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# Synthesis, $\alpha_1$-adrenoceptor antagonist activity, and SAR study of novel arylpiperazine derivatives of phenytoin

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**Abstract**—In the search for new antiarrhythmic agents, some active 2-methoxyphenylpiperazine derivatives of phenytoin were obtained as a chemical modification of compound **AZ-99** (3-ethyl-1-[2-hydroxy-3-(4-phenylpiperazin-1-yl)-propyl]-2,4-dioxo-5,5-diphenylimidazolidine). These compounds possessed structural properties similar to those of $\alpha_1$-adrenoceptor antagonists. In the present study, the affinities of the 2-methoxyphenylpiperazine derivatives (**1a**–**3a**) for $\alpha_1$- and $\alpha_2$-adrenoceptors were evaluated using radioligand ([$^3$H]prazosin, [$^3$H]clonidine) binding assays. In the next step, a new series of phenylpiperazine derivatives of phenytoin (**4a**–**16a**) containing 2-methoxyphenyl-, 2-ethoxyphenyl-, 2-pyridyl- or 2-furoylpiperazine moiety, as well as, various ester or alkyl substituents at 3-position of hydantoin ring were synthesized. The newly synthesized compounds were tested for their affinity to $\alpha_1$- and $\alpha_2$-adrenoceptors. They have shown affinities for $\alpha_1$-adrenoceptors at nanomolar to submicromolar range. Some compounds were moderately selective ligands of $\alpha_1$-adrenoceptors. Selected compounds (**3a**–**5a**, **7a**, **13a**, **14a**) were also evaluated for their $\alpha_1$-adrenoceptor antagonistic properties in functional bioassays. A SAR study indicated that the most active compounds contain 2-alkoxyphenylpiperazine moieties and methyl or 2-methylpropionate substituent at 3-N position in hydantoin. The exchange of 2-alkoxyphenyl moiety into 2-furoyl or 2-pyridyl group significantly decreased affinities for $\alpha_1$-adrenoceptors. Molecular modelling results obtained using conformational analysis CONFLEX and PM5 method for geometry optimization, allowed for comparison of the spatial properties of tested compounds with pharmacophore model created by Barbaro et al. for the ideal $\alpha_1$-adrenoceptor antagonist.
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## 1. Introduction

The adrenergic receptors, divided into three subclasses, $\alpha_1$, $\alpha_2$, and $\beta$, belong to the superfamily of G-protein coupled receptors that are targets of the catecholamines, such as adrenaline and noradrenaline.[1,2] The $\alpha_1$-adrenoceptors subclass, discovered as an independent group in 1974,[3] contains multiple subtypes which were identified based on pharmacological studies and cloning techniques. According to the pharmacological classification, and based on sensitivity to the prazosine, a potent and selective $\alpha_1$ antagonist, it has been proposed that $\alpha_1$-adrenoceptors can be divided into two types, $\alpha_{1H}$ (high sensitivity) and $\alpha_{1L}$ (low sensitivity) (see Fig. 1). Three subtypes of human $\alpha_{1H}$-adrenoceptors: $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$, have been identified by pharmacological and cloning studies. At this time, the $\alpha_{1L}$-adrenoceptors are recognised as a separate subtype of $\alpha_1$-adrenoceptors in various pharmacological assays but a discrete protein with $\alpha_{1L}$-adrenoceptor characteristics has yet to be cloned. Some lines of evidence suggest that $\alpha_{1L}$-adrenoceptors may represent a functional phenotype of $\alpha_{1A}$-adrenoceptors.[2,4]

The $\alpha_1$-adrenoceptors are involved in sympathetic and central nervous system functions,[1–7] and are responsible for many physiological effects including: vascular smooth muscle contraction, blood pressure regulation,[1,2]

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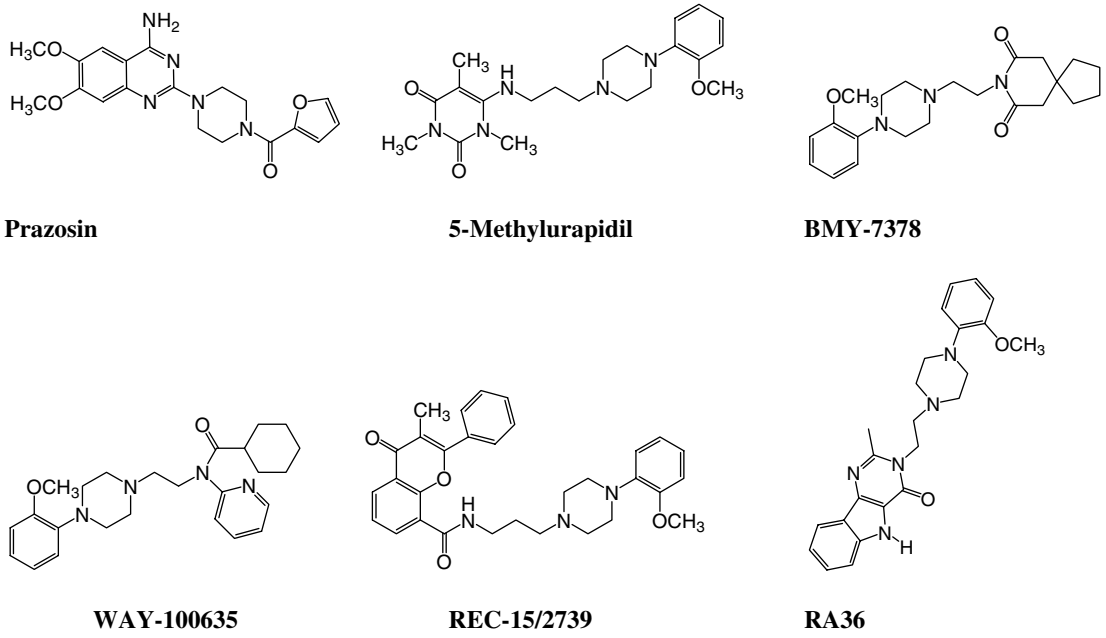


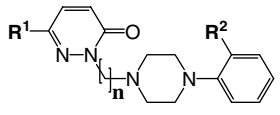
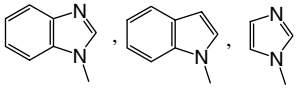
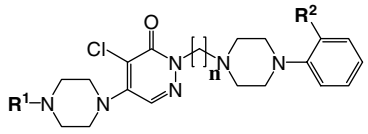
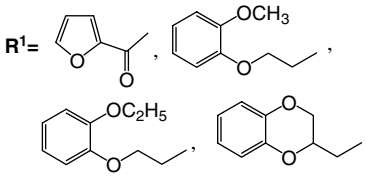
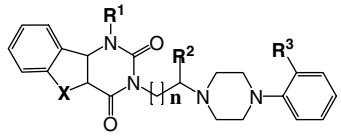
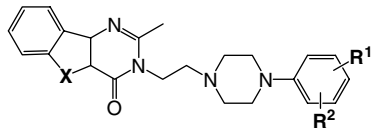
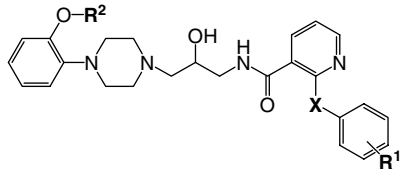

**Figure 1.** Structure of potent $\alpha_1$-adrenoceptor antagonists.

the human prostate smooth muscle contraction,[5] or regulation of cerebral microcirculation.[6]

Thus, $\alpha_1$-adrenoceptor antagonists can be useful in the treatment of hypertension, benign prostatic hyperplasia (BPH), lower urinary tract symptoms (LUTS), or cardiac arrhythmia.[1,2,5,6] In this context, the search for selective $\alpha_1$-adrenoceptor antagonists has been, and still is, an important topic in medicinal chemistry. In recent decades, various new $\alpha_1$-adrenoceptor antagonists have been designed and successfully synthesized.[8–19] Analysis of a number of chemical structures of selective $\alpha_1$-adrenoceptor antagonists[8] indicates that a large group of active compounds contain arylpiperazine moieties. Some known phenylpiperazine derivatives such as **5-methylurapidil**, **BMY-7378**, **WAY-100635**, **REC-15/2739**, and **RA36** (Fig. 1) can be considered as classical examples of potent $\alpha_1$-adrenoceptor antagonists useful in various pharmacological assays including binding studies, as well as, in vivo tests.[8,9] Furthermore, in recent years, new families of phenylpiperazine derivatives with high binding potency for $\alpha_1$-adrenoceptors have been synthesized.[9–17] The general structures of compounds and ranges of their affinities for $\alpha_1$-adrenoceptors, described by Romeo et al.,[9,16] Betti et al.,[10–13] Barbaro et al.,[14] Strappaghetti et al.,[15] and Kuo et al.,[17] are presented in Table 1. The large database of active compounds allowed to evaluate pharmacophore models of the $\alpha_1$-antagonist.[8,14] The most recent models, elaborated using radioligand binding data and computer aided methods with Catalyst have been proposed by Bremner et al.[8] and Barbaro et al.[14] Barbaro's model, especially useful for phenylpiperazine derivatives, has postulated five pharmacophore features: a positive ionisable atom (PI), three hydrophobic regions (HY1–HY3), and a hydrogen bond acceptor (HBA). Considering the structure of active arylpiperazine $\alpha_1$-antagonists (Fig. 1 and Table 1) in comparison with the pharmacophore model of Barbaro, five structural fragments, common for target compounds, corresponding to five pharmacophore features can be found (Fig. 2). As 2-substituted phenylpiperazine derivatives, all considered compounds possess a positive ionisable nitrogen atom and an *ortho*-substituted phenyl ring corresponding to PI and both HY1 and HY2, respectively. These compounds also contain an additional or fused aromatic moiety, ending the heterocyclic fragments, which can correspond to the HY3-feature. Additionally, they possess an alkyl spacer between piperazine nitrogen and nitrogen placed close to carbonyl oxygen (HBA) at a chain or at heterocyclic rings. The alkyl spacer may consist of 2–7 carbons and may additionally be substituted with alkyl or hydroxyl moieties, respectively. Some lines of evidence indicate that acetylation of the hydroxyl moieties significantly increases the affinity for $\alpha_1$-AR.[18]

On the other hand, our previous studies were focused on evaluating various biologically active phenytoin (DPH) derivatives.[19–21] We carried out the chemical modification of phenytoin, a known anticonvulsant and antiarrhythmic agent, in order to increase its antiarrhythmic potency and decrease its anticonvulsant action. We introduced various amine moieties into phenytoin structures to minimize its interaction with the CNS. Among others, we obtained 3-ethyl-1-[2-hydroxy-3-(4-phenylpiperazin-1-yl)-propyl]-2,4-dioxo-5,5-diphenylimidazolidine (**AZ-99**, Table 2) that was further modified to give a series of pharmacologically active compounds.[19] Chemical structures of the phenytoin derivatives display a similarity to many phenylpiperazine $\alpha_1$-adrenoceptor antagonists. These compounds possess phenylpiperazine moieties, a heterocyclic ring of hydantoin with two phenyl rings (position 5) and a 2-hydroxypropyl spacer between piperazine and 1-N-hydantoin moieties. Especially, three derivatives of **AZ-99**, compounds **1**–**3** (Table 2), show high similarity to $\alpha_1$-adrenoceptor

**Table 1.** Selected general structures of recently discovered $\alpha_1$-adrenoceptor antagonists

| Structures | | $\alpha_1$-Adrenoceptor affinity range $\sim K_i$[a] (nM) |
|---|---|---|
|  | **R¹=**  <br>$R^2$ = Cl, $OCH_3$<br>*n*=2, 3, 4, 5, 6, 7 | ~0.2–120[10,11] |
|  | **R¹=**  <br>$R^2$ = $OCH_3$, $OC_2H_5$, $OCH(CH_3)_2$<br>*n*=2, 3, 4, 5, 6, 7 | ~0.05–60[12–14] |
|  | $R^1$ = H, $CH_3$<br>$R^2$ = H, $CH_3$<br>$R^3$ = $OCH_3$<br>*n* = 1,2<br>X = NH, $NCH_3$, S, O | ~0.1–15[16] |
|  | X = NH, S<br>$R^1$ = 2-CH3O<br>$R^2$ = H, 5-Cl | ~0.5–20[9] |
|  | $R^1$ = H, 4-Cl, 3,4-$Cl_2$, 4-$CH_3$, 4-$C(CH_3)_2$, 3-$N(CH_3)_2$, 2-$CH_3$, 2-$OCH_3$, 4-$OCH_3$, 3-$CF_3$<br>$R^2$ = $CH_3$, $C_2H_5$, $CH(CH_3)_2$<br>X = O, S, NH | ~0.5–30.0[17] |

[a] Results of radioligand binding assays performed for $\alpha_1$-AR (rat cortex) or cloned $\alpha_1$-adrenoceptor subtypes ($\alpha_{1a}$, $\alpha_{1b}$, $\alpha_{1d}$), respectively (see Refs. 9–14, 16, 17).

antagonists as members of 2-methoxyphenylpiperazine derivatives family.

In our present work we decided to evaluate the compounds (**1**–**3**) for their $\alpha_1$- and $\alpha_2$-adrenoceptor affinity in radioligand binding assays. Furthermore, we designed and synthesized a series of new compounds (**4**–**16**) as further derivatives of **AZ-99** (Table 2) possessing 2-methoxyphenyl-, 2-ethoxyphenyl-, 2-furoyl-, or 2-pyridylpiperazine fragments, as well as, various substituents at position 3-N of the hydantoin ring. In the case of compound **8**, we modified, additionally, the hydroxypropyl chain by acetylation of the hydroxyl group. The new compounds were converted into hydrochloric salts **4a**–**16a** and initially tested for their affinities to both $\alpha_1$- and $\alpha_2$-adrenoceptors in radioligand binding assays. Selected compounds (**3a**–**5a**, **7a**, **13a**, and **14a**) were then tested for their antagonistic properties at the vascular $\alpha_1$-adrenoceptor within functional bioassays. Finally, we analysed the role of the modified chemical fragments in $\alpha_1$-antagonistic properties of compounds **1a**–**16a** within SAR studies including molecular modelling calculations.

## 2. Results

### 2.1. Synthesis

The synthesis of compounds **1a**–**3a** was described earlier.[19] Compounds **4a**–**16a** were obtained according to Scheme 1. Compounds **4**–**7** and **9**–**16** were synthesized by three-step alkylation using phenytoin (**17**) as a starting product. At first, phenytoin was alkylated at 3-N position giving methyl- or methyl ester derivatives **18**–**21**, respectively. The synthesis of compounds **18** and **19** was performed via the alkylation with $CH_3I$ in EtONa (**18**) or the reaction with methyl 2-bromoacetate in two phase-transfer catalytic conditions in the presence of potassium carbonate and benzyltriethylammonium chloride (TEBA) as phase-transfer catalyst (**19**), according to the methods described earlier.[20,21] Two new methyl esters, derivatives of 2-methylpropionate (**20**)

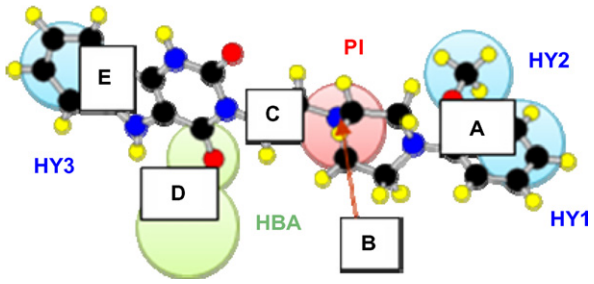
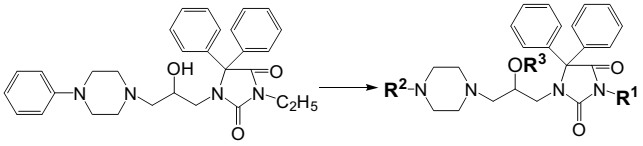



**Figure 2.** Structural features (A–E) of phenylpiperazine $\alpha_1$-adrenoceptor antagonists[9–17] compared to pharmacophore model of Barbaro et al. (PI, HY1–3, HBA).[14] (A) (Un)substituted phenylpiperazine phenyl ring corresponding to hydrophobic features (HY1 and HY2); (B) positive ionisable nitrogen of phenylpiperazine corresponding to PI; (C) (un)substituted alkyl spacer between two nitrogens. (D) Carbonyl moiety corresponding to HBA; (E) heterocyclic moiety with condensed or substituted phenyl ring corresponding to HY3 in Barbaro's model. Draft pharmacophore model mapped with an example phenylpiperazine antagonist (3-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)-4a,5-dihydro-1H-pyrimido[5,4-b]indole-2,4(3H,9bH)-dione) made basing on Ref. 14.

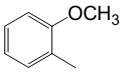
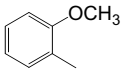
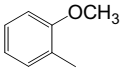
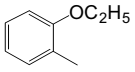
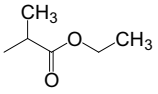
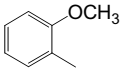
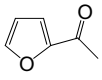
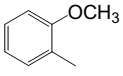
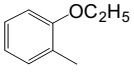
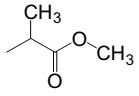
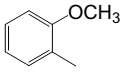
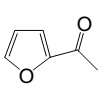
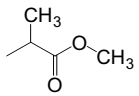
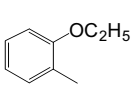
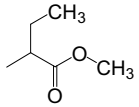
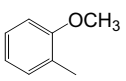
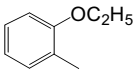
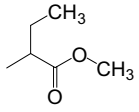
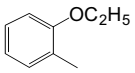
and 2-methyl butyrate (**21**), were synthesized by heating compound **17** in acetone with TEBA, $K_2CO_3$, and commercially available methyl 2-bromopropionate or methyl 2-bromobutyrate, respectively. The reactions were carried out in similar conditions to those for compound **19**.

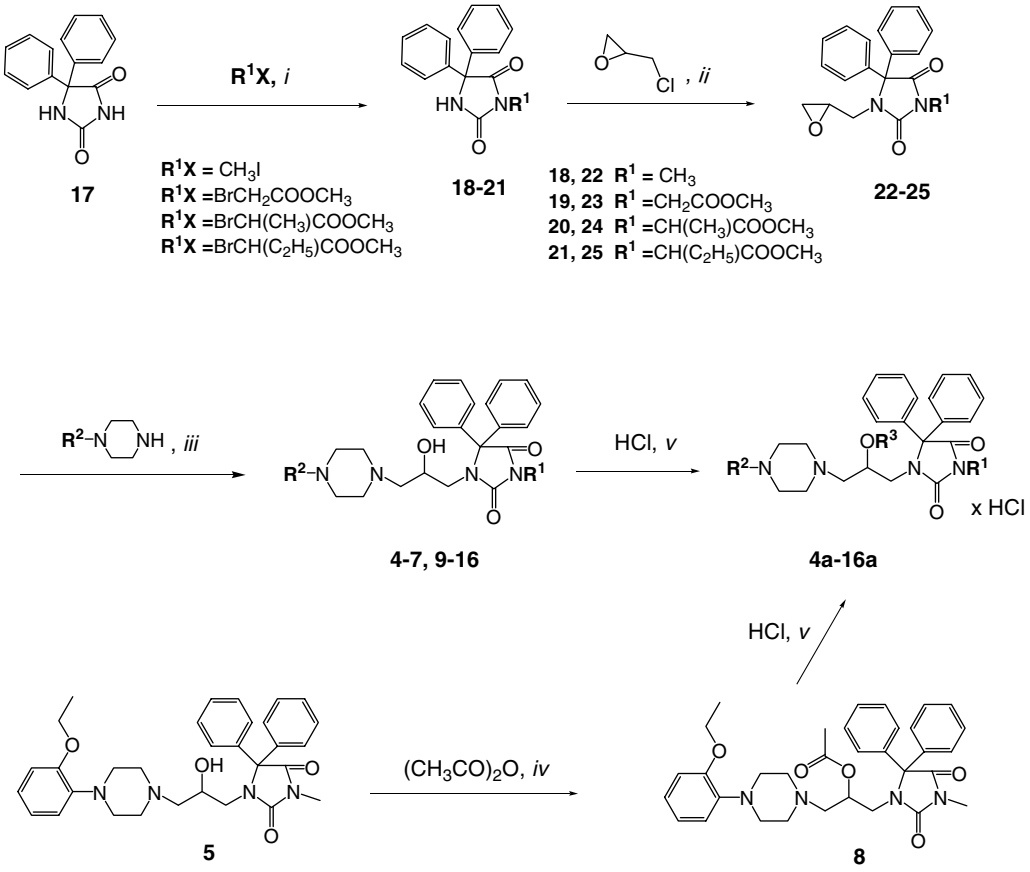
The next steps of synthesis were based on double-alkylating properties of epichlorohydrin. At first, epichlorohydrin was used, as an alkyl chloride, to introduce oxiranylmethyl moiety into the 1-N position of compounds **18**–**21** to give compounds **22**–**25**. The reaction was carried out at room temperature by stirring the reactants in phase-transfer catalytic conditions according to the method described earlier.[20,21] In the case of new compounds **24** and **25**, the previous method was modified by changing stirring time and methods of purification. The synthesis was performed under TLC control (toluene–acetone 40:3). Compound **24** was obtained as a result of long-time stirring for 54.5 h. The compound precipitated as pure crystals from condensed filtrate that was obtained after the separation of the solid phase from the reaction mixture. According to TLC control, synthesis of compound **25** needed 69 h of stirring. The pure compound **25** was obtained by crystallization from methanol.

**Table 2.** Compound **AZ-99** and its chemical modifications




**AZ-99** **Compounds 1-16**

| Compound | $R^1$ | $R^2$ | $R^3$ | Compound | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|---|---|---|
| **1** | $-C_2H_5$ |  | H | **9** | $-CH_2COOCH_3$ |  | H |
| **2** | $-CH_2COOC_2H_5$ |  | H | **10** | $-CH_2COOCH_3$ |  | H |
| **3** |  |  | H | **11** | $-CH_2COOCH_3$ |  | H |
| **4** | $-CH_3$ |  | H | **12** | $-CH_2COOCH_3$ |  | H |
| **5** | $-CH_3$ |  | H | **13** |  |  | H |
| **6** | $-CH_3$ |  | H | **14** |  |  | H |
| **7** | $-CH_3$ |  | H | **15** |  |  | H |
| **8** | $-CH_3$ |  |  | **16** |  |  | H |




**Scheme 1.** Synthesis of compounds **4a**–**16a**. Reagents and conditions: (i) EtONa or $K_2CO_3$, TEBA, EtOH; (ii) $K_2CO_3$, TEBA, acetone, 20 °C; (iii) mw-irradiation; (iv) reflux; (v) MeOH or EtOH, gaseous HCl.

Synthesis of the final products **4**–**7** and **9**–**16** was based on N-alkylating properties of the oxiranyl rings of compounds **22**–**25** in reactions with secondary amines. The oxiranyl rings of compounds **22**–**25** were opened via reactions with 2-alkoxyphenyl-, 2-furoyl-, or 2-pyridylpiperazine, respectively. The reactions were carried out with equimolar amounts of dry reagents under microwave irradiation using a standard household microwave oven.[19]

As a result of irradiation glassy residues were obtained. Contrary to compounds **11**, **15**, and **16**, for compounds **4**–**7**, **9**, **10**, and **12**–**14**, pure products were obtained by recrystallization of glass residues from ethanol or methanol, respectively. Synthesis of 3-(4-(2-ethoxyphenyl) piperazin-1-yl)-1-(3-methyl-2,4-dioxo-5,5-diphenylimidazolidin-1-yl)propan-2-yl acetate (**8**) was performed by acetylation of compound **5** using acetic anhydride to give a gluey residue.

Finally, all compounds (**4**–**16**) were converted into hydrochloric salts (**4a**–**16a**) using gaseous HCl.[19] Compounds **5**–**7**, **9**, **10**, and **12**–**14**, obtained as pure precipitates, were dissolved in anhydrous alcohol and saturated with HCl (method A). Compounds **4**, **6**, **11**, **15**, and **16** did not crystallize from alcohols, giving gluey residues. These residues were dissolved in methylene chloride and washed from the rest of starting piperazines by the use of diluted hydrochloric acid. After solvent evaporation, the residues of **4**, **6**, **11**, **15**, and **16** were dissolved in anhydrous alcohol for saturation with gaseous HCl (method B). In the case of compound **8a**, a glue residue of compound **8** was dissolved in anhydrous methanol for the saturation with HCl. All hydrochlorides (**4a**–**16a**) precipitated from alcohols to give pure bright crystals.

Additionally, syntheses of two compounds (**4** and **5**) were carried out using the microwave oven 'CEM-Discover', qualified for organic synthesis performed in various conditions including high pressure, reactants in solvent or in dry conditions. This professional microwave oven allows to create process conditions by the set of following parameters: temperature, starting time to achieve a desirable temperature and continuing irradiation time. During the process, temperature, power and pressure were automatically controlled by computer monitoring. The reactants were magnetically stirred in flat-bottom flask placed in microwave oven and covered by special polymer protector. Special protecting system does not allow open microwave oven until the temperature decreases to a safe value. In the case of compound **4**, reactants were irradiated for three periods of time controlling the colour of the melting mixture. After the first period of irradiation at 120 °C, about half of the reactants was melted. Irradiation was prolonged but the result was not satisfying as the part of reactants was still not melted. When the temperature was raised to 140 °C, in the third irradiation period, a little part of

white starting product was still present in the flask and reactant mixture was getting dark yellow. Despite mixing and computer-controlled conditions, an appearance of partial overheatings was observed. Therefore the irradiation process was interrupted.

In case of compound **5**, dry reactants were dissolved in $CH_2Cl_2$ and the solvent was evaporated to give a homogeneous reactant mixture as glassy residue. A flask with the residue was placed in microwave oven 'CEM-discover' and the process was performed in the same manner as that of compound **4**. Unfortunately, the reactant mixture was getting dark brown after the second irradiation period at 120 °C giving burnt product.

The experiments indicated that professional microwave oven did not allow to create universal repeatable conditions for synthesis of the presented phenylpiperazine derivatives. Furthermore, the longer time of irradiation seems to be dangerous for reactant mixture in professional microwave oven, too. Thus, short-irradiation-sessions (1–2 min) with reactants-semblance control and TLC monitored reaction progress are recommended to assure stable and efficient process conditions.

Summing up performance of the reactions especially in solvent-free conditions was not better in professional microwave oven comparing to that in domestic microwave oven. In the literature there are some references.[22–24] confirming our observations and conclusions concerning solvent-free reactions performed in domestic microwave ovens.

## 2.2. Pharmacology

**2.2.1. Radioligand binding results.** Compounds **1a**–**16a** were tested for their in vitro affinity to $\alpha_1$- and $\alpha_2$-adrenoceptors in rat cerebral cortex by radioligand binding assays using [$^3$H]prazosin and [$^3$H]clonidine as specific radioligands, respectively.[25] The affinities described by $K_i$ values (nM) are shown in Table 3. Selectivity towards $\alpha_1$-AR in respect of $\alpha_2$-AR was calculated as $K_{i\alpha2}/K_{i\alpha1}$. All compounds displayed lower affinities for $\alpha_1$-AR when compared to prazosin. A large group of compounds (**1a**–**5a**, **9a**, **10a**, **13a**, and **14a**) showed higher affinities for $\alpha_1$-AR than that of **AZ-99**, with $K_i$ values 100–300 nM. Compounds **7a** and **8a** showed affinities slightly lower than that of the lead compound **AZ-99**. The rest of the compounds (**6a**, **11a**, **12a**, **15a**, and **16a**) had a low affinity for both the $\alpha_1$- and $\alpha_2$-adrenoceptors. Especially, 2-furoyl derivative **6a** did not show any affinity for $\alpha_1$-AR at tested concentrations. Generally, the target compounds possessed a slightly higher selectivity for $\alpha_1$-AR over $\alpha_2$-AR. The most selective compound (**3a**) showed 8.48-fold higher affinity for $\alpha_1$-AR than for $\alpha_2$-AR (Table 3).

## 2.3. Functional bioassays results

The $\alpha_1$-adrenoceptor antagonistic activity of compounds **3a**–**5a**, **7a**, **13a**, and **14a** was studied in rat aorta from adult Wistar rats and was assessed by inhibition of phenylephrine-induced contractions. The investigated compounds, concentration-dependently, shifted the phenylephrine response to the right. For compound **4a** a Schild slope did not differ significantly from unity, indicating a competitive interaction with the $\alpha_1$-adrenoceptors, and thus allowing for the unambiguous determination of the $pA_2$ value. In other cases affinities were reported as $pK_B$ estimates, since Schild slopes were lower than unity. This means that the antagonism was not competitive and can suggest that the responses were mediated by more than one receptor. Other possibilities include a slow dissociation of the ligand from the receptor or an allosteric modulation of receptors.[26]

The following order of activity was found for tested compounds: **13a** > **5a** > **3a** > **4a** ≈ **14a** > **7a** (Table 4, Figs. 3 and 4). The strongest antagonistic activity was from compounds **13a** and **5a** with $pK_B$ estimates of 7.084 and 7.022, respectively. For compounds **3a**, **4a**, and **5a** $pK_B$ values ranged from 6.860 to 6.611. Compound **4a** gave a $pA_2$ value of 6.616 with a slope

**Table 3.** Binding properties of compounds **1a**–**16a**

| Compound | Affinity $K_i$ (nM) | | |
|---|---|---|---|
| | $\alpha_1$-AR | $\alpha_2$-AR | $\alpha_2/\alpha_1$ |
| **1a** | 292.7 ± 1.1 | 415.63 | 1.42 |
| **2a** | 160 ± 21.3 | 413.7 ±59.3 | 2.59 |
| **3a** | 135.7 ± 31.3 | 1150 ± 170 | 8.48 |
| **4a** | 160.7 ± 13.6 | 344.2 ± 44.3 | 2.15 |
| **5a** | 121.6 ± 14.9 | 534.7 ± 54 | 4.40 |
| **6a** | >100 000 | 28300 ± 5300 | — |
| **7a** | 691.9 ± 16.5 | 693.2 ± 100 | 1.01 |
| **8a** | 607 ± 74.7 | 1900 ± 200 | 3.13 |
| **9a** | 197.8 ± 25.4 | 800 ± 160 | 4.04 |
| **10a** | 251.6 ± 3.8 | 852 ± 70 | 3.39 |
| **11a** | 7600 ± 1150 | 5600 ± 900 | 0.74 |
| **12a** | 1100 ± 100 | 990 ± 250 | 0.9 |
| **13a** | 103.9 ± 4.2 | 448.6 ± 25.9 | 4.32 |
| **14a** | 167.7 ± 8 | 733.8 ± 90 | 4.38 |
| **15a** | 3100 ± 200 | 5600 ± 400 | 1.81 |
| **16a** | 7800 ± 500 | 23800 ± 900 | 3.05 |
| **AZ-99a** | 529 ± 9.5 | 564.1 ± 28.6 | 1.07 |
| **Prazosin** | 0.24 ± 0.05[a] | — | — |

Inhibition constants ($K_i$) were calculated according to the equation of Cheng and Prusoff. Radioligand binding assays to rats cortex membrane using [$^3$H]prazosin ($\alpha_1$) and [$^3$H]clonidine ($\alpha_2$), respectively.
[a] According to Ref. 11.

**Table 4.** Functional bioassay results for selected compounds **3a**–**5a**, **7a**, **13a**, and **14a**

| Compound | $pK_B/pA_2$ ± SEM (Slope ± SEM) |
|---|---|
| **3a** | $pK_B$ = 6.860 ± 0.07 |
| **4a** | $pA_2$ = 6.616 ± 0.12[a] (0.99 ± 0.13) |
| **5a** | $pK_B$ = 7.022 ± 0.06 |
| **7a** | $pK_B$ = 6.133 ± 0.05 |
| **13a** | $pK_B$ = 7.084 ± 0.05 |
| **14a** | $pK_B$ = 6.611 ± 0.08 |

Antagonistic potency of selected compounds, expressed as $pA_2$or $pK_B$ ± SEM values, in isolated rat thoracic aorta ($\alpha_1$-AR).
$pK_B$ values were calculated according to relationship $pK_B = \log(\text{concentration ratio} - 1) - \log(\text{molar antagonist concentration})$.
[a] $pA_2$ value was obtained from the linear regression of Schild plot. Each value was the mean ± SEM of 5–8 experimental results.

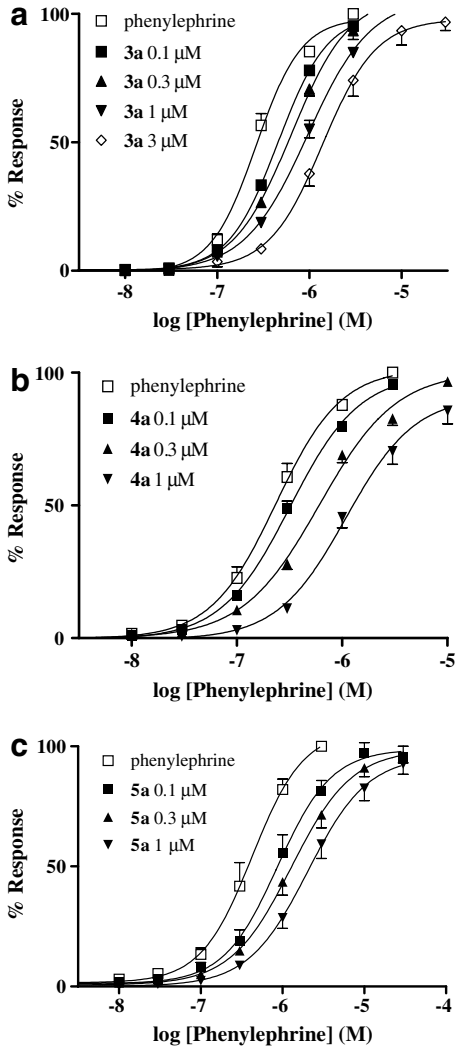



**Figure 3.** Concentration–response curves to phenylephrine in the rat aorta in the absence (□) or presence of (a) **3a** (■ 0.1, ▲ 0.3, ▼1 and ♦ 3 μM); (b) **4a** (■ 0.1, ▲ 0.3, ▼1 μM); (c) **5a** (■ 0.1, ▲ 0.3, ▼1 μM). Results are expressed as a percentage of the maximal response to phenylephrine in the first concentration–response curve. Each point represents the mean ± SEM ($n$ = 5–8).

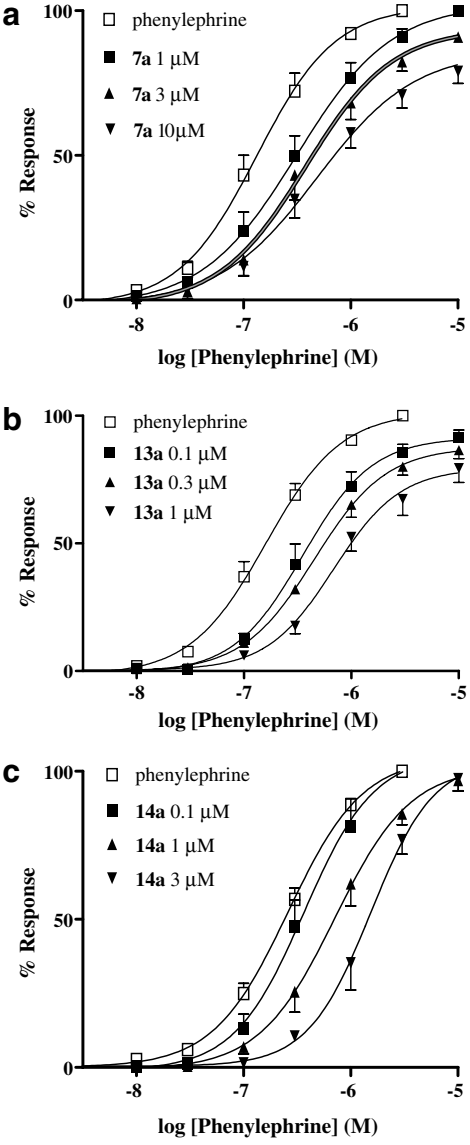



**Figure 4.** Concentration–response curves to phenylephrine in the rat aorta in the absence (□) or presence of (a) **7a** (■ 1, ▲ 3, ▼10 μM); (b) **13a** (■ 0.1, ▲ 0.3, ▼1 μM); (c) **14a** (■ 0.1, ▲ 1, ▼3 μM). Results are expressed as a percentage of the maximal response to phenylephrine in the first concentration–response curve. Each point represents the mean ± SEM ($n$ = 5–8).

0.99 ± 0.13. Compound **7a** showed the weakest antagonistic potency, giving a p$K_B$ estimate of 6.133. It is noticeable that the affinity from the functional test for compounds **3a**–**5a**, **7a**, **13a**, and **14a** was in the same concentration range as determined in the radioligand binding assay.

## 2.4. SAR studies

**2.4.1. Molecular modelling.** The goal of the present molecular modelling was to determine the 3D-structural properties of the phenylpiperazine derivatives **1a**–**16a** in order to compare them with ideal $\alpha_1$-antagonist properties according to the pharmacophore model of Barbaro.[14]

3D-structures of compounds **1a**–**16a** were built based on the crystal structure of phenylpiperazine phenytoin derivative **JH-9a** (Fig. 5). The structures were built by exchange of the appropriate substituents in **JH-9a** using the computer programme HyperChem 7.5 (Hypercube). The primary optimisation of the prepared structures was carried out using the semi-empirical method PM5, available by the MOPAC implement in CAChe 5.0. In the next step, a conformational analysis using the CONFLEX method was performed to give different conformer populations for each calculated compound **1a**–**16a**. Selected conformers were re-optimized using PM5. The lowest-energy conformer for each compound (**1a**–**16a**) was selected as representative for the real 3D-structure and was evaluated on its geometrical parameters. Four distances were analysed, important for $\alpha_1$-antagonistic properties that correspond to Barbaro's model (Fig. 6). In this context, the following distances were assessed between positive ionisable

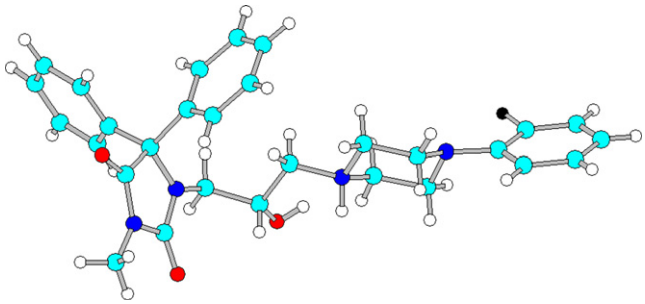
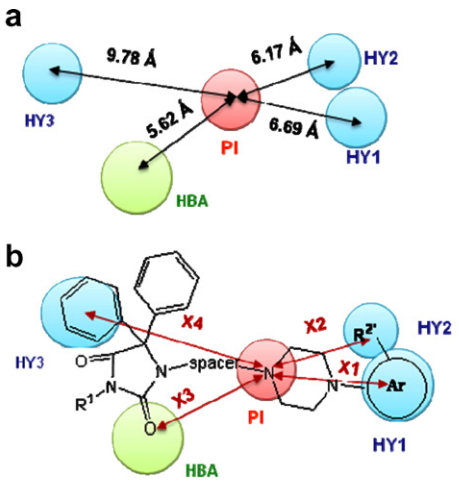


**Figure 5.** The starting point for molecular modelling calculations. Crystal structure of phenylpiperazine derivative of phenytoin **JH-9a** (1-(3-(4-(2-chlorophenyl)piperazin-1-yl)-2-hydroxypropyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione hydrochloride) determined by X-ray analysis. Element colours: C, cyan; H, white; N, blue; O, red; F, black.




| Cpd | X1[Å] | X2[Å] | X3[Å] | X4[Å] |
|---|---|---|---|---|
| **1a** | 6.52 | 5.17 | 5.06 | 7.97 |
| **2a** | 6.47 | 5.32 | 5.03 | 7.92 |
| **3a** | 6.54 | 5.47 | 5.35 | 7.86 |
| **4a** | 6.45 | 5.72 | 5.39 | 7.90 |
| **5a** | 6.49 | 6.12 | 5.38 | 8.00 |
| **6a** | 6.09 | - | 5.42 | 7.92 |
| **7a** | 6.50 | - | 5.18 | 7.98 |
| **8a** | 6.57 | 6.35 | 5.28 | 8.09 |
| **9a** | 6.49 | 5.78 | 5.29 | 7.98 |
| **10a** | 6.51 | 5.21 | 5.52 | 7.92 |
| **11a** | 6.01 | - | 5.16 | 7.90 |
| **12a** | 6.55 | - | 5.15 | 7.85 |
| **13a** | 6.51 | 5.61 | 5.59 | 7.81 |
| **14a** | 6.59 | 5.52 | 5.19 | 7.86 |
| **15a** | 6.50 | 5.08 | 5.51 | 7.91 |
| **16a** | 6.50 | 5.11 | 5.23 | 7.85 |

**Figure 6.** Structural parameters of compounds **1a**–**16a** in the comparison with pharmacophore model of Barbaro. (a) Spatial properties required for the ideal $\alpha_1$-antagonist described by Barbaro et al.[14] Distances between proton ionisable nitrogen (PI) and following regions: HY1, HY2, HY3, and HBA. (b) The corresponding distances for compounds **1a**–**16a** according to the molecular modelling calculation (CONFLEX conformational analysis, PM5-geometry optimization): protonated nitrogen—centre of aromatic (acyl-aromatic) piperazine substituent (X1); protonated nitrogen—substituent at aromatic ring (X2); protonated nitrogen—carbonyl oxygen (X3); protonated nitrogen—centre of most distant phenyl ring (X4).

nitrogen of piperazine and a centre of arylpiperazine aryl (furoyl in the case of compounds **6a** and **11a**) ring (X1) corresponding to PI–HY1, between positive ionisable nitrogen of piperazine and alkoxyl substituent at phenylpiperazine phenyl ring (X2) corresponding to PI–HY2; between positive ionisable nitrogen of piperazine and carbonyl oxygen in position 2 of hydantoin (X3) corresponding to PI–HBA and between positive ionisable nitrogen of piperazine and a centre of the most distant phenyl ring at hydantoin fragment (X4) as corresponding to PI–HY3 in Barbaro's model. Most of the considered compounds showed values of PI–HY1 (X1) distance in the range of 6.5–6.6 Å, similar to that of ideal $\alpha_1$-antagonist. Only two furoyl derivatives (**6a** and **11a**) showed slightly lower X1 distances in the range of 6.0 Å. In case of PI–HY2 (X2), most of the compounds displayed lower values than that of the ideal antagonist, in the range of 5.08–5.78 Å. The best agreement between the ideal $\alpha_1$-antagonist and the compounds was observed for the ethoxyphenyl derivatives **5a** and **8a**. Compounds **10a**, **13a**, and **15a** showed the best values of X3 distances (5.51–5.59 Å), similar to that of the ideal antagonist (5.63 Å). The rest of compounds possessed lower X3-values (5.03–5.42 Å). All of the obtained compounds **1a**–**16a** possess the most distant hydantoin phenyl rings placed significantly closer to the positive ionisable centre (7.85–8.09 Å) when compared to the ideal PI–HY3 value (9.78 Å) described by Barbaro et al.[14]

**2.4.2. SAR analysis.** In order to obtain the considered phenylpiperazine derivatives of phenytoin **1a**–**16a**, three structural fragments of the lead compound **AZ-99** were modified: the phenylpiperazine phenyl ring, the 3-N-substituent at the hydantoin moiety, and the 2-hydroxypropyl chain. In this context, we analysed the influence of each of these three structural fragments on affinities for $\alpha_1$-adrenoceptors (Tables 2 and 3). At first, we analysed two example groups of compounds differing only in the area of the phenylpiperazine aryl moiety (**4a**–**7a** and **9a**–**12a**). The first group contained 3-*N*-methyl derivatives (**4a**–**7a**) with 2-methoxyphenyl-, 2-ethoxyphenyl-, 2-pyridyl and 2-furoylpiperazine moieties, respectively. Similarly, the second group included 3-*N*-methyl acetate derivatives (**9a**–**12a**). The influence of the substituent at the 3-N-hydantoin ring on $\alpha_1$-adrenoceptors affinities can be seen in the case of 2-methoxyphenylpiperazine derivatives (**1a**–**4a**, **9a**, **13a**, and **15a**).

In the next step, the study of the relationship between the affinity of the compounds for $\alpha_1$-adrenoceptors and the $\alpha_1$-adrenoceptor antagonistic potency was carried out. Figure 7 demonstrates a comparison of functional bioassay results ($pK_B$, $pA_2$) to radioligand binding results ($pK_i$) for selected compounds (**3a**–**5a**, **7a**, **13a**, and **14a**). For these compounds a linear quantitative $pK_i$–$pK_B$ relationship was found (Fig. 7). Correlation of the affinities for $\alpha_1$-AR from binding studies with those obtained from functional studies in rat aorta was statistically significant ($R^2 = 0.89$, $p < 0.005$).

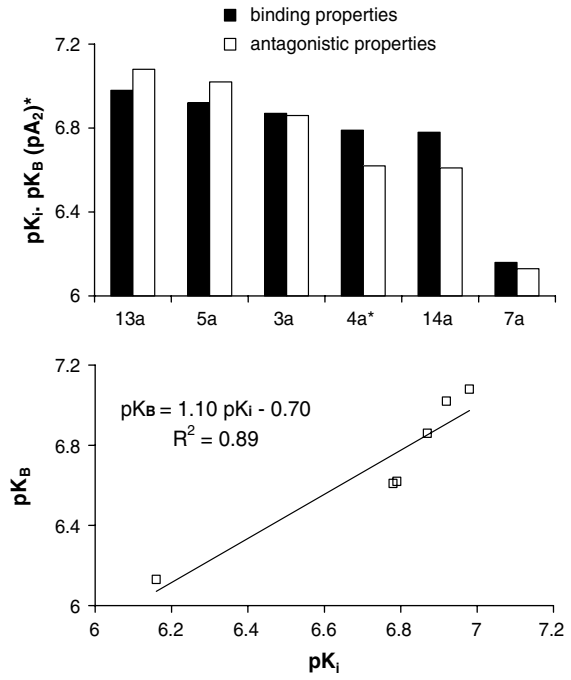



**Figure 7.** The relationship between $\alpha_1$-adrenoceptor affinities and antagonistic properties for compounds: **3a**–**5a**, **7a**, **13a**, and **14a**, expressed as $pK_i$ and $pK_B$ (*__4a__: $pK_B = pA_2$) values. Quantitative relationship $pK_i$–$pK_B$ is expressed by a linear equation. The correlation was calculated using Pearson worksheet function ($R^2 = 0.89$; $p < 0.005$).

## 3. Discussion

As a result of the chemical modification of compound **AZ-99**, the new group of compounds with a wide range of affinities to $\alpha_1$-adrenoceptors (103.9–100,000 nM) and a relatively weak selectivity for $\alpha_1$-adrenoceptors over $\alpha_2$-adrenoceptors has been synthesized and characterised. Based on radioligand binding results (Table 3), it can be concluded that the type of substituent at 4-N of piperazine significantly influences the affinity for $\alpha_1$-adrenergic receptors. The introduction of 2-methoxyl moiety into the phenylpiperazine phenyl ring (**1a**) enhanced the $\alpha_1$-binding potency of the lead compound **AZ-99**. These results are in agreement with many other reports, which have also confirmed the importance of 2-methoxyphenylpiperazine moiety for the $\alpha_1$-adrenoceptor antagonistic properties.[9–17] It is important to note that the work of Romeo et al.,[16] carried out on a group of pyrimido-2,4-dione phenylpiperazine derivatives, indicated an increase of the $\alpha_1$-AR affinity following the introduction of methoxyl moiety at position *ortho* in the phenylpiperazine phenyl ring. Although the compounds described by Romeo et al. were more active than our compounds **1a**–**16a**, each phenylpiperazine derivative showed lower affinity for the $\alpha_1$-adrenoceptor than the corresponding 2-methoxyphenyl derivative.

The lower activity of presented phenylpiperazine derivatives **1a**–**16a**, compared to reference $\alpha_1$-antagonists[9–17] with corresponding arylpiperazine moieties, can be in part explained based on molecular modelling results. The results indicated that all compounds **1a**–**16a** possess phenyl rings at hydantoin situated too close to positive ionisable nitrogen compared to that required for the ideal $\alpha_1$-adrenoceptor antagonist. In the case of the most distant phenyl rings the distance PI–HY4 (X4) is approx. 20% shorter. Furthermore, the second phenyl rings at hydantoin are situated much closer to PI (~5–6 Å). The rest of the distances (X1–X3) do not show too much difference when compared to the ideal value and in the case of the selected compounds high conformity is observed (X2 for **5a**; X3 for **13a**, see Fig. 6). Thus, we assume that the presence of two phenyl rings in close proximity to the PI-centre may cause some decrease in the $\alpha_1$-antagonistic properties in all of the investigated phenylpiperazine derivatives **1a**–**16a**.

In our study, the most active compounds (**2a**–**5a**, **9a**, **13a**, and **14a**) contained 2-alkoxyphenylpiperazine moieties, 2-methoxyphenyl or 2-ethoxyphenyl. Although, most of 2-alkoxyphenylpiperazine derivatives (**1a**–**5a**, **9a**, **10a**, **13a**, and **14a**) showed $\alpha_1$-affinities in the range of 100–300 nM, it is difficult to recognise which type of alkoxyl substituent is superior for target activity. In the group of 3-*N*-methyl derivatives, the 2-methoxyphenyl derivative (**4a**) showed a slightly lower affinity for $\alpha_1$-AR than the 2-ethoxyphenyl derivative (**5a**). In the case of the three pairs of 3-*N*-methyl ester derivatives (**9a** and **10a**, **13a** and **14a**, and **15a** and **16a**), the opposite situation was observed.

Comparing two groups of compounds containing the same substituents at 3-N-hydantoin rings and different at 4-*N*-piperazine fragments, similar effects on affinities for $\alpha_1$-AR can be observed for 2-pyridyl- and 2-furoyl moieties. In both groups, the $pK_i$ values are decreasing in the following order: 2-alkoxyphenyl- > 2-pyridyl- > 2-furoyl- and the difference between the affinities of 2-pyridyl- (**7a**) and analogical 2-furoyl derivative (**6a**) is much higher in the group of 3-*N*-methyl derivatives than in the group of 3-*N*-methyl acetate derivatives. The affinity of compound **7a** can be considered as a moderate value ($K_i \approx 0.7$ μM) while compound **6a** did not display any receptor binding. In the case of methyl 2-(3-(2-hydroxy-3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetate dihydrochloride (**12a**) and methyl 2-(3-(3-(4-(furan-2-carbonyl)piperazin-1-yl)-2-hydroxypropyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetate hydrochloride (**11a**), both compounds were weakly active with affinities for $\alpha_1$-AR in the micromolar range. Generally, the replacement of 2-alkoxyphenyl moiety with 2-pyridyl- or, particularly, 2-furoyl moieties is not beneficial for affinities to $\alpha_1$-AR in the presented group of phenytoin–arylpiperazine derivatives. This observation is comparable to results of Barbaro et al. in the group of 1,4-benzodioxan-arylpiperazine derivatives.[15] In the case of compounds with a propyl spacer, according to Barbaro et al.,[15] affinities for $\alpha_1$-adrenoceptors were decreasing in the following order: 2-methoxyphenyl- > phenyl- > 2-furoyl- > 2-pyridylpiperazine derivatives. In contrast to our results, the 1,4-benzodioxan derivatives showed higher activity with the 2-furoyl- than with the 2-pyridyl substituent. The decrease of activity for furoyl- and pyridyl derivatives can be explained based on our molecular

modelling results (Fig. 6). In the case of furoyl- and pyridyl derivatives (**6a**, **7a**, **11a**, **12a**), the structural parameters significantly differ from the ideal $\alpha_1$-antagonist parameters. These compounds do not possess any fragment that could map HY2 of Barbaro's model. Furthermore, furoyl derivatives **6a** and **11a**, contrary to the rest of the obtained compounds (**1a**–**5a**, **7a**–**10a**, and **12a**–**16a**), possess distinctly shorter PI–HY1 distances than that described for the ideal antagonist. Finally, the furoyl- and pyridyl derivatives (**6a**, **7a**, **11a**, and **12a**), similarly to all the tested group of compounds (**1a**–**16a**), showed significantly shorter PI–HY3 distances in comparison to the ideal $\alpha_1$-antagonist described by Barbaro et al.[14]

Although a favourable influence of 2-alkoxyphenylpiperazine moieties on $\alpha_1$-adrenoceptor antagonistic properties is obvious, we also obtained some 2-alkoxyphenylpiperazine derivatives (**8a**, **15a**, and **16a**) which were clearly less active than the lead compound **AZ-99**. This can suggest that other structural fragments can strongly limit affinities for $\alpha_1$-AR in the considered chemical group (**1a**–**16a**). Considering an importance of 3-N-substituents, the $\alpha_1$-adrenoceptor affinities for 2-methoxyphenylpiperazine derivatives (**1a**–**4a**, **9a**, **13a**, bf 15a) were analysed. The results showed that the methyl 2-propionate substituent (**13a**) was the most favourable. The other ester derivative, ethyl 2-(3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)propionate hydrochloride (**3a**), showed slightly lower affinity for $\alpha_1$-adrenoceptors but the highest selectivity to $\alpha_2$-AR. Interestingly, this compound (**3a**) was the most active antiarrhythmic agent in the adrenaline-induced model of arrhythmia.[19] The $\alpha_1$-adrenoceptor affinities for 2-methoxyphenyl derivatives were decreasing in the following order: methyl 2-propionate- (**13a**) > ethyl 2-propionate- (**3a**) > ethyl acetate- (**2a**) > methyl- (**4a**) > methyl acetate- (**9a**) > ethyl- (**1a**) > methyl 2-butyrate derivative (**15a**). Generally, compounds **1a**–**4a**, **9a**, and **13a** showed affinities in the range of $10^{-7}$ M. Only compound **15a** was significantly less active, displaying an affinity for $\alpha_1$-AR in the micromolar range (3.1 μM). This may suggest an unprofitable effect of large methyl 2-butyrate moiety at position 3-N in hydantoin. A similar situation can be observed in the case of 2-ethoxyphenylpiperazine derivatives (**5a**, **10a**, **14a**, and **16a**). Compound **16a**, possessing a methyl 2-butyrate fragment, was much less active than the rest of the compounds (**5a**, **10a**, and **14a**).

Malawska et al.[20] indicated that acetylation of the 2-hydroxypropyl spacer may improve the affinity for $\alpha_1$-adrenoceptors. They described two pairs of arylpiperazine derivatives of pirolidyn-2-one possessing 2-hydroxypropyl- or 2-acetoxypropyl moiety. In the case of 2-methoxyphenylpiperazine derivatives, the compound possessing a 2-acethoxypropyl group had a 1.68-fold higher affinity for $\alpha_1$-AR than its hydroxypropyl analogue. In the pair of 2-chlorophenylpiperazine derivatives, the 2-acethoxypropyl derivative was 2.23-fold more potent than its 2-hydroxypropyl analogue. In our present work, we obtained the opposite results. Considering the binding properties of the two corresponding 2-ethoxyphenyl derivatives **5a** and **8a**, differing in the area of the 2-hydroxypropyl spacer, an almost 5-fold decrease of affinity for $\alpha_1$-adrenoceptors was observed in the case of acetylated compound **8a** (Table 3).

In terms of selectivity, compounds **1a**–**16a** were weakly selective (0.74- to 8.48-fold) towards $\alpha_1$-AR over $\alpha_2$-AR (Table 3). The most active compounds (**2a**–**5a**, **9a**, **13a**, and **14a**) displayed a slightly higher selectivity than the lead compound **AZ-99**. The highest selectivity of compound **3a** may suggest that ethyl 2-propionate moiety at position 3 in hydantoin is responsible for the selectivity. This cannot be fully confirmed in the present work because compound **3a** is the only member of ethyl 2-propionate derivatives. In the case of similar compounds possessing methyl 2-propionate moieties (**13a** and **14a**), the $\alpha_2/\alpha_1$ selectivity is almost 2-fold lower. The 3-methyl derivatives group, including five compounds (**4a**–**8a**) shows heterogeneous $\alpha_2/\alpha_1$ selectivity. Similar trends can be observed in the group of methyl 2-acetate derivatives (**9a**–**12a**). The active 2-alkoxyphenylpiperazine derivatives (**9a** and **10a**) were 3- to 4-fold more potent at $\alpha_1$-ARs while the weakly active compounds (**11a** and **12a**) displayed higher affinity for $\alpha_2$- than for $\alpha_1$-ARs. Thus, a substituent at 3-N-hydantoin does not seem to be crucial for target selectivity. Likewise, the presence of 2-alkoxyphenyl does not affect selectivity. On the other hand, the introduction of 2-pyridyl- or 2-furoyl moiety reduces the selectivity towards $\alpha_1$- in relation to $\alpha_2$-adrenoceptors dramatically.

Based on the radioligand binding results, we selected a smaller group of compounds, including active 2-alkoxyphenylpiperazine derivatives (**3a**–**5a**, **13a**, and **14a**) and the moderately active compound **7a**, for functional bioassay to evaluate the nature of their interaction with $\alpha_1$-adrenoceptors. The obtained results showed $\alpha_1$-adrenoceptor antagonistic properties for all tested compounds, but only 1-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione hydrochloride (**4a**) showed competitive interaction with $\alpha_1$-adrenoceptors in rat aorta. Furthermore, the results of antagonistic potency for the tested compounds were in agreement with radioligand binding results. The most similar results were obtained for the ethyl 2-propionate derivative **3a**, which showed almost identical $pK_i$- and $pK_B$ values (Fig. 7). In addition, radioligand binding test and functional bioassay results were also consistent for the less active 2-pyridyl derivative **7a**. In the case of most active compounds (**13a** and **5a**) $pK_B$ values were slightly higher than corresponding $pK_i$ values. In contrast, for compounds **4a** and **14a** both the $pA_2$- and $pK_B$ values were slightly lower than the corresponding $pK_i$ values. The similarity of binding results to functional bioassays results, obtained for antagonists **3a**, **4a**, **5a**, **7a**, **13a** and **14a**, indicated an almost linear correlation for the $pK_i$–$pK_B$ relationship (Fig. 7) with a $R^2$-value of 0.89 ($p < 0.005$). Although the results of both functional and radioligand binding assays are not fully comparable because of different experimental conditions,[9] the

obtained consistency seems to confirm the accuracy of the performed tests.

## 4. Conclusion

The results of pharmacological tests confirmed our hypothesis that previously obtained derivatives (**1a**–**3a**) with antiarrhythmic properties possess antagonistic activity towards $\alpha_1$-adrenoceptors. Furthermore, through modifications of compound **AZ-99**, a series of new $\alpha_1$-adrenoceptor antagonists were synthesized (**4a**, **5a**, **9a**, **10a**, **13a**, and **14a**). The SAR study has distinctly indicated a favourable effect of 2-alkoxyphenylpiperazine moieties for $\alpha_1$-adrenoceptor antagonistic properties in the considered group. The results of both radioligand binding assays and functional bioassays demonstrate that methyl 2-(3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)propionate hydrochloride (**13a**) and 1-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)-2-hydroxypropyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione hydrochloride (**5a**) are the most active $\alpha_1$-adrenoceptor antagonists among tested arylpiperazine derivatives of phenytoin (**1a**–**16a**). As all 2-alkoxyphenylpiperazine derivatives with higher potency (**3a**, **4a**, **5a**, **13a**, and **14a**) displayed the similar magnitude of $\alpha_1$-adrenoceptor blocking activity, the SAR study was not able to estimate which of the two 2-alkoxyl groups, as well as, which of the corresponding four types of 3-N-substituents is distinctly preferable for the observed $\alpha_1$-adrenolytic properties. In contrast, the SAR study clearly indicated some structural disadvantages for decreasing the affinity for $\alpha_1$-AR: 2-furoyl- or 2-pyridylpiperazine group, methyl 2-butyrate moiety at position 3-N as well as the acetylation of 2-hydroxylpropyl chain. The molecular modelling results helped to explain the lowest activities of the 2-pyridyl and 2-furoyl derivatives. These results indicated that compounds **6a**, **7a**, **11a**, and **12a** poorly map the pharmacophore model of $\alpha_1$-adrenoceptor antagonist because the distances between PI-nitrogen and hydrophobic fragments (HY1 and HY3) are significantly shorter than those for the ideal $\alpha_1$-antagonist and the compounds do not possess any substituent corresponding to HY2 region. Furthermore, results of the molecular modelling showed that all of the investigated compounds (**1a**–**16a**) hardly map pharmacophore hydrophobic region HY3 as they possess appropriate aromatic rings situated too close to positive ionisable region comparing to the ideal $\alpha_1$-antagonist. This may be the main disadvantage limiting $\alpha_1$-adrenoceptor antagonistic properties in this group of phenylpiperazine derivatives of phenytoin.

As a continuation of this study, further modifications of compound **AZ-99** are necessary to improve its $\alpha_1$-adrenoceptor antagonistic activity. Furthermore, the antiarrhythmic properties of phenylpiperazine derivatives of phenytoin (**4a**–**16a**) presented here need to be evaluated. Also, the relationship between $\alpha_1$-antagonistic- and antiarrhythmic activity needs to be established.

## 5. Experimental

### 5.1. Chemistry

$^1$H NMR spectra were recorded on Varian Mercury VX 300 MHz PFG instrument in DMSO-$d_6$ at ambient temperature. Chemical shifts are given in parts per million relative to tetramethylsilane, coupling constants given in Hz. 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 precoated Merck silica gel 60 $F_{254}$ aluminium sheets, the used solvent systems were: (I) toluene/acetone 40:3; (II) toluene/acetone/methanol 5:5:1. Melting points were determined using Mel-Temp II apparatus and are uncorrected. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values unless stated otherwise (Table 5). Syntheses under microwave irradiation were performed in household microwave oven Samsung M1618. Syntheses of compounds **18**, **19**, **22**, and **23** were described earlier.[20,21]

**5.1.1. General method for preparation of esters 20 and 21.** Compounds **20** and **21** were obtained based on the procedure described for similar esters.[19] A mixture of DPH **17** (100 mmol, 25.2 g), TEBA (13 mmol, 3 g), and $K_2CO_3$ (290 mmol, 40 g) in acetone (500 mL) was heated under reflux for 30 min. Appropriate methyl bromoester (100 mmol) in acetone (100 mL) was added. The mixture was heated under reflux for 3–5 h and stirred at room temperature overnight. The inorganic precipitate was separated by filtration. The filtrate was evaporated and the residue was crystallised from methanol giving pure products **20** and **21**, respectively.

**5.1.1.1. Methyl 2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl)propionate (20).** One-hundred millimoles of educt was used. Bright crystals (19.8 g, 58.6 mmol, 59%) mp 106–108 °C, $R_f$(I): 0.26.

**5.1.1.2. Methyl 2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl)butyrate (21).** Eighty millimoles (20.16 g) of educt was used. White crystals (20.1 g, 56.8 mmol, 71%) mp 152–153 °C, $R_f$(I): 0.24.

**5.1.2. Preparation of methyl 2-(3-(oxiran-2-ylmethyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)esters 24 and 25.** Compounds **24** and **25** were obtained based on the procedures described earlier for similar esters.[19]

**5.1.2.1. Methyl 2-(3-(oxiran-2-ylmethyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)propionate (24).** A suspension of **20** (50 mmol, 16.19 g), $K_2CO_3$ (20 g), and TEBA (1.5 g) in acetone (100 mL) was stirred at room temperature for 30 min, then, a solution of freshly distilled epichlorohydrin (55 mmol, 5.06 g) in acetone (60 mL) was added dropwise. The suspension was stirred for the next 54 h, then, the precipitate was removed by filtration. The filtrate was condensed to 1/3 of the starting volume and kept at 0–4 °C overnight. The obtained solid was collected by filtration and dried to give bright crystals of **24** (13.2 g, 34 mmol, 67%) mp 121–122 °C, $R_f$(I): 0.30.

**Table 5.** Elemental analysis of compounds **4a**–**16a** and corresponding basic forms **4**–**16**

| Compound | Empirical formula | % C | | % H | | % N | |
|---|---|---|---|---|---|---|---|
| | | Calcd[a] | Found | Calcd | Found | Calcd | Found |
| **4** | $C_{30}H_{34}N_4O_4$ | 70.02 | 70.12 | 6.66 | 6.65 | 10.89 | 10.88 |
| **4a** | $C_{30}H_{34}N_4O_4 \times HCl$ | 65.38 | 65.64 | 6.40 | 6.40 | 10.17 | 10.08 |
| **5** | $C_{31}H_{36}N_4O_4$ | 70.43 | 70.29 | 6.86 | 6.85 | 10.60 | 10.57 |
| **5a** | $C_{31}H_{36}N_4O_4 \times HCl$ | 65.89 | 65.41 | 6.60 | 6.53 | 9.91 | 9.66 |
| **6a** | $C_{28}H_{34}N_4O_5 \times HCl$ | 62.39 | 62.21 | 5.80 | 5.78 | 10.39 | 10.21 |
| **7** | $C_{28}H_{31}N_5O_3$ | 69.26 | 69.24 | 6.43 | 6.44 | 14.42 | 14.15 |
| **7a** | $C_{28}H_{31}N_5O_3 \times 1.75\ HCl \times H_2O \times 0.33\ CH_3OH$ | 58.67 | 58.80 | 6.35 | 6.41 | 12.00 | 12.14 |
| **8a** | $C_{33}H_{37}N_4O_5 \times HCl \times 0.5\ H_2O$ | 64.43 | 64.39 | 6.39 | 6.44 | 9.11 | 9.14 |
| **9** | $C_{32}H_{36}N_4O_6$ | 66.59 | 66.55 | 6.37 | 6.26 | 9.71 | 9.36 |
| **9a** | $C_{32}H_{36}N_4O_6 \times HCl \times 0.5\ H_2O$ | 62.18 | 62.14 | 6.20 | 6.17 | 9.06 | 8.97 |
| **10** | $C_{33}H_{38}N_4O_6$ | 66.87 | 66.87 | 6.58 | 6.53 | 9.46 | 9.37 |
| **10a** | $C_{33}H_{38}N_4O_6 \times HCl \times H_2O$ | 61.82 | 61.79 | 6.45 | 6.46 | 8.74 | 8.65 |
| **11a** | $C_{30}H_{32}N_4O_7 \times HCl$ | 60.35 | 60.29 | 5.57 | 5.56 | 9.38 | 9.12 |
| **12** | $C_{30}H_{33}N_5O_5$ | 65.85 | 65.66 | 6.15 | 6.06 | 12.80 | 12.70 |
| **12a** | $C_{30}H_{33}N_5O_5 \times 2\ HCl \times 1.5\ H_2O$ | 56.00 | 56.19 | 5.95 | 5.91 | 10.88 | 10.91 |
| **13** | $C_{33}H_{38}N_4O_6$ | 67.56 | 67.37 | 6.53 | 6.56 | 9.55 | 9.43. |
| **13a** | $C_{33}H_{38}N_4O_6 \times HCl \times 0.25\ H_2O$ | 63.15 | 63.35 | 6.34 | 6.28 | 8.93 | 8.88 |
| **14** | $C_{34}H_{40}N_4O_6$ | 66.98 | 66.95 | 6.78 | 6.77 | 9.19 | 9.10 |
| **14a** | $C_{34}H_{40}N_4O_6 \times HCl \times 1.5\ H_2O$ | 61.48 | 61.64 | 6.68 | 6.64 | 8.44 | 8.52 |
| **15a** | $C_{34}H_{40}N_4O_6 \times HCl \times 1.33\ H_2O$ | 61.76 | 61.79 | 6.66 | 6.58 | 8.47 | 8.44 |
| **16a** | $C_{35}H_{42}N_4O_6 \times HCl$ | 64.55 | 64.65 | 6.66 | 6.57 | 8.60 | 8.63 |
| **20** | $C_{19}H_{18}N_2O_4$ | 67.44 | 67.4 | 5.36 | 5.51 | 8.28 | 8.26 |
| **21** | $C_{20}H_{20}N_2O_4$ | 68.17 | 68.47 | 5.72 | 5.65 | 7.95 | 8.06 |
| **24** | $C_{22}H_{22}N_2O_5$ | 66.99 | 66.98 | 5.62 | 5.67 | 7.10 | 7.09 |
| **25** | $C_{23}H_{24}N_2O_5$ | 67.63 | 67.78 | 5.92 | 5.89 | 6.86 | 6.83 |

[a] Theoretical results of elementary analysis were calculated for the given empirical formulas.

**5.1.2.2. Methyl 2-(3-(oxiran-2-ylmethyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)butyrate (25).** A suspension of **21** (20 mmol, 7.04 g), $K_2CO_3$ (8 g), and TEBA (0.6 g), in acetone (40 mL) was stirred at room temperature for 60 min, then, a solution of freshly distilled epichlorohydrin (22 mmol, 5.06 g) in acetone (24 mL) was added dropwise. The suspension was stirred for the next 68 h, then, the precipitate was removed by filtration. The filtrate was condensed by evaporation. The residue was purified by crystallisation from methanol to give white powder of **25** (5.8 g, 14 mmol, 71%) mp 110–112 °C, $R_f$(I): 0.29.

**5.1.3. Synthesis of arylpiperazine phenytoin derivatives (4a–16a).** Preparation of compounds **4**, **6**, **7**, **9**, **11**–**13**, and **15** was performed using commercially available free bases of 2-methoxyphenyl-, 2-furoyl-, and 2-pyridylpiperazine, respectively. In the case of synthesis of compounds **5**, **8**, **10**, **14**, and **16**, commercially available monohydrochloride salt of 2-ethoxyphenylpiperazine was previously converted into free base according to the method described earlier.[27]

**5.1.3.1. General procedure for preparation of arylpiperazine phenytoin derivatives (4a–7a, 9a, 10a, and 12a–14a ). Method A.** Equimolar (5–10 mmol) amounts of appropriate arylpiperazine and 2-(3-(oxiran-2-ylmethyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl derivative (**22**–**25**) were placed in a flat-bottomed flask and irradiated in a standard household microwave oven, using various times of irradiation for each prepared compound, respectively. The reactants were melted at higher irradiation-power (450 W) for 3–5 min, then the irradiation was continued at mean power (300–450 W) for the next 7–12 min under TLC control. A product was precipitated by methanol from the glassy residue giving pure crystals of desired compound in basic form (**4**–**7**, **9**, **10**, and **12**–**14**). The obtained basic form (**4**–**7**, **9**, **10**, and **12**–**14**) was dissolved in anhydrous methanol and was saturated with dried gaseous hydrogen chloride until acidic pH. The mixture was left at 0–4 °C overnight to give a precipitate of a desirable hydrochloride (**4a**–**7a**, **9a**, **10a**, and **12a**–**14a**).

*5.1.3.1.1. Hydrochloride of 1-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione (**4a**). Compound* **4***:* Compound **22** (1.56 g,) and 1-(2-methoxyphenyl)piperazine (0.96 g) were melted (450 W) for 3 min, then for 2 min (450 W), 4 min (300 W). White precipitate (1.2 g, 2.3 mmol, 46%) mp 124–125 °C, $R_f$(II): 0.66. $^1$H NMR for **4** (DMSO-$d_6$) $\delta$ [ppm]: 1.96–2.01 (m, 2H, Pp-$CH_2$), 2.19 (br s, 4H, Pp-2,6-H), 2.78 (br s, 4H, Pp-3,5-H), 2.97–3.03 (m, 4H, N3-$CH_3$, C*H*OH), 3.26–3.34 (m, 2H, N1-$CH_2$), 3.72 (s, 3H, $OCH_3$), 4.40 (d, $J$ = 4.95 Hz, 1H, OH) 6.81–6.83 (m, 2H, PpPh-4,6-H), 6.86–6.92 (m, 2H, 2× Ph-4-H), 7.22–7.27 (m, 4H, 2× Ph-2,6-H), 7.40–7.45 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H).

*Compound* **4a***:* Using compound **4** (1 g, 1.94 mmol) in 15 mL of methanol. White crystals of **4a** from ethanol (1.05 g, 1.91 mmol, 98%) mp 260–262 °C, $R_f$(II): 0.66. $^1$H NMR for **4a** (DMSO-$d_6$) $\delta$ [ppm]: 2.61–2.68 (m, 1H, C*H*OH), 2.80–2.95 (m, 6H, Pp-$CH_2$, Pp-2,6-H), 2.98 (s, 3H, N3-$CH_3$), 3.23–3.46 (m, 6H, Pp-3,5-H, N1-$CH_2$), 3.78 (s, 3H, $OCH_3$), 4.05 (br s, 1H, OH), 6.86–6.87 (m, 2H, PpPh-4,6-H), 6.94–7.03 (m, 2H, 2×

Ph-4-H), 7.21–7.32 (m, 4H, 2× Ph-2,6-H), 7.43–7.49 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H), 9.96 (br s, 1H, $NH^+$). IR (KBr) [$cm^{-1}$]: 3292 (OH), 2953 (CH), 2549 ($NH^+$), 1770 (C2═O), 1716 (C4═O), 1593 (Ar).

*5.1.3.1.2. Hydrochloride of 1-(3-(4-(2-ethoxyphenyl)-piperazin-1-yl)-2-hydroxypropyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione (**5a**). Compound **5**:* Compound **22** (1.55 g, 5 mmol) and 1-(2-ethoxyphenyl)piperazine (1.03 g, 5 mmol) were melted (450 W) for 3 min, then for 3 min (450 W), 4 min (300 W). White crystals (1.4 g, 2.7 mmol, 54%) mp 120–122 °C, $R_f$(II): 0.69. $^1H$ NMR for **5** (DMSO-$d_6$) $\delta$ [ppm]: 1.28 (t, $J$ = 7.05 Hz, 3H, $OCH_2CH_3$), 2.0 (d, $J$ = 5.8 Hz, 2H, Pp-$CH_2$), 2.19 (br s, 4H, Pp-2,6-H), 2.80 (br s, 4H, Pp-3,5-H), 2.90–3.02 (s, 1H, C*H*OH), 2.97 (s, 3H, N3-$CH_3$), 3.22–3.36 (m, 2H, N1-$CH_2$), 3.92 (q, $J$ = 6.98 Hz, 2H, $OCH_2CH_3$), 4.40 (d, $J$ = 4.95 Hz, 1H, OH) 6.80–6.87 (m, 4H, PpPh-4,6-H, 2× Ph-4-H), 7.22–7.27 (m, 4H, 2× Ph-2,6-H), 7.40–7.47 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H).

*Compound* **5a***:* Using compound **5** (1 g, 1.9 mmol) in 15 mL of methanol. Pure white crystals (1.05 g, 1.86 mmol, 98%) mp 245–246 °C, $R_f$(II): 0.69. $^1H$ NMR for **5a** (DMSO-$d_6$) $\delta$ [ppm]: 1.36 (t, $J$ = 7.02 Hz, 3H, $OCH_2CH_3$), 2.66–2.90 (m, 7H, Pp-$CH_2$, Pp-2,6-H, C*H*OH), 2.98 (s, 3H, N3-$CH_3$), 3.14–3.49 (m, 6H, Pp-3,5-H, N1-$CH_2$), 3.98 (q, $J$ = 6.88 Hz, 2H, $OCH_2CH_3$), 5.64 (br s, 1H, OH), 6.85–6.89 (m, 2H, PpPh-4,6-H), 6.91–6.99 (m, 2H, 2× Ph-4-H), 7.22–7.34 (m, 4H, 2× Ph-2,6-H), 7.44–7.49 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H), 9.70 (br s, 1H, $NH^+$). IR (KBr) [$cm^{-1}$]: 3274 (OH), 2973 (CH), 2452 ($NH^+$), 1770 (C2═O), 1714 (C4═O), 1589 (Ar).

*5.1.3.1.3. Dihydrochloride of 1-(2-hydroxy-3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione (**7a**). Compound **7**:* Compound **22** (3.1 g, 10 mmol) and 1-(2-pyridiny)lpiperazine (1.66 g, 10 mmol) were melted (450 W) for 3 min, then for 4 min (300 W). White crystals (1.29 g, 2.66 mmol, 27%) mp 95–96 °C, $R_f$ (II): 0.65. $^1H$ NMR for **7** (DMSO-$d_6$) $\delta$ [ppm]: 1.92–2.04 (m, 2H, Pp-$CH_2$), 2.12 (t, $J$ = 4.81 Hz, 4H, Pp-2,6-H), 2.97 (s, 3H, N3-$CH_3$), 3.00–3.16 (m, 1H, CHOH), 3.12–3.38 (m, 6H, Pp-3,5-H, N1-$CH_2$); 4.41 (d, $J$ = 4.95 Hz, 1H, OH), 6.56 (dd, $J_1$ = 6.5 Hz, $J_2$ = 4.95 Hz, 1H, Pd-5-H), 6.72 (d, $J$ = 8.8 Hz, 1H, Pd-3-H), 7.21–7.26 (m, 4H, 2× Ph-2,6-H), 7.32–7.50 (m, 7H, 2× Ph-3,4,5-H, Pd-4-H), 8.04 (dd, $J_1$ = 4.8 Hz, $J_2$ = 1.8 Hz, 1H, Pd-6-H).

*Compound* **7a***:* Using compound **7** (1 g, 2.1 mmol) and 15 mL of methanol. Bright crystals (1.1 g, 1.9 mmol, 91%) mp 108–110 °C, $R_f$(II): 0.65. $^1H$ NMR for **7a** (DMSO-$d_6$) $\delta$ [ppm]: 2.65–2.89 (m, 3H, C*H*OH, Pp-$CH_2$), 2.97 (s, 3H, N3-$CH_3$), 2.97–3.05 (m, 2H, Pp-2,6-$H_a$), 3.21–3.49 (m, 6H, Pp-2,6-$H_e$, Pp-3,5-$H_a$, N1-$CH_2$), 4.34–4.43 (m, 3H, Pp-3,5-$H_e$, OH), 6.91 (t, $J$ = 6.3 Hz, 1H, Pd-5-H), 7.14–7.28 (m, 5H, Pd-3-H, 2× Ph-2,6-H), 7.44–7.48 (m, 7H, 2× Ph-3,4,5-H, Pd-1-$NH^+$-H), 7.88 (t, $J$ = 7.7 Hz, 1H, Pd-4-H), 8.09 (dd, $J_1$ = 5.8 Hz, $J_2$ = 1.7 Hz, 1H, Pd-6-H), 10.24 (br s, 1H, $NH^+$). IR (KBr) [$cm^{-1}$]: 3404 (OH), 2946 (CH), 2458 ($NH^+$), 1771 (C2═O), 1707 (C4═O), 1541 (Ar).

*5.1.3.1.4. Hydrochloride of methyl 2-(3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetate (**9a**). Compound **9**:* Compound **23** (2.15 g, 6 mmol) and *N*-2-metoxyphenyl-piperazine (1.1 g, 6 mmol)) were melted (450 W) for 5 min, then for 9 min (300 W). White precipitate (1.7 g, 3 mmol, 50%) mp 100–103 °C, $R_f$(II): 0.67. $^1H$ NMR for **9** (DMSO-$d_6$) $\delta$ [ppm]: 1.99 (d, $J$ = 6.05 Hz, 2H, Pp-$CH_2$), 2.21 (br s, 4H, Pp-2,6-H), 2.79 (br s, 4H, Pp-3,5-H), 2.96–3.07 (m, 1H, C*H*OH), 3.21–3.36 (m, 2H, N1-$CH_2$), 3.68 (s, 3H, $OCH_3$), 3.73 (s, 3H, $COOCH_3$), 4.34 (s, 2H, N3-$CH_2$), 4.38–4.42 (m, 1H, OH), 6.81–7.05 (m, 4H, PpPh-4,6-H, 2× Ph-4-H), 7.29–7.34 (m, 4H, 2× Ph-2,6-H), 7.44–7.49 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H).

*Compound* **9a***:* Using compound **9** (1.3 g, 2.25 mmol) and 15 mL of methanol. White crystals (1.1 g, 1.78 mmol, 80%) mp 215–217 °C, $R_f$(II): 0.67. $^1H$ NMR for **9a** (DMSO-$d_6$) $\delta$ [ppm]: 2.75–2.81 (m, 3H, C*H*OH, Pp-$CH_2$), 2.92–3.08 (m, 4H, Pp-2,6-$CH_2$), 3.14–3.36 (m, 6H, N1-$CH_2$, Pp-3,5-H), 3.67 (s, 3H, $COOCH_3$), 3.77 (s, 3H, $OCH_3$), 4.37 (s, 2H, N3-$CH_2$), 5.23 (br s, 1H, OH), 6.87–6.90 (m, 2H, PpPh-4,6-H), 6.94–7.03 (m, 2H, 2× Ph-4-H), 7.28–7.44 (m, 4H, 2× Ph-2,6-H), 7.47–7.53 (m, 6H, PpPh-3,5-H, 2× Ph-3,5-H), 10.13 (br s, 1H, $NH^+$). IR (KBr) [$cm^{-1}$]: 3458 (OH), 2950 (CH), 2432 ($NH^+$), 1780 (C2═O), 1744 (C═O ester), 1728 (C4═O), 1603 (Ar).

*5.1.3.1.5. Hydrochloride of methyl 2-(3-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)-2-hydroxypropyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetate (**10a**). Compound **10**:* Compound **23** (1.99 g, 5 mmol) and 1-(2-ethoxyphenyl)piperazine (1.03 g, 5 mmol)) were melted (450 W) for 3 min, then for 3 min (450 W), for 6 min (300 W). White precipitate (1.35 g, 2.28 mmol, 46%) mp 126–128 °C, $R_f$(II): 0.78. $^1H$ NMR for **10** (DMSO-$d_6$) $\delta$ [ppm]: 1.28–1.33 (t, $J$ = 7.05 Hz, 3H, $OCH_2CH_3$), 1.99 (d, $J$ = 6.1 Hz, 2H, Pp-$CH_2$), 2.20 (br s, 4H, Pp-2,6-H), 2.81 (br s, 4H, Pp-3,5-H), 3.03–3.09 (m, 1H, C*H*OH), 3.22–3.35 (m, 2H, N1-$CH_2$), 3.68 (s, 3H, $OCH_3$), 3.93 (q, $J$ = 6.9 Hz, 2H, $OCH_2CH_3$), 4.34 (s, 2H, N3-$CH_2$), 4.41 (d, $J$ = 4.95 Hz, 1H, OH), 6.78–6.87 (m, 4H, PpPh-4,6-H, 2× Ph-4-H), 7.30–7.34 (m, 4H, 2× Ph-2,6-H), 7.41–7.49 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H).

*Compound* **10a***:* Using compound **10** (1.0 g, 1.7 mmol) and 15 mL of methanol. White crystals (1.07 g, 1.67 mmol, 98%) mp 130–131 °C, $R_f$(II): 0.78. $^1H$ NMR for **10a** (DMSO-$d_6$) $\delta$ [ppm]: 1.34 (t, $J$ = 6.88 Hz, 3H, $OCH_2CH_3$), 2.05–2.07 (m, 2H, Pp-$CH_2$), 2.77–3.06 (m, 5H, Pp-2,6-H, C*H*OH), 3.31–3.58 (m, 6H, Pp-3,5-H, N1-$CH_2$), 3.66 (s, 3H, $OCH_3$), 3.98 (q, $J$ = 6.97 Hz, 2H, $OCH_2CH_3$), 4.37 (s, 2H, N3-$CH_2$), 5.63 (br s, 1H, OH), 6.85–6.91 (m, 2H, PpPh-4,6-H), 6.94–6.99 (m, 2H, 2× Ph-4-H), 7.29–7.37 (m, 4H, 2× Ph-2,6-H), 7.44–7.53 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H), 9.88 (br s, 1H, $NH^+$); IR (KBr) [$cm^{-1}$]: 3357 (OH), 2980, 2940 (CH), 2427 ($NH^+$), 1777 (C2═O), 1751 (C═O ester), 1722 (C4═O), 1592 (Ar).

*5.1.3.1.6. Dihydrochloride of methyl 2-(3-(2-hydroxy-3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetate (**12a**). Compound **12**:*

Compound **23** (1.9 g, 5 mmol) and 1-(2-pyridinyl)piperazine (0.83 g, 5 mmol)) were melted (450 W) for 4 min, then for 9 min (300 W). White precipitate (1.4 g, 2.56 mmol, 51%) mp 134–135 °C, $R_f$(II): 0.63. $^1$H NMR for **12** (DMSO-$d_6$) $\delta$ [ppm]: 1.97 (d, $J$ = 6.05 Hz, 2H, Pp-$CH_2$), 2.15 (br s, 4H, Pp-2,6-H), 3.04–3.16 (m, 1H, C*H*OH), 3.23–3.36 (m, 6H, Pp-3,5-H, N1-$CH_2$), 3.66 (s, 3H, $OCH_3$), 4.34 (s, 2H, N3-$CH_2$), 4.45 (br s, 1H, OH), 6.57 (dd, $J_1$ = 6.6 Hz, $J_2$ = 4.95 Hz, 1H, Pd-5-H), 6.73 (d, $J$ = 8.8 Hz, 1H, Pd-3-H), 7.29–7.34 (m, 6H, 2× Ph-2,4,6-H), 7.44–7.50 (m, 5H, 2× Ph-3,5-H, Pd-4-H), 8.04 (dd, $J_1$ = 4.95 Hz, $J_2$ = 1.4 Hz, 1H, Pd-6-H).

*Compound* **12a**: Using compound **12** (1.1 g, 2.0 mmol) and 15 mL of methanol. White crystals (1.1 g, 1.71 mmol, 85%) mp 94–95 °C, $R_f$(II): 0.63. $^1$H NMR for **12a** (DMSO-$d_6$) $\delta$ [ppm]: 2.77–2.91 (m, 3H, Pp-$CH_2$, C*H*OH), 3.04–3.14 (m, 4H, Pp-2,6-H), 3.30–3.56 (m, 6H, Pp-3,5-H, N1-$CH_2$), 3.66 (s, 3H, $OCH_3$), 4.36 (s, 2H, N3-$CH_2$), 4.39–4.51 (m, 1H, OH), 6.94 (t, $J$ = 6.33 Hz, 1H, Pd-5-H), 7.27–7.35 (m, 5H, 2× Ph-2,6-H, Pd-3-H), 7.44–7.53 (m, 7H, 2× Ph-3,4,5-H, Pd-1-$NH^+$), 7.92 (t, $J$ = 7.7 Hz, 1H, Pd-4-H), 8.08 (dd, $J_1$ = 5.78 Hz, $J_2$ = 1.38 Hz, 1H, Pd-6-H), 10.39 (br s, 1H, $NH^+$); IR (KBr) [$cm^{-1}$]: 3441 (OH), 2949, 2936 (CH), 2464 ($NH^+$), 1779 (C2═O), 1754 (C═O ester), 1704 (C4═O), 1590 (Ar).

*5.1.3.1.7. Hydrochloride of methyl 2-(3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)propionate (**13a**). Compound* **13**: Compound **24** (1.97 g, 5 mmol) and 1-(2-methoxyphenyl)piperazine (0.91 g, 5 mmol)) were melted (450 W) for 4 min, then for 2 min (450 W), for 4 min (300 W). White precipitate (1.6 g, 2.73 mmol, 55%) mp 115–118 °C, $R_f$(II): 0.75. $^1$H NMR for **13** (DMSO-$d_6$) $\delta$ (ppm): 1.46 (d, $J$ = 7.15 Hz, 3H, N3-$CHCH_3$), 2.01–2.09 (m, 2H, Pp-$CH_2$), 2.21 (br s, 4H, Pp-2,6-H), 2.80 (br s, 4H, Pp-3,5-H), 3.10–3.18 (m, 1H, C*H*OH), 3.27–3.34 (m, 2H, N1-$CH_2$), 3.68 (s, 3H, $OCH_3$), 3.76 (s, 3H, $COOCH_3$), 4.42 (d, $J$ = 4.95 Hz, 1H, OH), 4.86 (q, $J$ = 7.20 Hz, 1H, N3-$CHCH_3$) 6.78–6.89 (m, 4H, PpPh-4,6-H, 2× Ph-4-H), 7.20–7.34 (m, 4H, 2× Ph-2,6-H), 7.40–7.52 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H).

*Compound* **13a**: Using compound **13** (1.0 g, 1.7 mmol) in 15 mL of methanol. White crystals (1.0 g, 1.6 mmol, 94%) mp 168–169 °C, $R_f$(II): 0.75. $^1$H NMR for **13a** (DMSO-$d_6$) $\delta$ [ppm]: 1.54 (d, $J$ = 7.15 Hz, 3H, N3-CH $CH_3$), 2.74–3.07 (m, 7H, C*H*OH, Pp-$CH_2$, Pp-2,6-H), 3.38–3.47 (m, 6H, Pp-3,5-H, N1-$CH_2$), 3.63 (s, 3H, $COOCH_3$), 3.81 (s, 3H, $OCH_3$), 4.01 (br s, 1H, OH), 4.89 (q, $J$ = 7.15 Hz, 1H, N3-$CHCH_3$), 6.91–6.98 (m, 2H, PpPh-4,6-H), 7.00–7.07 (m, 2H, 2× Ph-4-H), 7.28–7.39 (m, 4H, 2× Ph-2,6-H), 7.51–7.56 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H), 9.97 (br s, 1H, $NH^+$). IR (KBr) [$cm^{-1}$]: 3415 (OH), 2950 (CH), 2418 ($NH^+$), 1773 (C2═O), 1752 (C═O ester), 1716 (C4═O), 1609 (Ar).

*5.1.3.1.8. Hydrochloride of methyl 2-(3-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)-2-hydroxypropyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)propanoate (**14a**). Compound* **14**: **24** (1.97 g, 5 mmol) and 1-(2-ethoxyphenyl)piperazine (1.03 g, 5 mmol)) were melted (450 W) for 3 min, then for 1 min (450 W), for 3 min (300 W). White precipitate (2.0 g, 3.28 mmol, 66%) mp 134–135 °C, $R_f$(II): 0.71. $^1$H NMR for **14** (DMSO-$d_6$)$\delta$ (ppm): 1.32 (t, $J$ = 6.88 Hz, 3H, $OCH_2CH_3$), 1.49 (d, $J$ = 7.15 Hz, 3H, N3-$CHCH_3$), 2.03–2.10 (m, 2H, Pp-$CH_2$), 2.23 (br s, 4H, Pp-2,6-H), 2.85 (br s, 4H, Pp-3,5-H), 3.14–3.21 (m, 1H, C*H*OH), 3.29–3.35 (m, 2H, N1-$CH_2$,), 3.64 (s, 3H, $OCH_3$), 3.96 (q, $J$ = 7.0 Hz, 2H, $OCH_2CH_3$), 4.41 (br s, 1H, OH), 4.88 (q, $J$ = 7.24 Hz, 1H, N3-$CHCH_3$) 6.84–6.91 (m, 4H, PpPh-4,6-H, 2× Ph-4-H), 7.29–7.36 (m, 4H, 2× Ph-2,6-H), 7.46–7.54 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H).

*Compound* **14a**: Using compound **14** (1.0 g, 1.7 mmol) in 15 mL of methanol. White crystals of **14a** (1.1 g, 1.64 mmol, 97%) mp 148–150 °C, $R_f$(II): 0.71. $^1$H NMR for **14a** (DMSO-$d_6$) $\delta$ [ppm]: 1.35 (t, $J$ = 6.80 Hz, 3H, $OCH_2CH_3$), 1.51 (d, $J$ = 7.44 Hz, 3H, N3-$CHCH_3$), 2.71–2.88 (m, 4H, Pp-$CH_2$, Pp-2,6-$H_a$), 3.00–3.03 (m, 3H, C*H*OH, Pp-2,6-$H_e$), 3.35–3.43 (m, 6H, Pp-3,5-H, N1-$CH_2$), 3.61 (s, 3H, $COOCH_3$), 3.98–4.05 (m, 3H, $OCH_2CH_3$, OH), 4.88 (q, $J$ = 7.18 Hz, 1H, N3-$CHCH_3$), 6.86–6.98 (m, 4H, PpPh-4,6-H, 2× Ph-4-H), 7.25–7.36 (m, 4H, 2× Ph-2,6-H), 7.47–7.52 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H), 9.82 (br s, 1H, $NH^+$). IR (KBr) [$cm^{-1}$]: 3417 (OH), 2956 (CH), 2347 ($NH^+$); 1772 (C2═O), 1750 (C═O ester), 1716 (C4═O), 1611 (Ar).

**5.1.3.2. General procedure for preparation of arylpiperazine phenytoin derivatives (6a, 11a, 15a, and 16a). Method B.** Equimolar (5–10 mmol) amounts of appropriate arylpiperazine and 2-(3-(oxiran-2-ylmethyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl derivative (**22**–**25**) were placed in a flat-bottomed flask and irradiated in a standard household microwave oven at similar conditions to those of method A. After irradiation, the glassy residue was dissolved by heating with alcohol. The solution was left at 0–4 °C for 7–14 days. Since no precipitate appeared, the solvent was removed by evaporation. A residue was dissolved in $CH_2Cl_2$ (20–25 mL) and washed with hydrochloric acid (1%, 3 × 15 mL). The organic phase was evaporated. A residue was dissolved in anhydrous alcohol and was saturated with dried gaseous hydrogen chloride until acidic pH. The mixture was left at 0–4 °C overnight to give a desired precipitate of hydrochloride (**6a**, **11a**, **15a**, and **16a**).

*5.1.3.2.1. Hydrochloride. of 1-(3-(4-(furan-2-carbonyl)piperazin-1-yl)-2-hydroxypropyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione (**6a**).*Compound **22** (1.55 g, 5 mmol) and 1-(2-furoyl)piperazine (0.9 g, 5 mmol) were melted (450 W) for 4 min, then irradiation was continued for 2 min (300 W), 5 min (450 W). White crystals of **6a** from methanol (1.80 g, 3.24 mmol, 65%) mp 280–281 °C, $R_f$(II): 0.57. $^1$H NMR for **6a** (DMSO-$d_6$) $\delta$ [ppm]: 2.47–2.48 (m, 1H, C*H*OH), 2.57–2.64 (m, 2H, Pp-$CH_2$), 2.73–2.89 (m, 2H, Pp-3,5-$H_a$), 2.97 (s, 3H, N3-$CH_3$), 2.97–3.10 (m, 2H, Pp-3,5-$H_e$), 3.30–3.42 (m, 4H, Pp-4,6-H), 4.31–4.41 (m, 2H, N1-$CH_2$), 5.68 (br s, 1H, OH), 6.64 (dd, $J_1$ = 3.6, $J_2$ = 1.7 Hz, 1H, fur-4-H), 7.07 (d, $J$ = 3.0 Hz, 1H, fur-3-H), 7.21–7.28 (m, 4H, 2× Ph-2,6-H), 7.43–7.45 (m, 6H, 2× Ph-3,4,5-H), 7.88 (d, $J$ = 0.8 Hz, 1H, fur-5-H), 10.29 (br s, 1H,

$NH^+$); IR (KBr) ($cm^{-1}$): 3421(OH), 2944 (CH), 2433 ($NH^+$); 1767 (C2═O), 1741 (C═O furoilo), 1717 (C4═O), 1581 (Ar).

*5.1.3.2.2. Hydrochloride of methyl 2-(3-(3-(4-(furan-2-carbonyl)piperazin-1-yl)-2-hydroxypropyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetate (**11a**).* Compound **23** (1.9 g, 5 mmol) and 1-(2-furoyl)piperazine (0.9 g, 5 mmol) were melted (450 W) for 2 min, then for 2 min (300 W), for 2 min (450 W). White crystals of **11a** from methanol (0.83 g, 1.39 mmol, 28%) mp 230–231 °C, $R_f$(II): 0.59. $^1$H NMR for **11a** (DMSO-$d_6$) $\delta$ [ppm]: 2.49–2.71 (m, 3H, C*H*OH, Pp-$CH_2$), 2.80–3.14 (m, 2H, Pp-3,5-$H_a$), 3.24–3.63 (m, 6H, Pp-3,5-$H_e$, Pp-2,6-H), 3.66 (s, 3H, $OCH_3$), 4.30 (m, 2H, N1-$CH_2$), 4.36 (s, 2H, N3-$CH_2$), 5.71 (br s, 1H, OH), 6.65 (dd, $J_1$ = 3.34 Hz, $J_2$ = 1.7 Hz, 1H, fur-4-H), 7.07 (d, $J$ = 3.6 Hz, 1H, fur-3-H), 7.27–7.36 (m, 4H, 2× Ph-2,6-H), 7.42–7.52 (m, 6H, 2× Ph-3,4,5-H), 7.88 (d, $J$ = 1.7 Hz, 1H, fur-5-H), 10.20 (br s, 1H, $NH^+$). IR (KBr) [$cm^{-1}$]: 3211 (OH), 2962 (CH), 2427 ($NH^+$), 1776 (C2═O), 1748 (C═O ester), 1718 (C4═O), 1571 (Ar).

*5.1.3.2.3. Hydrochloride of methyl 2-(3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)butyrate (**15a**).* Compound **25** (2.05 g, 5 mmol) and 1-(2-methoxyphenyl)piperazine (0.9 g, 5 mmol) were melted (450 W) for 4 min, for 4 min (300 W), for 2 min (450 W). White crystals of **15a** from methanol (1.22 g, 1.85 mmol, 37%) mp 182–184 °C, $R_f$(II): 0.76. $^1$H NMR for **15a** (DMSO-$d_6$) $\delta$ [ppm]: 0.72 (t, $J$ = 7.30 Hz, 3H, N3-$CHCH_2CH_3$), 1.95–2.08 (m, 2H, $N_3$-CH-$CH_2$-$CH_3$), 2.70–3.04 (m, 7H, C*H*OH, Pp-$CH_2$, Pp-2,6-H), 3.28–3.46 (m, 6H, Pp-3,5-H, N1-$CH_2$), 3.64 (s, 3H, $COOCH_3$), 3.77 (s, 3H, $OCH_3$), 4.41 (br s, 1H, OH), 4.65 (t, $J$ = 5.30 Hz, 1H, N3-$CHCH_2CH_3$), 6.87–6.88 (m, 2H, PpPh-4,6-H), 6.94–7.03 (m, 2H, 2× Ph-4-H), 7.25–7.36 (m, 4H, 2× Ph-2,6-H), 7.45–7.53 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H), 9.99 (br s, 1H, $NH^+$). IR (KBr) [$cm^{-1}$]: 3401 (OH), 2969 (CH), 2426 ($NH^+$), 1770 (C2═O), 1745 (C═O ester), 1711 (C4═O), 1608 (Ar).

*5.1.3.2.4. Hydrochloride of methyl 2-(3-(2-hydroxy-3-(4-(2-ethoxyphenyl)piperazin-1-yl)propyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)butyrate (**16a**).* Compound **25** (2.87 g, 7 mmol) and 1-(2-methoxyphenyl)piperazine (1.44 g, 7 mmol) were melted (450 W) for 3 min, then for 2 min (450 W). White crystals of **16a** from methanol (1.20 g, 1.84 mmol, 26%) mp 203–205 °C, $R_f$(II): 0.70. $^1$H NMR for **16a** (DMSO-$d_6$) $\delta$ [ppm]: 0.72–0.75 (m, 3H, $CHCH_2CH_3$), 1.37 (t, $J$ = 6.92 Hz, 3H, $OCH_2CH_3$), 1.98–2.07 (m, 2H, $CHCH_2CH_3$), 2.60–3.03 (m, 7H, C*H*OH, Pp-$CH_2$, Pp-2,6-H), 3.16–3.39 (m, 6H, N1-$CH_2$, Pp-3,5-H), 3.64 (s, 3H, $OCH_3$), 3.98 (q, $J$ = 6.92 Hz, 2H, $OCH_2CH_3$), 4.68–4.72 (m, 1H, $CHCH_2CH_3$), 5.68 (br s, 1H, OH), 6.85–6.86 (m, 2H, PpPh-4,6-H), 6.91–6.98 (m, 2H, 2× Ph-4-H), 7.25–7.37 (m, 4H, 2× Ph-2,6-H), 7.44–7.53 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H), 9.92 (br s, 1H, $NH^+$). IR (KBr) [$cm^{-1}$]: 3255 (OH), 2977 (CH), 2486 ($NH^+$), 1776 (C2═O), 1754 (C═O ester), 1726 (C4═O), 1591 (Ar).

**5.1.3.3. Hydrochloride of 3-(4-(2-ethoxyphenyl)piperazin-1-yl)-1-(3-methyl-2,4-dioxo-5,5-diphenylimidazolidin-1-yl)propan-2-yl acetate (8a).** To compound **5** (1.9 mmol, 1 g) in a flat-bottomed flask acetic anhydride (8 mL) was added. The mixture was refluxed for 2 min and was left at 0–4 °C for 7 days. As no precipitate appeared, a glue residue was dissolved in anhydrous methanol and was saturated with dried hydrogen chloride. The mixture was left at 0–4 °C overnight to afford white crystals of **8a** (0.65 g, 1.1 mmol, 56%) mp 156–157 °C, $R_f$(II): 0.89. $^1$H NMR for **8a** (DMSO-$d_6$) $\delta$ [ppm]: 1.36 (t, $J$ = 6.92 Hz, 3H, $OCH_2CH_3$), 1.88 (s, 3H, $CH_3CO$), 2.67–2.79 (m, 1H, CH), 2.83–2.98 (m, 2H, Pp-$CH_2$), 2.98 (s, 3H, N3-$CH_3$), 3.11–3.14 (m, 4H, Pp-2,6-H), 3.17–3.37 (m, 4H, Pp-3,5-H), 3.57–3.73 (m, 2H, N1-$CH_2$), 3.98 (q, $J$ = 6.92 Hz, 2H, $OCH_2CH_3$), 6.85–6.86 (m, 2H, PpPh-4,6-H), 6.89–6.99 (m, 2H, 2× Ph-4-H), 7.19–7.32 (m, 4H, 2× Ph-2,6-H), 7.44–7.46 (m, 6H, 2× Ph-3,5-H, PpPh-3,5-H), 10.74 (br s, 1H, $NH^+$); IR (KBr) [$cm^{-1}$]: 2998 (CH), 2409 ($NH^+$), 1768 (C2═O), 1736 ($CH_3C$═O), 1714 (C4═O), 1598 (Ar).

## 5.2. Pharmacology

**5.2.1. General information.** The pharmacological studies were carried out on male Wistar rats ((KRF.(WI).WU), Animal House, Faculty of Pharmacy, Jagiellonian University Medical College, Cracow) weighing 170–350 g. Treatment of laboratory animals in the present study was in accordance with the respective Polish regulations. All procedures were conducted according to guidelines of ICLAS (International Council on Laboratory Animal Science) and approved by the Local Ethics Committee on Animal Experimentation.

Source of compounds: phenylephrine hydrochloride, acetylcholine hydrochloride, (±)-noradrenaline hydrochloride (Sigma, Aldrich Chemie Gmbh); thiopental sodium (Biochemie Gmbh, Vienna); [$^3$H]prazosin, [$^3$H]clonidine (Amersham). Other reagents were of analytical grade from local sources.

**5.2.2. Radioligand binding test.** The compounds were evaluated for their affinity to $\alpha_1$- and $\alpha_2$-adrenoceptors by determining for each of them its ability to displace [$^3$H]prazosin or [$^3$H]clonidine from specific binding sites in rat cerebral cortex. [$^3$H]prazosin (19.5 Ci/mmol, $\alpha_1$-adrenergic receptor) and [$^3$H]clonidine (70.5 Ci/mmol, $\alpha_2$-adrenergic receptor) were used. Rat brains were homogenised in 20 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.6), and centrifuged at 20,000g for 20 min (0–4 °C). The cell pellet was resuspended in Tris–HCl buffer and centrifuged again. Radioligand binding assays were performed in plates (MultiScreen/Millipore). The final incubation mixture (final volume 300 μL) consisted of 240 μL membrane suspension, 30 μL of [$^3$H]prazosin (0.2 nM) or [$^3$H]clonidine (2 nM) solution, and 30 μL of buffer containing from seven to eight concentrations ($10^{-11}$–$10^{-4}$ M) of tested compounds. For measuring unspecific binding, phentolamine −10 μM (in the case of [$^3$H]prazosin) and clonidine −10 μM ( in the case of [$^3$H]clonidine) were applied. The incubation was terminated by rapid filtration over glass fibre filters (Whatman GF/C) using a vacuum manifold (Millipore). The filters were then washed

twice with the assay buffer and placed into scintillation vials with liquid scintillation cocktail. Radioactivity was measured using WALLAC 1409 DSA—liquid scintillation counter. All assays were done in duplicate.

Radioligand binding data were analysed[25] using iterative curve fitting routines (GraphPAD/Prism, Version 3.0—San Diego, CA, USA) $K_i$ values were calculated from the Cheng and Prusoff equation.[28]

**5.2.3. Functional bioassay.** Isolated rat aorta was used in order to test antagonistic activity of investigated compounds for $\alpha_1$-adrenoceptors. The male Wistar rats weighing 200–350 g were anaesthetised with thiopental sodium (75 mg/kg ip) and the aorta was dissected and placed into a Krebs–Henseleit solution and cleaned of surrounding fat tissue. The thoracic aorta was denuded of endothelium and cut into approximately 4-mm long rings. The aorta rings were incubated in 30-mL chambers filled with a Krebs–Henseleit solution (NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 2.25 mM, $MgSO_4$ 1.64 mM, $KH_2PO_4$ 1.18 mM, $NaHCO_3$ 24.88 mM, glucose 10 mM, $C_3H_3O_3Na$ 2.2 mM, and EDTA 0.05 mM) at 37 °C and pH 7.4 with constant oxygenation ($O_2/CO_2$, 19:1). Two stainless steel pins were inserted through the lumen of each arterial segment: one pin was attached to the bottom of the chamber and the other to an isometric FDT10-A force displacement transducer (BIOPAC Systems, Inc., COMMAT Ltd, Turkey). The aorta rings were stretched and maintained at optimal tension of 2 g and allowed to equilibrate for 2 h. The lack of endothelium was confirmed by the absence of acetylocholine (1 μM) vasorelaxant action in aortic rings precontracted by noradrenaline (0.1 μM).

Cumulative concentration–response curves to phenylephrine ($3 \times 10^{-9}$–$3 \times 10^{-6}$ M) were obtained by the method of van Rossum.[29] Following the first phenylephrine curve, aorta rings were incubated with one of three to four concentrations of tested compounds (one concentration of the antagonist was used in each arterial ring in every experiment) for 20 min and the next cumulative concentration curve to phenylephrine was obtained. In order to avoid fatigue of the aorta preparation, a 60-min recovery period was allowed between phenylephrine curves.

Concentration–response curves were analysed using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA). Contractile responses to vasoconstrictor (in the presence or absence of tested compounds) are expressed as a percentage of the maximal phenylephrine effect ($E_{max}$ = 100%), reached in the concentration–response curves obtained before incubation with the tested compounds. Data are the means ± SEM of at least five separate experiments. Schild analysis was performed, and when the slope was not significantly different from unity, the $pA_2$ value was determined.[30] When the slope was significantly different from unity, the affinity was estimated with the equation $pK_B$ = log(concentration ratio − 1) − log(molar antagonist concentration), where the concentration ratio is the ratio of equieffective agonist concentrations in the absence and in the presence of the antagonist.

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