_1$-adrenoceptors stimulation modulates cardiac repolarizing hERG/$I_{Kr}$ potassium currents via protein kinase A (PKA) and protein kinase C—dependent and PKC-independent phosphorylation sites in hERG. Stimulation of $\alpha_{1A}$-adrenergic receptors produced a reduction in current amplitude of the rapidly activating delayed rectifier $K^+$ current in cardiomyocytes what may induce ventricular arrhythmias, in particular in patients with ischemic heart disease or hereditary long QT syndrome. The possible proarrhythmic action of hERG current inhibition might be counteracted by the prevention of arrhythmia via $\alpha_{1A}$-adrenoceptor blockade, since $\alpha_{1A}$-adrenergic stimulation may induce arrhythmias through modification of hERG channel activation. Thus, hERG currents are a primary target for the pharmacological management of some arrhythmias. Pharmacological blockade of $I_{Kr}$ causes lengthening of the cardiac action potential, which may produce a beneficial class III antiarrhythmic effect of amiodarone and verapamil. Class III antiarrhythmic drugs with hERG channel blocking properties are effective in the treatment of supraventricular and ventricular tachycardia. From the other side reduction in hERG currents due to either genetic defects or adverse effects can lead to hereditary or acquired long QT syndromes characterized by action potential prolongation, lengthening of the Q–$T_c$ interval on the surface ECG, and an increased risk for 'torsade the pointes' arrhythmias and sudden death. In several cardiovascular drugs such as prazosin, doxazosin, terazosin or carvedilol hERG current block does not generally lead to severe cardiac arrhythmias with the risk of sudden cardiac death which may be due to additional pharmacological effects on ion currents or adrenergic receptors.[33–36]

Although our theoretical studies on hERG inhibition properties of the phenylpiperazine derivatives of phenytoin have not been compared with corresponding experimental data, some effects of the compounds in tests in vivo can suggest their influence on hERG $K^+$ currents. A prolongation of the QT interval may be the main experimental evidence for hERG $K^+$ blocking action in the group of the tested compounds. Results of primary tests in vivo in rats indicated that most of the phenylpiperazine hydantoin derivatives (**2a**–**21a**) tend to prolong the QT-interval (Tables 3 and 4). This observation is in agreement with results of our studies in silico which ascribed hERG-inhibition properties to all tested compounds (**2a**, **3a**, **6a–10a, 14a, 19a** and **21a**) with significant $IC_{50}$ values ($\log IC_{50} < -7$). The order of ability to prolong the QT interval in Wistar rats was as follows: **14a** ⩾ **2a** ≈ **6a** > **10a** ≈ **21a** > **3a** ≈ **9a** > **7a** > **19a** ≈ **8a**. Interestingly, compound **14a** caused the distinctly strongest prolongation of the QT interval, but its predicted potency to diminish hERG $K^+$ currents was relatively lower than those calculated for the other compounds (Table 5). In turn, the most potent hERG-blocker in silico, **21a**, slightly prolonged the QT interval in vivo (18–21%, $p < 0.05$, Table 4), and compound **19a** ($\log IC_{50} = -8.33$) did not significantly influenced this interval. Observed pharmacological properties of tested compounds could be partially explained by both, hERG-action and $\alpha_1$-addrenoceptor antagonistic properties. Compound **14a** is a significantly weaker $\alpha_1$-adrenoceptor antagonist than **21a** and much weaker than compound **19a**. As the results of hERG-blocking properties in silico have only theoretical meaning, it is highly probable that a direct hERG-blocking potency[34] of compound **14a** in real conditions is higher than those of **21a** and **19a**. In the context of interactions between adrenergic system and hERG currents, the real hERG-blocking properties of compound **14a** could be an effect of synergistic action of its direct hERG inhibition (suggested by the studies in silico) and partial indirect hERG -inhibition by adrenergic receptors. Our previous studies for compound **14a** indicated its only moderate, antagonistic properties at $\alpha_{1A}$-adrenoceptors ($pA_2 = 6.05$).[29] Thus, in the presence of compound **14a**, in contrary to the potent antagonists **21a** and **19a**, it is possible some stimulation of $\alpha_{1A}$-adrenoceptors induced by native ligands in the rat heart.

Therefore, an additional hERG-inhibition action might be caused by following results of $\alpha_{1A}$-adrenoceptor signal transduction pathways: (i) activation of PKC and PKA, and (ii) a decrease of concentration of PIP2, a phospholipid enhancing hERG $K^+$ in myocytes,[33,34] which is converted into IP3 during $\alpha_{1A}$-adrenoceptors action. In the case of **21a**, and particularly, **19a**, the blockade of $\alpha_1$-ARs does not produce an activation of PKC and PKA within the $\alpha_1$-AR transduction pathways, and in consequence, it restores higher concentration of PIP2. The higher concentration of PIP2 may enhance hERG currents to cause an effect opposite to direct hERG inhibition action which is hypothetized for compounds **19a** and **21a**, based on results of our studies in silico. Thus, their actual hERG-inhibitory effect may be weaker than that of compound **14a**, what can be observed as a much slighter QT-prolongation in the case of **21a**, and particularly, in the case of **19a**. However, the data coming from our studies are not sufficient to confirm or reject this hypothesis, particularly, as the investigated compounds can be involved into other interactions with functional proteins contributing to the cardiovascular regulation of the heart rhythm. Especially, the experimental studies on hERG-inhibition in this chemical group could explain more and we will focus on them in the future.

Interestingly, most of the tested phenylpiperazine derivatives **2a–21a** showed higher antiarrhythmic potency than that of known $\alpha_1$-adrenolytic, tolazoline (Table 5) which does not involve hERG $K^+$ channels in its action. As we know the hERG $K^+$ blockade could modulate the antiarrhythmic effect of an inhibition of $\alpha_1$-adrenoceptors in cardiovascular system. The discovery of adrenergic mechanisms of hERG channel regulation as well as the development of strategies to enhance hERG currents and to modify intracellular hERG protein processing may provide novel antiarrhythmic options in the repolarization disorders.

The obtained results of tests in vivo, and in silico showed that antiarrhythmic properties of the tested phenylpiperazine derivatives **2a–21a** were not homogenous. At least two groups of promising antiarrhythmic agents can be selected including 2-alkoxyphenylpiperazine derivatives (**2a**, **3a**, **6a–10a, 19a** and **21a**) and 2-halogenphenylpiperazine derivatives (**13a, 14a**). In the first group, the antiarrhythmic activity arises mainly from $\alpha_1$-adrenoceptor antagonistic action of the compounds. Although their predicted strong hERG $K^+$ blocking properties could affected the antiarrhythmic abilities, it can be confirmed only in the case of compounds **2a**, **6a** and **21a** which caused the QT-prolongation in rats in vivo (Table 3 and 4). In this context, the most of compounds **2a**, **3a**, **6a–10a, 19a** and **21a** displayed highly selective antiarrhythmic action in the model of adrenaline induced arrhythmia distinctly corresponding to their individual affinities for $\alpha_1$-AR. However they were not active in the $BaCl_2$-induced arrhythmia and also weakly influenced the time of repolarisation (QT-interval). The derivatives of 2-halogenophenylpiperazine (**13a, 14a**), possessing moderate affinities for $\alpha_1$-AR (p*K*i <6.5), did not display selective antiarrhythmic activities. Interestingly, in the case of compounds **13a** and **14a**, a decrease of activities in the adrenaline model of arrhythmia was accompanied by the significant prophylactic antiarrhythmic activities in $BaCl_2$-induced arrhythmia. Compounds **13a** and **14a** showed also a distinct prolongation of the QT interval, much higher than that of alkoxyphenylpiperazine derivatives (**2a**, **3a**, **6a–10a, 19a** and **21a**). .

## 3. Conclusion

The performed studies allowed us to identify and gain information on the mechanisms of the antiarrhythmic activity of a series of phenylpiperazine derivatives **2a–21a**. Among new synthesized compounds **12a–21a**, 2-alkoxyphenylpiperazine derivatives with butyl linker (**19a** and **21a**) were the most potent $\alpha_1$-adrenoceptor antagonists with the affinities in low-nanomolar range. Most of the tested 2-alkoxyphenylpiperazine derivatives displayed strong and selective antiarrhythmic activities in the adrenaline induced arrhythmia model. Their effective dose $ED_{50}$, lower than that of tolazoline, showed a significant correlation with affinities for $\alpha_1$-AR (p$K_i$) evaluated in radioligand binding assays. Analysis of the pharmacological profile of the most active compounds was performed based on the radioligand binding assay, tests in vivo in male Wistar rats and theoretical studies in silico on their hERG $K^+$ blocking properties. The results indicated that both $\alpha_1$-AR- and hERG $K^+$-antagonistic properties could determine the pharmacological profile of the compounds, which has some similarities to class III and Ia of the antiarrhythmic agents. The presented studies proposed two levels of primary pharmacological screening which seems to be sufficient for the successful selection of promising antiarrhythmic agents for further pharmacological assays. Based on this, 1-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione hydrochloride (**19a**) was found as the most potent antiarrhythmic agent suitable for more advanced pharmacological assays.

## 4. Experimental

### 4.1. Chemistry

$^1$H NMR spectra were recorded on a Varian Mercury VX 300 MHz PFG instrument (Varian Inc., Palo Alto, CA, USA) in DMSO-$d_6$ at ambient temperature using the solvent signal as an internal standard. IR spectra were recorded on a Jasco FT/IR-410 apparatus using KBr pellets and are reported in $cm^{-1}$. Thin-layer chromatography was performed on pre-coated Merck Silica Gel 60 $F_{254}$ aluminium sheets, the used solvent systems were: (I) toluene/acetone 40:3; (II) toluene/acetone/methanol 15:5:1; (III) toluene/acetone/methanol 5:5:1. Melting points were determined using Mel-Temp II apparatus and are uncorrected. Elemental analyses were carried out on a Vario EL III Model Elemental Analyzer. Microwave aided reactions were performed in microwave reactor for organic synthesis 'Plazmatronika' in solvents or in household microwave oven 'Samsung M1618' in case of solvent-free condition. Commercial phenylpiperazines (**28**–**30**, **35** and **36**) were used. Synthesis of compounds **2**–**11**, **22**–**27** and **34** was described within other works.[25,28]

#### 4.1.1. General procedure for bromoalkyl derivatives (31–33)

*Method A*: A mixture of the appropriate N3-substituted derivative of 5,5-diphenylhydantoin **23–25** (15 mmol), TEBA (2 mmol, 0.45 g) and potassium carbonate (44 mmol, 6 g) in acetone (30 mL) was stirred under reflux for 30 min, then an appropriate dibromoalkane (20 mmol) in acetone (15 mL) was added. The mixture was stirred at room temperature for 64–92 h, according to a progress controlled by TLC (I). Then, inorganic precipitate was filtered off, the mother liquor was evaporated. The residue was crystallized with methanol or isopropanol.

*Method B*: A suspension of the appropriate N3-substituted derivative of 5,5-diphenylhydantoin **23–25** (15 mmol), TEBA (2 mmol, 0.45 g) and potassium carbonate (44 mmol, 6 g) in acetone (30 mL) was prepared in the oval flask 'Plazmatronika'. The suspension was placed in the microwave reactor, stirred under reflux for 15 min using 45% of power of irradiation at 40–45 °C. Then, an appropriate dibromoalkane (20 mmol) in acetone (15 mL) was added. The mixture was stirred under microwave irradiation using following program: 1× cycle 1 (time: 30 min, temperature: 40–50 °C, max power: 40%) and 4× cycles 2 (time: 30 min, temperature: 40–60 °C, max power: 40%) according to a progress controlled by TLC (I). Then, inorganic precipitate was filtered off, the filtrate

was evaporated and the residue was crystallized with methanol or isopropanol.

**4.1.1.1. 1-(3-Bromopropyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione (31).** *Method A*: The reactants were stirred for 72 h. Pure white crystals of product **31** were crystallized with methanol. Yield 74%; mp 116–117 °C, TLC: $R_f$ (I):0.67. Anal. Calcd for $C_{19}H_{19}BrN_2O_2$: C, 58.93; H, 4.95; N, 7.23. Found: C, 59.01; H, 4.91; N, 7.15. $^1$H NMR $\delta$ (ppm): 1.22 (qu, $J$ = 7.44 Hz, 2H, N1-$CH_2$-$CH_2$), 2.98 (s, 3H, N-$CH_3$), 3.15 (t, $J$ = 6.70 Hz, 2H, N1-$CH_2$), 3.40–3.45 (t def., 2H, Br-$CH_2$), 7.20–.44 (m, 10 H, 2× Ph)

**4.1.1.2. 1-(4-Bromobutyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione (32).** Method A and method B were used. The reactants were stirred for 64 h (method A). Pure white crystals of product **32** were crystallized with methanol. Yield 57% (method A), 66% (method B); mp 120–122 °C, TLC: $R_f$ (I):0.62. Anal. Calcd for $C_{20}H_{21}BrN_2O_2$: C, 59.86; H, 5.27; N, 6.98. Found: C, 59.80; H, 5.30; N, 7.12. $^1$H NMR $\delta$ (ppm): 0.82 (qu, $J$ = 7.60 Hz, 2H, N1-$CH_2$-$CH_2$), 1.38 (qu, $J$ = 6.54 Hz, 2H, Br-$CH_2$-$CH_2$), 2.97 (s, 3H, N-$CH_3$), 3.19 (t, $J$ = 6.70 Hz, 2H, N1- $CH_2$), 3.30–.35 (m, 2H, Br-$CH_2$), 7.20–7.25 (m, 4H, 2× Ph-3,5-H), 7.37–7.45 (m, 6H, 2× Ph-2,4,6-H)

**4.1.1.3. Methyl 2-(1-(3-bromopropyl)-2,4-dioxo-5,5-diphenylimidazolidin-3-yl)propionate (33).** *Method A:* The reactants were stirred for 92 h. Pure white crystals of product **33** were crystallized with *i*-PrOH. Yield 75%; mp 89–90 °C, TLC: $R_f$ (I):0.64. Anal. Calcd for $C_{22}H_{23}BrN_2O_4$: C, 57.53; H, 5.05; N, 6.10. Found: C, 57.68; H, 5.01; N, 6.13. $^1$H NMR $\delta$ (ppm): 1.18-1.33 (m, 2H, N1-$CH_2$-$CH_2$), 1.47 (d, $J$ = 7.18 Hz, 3H, CHC$H_3$), 3.15 (t, $J$ = 6.70 Hz, 2H, N1-$CH_2$), 3.41–3.46 (t def., 2H, Br-$CH_2$), 3.65 (s, 3H, $OCH_3$), 4.86 (q, $J$ = 7.18 Hz, 1H, C$H$CH$_3$), 7.22–7.28 (m, 4H, 2× Ph-3,5-H), 7.42–7.51 (m, 6H, 2× Ph-2,4,6-H)

### 4.1.2. General procedure for N1-phenylpiperazinehydroxy propyl-5,5-diphenylhydantoin derivatives (12a–17a)

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 earlier.[21]

*Method C*: A mixture of oxiran derivative **26** or **27** (5 mmol) and suitable phenylpiperazine **28–30** (5 mmol) was dissolved in $CH_2Cl_2$ (20 ml) in a flat-bottomed 50 ml flask. The solvent was evaporated. The homogeneous residue covered with a small inverted funnel. The flask was placed in a standard household microwave oven and irradiated for several 1–2 min-cycles using various irradiation powers, according to TLC-controlled progress of the reaction. If longer heating did not cause any increase of the product spot (TLC) intensity, the process was interrupted. The reaction mixture was crystallized from MeOH to afford pure compounds (**12**–**17**). The compounds (**12**–**17**) were converted into hydrochlorides (**12a**–**17a**) by saturation with gaseous HCl in dry methanol solution (50 mg/mL) until acidic pH. The solutions were cooled at 4 °C overnight for precipitation. The solids of pure hydrochlorides were separated by filtration.

*Method D*: A mixture of oxiran derivative **26** or **27** (5 mmol) and suitable phenylpiperazine **28–30** (5 mmol) was placed in a flat-bottomed 50 ml flask immediately under microwave irradiation to melt at first. Then, the procedure went according to the method C.

**4.1.2.1. 1-[2-Hydroxy-3-(4-phenylpiperazin-1-yl)-propyl]-2,4-dioxo-3-methyl-5,5-diphenylimidazolidine hydrochloride (12a).** *Method C:* A mixture of **26** (1.55 g) and N-phenylpiperazine (0.81 g) was irradiated (450 W) for 9 min (3 × 3 min). The irradiation was continued for the next 4 min at 600 W(2 × 2 min). White crystals of compound **12**. Yield 84%, mp 136–138 °C; TLC: $R_f$ (III): 0.71; Anal. Calcd for $C_{29}H_{32}N_4O_3$: C, 71.81; H, 6.66; N, 11.56. Found: C, 71.86; H, 6.64; N, 11.27. $^1$H NMR $\delta$ (ppm) of base **12**: 1.98–2.01 (dd, $J_1$ = 6.05 Hz, $J_2$ = 3.86 Hz, 2H, Pp-$CH_2$), 2.18–2.21 (t, $J$ = 4.81, 4H, Pp-2,6-H), 2.90–3.01(m, 7H, Pp-3,5-H, C$H$OH, N1-$CH_2$); 3.31 (s, 3H, N-$CH_3$); 4.43–4.45 (d, $J$ = 4.95 Hz, 1H, OH) 6.70–6.75 (t def., 1H, PpPh-4-H), 6.84–6.87 (d def, 2H, PpPh-2,4-H), 7.13–7.18 (t def., 2H, PpPh-3,4-H); 7.19–7.26 (m, 4H, 2× Ph-3,5-H); 7.41–7.47 (m, 6H, 2× Ph-2,4,6-H).

White crystals of **12a**. Yield 38%, mp 266–268 °C, TLC: $R_f$(III): 0.71. Anal. Calcd for $C_{29}H_{33}N_4O_3Cl$: C, 66.85; H, 6.38; N, 10.75. Found: C, 66.90; H, 6.36; N, 10.68. $^1$H NMR for **12a** $\delta$ (ppm): 2.64–2.95 (m, 7H, Pp-$CH_2$, Pp-2,6-H, C$H$OH), 2.98 (s, 3H, N3-$CH_3$), 3.14–3.45 (m, 4H, Pp-3,5-H), 3.66–3.77 (m, 2H, N1-$CH_2$), 4.22 (br s, 1H, OH), 6.81 (t, $J$ = 7.20 Hz, 1H, PpPh-4-H), 6.93 (d, $J$ = 8.30 Hz, 2H, PpPh-2,6-H), 7.03–7.05 (m, 2H, 2×Ph-4-H), 7.21–7.37 (m, 4H, 2×Ph-2,6-H), 7.44–7.49 (m, 6H, 2×Ph-3,5-H, PpPh-3,5-H), 9.80 (br s, 1H, $NH^+$). IR: 3242 (OH), 2944, 2895, 2848 (CH), 2541 ($NH^+$); 1772 (C2═O), 1716 (C4═O), 1599 (Ar).

**4.1.2.2. 1-[3-(4-(2-Chlorophenyl)-piperazin-1-yl)-2-hydroxypropyl]-3-methyl-2,4-dioxo-5,5-diphenylimidazolidine hydrochloride (13a).** *Method C:* A mixture of **26** (1.55 g) and 2-Cl-phenylpiperazine (0.9 g) was irradiated (450 W) for 6 min (3 × 2 min). The irradiation was continued for the next 6 min at 600 W(3 × 2 min). White crystals of compound **13**. Yield 69%, mp 148–149 °C; TLC: $R_f$(III): 0.74; Anal. Calcd for $C_{29}H_{31}ClN_4O_3$: C, 67.11; H, 6.02; N, 10.79. Found: C, 67.42; H, 6.03; N, 10.82. $^1$H NMR for **13** $\delta$ (ppm): 1.95–2.07 (m, 2H, Pp-$CH_2$), 2.22 (br s, 4H, Pp-2,6-H), 2.80 (br s, 4H, Pp-3,5-H), 2.90 (s, 1H, C$H$OH), 2.97 (s, 3H, N3-$CH_3$), 3.28–3.35 (t, $J$ = 9.60 Hz, 2H, N1-$CH_2$), 4.43–4.44 (d, $J$ = 5.00 Hz, 1H, OH), 6.96–7.02 (m, 1H, PpPh-4-H), 7.07–7.11 (m, 2H, 2× Ph-4-H), 7.22–7.27 (m, 4H, 2× Ph-2,6-H), 7.32–7.37 (m, 1H, PpPh-6-H), 7.41–7.47 (m, 6H, 2×Ph-3,5-H, PpPh-3,5-H).

White crystals of **13a**. Yield 89%, mp 280–281 °C, TLC: $R_f$(III): 0.74. Anal. Calcd for $C_{29}H_{32}Cl_2N_4O_3$: C, 62.70; H, 5.81; N, 10.09. Found: C, 62.70; H, 5.83; N, 9.96. $^1$H NMR for **13a** $\delta$ (ppm): 2.68–2.71 (m, 1H, C$H$OH), 2.85–3.07 (m, 6H, $PpCH_2$, Pp-2,6-H), 2.99 (s, 3H, N3-$CH_3$), 3.14–3.22 (d def., 2H, N1$CH_2$), 3.26–3.47 (m, 4H, Pp-3,5-H), 5.63 (br s, 1H, OH), 7.11–7.16 (m, 2H, PpPh-4,6-H), 7.22–7.34 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H), 7.42–7.49 (m, 6H, 2× Ph-2,4,6-H), 9.60 (br s, 1H, $NH^+$). IR: (KBr) ($cm^{-1}$): 3230 (OH), 2984 (CH), 2534 ($NH^+$), 1775 (C2═O), 1718 (C4═O), 1586 (Ar).

**4.1.2.3. 1-[3-(4-(2-Fluorophenyl)-piperazin-1-yl)-2-hydroxypropyl]-3-methyl-2,4-dioxo-5,5-diphenylimidazolidine hydrochloride (14a).** *Method D:* A mixture of **26** (1.55 g) and 2-fluorophenylpiperazine (0.93 g) was irradiated (450 W) for 3 min. The irradiation was continued for the next 4 min at 300 W (2 × 2 min). White crystals of compound **14**. Yield 61%, mp 149–150 °C; TLC: $R_f$(III): 0.82; Anal. Calcd for $C_{29}H_{31}FN_4O_3$: C, 69.30; H, 6.22; N, 11.15. Found: C, 69.29; H, 6.20; N, 11.15. $^1$H NMR for **14** $\delta$ (ppm): 1.94–2.06 (m, 2H, Pp-$CH_2$), 2.21 (br s, 4H, Pp-2,6-H), 2.83 (br s, 4H, Pp-3,5-H), 2.97 (m, 4H, C$H$OH, N3-$CH_3$), 3.21–3.37 (m, 2H, N1-$CH_2$); 4.41 (d, $J$ = 4.95 Hz, 1H, OH) 6.93–7.10 (m, 4H, PpPh-4,6-H, 2×Ph-4-H), 7.22–7.27 (m, 4H, 2× Ph-2,6-H), 7.41–7.47 (m, 6H, 2×Ph-3,5-H, PpPh-3,5-H).

White crystals of **14a**. Yield 91%, mp 278–279 °C, TLC: $R_f$(III): 0.82. Anal. Calcd for $C_{29}H_{32}ClFN_4O_3$: C, 64.62; H, 5.98; N, 10.39. Found: C, 64.15; H, 5.93; N, 10.30. $^1$H NMR for **14a** $\delta$ (ppm): 2.63–2.80 (m, 1H, C$H$OH), 2.82–3.18 (m, 6H, Pp-$CH_2$, Pp-2,6-H), 2.98 (s, 3H, N3-$CH_3$), 3.27–3.42 (m, 6H, N1-$CH_2$, Pp-3,5-H), 5.83 (br s, 1H, OH), 6.98–7.90 (m, 8H, PpPh-4,6-H, 2×Ph-2,4,6-H),

7.42–7.48 (m, 6H, PpPh-3,5-H, 2×Ph-3,5-H), 10.17 (br s, 1H, $NH^+$). IR: (KBr) ($cm^{-1}$): 3455, 3163 (OH), 2945, 2831 (CH), 2634 ($NH^+$), 1767 (C2═O), 1713 (C4═O), 1599 (Ar).

**4.1.2.4. (1-(2-Hydroxy-3-(4-phenylpiperazin-1-yl)-propyl)-2,4-dioxo-5,5-diphenylimidazolidin-3-yl)-acetic acid methyl ester hydrochloride (15a).** *Method D:* A mixture of **27** (1.9 g) and N-phenylpiperazine (0.81 g) was irradiated at 450 W for 2 min The irradiation was continued for the next 6 min at 300 W (3 × 2 min). White crystals of compound **15**. Yield 44%, mp 132–134 °C; TLC: $R_f$(III): 0.79. Anal. Calcd for $C_{31}H_{34}N_4O_5$: C, 68.61; H, 6.32; N, 10.32. Found: C, 68.39; H, 6.29; N, 10.11. $^1$H NMR for **15** $\delta$ (ppm): 1.98 (d, $J$ = 6.05 Hz, 2H, Pp-$CH_2$), 2.15–2.30 (m, 4H, Pp-2,6-H), 2.93–2.96 (m, 5H, Pp-3,5-H, C*H*OH), 3.32 (s, 2H, N1-$CH_2$,), 3.67 (s, 3H, $OCH_3$), 4.34 (s, 2H, N3-$CH_2$), 4.47 (d, $J$ = 5.00 Hz, 1H, OH), 6.73 (t, $J$ = 7.30 Hz, 1H, PpPh-4-H), 6.86 (d, $J$ = 8.0 Hz, 2H, PpPh-2,6-H), 7.16 (t, $J$ = 7.90 Hz, 2H, 2× Ph-4-H), 7.28–7.40 (m, 4H, 2× Ph-2,6-H), 7.40–7.55 (m, 6H, 2×Ph-3,5-H, PpPh-3,5-H).

White crystals of **15a**. Yield 94%, mp 210–211 °C, TLC: $R_F$(III): 0.79. Anal. Calcd for $C_{31}H_{35}ClN_4O_5$: C, 64.30; H, 6.09; N, 9.68. Found: C, 64.25; H, 6.09; N, 9.33. NMR for **15a** $\delta$ (ppm): 2.76 (br s, 3H, Pp-$CH_2$, C*H*OH), 2.98–3.15 (m, 4H, Pp-2,6-H), 3.35–3.43 (m, 4H, Pp-3,5-H), 3.64–3.75 (m, 2H, N1-$CH_2$), 3.66 (s, 3H, $OCH_3$), 4.20–4.23 (d def., 1H, OH), 4.37 (s, 2H, N3-$CH_2$), 6.82–6.87 (t def., 1H, PpPh-4-H), 6.94–6.97 (d def., 2H, PpPh-2,6-H), 7.21–7.36 (m, 6H, 2×Ph-2,4,6-H), 7.47–7.53 (m, 6H, PpPh-3,5-H, 2× Ph-3,5-H), 10.09 (br s, 1H, $NH^+$). IR: (KBr) ($cm^{-1}$): 3388 (OH), 2980, 2950 (CH), 2533 ($NH^+$), 1775 (C2═O), 1749 (C═O ester), 1719 (C4═O), 1597 (Ar)

**4.1.2.5. (1-(3-(4-(2-Chlorophenyl)-piperazin-1-yl)-2-hydroxy-propyl)-2,4-dioxo-5,5-diphenylimidazolidin-3-yl)-acetic acid ethyl ester hydrochloride (16a).** *Method D:* A mixture of **27** (1.9 g) and *N*-2-chlorophenylpiperazine (0.9 g) was irradiated at 450 W for 3 min The irradiation was continued for the next 6 minutes at 300 W (3 × 2 min). White crystals of compound **16**. Yield 49%, mp 120–122 °C; TLC: $R_f$(III): 0.73. Anal. Calcd for $C_{31}H_{33}ClN_4O_5$: C, 64.52; H, 5.76; N, 9.72. Found: C, 64.58; H, 5.72; N, 9.30. $^1$H NMR for **16** $\delta$ (ppm): 2.02 (d def., 2H, Pp-$CH_2$), 2.23 (br s, 4H, Pp-2,6-H), 2.80 (br s, 4H, Pp-3,5-H), 3.01–3.07 (m, 1H, C*H*OH), 3.21–3.32 (m, 2H, N1-$CH_2$,), 3.68 (s, 3H, $OCH_3$), 4.37 (s, 2H, N3-$CH_2$), 4.45 (d, $J$ = 4.70 Hz, 1H, OH), 6.96 (t, $J$ = 7.50 Hz, 1H, PpPh-4-H), 7.08 (d, $J$ = 8.00 Hz, 1H, PpPh-6-H), 7.23–7.37 (m, 6H, 2× Ph-2,4,6-H), 7.41–7.59 (m, 6H, 2×Ph-3,5-H, PpPh-3,5-H).

White crystals of **16a**. Yield 97%, mp 190-192 °C, TLC: $R_f$(III): 0.73. Anal. Calcd for $C_{31}H_{34}Cl_2N_4O_5$: C, 60.69; H, 5.59; N, 9.13. Found: C, 60.45; H, 5.49; N, 9.20. $^1$H NMR for **16a** $\delta$ (ppm): 2.79 (br s, 3H, Pp-$CH_2$, C*H*OH), 2.99–3.16 (m, 4H, Pp-2,6-H), 3.22–3.43 (m, 4H, Pp-3,5-H), 3.36 (s, 2H, N1-$CH_2$), 3.66 (s, 3H, $OCH_3$), 4.26 (s., 1H, OH), 4.37 (s, 2H, N3-$CH_2$), 7.06–7.16 (m, 2H, PpPh-4,6-H), 7.29–7.39 (m, 5H, 2×Ph-3,5-H, PpPh-3-H), 7.41–7.50 (m, 7H, 3×Ph-2,4,6-H, PpPh-5-H), 10.08 (br s, 1H, $NH^+$). IR (KBr) ($cm^{-1}$): 3455, 3257 (OH), 2952 (CH), 2580 ($NH^+$), 1775 (C2═O), 1747 (C═O ester), 1721 (C4═O), 1620, 1588 (Ar).

**4.1.2.6. (1-[3-(4-(2-Fluorphenyl)-piperazin-1-yl)-2-hydroxypropyl)-2,4-dioxo-5,5-diphenylimidazolidin-1-yl)-acetic acid ethyl ester hydrochloride (17a).** *Method D:* A mixture of **27** (1.9 g) and N-2-fluorophenylpiperazine (0.93 g) was irradiated at 450 W for 4 min The irradiation was continued for the next 6 minutes at 300 W (2 × 3 min). White crystals of compound **17**. Yield 36%, mp 87–88 °C; TLC: $R_f$(III): 0.81. Anal. Calcd for $C_{31}H_{33}FN_4O_5$: C, 66.41; H, 5.93; N, 10.00. Found: C, 66.92; H, 6.09; N, 10.26. $^1$H NMR for **17** $\delta$ (ppm): 2.00 (d, $J$ = 6.05 Hz, 2H, Pp-$CH_2$), 2.23 (br s, 4H, Pp-2,6-H), 2.84 (br s, 4H, Pp-3,5-H), 2.96–3.08 (m, 1H, C*H*OH), 3.22–3.36 (m, 2H, N1-$CH_2$,), 3.68 (s, 3H, $OCH_3$), 4.34 (s, 2H, N3-$CH_2$), 4.44 (br s, 1H, OH), 6.88–7.11 (m, 4H, PpPh-4,6-H, 2× Ph-4-H), 7.30–7.38 (m, 4H, 2× Ph-2,6-H), 7.40–7.49 (m, 6H, 2×Ph-3,5-H, PpPh-3,5-H).

White crystals of **17a**. Yield 89.4%; mp 209–210 °C; TLC: $R_f$(III): 0.81. Anal. Calcd for $C_{31}H_{34}ClFN_4O_5 \times H_2O$: C, 60.53; H, 5.89; N, 9.11. Found: C, 60.39; H, 5.84; N, 9.11. $^1$H NMR for **17a** $\delta$ (ppm): 2.76–2.86 (m, 4H, Pp-$CH_2$, Pp-2,6-$H_a$), 3.04–3.14 (m, 5H, Pp-2,6-$H_e$, C*H*OH, Pp-3,5-$H_a$) 3.17–3.37 (m, 4H, Pp-3,5-$H_e$, N1-$CH_2$), 3.66 (s, 3H, $OCH_3$), 4.37 (s, 2H, N3-$CH_2$), 5.70 (br s, 1H, OH), 6.99–7.19 (m, 4H, PpPh-4,6-H, 2×Ph-4-H), 7.29–7.36 (m, 4H, 2×Ph-2,6-H), 7.47–7.53 (m, 6H, 2×Ph-3,5-H, PpPh-3,5-H), 10.09 (br s, 1H, $NH^+$). IR (KBr) ($cm^{-1}$): 3450, 3244 (OH), 2951 (CH), 2586 ($NH^+$), 1776 (C2═O), 1745 (C═O ester), 1722 (C4═O), 1612 (Ar).

### 4.1.3. General procedure for phenylpiperazine derivatives 18a–21a

An appropriate phenylpiperazine (5 mmol), $K_2CO_3$ (2 g), TEBA (15 g) were suspended in acetone (20 ml) and stirred under reflux for 30 min. *N*-1-bromoalkyl-5,5-diphenylhydantoin derivatives **31**–**34** (6 mmol) dissolved in acetone (15 ml) were added. The mixture was stirred at room temperature for 72–110 h. The inorganic precipitate was separated by filtration and washed with acetone. Combined filtrates were evaporated. The obtained residue was dissolved in methylene chloride (15 ml), washed with 1% solution of HCl (2 × 15 ml) and water (2 × 10 ml), dried with $Na_2SO_4$ anhydrous.

*Method E*: After evaporation of the solvent, the pure compounds (**20** and **21**) were obtained from the residue by crystallization with methanol. Compounds (**20** and **21**), obtained as crystals in basic forms were converted into hydrochlorides using the method described for **12a–17a**.

*Method F*: For compounds (**18** and **19**) which did not crystallize with methanol, the alcohol solution was immediately saturated with gaseous HCl to give a precipitate of compounds **18a** and **19a** in hydrochloride form.

**4.1.3.1. 1-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione hydrochloride (18a).** *Method F*: N-2-methoxyphenylpiperazine (0.96 g) and compound **31** (2.30 g) were stirred for 90 h. White crystals of compound **18a**. Yield 69%, mp 266–267 °C, TLC: $R_f$ (II): 0.74. Anal. Calcd for $C_{30}H_{35}ClN_4O_3$: C, 67.34; H, 6.59; N, 10.47. Found: C, 67.60; H, 6.34; N, 10.27. $^1$H NMR $\delta$ (ppm): 1.2 (qu def., 2H, $CH_2CH_2CH_2$), 2.76–2.97 (m, 4H, Pp-$CH_2$, Pp-2,6-$H_a$), 2.99 (s, 3H, N3-$CH_3$), 3.10–3.12 (m, 2H, Pp-2,6-$H_e$), 3.32–3.44 (m, 6H, Pp-3,5-H, N1-$CH_2$), 3.76 (s, 3H, $OCH_3$), 6.87–6.88 (m, 2H, PpPh-4,6-H), 6.96–7.01 (m, 2H, 2×Ph-4-H), 7.23–7.69 (m, 4H, 2×Ph-2,6-H), 7.43–7.48 (m, 6H, 2×Ph-3,5-H, Pp-3,5-H), 10.12 (br s, 1H, $NH^+$). IR (KBr) ($cm^{-1}$): 2950 (CH), 2398 ($NH^+$), 1768 (C2═O), 1710 (C4═O), 1592 (Ar).

**4.1.3.2. 1-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione hydrochloride (19a).** *Method F*: N-2-Methoxyphenylpiperazine (0.96 g) and compound **32** (2 g) were stirred for 72 h. White crystals of compound **19a**. Yield 42%, mp 130–131 °C, TLC: $R_f$ (II): 0.74. Anal. Calcd for $C_{31}H_{37}ClN_4O_3 \times 0.8$ $H_2O$: C, 66.07; H, 6.90; N, 9.96. Found: C, 66.09; H, 6.89; N, 9.97. $^1$H NMR $\delta$ (ppm): 0.76 (qu, $J$ = 7.40 Hz, 2H, Pp-$CH_2CH_2$), 1.37 (qu, $J$ = 7.40 Hz, 2H, N1-$CH_2CH_2$), 2.74 (m, 2H, Pp-$CH_2$), 2.90–3.03 (m, 4H, Pp-2,6-H), 2.98 (s, 3H, N3-$CH_3$), 3.30–3.43 (m, 6H, Pp-3,5-H, N1-$CH_2$), 3.76 (s, 3H, $OCH_3$), 6.84–7.03 (m, 4H, PpPh-2,6-H, 2×Ph-4-H), 7.19–7.27 (m, 4H, 2×Ph-2,6-H), 7.37–7.48 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H), 10.92 (br s, 1H, $NH^+$). IR (KBr) ($cm^{-1}$): 2942 (CH), 2401 ($NH^+$), 1769 (C2═O), 1712 (C4═O), 1595 (Ar)

**4.1.3.3. Methyl 2-(2,4-dioxo-5,5-diphenyl-1-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)propyl)imidazolidin-3-yl)propionate hydrochloride (20a).** *Method E*: N-2-ethoxyphenylpiperazine (1.03 g) and compound **34** (2.4 g) were stirred for 96 h. White crystals of compound **20**. Yield 32%, mp 138–139 °C, TLC: $R_f$ (II): 0.75. Anal. Calcd for $C_{34}H_{40}N_4O_5$: C, 69.84; H, 6.90; N, 9.58. Found: C, 69.64; H, 6.79; N, 9.61. $^1$H NMR for **20** $\delta$ (ppm): 1.19–1.25 (qu def., 2H, $CH_2CH_2CH_2$), 1.32 (t, $J$ = 6.90 Hz, 3H, $OCH_2CH_3$), 1.49 (d, $J$ = 7.18 Hz, 3H, N3-$CHCH_3$), 1.98 (t, $J$ = 6.80 Hz, 2H, Pp-$CH_2$), 2.26 (br s, 4H, Pp-2,6-H), 2.83 (br s, 4H, Pp-3,5-H), 3.26–3.35 (m, 2H, N1-$CH_2$,), 3.64 (s, 3H, $OCH_3$), 3.97 (q, $J$ = 7.00 Hz, 2H, $OCH_2CH_3$), 4.86 (q, $J$ = 7.22 Hz, 1H, N3-$CHCH_3$), 6.81–6.94 (m, 4H, PpPh-4,6-H, 2× Ph-4-H), 7.27–7.36 (m, 4H, 2× Ph-2,6-H), 7.41–7.52 (m, 6H, 2×Ph-3,5-H, PpPh-3,5-H). White crystals of compound **20a**. Yield 51%, mp 149–150 °C, $R_f$ (II): 0.75. Anal. Calcd for $C_{34}H_{41}ClN_4O_5 \times 1.7$ $H_2O$: C, 63.29; H, 6.86; N, 8.60. Found: C, 62.69; H, 6.84; N, 8.50. $^1$H NMR for **20a** $\delta$ (ppm): 1.21–1.28 (qu def., 2H, $CH_2CH_2CH_2$), 1.32 (t, $J$ = 6.92 Hz, 3H, $OCH_2CH_3$), 1.48 (d, $J$ = 7.44 Hz, 3H, N3-$CHCH_3$) 2.77–2.96 (m, 4H, Pp-$CH_2$, Pp-2,6-$H_a$), 3.01–3.29 (m, 4H, Pp-2,6-$H_e$, N1-$CH_2$), 3.38–3.50 (m, 4H, Pp-3,5-H), 3.65 (s, 3H, $OCH_3$), 3.97 (q, $J$ = 6.92 Hz, 2H, $OCH_2CH_3$), 4.87 (q, $J$ = 7.20 Hz, 1H, N3-$CHCH_3$), 6.82–6.99 (m, 4H, Pp-4,6-H, 2×Ph-4-H), 7.21–7.39 (m, 4H, 2×Ph-2,6-H), 7.42–7.52 (m, 6H, 2×Ph-3,5-H, PpPh-3,5-H), 10.99 (br s, 1H, $NH^+$). IR (KBr) ($cm^{-1}$): 2950 (CH), 2346 ($NH^+$), 1770 (C2═O), 1741 (C═O ester), 1718 (C4═O), 1592 (Ar).

**4.1.3.4. Methyl 2-(2,4-dioxo-5,5-diphenyl-1-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)imidazolidin-3-yl)acetate hydrochloride (21a).** *Method E*: N-2-methoxyphenylpiperazine (0.96 g) and compound **33** (2.4 g) were stirred for 96 h. White crystals of compound **21**. Yield 51%, mp 130–131 °C, TLC: $R_f$ (II): 0.58. Anal. Calcd for **21** $C_{33}H_{38}N_4O_5$: C, 69.45; H, 6.71; N, 9.82. Found: C, 69.31; H, 6.70; N, 9.80. $^1$H NMR $\delta$ (ppm): 0.78 (qu, $J$ = 7.30 Hz, 2H, Pp-$CH_2CH_2$), 1.05 (t, $J$ = 7.05 Hz, 2H, N1-$CH_2CH_2$), 1.94 (t, $J$ = 6.80 Hz, 2H, Pp-$CH_2$), 2.27 (br s, 4H, Pp-2,6-H), 2.81 (br s, 4H, Pp-3,5-H), 3.34 (m, 2H, N1-$CH_2$), 3.70 (s, 3H, $COOCH_3$), 3.73 (s, 3H, $OCH_3$), 4.34 (s, 2H,N3-$CH_2$), 6.82–6.92 (m, 4H, PpPh-3,4,5,6-H), 7.27–7.29 (m, 4H, 2×Ph-3,5-H), 7.39–7.49 (m, 6H, 2× Ph-2,4,6-H). White crystals of compound **21a**. Yield 81%, mp 178–180 °C, TLC: $R_f$ (II): 0.58. Anal. Calcd for $C_{33}H_{39}ClN_4O_5$: C, 65.28; H, 6.47; N, 9.23. Found: C, 65.20; H, 6.46; N, 9.19. $^1$H NMR $\delta$ (ppm): 0.79 (br s, 2H, Pp-$CH_2CH_2$), 1.39 (br s, 2H, N1-$CH_2CH_2$), 2.79 (m, 2H, Pp-$CH_2$), 2.90–3.14 (m, 4H, Pp-2,6-H), 3.32–3.43 (m, 6H, Pp-3,5-H, N1-$CH_2$), 3.71 (s, 3H, $OCH_3$), 3.76 (s, 3H, $OCH_3$), 4.35 (s, 2H, N3$CH_2$), 6.87–7.20 (m, 4H, PpPh), 7.15–7.48 (m, 10H, 2xPh), 10.36 (br s, 1H, $NH^+$). IR (KBr) ($cm^{-1}$): 2948(CH), 2408 ($NH^+$), 1771 (C2═O), 1742 (C═O ester), 1716 (C4═O), 1590 (Ar).

## 4.2. Pharmacology

### 4.2.1. Materials

Compounds. Barium chloride (POCh, Poland), [$^3$H]clonidine (Amersham), epinephrine (adrenalinum hydrochloricum, Polfa, Poland), [$^3$H]prazosin (Amersham), heparin sodium (Polfa, Poland), thiopental sodium (Biochemie, GmbH, Vienna, Austria).

### 4.2.2. Animals

The experiments were carried out on male Wistar rats (180–250 g). Animals were housed in constant temperature facilities exposed to 12:12 h light–dark cycle and maintained on a standard pellet diet and tap water given ad libitum. Control and experimental groups consisted of 6 animals each. All procedures were performed according to the Animal Care and Use Committee Guidelines, and approved by the Ethical Committee of Jagiellonian University, Kraków.

### 4.2.3. Statistical analysis

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

### 4.2.4. Radioligand binding assay

The compounds were evaluated for their affinity for $\alpha_1$-adrenergic receptors by determining their ability to displace [$^3$H]prazosin from its specific binding sites in rat cerebral cortex. [$^3$H]prazosin (19.5 Ci/mmol) was used.

The tissue was homogenized in 20 vol of ice-cold 50 mM Tris–HCl buffer (pH 7.6 at 25 °C) and centrifuged at 20000×$g$ for 20 min. The cell pellet was resuspended in Tris–HCl buffer and centrifuged again. The final pellet was resuspended in Tris–HCl buffer (10 mg of wet weight/ml). Two hundred and forty μl of the tissue suspension, 30 μl of [$^3$H]prazosin and 30 μl of analyzed compound were incubated at 25 °C for 30 min. To determine unspecific binding 10 μM phentolamine was used. Automated pipetting system epMotion 5070 (Eppendorf, Germany) was used for the pipetting steps.

After incubation reaction mix was filtered immediately onto presoaked GF/B glass fiber filter mate using 96-well FilterMate Harvester (PerkinElmer, USA).

The radioactivity retained on the filter was counted in MicroBeta TriLux 1450 scintillation counter (PerkinElmer, USA). Non-linear regression of the normalized (percent radioligand binding compared to that observed in the absence of test or reference compound - total binding) raw data representing radioligand binding was performed in GraphPad Prism 3.0 (GraphPad Software) using the built-in three parameter logistic model describing ligand competition binding to radioligand-labeled sites.[37,38]

### 4.2.5. Antiarrhythmic activity

**4.2.5.1. Adrenaline-induced arrhythmia according to the Szekeres[31].** The arrhythmia was evoked in rats anesthetised with thiopental (60 mg/kg, ip) by iv injection of adrenaline (20 μg/kg). The tested compounds were administered iv 15 min before adrenaline. The criterion of antiarrhythmic activity was the lack of premature beats and inhibition of cardiac arrhythmia in comparison with the control group.

**4.2.5.2. Barium chloride-induced arrhythmia according to the Szekeres[31].** Barium chloride solution was injected into the caudal vein of rat (32 mg/kg, in a volume of 1 ml/kg). The tested compounds were given iv 15 min before the arrhythmogen. The criterion of antiarrhythmic activity was a gradual disappearance of the arrhythmia and restoration of the sinus rhythm.

### 4.2.6. The effect on the normal electrocardiogram (ECG)

Electrocardiographic investigations were carried out using Multicard 30 apparatus, standard lead II and paper speed of 50 mm/s. The tested compounds were administered intravenously (iv) at a dose of 10 mg/kg. The ECG was recorded just before and 1, 5, 15 min following the administration of compounds.

### 4.2.7. Influence on the blood pressure

Male Wistar normotensive rats were anesthetized with thiopental (50–75 mg/kg) by ip injection. The right carotid artery was cannulated with polyethylene tubing filled with heparin solution (in saline) to enable the blood pressure measurements using the Datamax apparatus (Columbus Instruments). The studied compounds were injected at a dose of 10 mg/kg iv into the caudal vein, after a 5 min of stabilization period, in a volume equivalent to 1 ml/kg.

### 4.3. hERG $K^+$ Channel blockage calculation

The 3D structure of each compound was created by Corina online version[39] and then read into Maestro.[40] After checking on the types of atoms, the structures were neutralized and optimized with OPLS-2005 force field by LigPrep.[41] To predict the ability of hERG $K^+$ channel blockage QikProp program[42] was applied. Activity against hERG channels was expressed as $\log IC_{50}$ and referred to the selected experimental data.[43,44]

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.02.009.

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