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Synthesis and SAR-study for novel arylpiperazine derivatives of 5-arylidenehydantoin with α_1-adrenoceptor antagonistic properties

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ABSTRACT

The study is focused on a series of 5-arylidenehydantoin derivatives with a phenylpiperazine-hydroxypropyl fragment at N3 of the hydantoin ring. The compounds were assessed on their affinity for α_1-adrenoceptors and evaluated in functional bioassays for their antagonistic properties. Crystal structures of (*Z*)-5-(4-chlorobenzylidene)-3-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)-2-hydroxypropyl)imidazolidine-2,4-dione (**7**) and hydrochloride of (*Z*)-5-(4-chlorobenzylidene)-3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)imidazolidine-2,4-dione (**10a**) were solved using the X-ray diffraction method. Classical molecular mechanics (MMFFs force field, MCMM, MacroModel) were used to predict 3D structure of compounds **5a**–**18a** using a crystal structure of **7**. SAR analysis was performed on the basis of Barbaro's pharmacophore model and structural properties of previously investigated α_1-adrenoceptor antagonists possessing a hydantoin fragment. Most of the compounds exhibited significant affinities for α_1-ARs in nanomolar range (40–290 nM). The highest activities (K_i <75 nM) were observed for compounds possessing a 2-alkoxyphenylpiperazine fragment and two methoxy substituents at the benzylidene moiety. The results indicated that chemical properties, number and positions of substituents at the 5-arylidene fragment influenced the power of α_1-affinities as follows: 3,4-di CH_3O>2,4-di CH_3O>4-Cl>2,3-di CH_3O>H>4-N$(CH_3)_2$.

1. Introduction

The α_1-adrenoceptors, belonging to the earliest identified G-protein coupled adrenergic receptors family, have been and still are an important drug target because of their various physiological functions linked to their wide distribution in many mammalian tissues.[1–3] Recent studies have given a new knowledge about α_1-AR functions in vivo, especially in the cardiovascular system[4] and brain.[5] Studies on mice carrying genetic modifications of the α_1-AR genes[6,7] have provided evidence that α_1-ARs contributing to the regulation of blood pressure play a role in cardiac pathological hypertrophy or physiological hypertrophy associated with postnatal growth and the α_1-ARs maintain normal heart function.[6] Other interesting features of the α_1-AR are related to their effects on heart contractile function, cardiac rhythm and protection from ischemic injury.[7] Antagonists of α_1-AR have been a promising tool in the treatment of benign prostatic hyperplasia (BPH) as they are able to reduce the 'dynamic' component of bladder outlet obstruction and they may reduce the irritant symptoms of the disease.[8,9]

Although α_1-adrenergic receptors have been intensively investigated for last decades, crystal structure of this GPCRs subfamily is still not available in the Protein Data Bank. Thus, all the information on their 3D-structure as well as the ligand binding pocket comes from molecular modelling supported by genetic and pharmacological assays. Various approaches[10–15] have been involved to develop the knowledge about α_1-ARs and their interactions with agonists and antagonists. At the absence of experimentally-identified crystal structure of the receptors, ligand-based (pharmacophore identification and QSAR modelling) and structure-based (comparative modelling and molecular docking) studies are the best way to characterize spatial properties of α_1-AR and its interactions with ligands.[10] In this context, it is necessary to search for new chemical groups of α_1-adrenoceptor antagonists with well-identified pharmacological profile useful to create various pharmacophore models which allow to obtain wider and more reliable knowledge about this GPCRs subfamily. New methods, such as: theoretical proton affinities of receptor ligands,[11] 3D-QSAR[12] and

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QSAR analyses with large number of molecular descriptors,[13,14] including docking studies[15,16] have been elaborated to increase a library of new strong α_1-adrenoceptor antagonists.

A number of designed and synthesized α_1-adrenoceptor antagonists could be divided into two main groups: piperazine- and non-piperazine derivatives, with predominant number of derivatives of piperazine. Investigations carried out during the last decade gave new very potent antagonists from both groups (Fig. 1), including non-piperazine analogues of WB4101 (such as **1**),[17] phenylpiperazine uroselective antagonists (such as **2**),[12] pyrrolopyrimidinedione derivatives (such as **3**)[15] and pyridazinone derivatives (such as **4**).[18]

As a variety of chemical structures of α_1-antagonists was found, several pharmacophore models have been postulated[10] in respect to pharmacological profile identified among different groups of antagonists. Our previous works[19–21] referred to the pharmacophore models of α_1-adrenoceptor antagonists, with special accent on models of Barbaro[19,20] and Bremner,[21] respectively. It is worth to add to this group Fang's pharmacophore model dedicated to uroselective α_1-AR antagonists.[10,12]

On the other hand, our previous studies[19–21] were focused on phenylpiperazine derivatives of phenytoin which displayed α_1-antagonistic activity in submicromolar range. Although the structure of this chemical group included all pharmacophoric features described by Barbaro (Fig. 2),[22,23] we supposed that α_1-antagonistic properties of this group was limited by some unfavourable positions of the pharmacophore fragments, particularly by not sufficient distances between a positive ionisable centre and both third hydrophobic centre (HY3) and hydrogen bond acceptor (HBA). Furthermore, a presence of two phenyl rings at position 5 of the hydantoin could decrease the activity, too. Taking this into account, we decided to perform further chemical modifications to eliminate the unprofitable properties by (i) replacement of 5,5-diphenyl fragment with various (un)substituted benzylidene moieties and (ii) an exchange of phenylpiperazine-hydroxypropyl fragment from position 1 of the hydantoin into position 3. Thus, a series of phenylpiperazine derivatives of arylidenehydantoin (Table 1) with expected higher α_1-adrenoceptor antagonistic properties was designed. Within this study, the synthesis of the new derivatives **5–18** was performed. The new compounds were examined on their affinity at α_1-AR in radioligand binding assays. Moreover antagonistic properties for selected compounds were tested in functional bioassay. Crystallographic analysis of selected compounds was performed. Spatial and chemical properties of the whole population **5–18** were investigated using molecular modelling calculations on the basis of crystallographic study results and pharmacophore hypotheses.

2. Results and discussion

2.1. Synthesis

Compounds **5–18** were obtained within three-step synthesis according to the Scheme 1. The initial step, Knoevenagel condensation of the hydantoin **19** with (un)substituted benzaldehydes **20–25**, leading to 5-arylidenehydantoins **26–31** has been described before.[24,25]

In the next step intermediates **26–31** were alkylated by Mitsunobu reaction by use of racemic oxiran-2-ylmethanol (1.5 equiv), triphenylphosphine (1.0 equiv) and diethyl azodicarboxylate (DEAD, 1.0 equiv), with DMF as a solvent. Water is a side product of the Mitsunobu reaction between alcohol and amides and decreases a reaction rate during the process, significantly limiting products yield. The progress of the reaction was observed only when the anhydrous DMF (containing less than 0.005% water) was used. In attempts to optimize the reaction conditions in a few cases DEAD was replaced with diisopropyl azodicarboxylate (DIAD), however, the purification of the product run into problems due to the produced side product, eluted during the column chromatography together with the target compound. The products **32–37** were obtained, using DEAD, with yields in range of 22–34%, the best yield was achieved in case of 3,4-dimethoxybenzylidene derivative **37** (the synthetic details of **32–34** are given elsewhere).

Compounds **5–18** were obtained by N-alkylation of (un)substituted phenylpiperazines with suitable oxiran derivatives (**32**–**37**) under microwave irradiation[19,20] in solvent-free conditions. The final hydrochlorides **5a–18a** were obtained by an instillation of concentrated HCl into the methanol solution of compounds **5–18** until acidic pH.

Compounds **5a**–**18a** possessing one chiral centre were obtained and tested as racemates.

2.2. Pharmacology

2.2.1. Radioligand binding study

Compounds **5a–18a** were tested for their in vitro affinity for α_1-adrenoceptors on rat cerebral cortex by the radioligand binding assays using [^{3}H]-prazosin as a specific radioligand. The affinities described by K_i values (nM) are shown in the Table 1. The presented group of arylidenehydantoin derivatives displayed a wide range of binding abilities at α_1-adrenoceptors with K_i values between 44 and 8700 nM. Three groups with activity at α_1-AR may be observed: (i) potent agents (**5a**–**11a**) with K_i <100 nM; (ii) compounds **12a–16a** with moderate affinities (100 nM <K_i

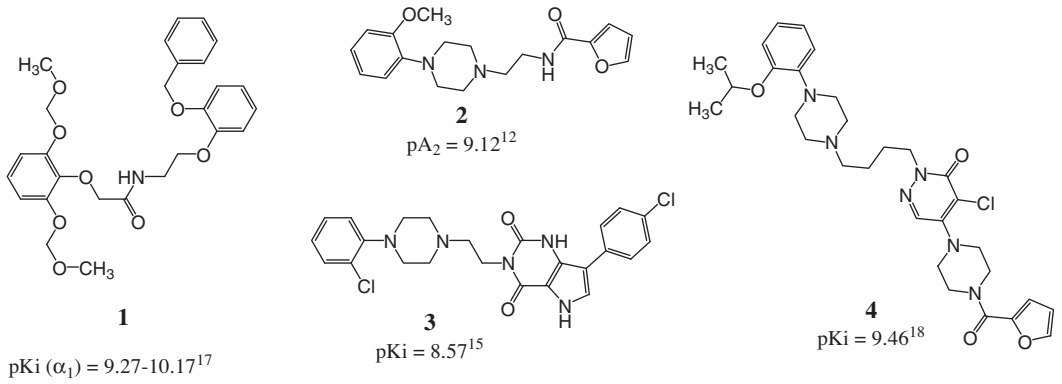
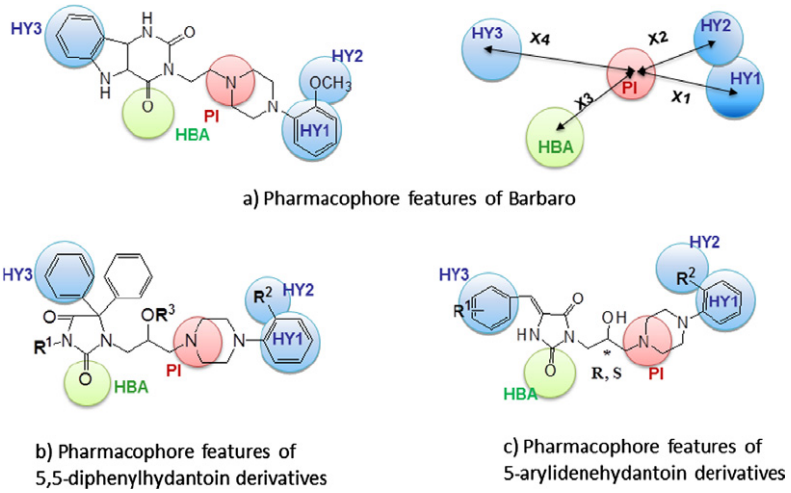


Figure 1. Members of piperazine- (**2**–**4**) or non-piperazine (**1**) derivatives of potent α_1-adrenoceptor antagonists. Example structures described during the last decade.[12,15,17,18]



a) Pharmacophore features of Barbaro

b) Pharmacophore features of 5,5-diphenylhydantoin derivatives

c) Pharmacophore features of 5-arylidenehydantoin derivatives

Chemical groups	Distances X1-X4 [Å]			
	X1 (HY1-PI)	X2 (HY2-PI)	X3 (HBA-PI)	X4(HY3-PI)
Ideal α_1-adrenoceptor Antagonist[22]	5.69	6.17	5.62	9.78
Phenytoin derivatives[19,20]	5.68-5.69	6.18-6.20	5.11-6.00	7.60-8.08
5-arylidenehydantoin derivatives **5a-18a**	5.56-5.63	5.58-5.73*	5.52-5.53	10.51-10.56
X-ray structure of **10a**	5.67	5.05	5.71	10.77**
	5.66	5.05	5.70	10.76***
X-ray structure of **7**	5.65	5.04	4.21	10.25

Figure 2. The comparison of pharmacophore features of potent α_1-adrenoceptor antagonists, based on Barbaro's model (a), for phenylpiperazine phenytoin derivatives (b)[19,20] and arylidenehydantoin derivatives **5a–18a** (c). Below, the most important distances, in respect to ideal values postulated by Barbaro et al., are presented, including calculated values and those from X-ray analysis (**isomer *R*, ***isomer *S*). *Excluding compounds: **12a**, **15a** and **17a**.

<1 μM) and (iii) compounds displaying weak activity (**17a, 18a**) with K_i >3 μM.

2.2.2. Functional bioassays results

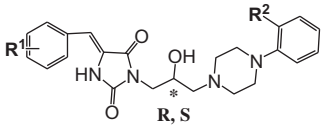
The α_1-adrenoceptor antagonistic activity of compounds **5a–11a** was studied in adult Wistar rat aorta and was assessed by inhibition of phenylephrine-inducted contractions. The investigated compounds, concentration-dependently, shifted the phenylephrine response to the right. For two compounds, that is **5a** and **8a**, a Schild slope did not differ significantly from unity, indicating a competitive interaction with the α_1-adrenoceptors, and thus allowing for unambiguous determination of the pA_2 value. In other cases, affinities were reported as pK_B estimates since Schild slope were lower than unity. It means, the antagonism for compounds **6a**, **7a, 9a**, **10a**, and **11a** was not competitive and may suggest that the responses were also mediated by some other receptors or they can be allosteric modulators of receptors. Other possibilities include also a slow dissociation of the ligand from the receptor.[26]

The following order of activity for tested compounds was found: **7a** > **6a** > **11a** > **10a** > **5a** > **8a** > **9a** (Table 2, Figs. 3 and 4). The strongest antagonistic activity was identified for compound **7a** with a pK_B value of 9.01, but its Schild slope differ significantly from unity so antagonism was not competitive, and suggest composite activity of compound **7a**. The weakest antagonistic activity showed compound **9a**, giving a pK_B estimate of 7.53.

2.3. X-ray analysis

As no experimental crystal structure of α_1-adrenoceptor (with)out antagonist has been ever identified, molecular modelling methods focused on the search for pharmacophoric features of investigated compounds seem to be a good way to characterize spatial properties of α_1-AR and its interactions with ligands. From the structural point of view, all studied molecules can be expressed in terms of three important elements. Accordingly,

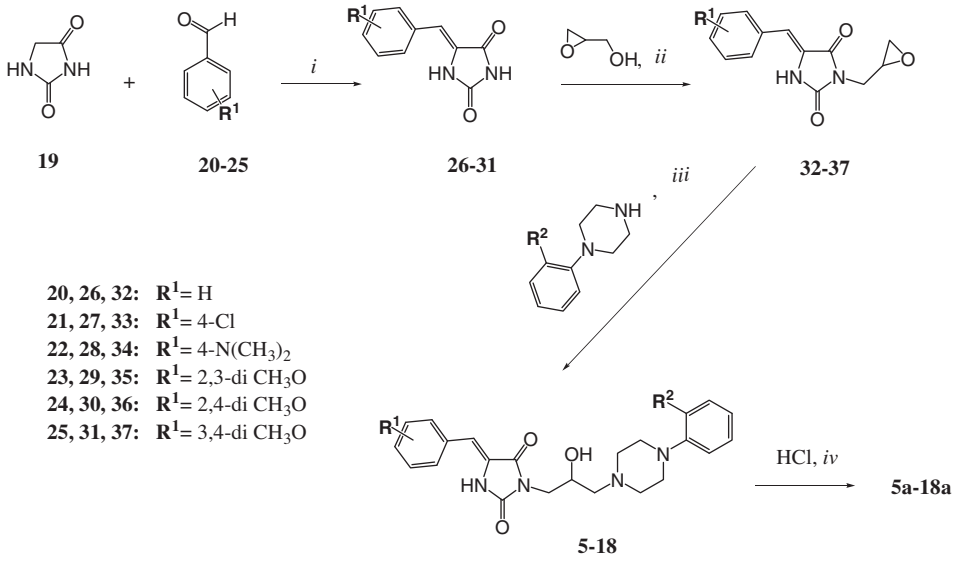
Table 1
Structure and binding properties at α_1-adrenoceptors of arylpiperazine derivatives of 5-arylidenehydantoin (**5a–18a**)



x HCl

5a-18a

Compd	R^1	R^2	α_1-AR K_i (nM) [^{3}H]-prazosin
5a	3,4-di CH_3O	OCH_3	44.5 ± 4.5
6a	2,4-di CH_3O	OCH_3	52.2 ± 1.5
7a	4-Cl	OC_2H_5	59.1 ± 0.1
8a	2,3-di CH_3O	OCH_3	70.0 ± 3.4
9a	2,3-di CH_3O	OC_2H_5	72.1 ± 4.3
10a	4-Cl	OCH_3	86.3 ± 5.2
11a	H	OC_2H_5	91.4 ± 6.0
12a	3,4-di CH_3O	H	197.6 ± 9.3
13a	2,4-di CH_3O	OC_2H_5	231.0 ± 10.9
14a	H	OCH_3	285.9 ± 12.5
15a	2,3-di CH_3O	H	318.8 ± 20.3
16a	4-$N(CH_3)_2$	OCH_3	834.9 ± 33.0
17a	4-Cl	H	3700 ± 300
18a	4-$N(CH_3)_2$	OC_2H_5	8700 ± 200
Phentolamine			10.2 ± 0.4



Scheme 1. Synthesis of compounds **5a–18a**. Reagents and conditions: (*i*) CH_3COONa, CH_3COOH, reflux; (*ii*) DIAD (method A) or DEAD (method B), TPP, dry DMF, 0 °C; (*iii*) microwave irradiation; (*iv*) 3–4 drops of 36% HCl/EtOH.

Table 2
Functional bioassay results for selected compounds **5a–11a**

Compd	pK_B/pA_2 ± SEM (slope ± SEM)
5a	pA_2 = 7.56 ± 0.03[a] (0.970 ± 0.001)
6a	pK_B = 8.95 ± 0.05
7a	pK_B = 9.01 ± 0.03
8a	pA_2 = 7.55 ± 0.01[a] (1.070 ± 0.003)
9a	pK_B = 7.53 ± 0.06
10a	pK_B = 7.58 ± 0.05
11a	pK_B = 7.66 ± 0.04

Antagonistic potency of selected compounds, expressed as pA_2 or pK_B ± SEM values, in isolated rat thoracic aorta (α_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.

arylidenehydantoin and arylpiperazine moieties create two boundary fragments joined by the aliphatic three atom distance arm. Conformation of two first elements is rather predictable and stable, however, the structure of the whole molecule depends on the flexible aliphatic chain conformation. For this reason the conformational analysis of our compounds is indispensable for the pharmacophore studies. Although neither crystallographic nor calculated low-energy conformations of ligands do not describe their bioactive pose inside the binding pocket, the latter can be used to reproduce bioactive conformations with a good approximation[27,28] and crystallographic studies of representative species in both unprotonated and ionic form seem to be a good starting point for these considerations. Thus, monocrystals of **7** (base) and **10a** (hydrochloride) were obtained and X-ray studies for both of them were carried out.

As it is evident from Figure 5, aliphatic chain does not adopt the same conformation in both structures. In the molecule **7** (basic form) the chain is bent, while in the protonated molecule **10a** (in a form of salt) it creates an extended shape. That difference may be illustrated by the values of torsion angles C7–C8–C9–N1A equalling −170.5° and −169.9°, respectively, for two independent molecules of **10a** while only 57.6° in **7**. Therefore, protonation of the piperazine nitrogen seems to favour an extended molecule conformation in the crystal. It is also of special interest to compare the formation of the crystal network in the studied crystals (Fig. 6). Out of two carbonyls of the hydantoin core, the oxygen O4 in both structures is involved in the strong hydrogen bond of N1-H···O4 assembling molecules in a ribbon shape. The second carbonyl and the hydroxyl group of aliphatic chain are linking the unprotonated molecule of **7** inside the ribbon via intermolecular O8-H···O2 bond. In case of the charged species **10a**, the oxygen O2 is not involved in any intermolecular interactions. In this crystal, two charge-assisted H-bonds were identified, both with chlorine ion as a proton acceptor (Fig. 6).

2.4. Molecular modelling study

To analyse structural features of the described compounds, the lowest energy conformation search was performed, both for (*R*) and (*S*) configuration of the secondary alcohol carbon. All compounds were tested in a protonated form (salt) and adopted under these conditions an extended conformation as the global minimum energy ('A', black carbon structures on Fig. 7). This observation is in agreement with the conformation seen in the crystal structure of **10a** (Fig. 5). However, in almost all cases the second competitive conformation has been found as an energetic local minimum, with potential energy not exceeding 0.6 kJ/mol over the global minimum ('B', brown carbon structures, Fig. 7). The optimized geometry of the two obtained conformations is determined by asymmetric carbon configuration as well as an intermolecular hydrogen bond of O–H···O, seen, in modelled structures, between the hydroxy group and one of carbonyls of the hydantoin core. Within series of both enantiomers, the 4-carbonyl group interacts with hydroxyl, shaping the global minimum conformation 'A', while in the other, energetically higher group of conformers 'B', hydrogen bond is created by carbonyl in the position 2 of the hydantoin. From the view of crystallographic data, similar conformation is presented by the molecule **7** (Fig. 5).

Basing on the modelled 3D-structures of compounds **5a–18a** and crystallographic data for **7** and **10a**, distances between positive ionisable centre (PI) and the most important pharmacophoric fragments (HBA, HY1, HY2, HY3) corresponding to Barbaro's model were estimated (Fig. 2). The distances were almost identical in case of both possible enantiomers. Furthermore, compounds **5a–18a** displayed only insignificant differences in all investigated distances (X1–X4; Fig. 2), what can be explained by their structural similarity and limited conformational flexibility in respect to the

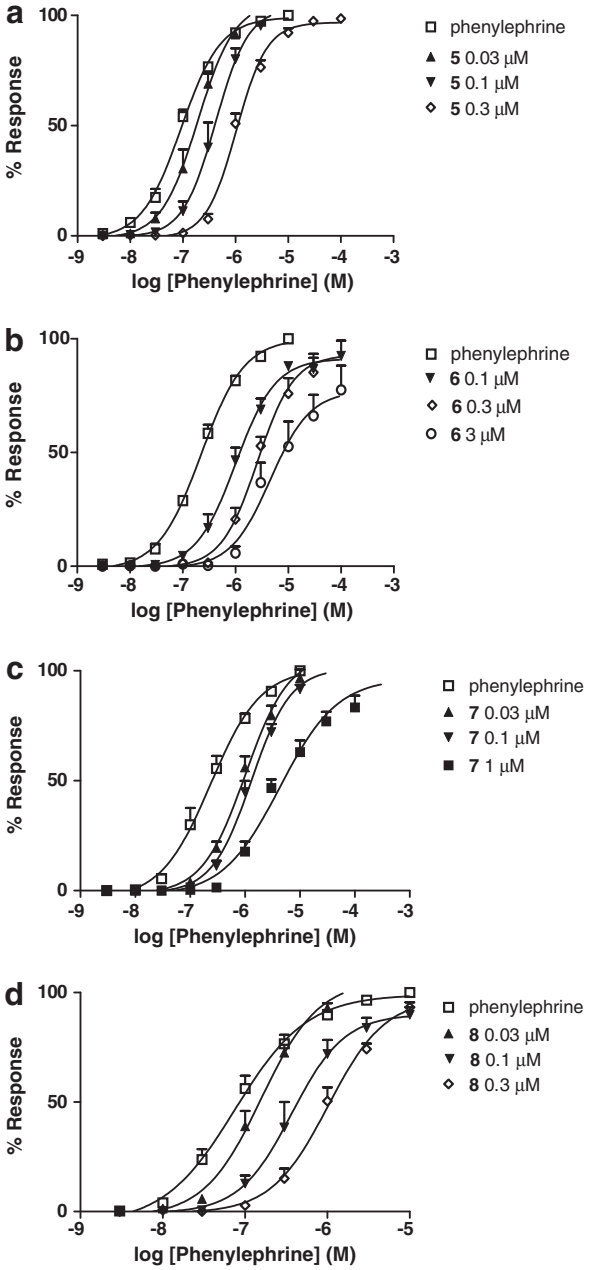


Figure 3. Concentration–response curves to phenylephrine in the rat aorta in the absence (□) or presence of (a) **5a** (▲ 0.03, ▼ 0.1, ◇ 0,3 μM); (b) **6a** (▼ 0.1, ◇ 0,3, ○ 3 μM); (c) **7a** (▲ 0.03, ▼ 0.1, ■ 1 μM); (d) **8a** (▲ 0.03, ▼ 0.1, ◇ 0,3 μM). Results are expressed as a percentage of the maximal response to phenylephrine in the first concentration–response curve. Each point represents the mean ± SEM (n = 5–8).

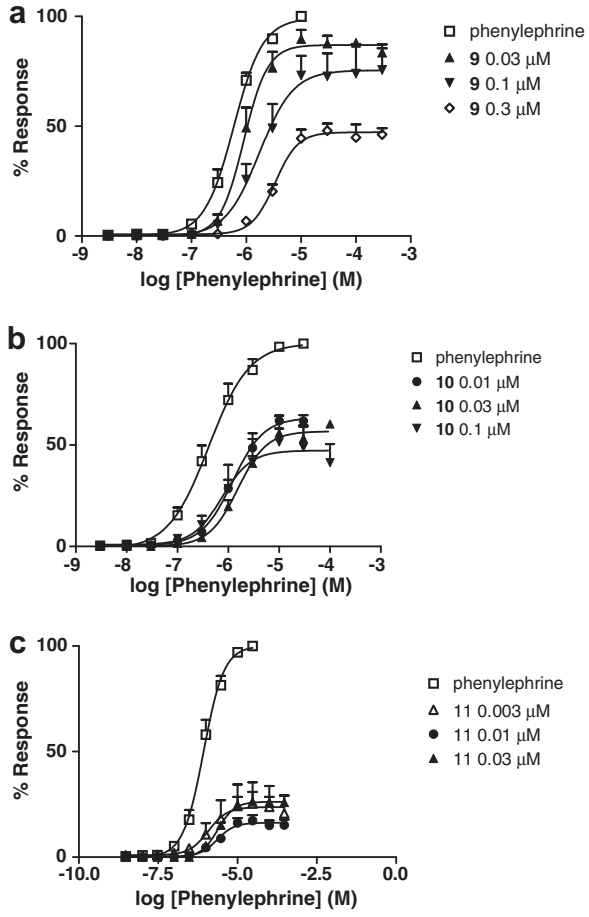


Figure 4. Concentration–response curves to phenylephrine in the rat aorta in the absence (□) or presence of (a) **9a** (▲ 0.03, ▼ 0.1, ◇ 0,3 μM); (b) **10a** (● 0.01, ▲ 0.03,▼ 0.1 μM); (c) **11a** (▲ 0.003,● 0.01, ▲ 0.03 μM). Results are expressed as a percentage of the maximal response to phenylephrine in the first concentration–response curve. Each point represents the mean ± SEM (n = 5–8).

rigid arylidene moiety together with a short branched linker (2-hydroxypropyl). The distances from modelled molecules **5a**–**18a** were compared to those measured for crystallographic structures **7** and **10a**. The only important difference was that X2 is connected with an adjustment of the phenylpiperazine phenyl ring due to close packing in the crystal. Results obtained for compounds **5a**–**18a** were compared with those described previously for tested phenylpiperazine derivatives of phenytoin[19,20] as well as the 'ideal' distances described by Barbaro et al.[22] (Fig. 2).

2.5. Structure–activity relationship analysis

A series of phenylpiperazine derivatives of 5-arylidenehydantoin consists of similar compounds **5a**–**18a**. They differ in their arylidene- and phenylpiperazine phenyl moieties which are substituted with various small substituents, possessing electron donor or weak electron acceptor properties. Alkoxy substituents at the phenylpiperazine phenyl ring are placed at the *ortho* position what has been indicated as a beneficial attribute for antagonistic interaction with α_1-adrenoceptors.[12–18,22,23] Three compounds with an unsubstituted phenylpiperazine (**12a**, **15a** and **17a**) were investigated as well. Results of the pharmacological studies for the compounds in comparison to their 2-alkoxy analogues (**12a** and **5a**, **15a** and **8a**, **17a** and **7a**) distinctly confirmed the importance of *o*-alkoxy substituents at the phenylpiperazine phenyl ring since their absence caused significant decreases of activity (4–60-fold increases of K_i values, Table 1). This is in a good agreement with pharmacophore model of Barbaro et al.[22] as the position *ortho*, occupied by hydrophobic alkoxy moiety, fit in the pharmacophore feature HY2 (Fig. 2). In the most cases, the chemical modifications performed within these studies gave derivatives with higher affinities for α_1-adrenoceptors comparing to previously investigated derivatives of phenytoin with similar phenylpiperazine-hydroxypropyl fragments.[19–21] This suggests that both, the replacement of the 5,5-diphenyl fragment with an arylidene one and an exchange of phenylpiperazine-alkyl fragment from 1 into 3 position of the hydantoin, are profitable for the target interactions with α_1-AR. Results of the functional bioassays confirmed the antagonistic properties of the most active compounds (**5a**–**11a**). This behaviour of compounds during the pharmacological tests can be explained on the basis of pharmacophore hypotheses (Fig. 2). Considering five pharmacophore features (PI, HY1–HY3

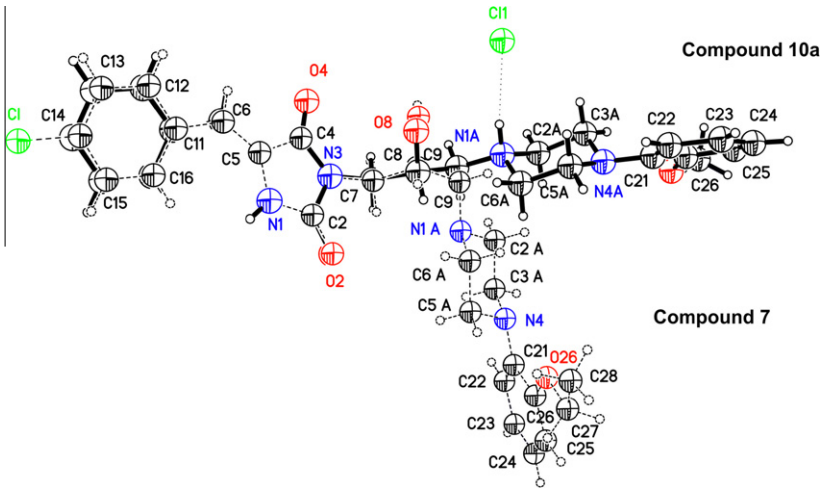


Figure 5. Superimposition of **7** and **10a** structures with respect to hydantoin ring atoms.

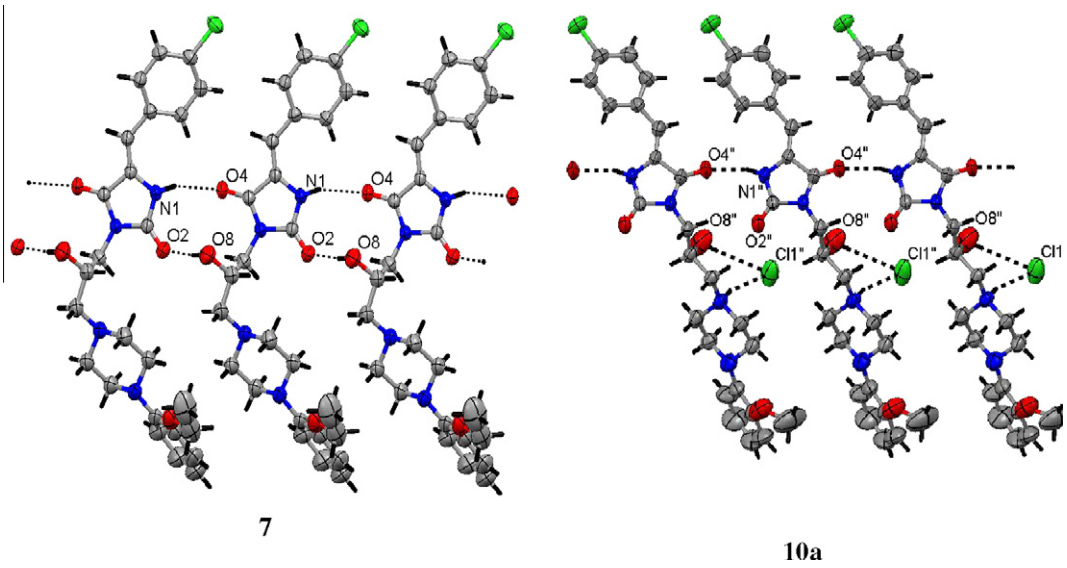


Figure 6. Hydrogen bonds net in the structures of unprotonated (**7**) and protonated (**10a**) compounds.

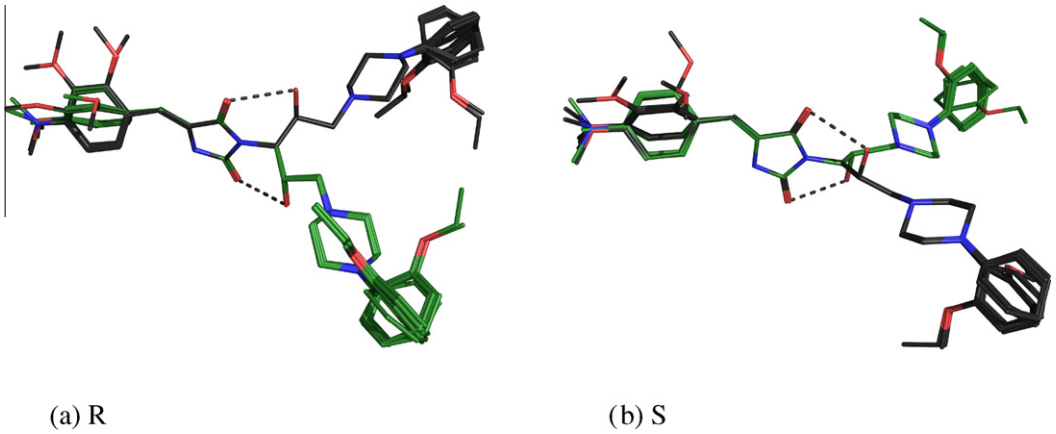


Figure 7. Superimposition of global ('A', black carbons) and local ('B', green carbons) energy minimum conformations of (a): (*R*)-**5a**–**18a** and (b): (*S*)-**5a**–**18a**. For clarity hydrogen atoms are hidden.

and HBA), it can be seen that both arylidenehydantoin derivatives (**5a**–**18a**) and previously investigated phenytoin derivatives,[19–21] include in their structures chemical moieties which fit in well with the pharmacophore model. Both groups display high accordance with an ideal antagonist of α_1-AR in terms of distances between the positive ionisable centre (PI) and hydrophobic fragments HY1 and HY2 (X1, X2, Fig 2). However, arylidenehydantoin derivatives **5a**–1**8a** seem to be closer to the ideal α_1-antagonist than their phenytoin analogues if one considers distances between the hydrogen bond acceptor (HBA) and PI (X3) as well as the distance from PI to the third hydrophobic fragment HY3 (X4). Particularly, arylidene derivatives (**5a**–**18a**) possess only one aromatic fragment corresponding with the HY3 moiety postulated by Barbaro et al.[22,23] which is placed in 10.51–10.56 Å from PI of piperazine, whereas phenytoin derivatives possess two possible phenyl rings corresponding to HY3, both placed too close to the PI (7.60–8.08 Å) comparing to an ideal distance X4 (9.78 Å, Fig. 2). This increase of the distance (X4) arose from both a spacer between the aromatic ring and the hydantoin position 5 in the case of arylidene fragment and from the presence of the amine-alkyl at position 3. In the case of phenytoin derivatives of hydroxypropyl-phenylpiperazine described previously,[19–21] the location of the amine-alkyl fragment at position 1, on the same side of the hydantoin with both phenyl rings, does not allow to prolong the distance X4, particularly, because of the limited flexibility of a rather short hydroxypropyl linker.

Although the role of alkoxy substituents at *o*-position of the phenylpiperazine phenyl ring has been underlined as beneficial for α_1-AR antagonist properties,[12–18,22,23] two *o*-alkoxyphenylpiperazine derivatives, **16a** and **18a**, showed only low or moderate affinities for the receptor. Furthermore, these weak antagonists (**16a, 18a**) show all pharmacophoric features of an ideal α_1-AR antagonist according to the model of Barbaro. These results indicated that either *o*-alkoxy (HY2) feature or the presence of all pharmacophoric features (HY1–3, PI and HBA) are not sufficient for high affinities for α_1-AR in the presented group of chemical compounds (**5a**–**18a**). It is observed that chemical properties of substituents at the arylidene aromatic ring (HY3) as well as their number and position significantly affect affinities of the phenylpiperazine arylidenehydantoins for α_1-AR. In spite of electron donor properties of the substituents at the arylidene rings, it is evident that a presence of two methoxy substituents (**5a**, **6a**, **8a**, **9a**, **12a**, **13a**, **15a**) is much more favourable than that of dimethylamine at position *para* (**16a** and **18a**). The position of both methoxy substituents seems to be important as well. In this context, the substituents at positions 3 and 4 are the most advantageous, and the α_1-adrenoceptor affinity decrease is seen in following order: 3,4-di-CH_3O>2,4-diCH_3O>4-Cl>2,3-diCH_3O>H> 4-N$(CH_3)_2$. The functional bioassay revealed that two alkoxy substituents located at position *meta* and *para* (**5a**) or *ortho* (**8a**) of the arylidene ring could be responsible for competitive antagonistic action on α_1-AR with pA_2 values in the range of 7.55 (Table 2), what is corresponding to their affinities for α_1-adrenoceptors identified within the radioligand binding assay (Table 1). Although the *p*-chloroarylidene derivative **7a** and the 2,4-dimethoxyarylidene compound **6a** demonstrated the most potent antagonistic action among the tested compounds, the antagonism was not competitive. Parallel antagonistic interactions with other protein targets may explain their unexpected high pK_B values in the range of 9, exceeding the affinities of **6a** and **7a** for α_1-AR noticed in radioligand binding tests (pK_i ~7.2).

3. Conclusion

In conclusion, a new series of phenylpiperazine derivatives of 5-arylidenehydantoin (**5a**–**18a**) was prepared by using of Mitsunobu reaction and microwave aided N-alkylations. The experimental and theoretical studies on spatial properties of the new promising α_1-adrenoceptor antagonists together with pharmacological studies, including radioligand binding tests and functional bioassays, provided new information in the field of structure–activity relationship. The SAR-studies enabled to analyse and revise previous pharmacophore models and identify new structural properties which affect the receptor affinities within the group of compounds **5a**–**19a**. In the investigated group, (*Z*)-5-(3,4-dimethoxybenzylidene)-3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl) propyl)imidazolidine-2,4-dione (**5a**) was found as the most potent competitive α_1-adrenoceptor antagonist. The SAR-analysis confirmed an important role of previously described five pharmacophore features (HY1–3, PI and HBA) and indicated a crucial role of substituents at the arylidene phenyl ring (HY3) for α_1-adrenoceptor affinities, including a profitable influence of two methoxy substituents and deactivating role of dimethylamine moiety at position *para*. The data coming from this work will support further development of studies on α_1-adrenoceptors with special attention to characteristics of their antagonists binding pockets. In this context, pharmacophore models are particularly important since the 3D structure of any α_1-ARs has not been experimentally determined till now.

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 ($CDCl_3$) or 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) CH_2Cl_2, (II) CH_2Cl_2/acetone 19:1; (III) CH_2Cl_2/acetone 18:2; (IV) CH_2Cl_2/acetone 17:3; (V) toluene/acetone/methanol 5:5:1. Melting points were determined using Mel-Temp II apparatus and are uncorrected. Elemental analyses were within ±0.4% of the theoretical values unless stated otherwise. Syntheses under microwave irradiation were performed in household microwave oven Samsung M1618. Syntheses of compounds **26**–**31**, and **32**–**34** are described elsewhere.[25,26]

4.1.1. General procedure for preparation of 5-arylidene-3-(oxiran-2-ylmethyl)imidazolidine-2,4-diones (35–37)

5-Arylideneimidazolidine-2,4-dione (**29–31**) (10 mmol), oxiran-2-ylmethanol (15 mmol, 1.11 g) and triphenylphosphine (10 mmol, 2.62 g) were added to 50 mL of dry DMF at 0 °C. The mixture was stirred on the ice-bath until the reactants were totally dissolved (~20 min). A solution of diethyl azodicarboxylate DEAD (10 mmol, 1.74 g) in dry DMF (10 mL) was added to the mixture dropwise for 45 min and stirring was continued at room temperature for further 3 h. Then, the mixture was poured into 100 mL of cold water to precipitate and left at 0–4 °C overnight. After filtration, pure product (**35–37**) was obtained from precipitate by column chromatography on silica gel with eluents I–IV and additional crystallization of combined fractions containing desirable product (**35–37**) by the use of alcohol.

4.1.1.1. (*Z*)-5-(2,3-Dimethoxybenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (35). (*Z*)-5-(2,3-Dimethoxybenzylidene)imidazolidine-2,4-dione **29** (2.48 g) was used. Crystallization with isopropanol gave white crystals of **35** (680 mg, 2.21 mmol). Yield 22.3%, mp 161–164 °C; TLC: R_f (IV): 0.70; Anal.

Calcd for $C_{15}H_{16}N_2O_5$: C, 59.20; H: 5.30; N, 9.21. Found: C, 58.95; H, 5.70; N, 9.26. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.70 (dd, J_1 = 4.87 Hz, J_2 = 2.56 Hz, 1H, N3-CH_2CHCH_a(H)), 2.81–2.84 (m, 1H, N3-CH_2CHCH_b(H)), 3.22–3.27 (m, 1H, N3-CH_2CHCH_2), 3.71 (dd, J_1 = 14.36 Hz, J_2 = 5.14 Hz, 1H, N3-CH_c(H)), 3.80 (s, 3H, OCH_3), 3.85 (d, J = 5.13 Hz, 1H, N3-H_d(H)), 3.90 (s, 3H, OCH_3), 6.65 (s, 1H, Ph-CH=), 6.90–6.97 (m, 2H, Ph-4,5-H), 7.09–7.14 (m, 1H, Ph-6-H), 8.25 (s, 1H, N1H). IR KBr (cm^{-1}): 3230 (N1H), 1762 (C2=O), 1714 (C4=O), 1662 (ArCH=), 1577 (Ar).

4.1.1.2. (*Z*)-5-(2,4-Dimethoxybenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (36). (*Z*)-5-(2,4-Dimethoxybenzylidene)imidazolidine-2,4-dione **30** (2.48 g) was used. Crystallization with isopropanol gave white crystals of **36** (790 mg, 2.57 mmol). Yield 26.0%, mp 166–168 °C; R_f (IV): 0.65; Anal. Calcd for $C_{15}H_{16}N_2O_5$: C, 59.21; H, 5.30; N, 9.21. Found: C, 59.14; H, 5.46; N, 9.29. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.68–2.70 (m, 1H, N3-CH_2CHCH_a(H)), 2.79–2.84 (m, 1H, N3-CH_2CHCH_b(H)), 3.21–3.26 (m, 1H, N3-CH_2CHCH_2), 3.71 (dd, J_1 = 14.36 Hz, J_2 = 4.87 Hz, 2H, N3-CH_2), 3.85 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 6.51–6.58 (m, 2H, Ph-3,5-H), 6.70 (s, 1H, Ph-CH=), 7.24–7.27 (m, 1H, Ph-6-H), 7.89 (s, 1H, N1H).

4.1.1.3. (*Z*)-5-(3,4-Dimethoxybenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (37). (*Z*)-5-(3,4-Dimethoxybenzylideneimidazolidine-2,4-dione **31** (2.48 g) was used. Crystallization with isopropanol gave white crystals of **37** (1.04 g, 3.39 mmol). Yield 34.2%, mp 190–192 °C; R_f (IV): 0.63; Anal. Calcd for $C_{15}H_{16}N_2O_5$: C, 59.21; H, 5.30; N, 9.21. Found: C, 58.95; H, 5.73; N, 9.16. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.68 (dd, J_1 = 4.87 Hz, J_2 = 2.56 Hz, 1H, N3-CH_2CHCH_a(H)), 2.80–2.82 (m, 1H, N3-CH_2CHCH_b(H)), 3.21–3.27 (m, 1H, N3-CH_2CHCH_2), 3.73–3.89 (m, 2H, N3-CH_2), 3.92 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 6.73 (s, 1H, Ph-CH=), 6.91–6.94 (m, 2H, Ph-2,5-H), 7.04–7.07 (m, 1H, Ph-6-H), 8.43 (s, 1H, N1H). IR KBr (cm^{-1}): 3244 (N1H), 3074 (=C–H), 1764 (C2=O), 1704 (C4=O), 1665 (ArCH=), 1598 (Ar).

4.1.2. General procedure for preparation of hydrochlorides of phenylpiperazine derivatives of 5-arylideneimidazolidine-2,4-diones (5a–18a)

An appropriate 5-arylidene-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (**32–37**) (0.5–1.3 mmol) and arylpiperazine (**38–40**) (0.55–1.43 mmol) were dissolved in CH_2Cl_2 (20 mL) in a flat-bottomed flask. The solvent was evaporated. A residue was irradiated in a standard household microwave oven at various power (450–750 W) and time intervals (7–25 min) of irradiation for each prepared compound (**5**–**18**), respectively. The reaction progress was controlled with TLC (V). After irradiation, the glassy residue was crystallized with ethanol to give a pure product in basic form (**5–18**). To convert into a hydrochloride, HCl concentrated (2–4 drops) was added to the basic form of product **5–18** (100–200 mg) dissolved in dry methanol (5–10 mL). The solution was stirred for 1–3 min and left at 0–4 °C overnight to precipitate. The precipitate of pure product (**5a–18a**) was separated by filtration.

4.1.2.1. Hydrochloride of (*Z*)-5-(3,4-Dimethoxybenzylidene)-3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)imidazolidine-2,4-dione (5a). (*Z*)-5-(3,4-Dimethoxybenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **37** (1 mmol, 0.30 g) and *N*-2-methoxyphenylpiperazine **39** (1.1 mmol, 0.21 g) were used. The irradiation: 450 W for 3 min, 600 W for 6 min (3 × 2 min) and 750 W for 2 min (2 × 1 min). Bright-yellow crystals of pure compound **5** (420 mg, 0.85 mmol). Yield 85%, mp 110–112 °C; TLC: R_f (V): 0.52; Anal. Calcd for $C_{26}H_{32}N_4O_6$ (**5**): C, 62.89; H, 6.50; N, 11.29. Found: C, 62.22; H, 6.77; N, 11.19. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.43–2.56 (m, 2H, Pp-CH_2), 2.63–2.65 (m, 2H, Pp-2,6-H_a), 2.81–2.86 (m, 2H, Pp-2,6-H_e), 3.06 (br s, 4H, Pp-3,5-H), 3.65–3.77 (m, 2H, N3-CH_2), 3.85 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3) 4.05–4.13 (m, 1H, C*H*–OH), 6.72 (s, 1H, Ph-CH=), 6.84–7.05 (m, 7H, PpPh-3,4,5,6-H, Ph-2,5,6-H), 8.13 (s, 1H, N1H). IR KBr (cm^{-1}): 3421 (OH), 1762 (C2=O), 1709 (C4=O), 1659 (ArCH=), 1597 (Ar). Compound **5** (0.20 g, 0.40 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **5a** (0.18 g, 0.34 mmol). Yield 85%, mp 245–249 °C; TLC: R_f (V): 0.52; Anal. Calcd for $C_{26}H_{32}N_4O_6 \times$ HCl (**5a**): C, 58.59; H, 6.24; N, 10.51. Found: C, 58.59; H, 6.24; N, 10.39. ^{1}H NMR for **5a** (DMSO-d_6) δ (ppm): 2.96–3.43 (m, 6H, Pp-CH_2, Pp-2,6-H), 3.46–3.,66 (m, 6H, Pp-3,5-H, N3-CH_2), 3.77 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.30 (br s, 1H, C*H*–OH), 5.97 (br s, 1H, OH), 6.51 (s, 1H, Ph-CH=), 6.85–7.03 (m, 5H, PpPh-3,4,5,6-H, Ph-5-H), 7.16 (d, J = 2.05 Hz, 1H, Ph-2-H), 7.24 (dd, J_1 = 8.46 Hz, J_2 = 1.79 Hz, 1H, Ph-6-H), 10.11 (br s, 1H, NH^+), 10.78 (s, 1H, N1-H). IR KBr (cm^{-1}): 3463 (OH), 3260 (N1H), 3061 (=C–H), 2461 (NH^+), 1774 (C2=O), 1715 (C4=O), 1665 (ArCH=), 1599 (Ar).

4.1.2.2. Hydrochloride of (*Z*)-5-(2,4-dimethoxybenzylidene)-3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)imidazolidine-2,4-dione (6a). (*Z*)-5-(2,4-Dimethoxybenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **36** (1 mmol, 0.30 g) and *N*-2-methoxyphenylpiperazine **39** (1.1 mmol, 0.21 g) were used. The irradiation: 450 W for 3 min, 600 W for 4 min (2 × 2 min) and 750 W for 10 min (5 × 2 min). Bright-yellow crystals of pure compound **6** (370 mg, 0.74 mmol). Yield 74.5%, mp 182–185 °C; TLC: R_f (V): 0.61; Anal. Calcd $C_{26}H_{32}N_4O_6$ (**6**): C, 62.89; H, 6.50; N, 11.29. Found: C, 62.61; H, 6.65; N, 11.26. ^{1}H NMR($CDCl_3$) δ (ppm) = 2.44–2.57 (m, 2H, Pp-CH_2), 2.62–2.66 (m, 2H, Pp-2,6-H_a), 2.82–2.87 (m, 2H, Pp-2,6-H_e), 3.08 (br s, 4H, Pp-3,5-H), 3.66–3.85 (m, 2H, N3-CH_2), 3.85 (s, 6H, 2 × OCH_3), 3.93 (s, 3H, OCH_3), 4.04–4.11 (m, 1H, C*H*–OH), 6.65–6.58 (m, 2H, Ph-3,5-H), 6.70 (s, 1H, Ph-CH=), 6.84–7.02 (m, 4H, PpPh-3,4,5,6-H), 7.26 (d, J = 8.72 Hz, Ph-6-H), 7.84 (s, 1H, N1H). IR KBr (cm^{-1}): 3426 (OH), 3222 (N1H), 1759 (C2=O), 1711 (C4=O), 1663 (ArCH=), 1607 (Ar). Compound **6** (0.20 g, 0.40 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **6a** (0.22 g, 0.41 mmol). Yield 95%, mp 182–185 °C; TLC: R_f (V): 0.53; Anal. Calcd for $C_{26}H_{32}N_4O_6 \times$ HCl (**6a**): C, 58.59; H, 6.24; N, 10.51. Found: C, 58.73; H, 6.60; N, 10.48. ^{1}H NMR (DMSO-d_6) δ (ppm) = 2.97–3.65 (m, 12H, Pp-CH_2, Pp-2,3,5,6-H, N3-CH_2), 3.77 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.30 (br s, 1H, C*H*–OH), 5.96 (br s, 1H, OH), 6.55–6.61 (m, 2H, Ph-3,5), 6.72 (s, 1H, Ph-CH=), 6.85–7.03 (m, 4H, PpPh-3,4,5,6-H), 7.60 (d, J = 8.46 Hz, 1H, Ph-6-H), 10.22 (br s, 1H, NH^+), 10.59 (s, 1H, N1H). IR KBr (cm^{-1}): 3462 (OH), 3263 (N1H), 2460 (NH^+), 1763 (C2=O), 1712 (C4=O), 1656 (ArCH=), 1605 (Ar).

4.1.2.3. Hydrochloride of (*Z*)-5-(4-chlorobenzylidene)-3-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)-2-hydroxypropyl)imidazolidine-2,4-dione (7a). (*Z*)-5-(4-Chlorobenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **33** (13 mmol, 0.36 g) and *N*-2-ethoxyphenylpiperazine **40** (1.4 mmol, 0.29 g) were used. The irradiation: 450 W for 3 min, 600 W for 4 min (2 × 2 min) and 750 W for 20 min (4 × 5 min). White crystals of pure compound **7** (360 mg, 0.74 mmol). Yield 57.1%, mp 200–203 °C; TLC: R_f (V): 0.62; Anal. Calcd for $C_{25}H_{29}N_4O_4Cl$, (**7**): C, 61.91; H, 6.03; N, 11.55. Found: C, 61.61; H, 6.18; N, 11.36. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.44 (t, J = 7.05 Hz, 3H, OCH_2CH_3), 2.43–2.58 (m, 2H, Pp-CH_2), 2.64–2.66 (m, 2H, Pp-2,6-H_a), 2.82–2.85 (m, 2H, Pp-2,6-H_e), 3.10 (br s, 4H, Pp-3,5-H), 3.65–3.81 (m, 2H, N3-CH_2), 4.02–4.14 (m, 3H, C*H*–OH, OCH_2CH_3), 6.70 (s, 1H, Ph-CH=), 6.82–6.99 (m, 4H, PpPh-3,4,5,6-H), 7.37–7.43 (m, 4H, Ph-2,3,5,6-H), 8.60 (br s,

1H, N1H). IR KBr (cm^{-1}): 3452 (OH), 3320 (N1H), 3058 (=C–H), 1758 (C2=O), 1661 (ArCH=), 1590 (Ar). Compound **7** (0.20 g, 0.41 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **7a** (0.21 g, 0.38 mmol). Yield 92.8%, mp 237–243 °C; TLC: R_f (V): 0.67; Anal. Calcd for $C_{24}H_{27}N_4O_4Cl \times HCl \times 1.5H_2O$ (**7a**): C, 54.75; H, 6.06; N, 10.22. Found: C, 54.56; H, 5.93; N, 10.14. ^1H NMR for **7a** (DMSO-d_6) δ (ppm): 1.33 (t, J = 6.92 Hz, 3H, OCH_2CH_3), 2.99–3.36 (m, 6H, Pp-CH_2, Pp-2,6-H), 3.41–3.67 (m, 6H, Pp-3,5-H, N3-CH_2), 4.02 (q, J = 6.92 Hz, 2H, OCH_2CH_3), 4.31–4.34 (m, 1H, C*H*–OH), 6.12 (br s, 1H, OH), 6.52 (s, 1H, Ph-CH=), 6.84–7.00 (m, 4H, PpPh-3,4,5,6-H), 7.46 (d, J = 8.46 Hz, 2H, Ph-3,5-H), 7.67 (d, J = 8.46 Hz, 2H, Ph-2,6-H), 10.26 (br s, 1H, NH^+), 10.90 (s, 1H, N1H). IR KBr (cm^{-1}): 3227 (N1H), 3073 (=C–H), 2442 (NH^+), 1769 (C2=O), 1710 (C4=O), 1664 (ArCH=), 1591 (Ar).

4.1.2.4. Hydrochloride of (*Z*)-5-(2,3-dimethoxybenzylidene)-3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)imidazolidine-2,4-dione (8a). (*Z*)-5-(2,3-Dimethoxybenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **35** (1 mmol, 0.30 g) and *N*-2-methoxyphenylpiperazine **39** (1.1 mmol, 0.21 g) were used. The irradiation: 450 W for 3 min, 600 W for 4 min (2 × 2 min) and 750 W for 6 min (3 × 2 min). White crystals of pure compound **8** (300 mg, 0.60 mmol). Yield 60.4%, mp 158–160 °C; TLC: R_f (V): 0.57;Anal. Calcd for $C_{26}H_{32}N_4O_6$ (**5**): C, 62.89; H, 6.50; N, 11.29. Found: C, 62.51; H, 6.73; N, 11.22. ^1H NMR ($CDCl_3$) δ (ppm): 2.44–2.57 (m, 2H, Pp-CH_2), 2.63–2.66 (m, 2H, Pp-2,6-H_a), 2.83–2.88 (m, 4H, Pp-2,6-H_e), 3.08 (br s, 4H, Pp-3,5-H), 3.66–3.77 (m, 2H, N3-CH_2), 3.80 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.05–4.12 (m, 1H, C*H*–OH), 6.65 (s, 1H, Ph-CH=), 6.84–7.02 (m, 6H, PpPh-3,4,5,6-H, Ph-4,5-H), 7.08–7.14 (m, 1H, Ph-6-H), 8.21 (s, 1H, N1H). IR KBr (cm^{-1}): 3502 (OH), 3270 (N1H), 3080(=C–H), 1761 (C2=O), 1707 (C4=O), 1656 (ArCH=), 1595 (AR). Compound **8** (0.20 g, 0.40 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **8a** (0.20 g, 0.37 mmol). Yield 91%, mp 215–218 °C; TLC: R_f (V): 0.53; Anal. Calcd for $C_{26}H_{32}N_4O_6 \times HCl \times 0.5H_2O$ (**8a**): C, 57.61; H, 6.32; N, 10.34. Found: C, 58.01; H, 6.56; N, 10.35. ^1H NMR for **8a** (DMSO-d_6) δ (ppm) = 2.98–3.35 (m, 6H, Pp-CH_2, Pp-2,6-H), 3.41–3.72 (m, 6H, Pp-3,5-H, N3-CH_2), 3.74 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.31–4.34 (m, 1H, C*H*–OH), 4.45 (br s, 1H, OH), 6.70 (s, 1H, Ph-CH=), 6.85–7.03 (m, 4H, PpPh-4,6-H, Ph-4,5-H), 7.04–7.13 (m, 2H, PpPh-3,5-H), 7.28 (dd, J_1 = 7.44 Hz, J_2 = 1.80, 1H, Ph-6-H), 10.25 (br s, 1H, NH^+), 10.72 (s, 1H, N1H). IR KBr (cm^{-1}): 3411 (OH), 3014 (=C–H), 2568 (NH^+), 1766 (C2=O), 1714 (C4=O), 1664 (ArCH=), 1577 (Ar)

4.1.2.5. Hydrochloride of (*Z*)-5-(2,3-dimethoxybenzylidene)-3-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)-2-hydroxypropyl)imidazolidine-2,4-dione (9a). (*Z*)-5-(2,3-Dimethoxybenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **35** (1.3 mmol, 0.40 g) and *N*-2-ethoxyphenylpiperazine **40** (1.4 mmol, 0.29 g) were used. The irradiation: 450 W for 3 min, 600 W for 4 min (2 × 2 min) and 750 W for 6 min (3 × 2 min). White crystals of pure compound **9** (350 mg, 0.69 mmol). Yield 52.7%, mp 127–130 °C; R_f (V): 0.65; Anal. Calcd for $C_{27}H_{34}N_4O_6$ (**9**): C, 63.51; H, 6.71; N, 10.98. Found: C, 63.00; H, 6.80; N, 10.90. ^1H NMR ($CDCl_3$) δ (ppm): 1.44 (t, J = 6.92 Hz, 3H, OCH_2CH_3), 2.44–2,57 (m, 2H, Pp-CH_2), 2,64–2.65 (m, 2H, Pp-2,6-H_a), 2.82–2.88 (m, 2H, Pp-2,6-H_e), 3.10 (br s, 4H, Pp-3,5-H), 3.65–3.77 (m, 2H, N3-CH_2), 3.80 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.02–4.12 (m, 3H, OCH_2CH_3, C*H*–OH), 6.65 (s, 1H, Ph-CH=), 6.82–6.99 (m, 6H, PpPh-3,4,5,6-H, Ph-4,5-H), 7.09–7.14 (m, 1H, Ph-6-H), 8.21 (s, 1H, N1H). IR KBr (cm^{-1}): 3473 (OH), 3325 (N1H), 3077 (=C–H), 1760 (C2=O), 1709 (C4=O), 1660 (ArCH=), 1595 (Ar). Compound **9** (0.20 g, 0.39 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **9a** (0.21 g, 0.38 mmol). Yield 97%, mp 205–210 °C; TLC: R_f (V): 0.58; Anal. Calcd for $C_{27}H_{34}N_4O_6 \times HCl \times 0.5H_2O$ (**9a**): C, 58.32; H, 6.53; N, 10.08. Found: C, 58.23; H, 6.73; N,1 0.02. ^1H NMR for **9a** (DMSO-d_6) δ (ppm):1.34 (t, J = 6.92 Hz, 3H, OCH_2CH_3), 3.00–3.36 (m, 6H, Pp-CH_2, Pp-2,6-H), 3.41–3.72 (m, 6H, Pp-3,5-H, N3-CH_2), 3.74 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.97–4.04 (m, 2H, OCH_2CH_3), 4.31–4.34 (m, 1H, C*H*–OH), 4.59 (br s, 1H, OH), 6.70 (s, 1H, Ph-CH=), 6.84–7.01 (m, 4H, PpPh-4,6-H, Ph-4,5-H), 7.03–7.13 (m, 2H, Ph-4,5-H), 7.28 (dd, J_1 = 7.44, J_2 = 1.80, 1H, Ph-6-H), 10.25 (br s, 1H, NH^+), 10.72 (s, 1H, N1H). IR KBr (cm^{-1}): 3369 (N1H), 2424 (NH^+), 1764 (C2=O), 1714 (C4=O), 1663 (ArCH=), 1576 (Ar).

4.1.2.6. Hydrochloride of (*Z*)-5-(4-chlorobenzylidene)-3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)imidazolidine-2,4-dione (10a). (*Z*)-5-(4-Chlorobenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **33** (1 mmol, 0.28 g) and *N*-2-methoxyphenylpiperazine **39** (1.1 mmol, 0.21 g) were used. The irradiation: 450 W for 3 min, 600 W for 10 min (2 × 3 min and 2 × 2 min). White crystals of pure compound **10** (330 mg, 0.70 mmol). Yield 70.3%, mp 161–164 °C; TLC: R_f (V): 0.35; Anal. Calcd for $C_{24}H_{27}N_4O_4Cl$ (**10**): C, 61.21; H, 5.78; N, 11.90 Found: C, 61.46; H, 6.11; N, 11.89. ^1H NMR (DMSO-d_6) δ (ppm): 2.31–2.53 (m, 6H, Pp-CH_2, Pp-2,6-H), 2.85 (br s, 4H, Pp-3,5-H), 3.44–3.52 (m, 2H, N3-CH_2), 3.72 (s, 3H, OCH_3), 3.98- 4.01 (m, 1H, C*H*–OH), 4.97 (d, J = 5.38 Hz, 1H, OH), 6.50 (s, 1H, Ph-CH=), 6.68–6.78 (m, 2H, PpPh-4,5-H), 6.85–6.89 (m, 2H, PpPh-3.6-H), 7.42 (d, J = 8.46 Hz, 2H, Ph-3,5-H), 7.64 (d, J = 8.72 Hz, 2H, Ph-2,6-H), 10.79 (s, 1H, N1H). IR KBr (cm^{-1}): 3455 (OH), 3319 (N1H), 3073 (=C–H), 1756 (C2=O), 1711 (C4=O), 1661 (ArCH=), 1590 (Ar). Compound **10** (0.20 g, 0.42 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **10a** (0.21 g, 0.41 mmol). Yield 95.8%, mp 262–265 °C, R_f (V): 0.57; Anal. Calcd for $C_{24}H_{27}N_4O_4Cl \times HCl \times 0.5H_2O$ (**10a**): C, 55.82; H, 5.66; N, 10.85. Found: C, 55.40; H, 5.74; N, 10.68. ^1H NMR for **10a** (DMSO-d_6) δ (ppm): 2.97–3.39 (m, 6H, Pp-CH_2, Pp-2,6-H), 3.41–3.65 (m, 6H, Pp-3,5-H, N3-CH_2), 3.77 (s, 3H, OCH_3), 4.30–4.32 (m, 1H, C*H*–OH), 4.98 (br s, 1H, OH), 6.53 (s, 1H, Ph-CH=), 6.85–7.03 (m, 4H, PpPh-3,4,5,6-H), 7.46 (d, J = 8.46 Hz, 2H, Ph-3,5-H), 7.67 (d, J = 8.72 Hz, 2H, Ph-2,6-H), 10.19 (br s, 1H, NH^+), 10.90 (s, 1H, N1H). IR KBr (cm $^{-1}$): 3472 (OH), 3269 (N1H), 3060 (=C–H), 2539 (NH^+), 1769 (C2=O), 1714 (C4=O), 1659 (ARCH=), 1588 (Ar).

4.1.2.7. Hydrochloride of (*Z*)-5-benzylidene-3-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)-2-hydroxypropyl)imidazolidine-2,4-dione (11a). (*Z*)-5-Benzylidene-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **32** (0.5 mmol, 0.12 g) and *N*-2-ethoxyphenylpiperazine **40** (0.55 mmol, 0.11 g) were used. The irradiation: 450 W for 2 min, 600 W for 3 min and 750 W for 15 min (3 × 5 min). White crystals of pure compound **11** (180 mg, 0.41 mmol). Yield 66.5%, mp 206–209 °C; TLC: R_f (V): 0.68; Anal. Calcd for $C_{24}H_{28}N_4O_4$ (**11**): C, 66.65; H, 6.71; N, 12.44. Found: C, 66.45; H, 6.84; N, 12.36. ^1H NMR ($CDCl_3$) δ (ppm): 1.45 (t, J = 6.92 Hz, 3H, OCH_2CH_3), 2.44–2.57 (m, 2H, Pp-CH_2), 2.61–2.65 (m, 2H, Pp-2,6-H_a), 2.81–2.86 (m, 4H, Pp-2,6-H_e), 3.09 (br s, 4H, Pp-3,5-H), 3.66–3.82 (m, 2H, N3-CH_2), 4.02–4.14 (m, 3H, OCH_2CH_3, C*H*–OH), 6.77 (s, 1H, Ph-CH=), 6.82–6.99 (m, 4H, PpPh-3,4,5,6-H), 7.26–7.47 (m, 5H, Ph-2,3,4,5,6-H), 8.27 (br s, 1H, N1H). IR KBr (cm^{-1}): 3448 (OH), 3330 (N1H), 3056 (=C–H), 1752 (C2=O), 1710 (C4=O), 1659 (ArCH=), 1592 (Ar). Compound **11** (0.13 g, 0.29 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **11a** (0.14 g, 0.27 mmol). Yield 93%, mp 227–231 °C; TLC: R_f (V): 0.60; Anal. Calcd for $C_{25}H_{30}N_4O_4 \times HCl \times 1.3H_2O$ (**11a**): C, 58.83; H, 6.64; N, 10.98. Found: C, 58.67; H, 6.70; N, 11.01. ^1H NMR for **11a** (DMSO-d_6) δ (ppm): 1.34 (t, J = 6.92 Hz, 3H, OCH_2CH_3), 3.00–3.55 (m, 6H, Pp-CH_2, Pp-2,6-H), 3.42–3.67 (m, 6H, Pp-3,5-H, N3-CH_2),

4.00 (q, J = 6.93 Hz, 2H, OCH_2CH$_3$), 4.32–4.35 (m, 1H, CH–OH), 6.39 (br s, 1H, OH), 6.53 (s, 1H, Ph-CH=), 6.84–7.00 (m, 4H, PpPh-3,4,5,6-H), 7.31–7.43 (m, 3H, Ph-3,4,5-H), 7.65 (d, J = 7.18 Hz, 2H, Ph-2,6-H), 10.30 (br s, 1H, NH$^+$), 10.83 (s, 1H, N1H). IR KBr (cm^{-1}): 3413 (OH), 3239 (N1H), 3062 (=C–H), 2463 (NH$^+$), 1767 (C2=O), 1708 (C4=O), 1660 (ArCH=), 1573 (Ar).

4.1.2.8. Hydrochloride of (*Z*)-5-(3,4-dimethoxybenzylidene)-3-(2-hydroxy-3-(4-phenylpiperazin-1-yl)propyl)imidazolidine-2,4-dione (12a). (*Z*)-5-(3,4-Dimethoxybenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **37** (1 mmol, 0.30 g) and *N*-phenylpiperazine **38** (1.1 mmol, 0.18 g) were used. The irradiation: 450 W for 3 min, 600 W for 3 min and 750 W for 15 min (3 × 5 min). Bright-yellow crystals of pure compound **12** (370 mg, 0.79 mmol). Yield 79.3%, mp 154–158 °C; TLC: R_f (V): 0.63; Anal. Calcd for **C**$_{25}$H$_{30}$N$_4$O$_5$ (**12**): C, 64.36; H, 6.48; N, 12.01. Found: C, 64.01; H, 6.72; N, 12.03. ^{1}H NMR (CDCl$_3$) δ (ppm): 2.43–2.51 (m, 2H, Pp-CH$_2$), 2.53–2.62 (m, 2H, Pp-2,6-H$_a$), 2.75–2.81 (m, 2H, Pp-2,6-H$_e$), 3.13–3.22 (m, 4H, Pp-3,5-H), 3.60–3.80 (m, 2H, N3-CH$_2$), 3.92 (s, 3H, OCH$_3$), 3.93 (s, 3H, OCH$_3$), 4.05–4.13 (m, 1H, CH–OH), 6.72 (s, 1H, Ph-CH=), 6.83–6.93 (m, 5H, PpPh-2,4,6-H, Ph-2,5-H), 7.04 (dd, J_1 = 8.47 Hz, J_2 = 1.8 Hz, 1H, Ph-6-H), 7.23–7.28 (m, 2H, PpPh-3,5-H), 8.19 (s, 1H, N1H). Compound **12** (0.20 g, 0.43 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **12a** (0.18 g, 0.34 mmol). Yield 79%, mp 230–235 °C; TLC: R_f (V): 0.58; Anal. Calcd for C$_{25}$H$_{30}$N$_4$O$_5$ × HCl × 1.5H$_2$O (**12a**): C, 56.65; H, 6.47; N, 10.57. Found: C, 56.65; H, 6.79; N, 10.62. ^{1}H NMR for **12a** (DMSO-d_6) δ (ppm): 3.11–3.34 (m, 6H, Pp-CH$_2$, Pp-2,6-H), 3.41–3.68 (m, 6H, Pp-3,5-H, N3-CH$_2$), 3.78 (s, 3H, OCH$_3$), 3.82 (s, 3H, OCH$_3$), 4.32–4.34 (m, 1H, CH–OH), 6.23 (br s, 1H, OH), 6.50 (s, 1H, Ph-CH=), 6.84 (t, J = 7.18 Hz, 1H, PpPh-4-H), 6.97 (d, J = 8.21 Hz, 3H, PpPh-2,6-H, Ph-5-H), 7.15 (d, J = 2,05 Hz, 1H, Ph-2-H), 7.21–7.27 (m, 3H, PpPh-3,5-H, Ph-6-H), 10.35 (br s, 1H, NH$^+$), 10.79 (s, 1H, N1H). IR KBr (cm^{-1}): 3413 (OH), 2446 (NH$^+$), 1766 (C2=O), 1709 (C4=O), 1656 (ArCH=), 1598 (Ar).

4.1.2.9. Hydrochloride of (*Z*)-5-(2,4-dimethoxybenzylidene)-3-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)-2-hydroxypropyl)imidazolidine-2,4-dione (13a). (*Z*)-5-(2,4-Dimethoxybenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **36** (1 mmol, 0.30 g) and *N*-2-ethoxyphenylpiperazine **40** (1.1 mmol, 0.23 g) were used. The irradiation: 450 W for 3 min, 600 W for 4 min (2 × 2 min) and 750 W for 10 min (5 × 2 min). Bright-yellow crystals of pure compound **13** (370 mg, 0.72 mmol). Yield 72.5%, mp 174–178 °C; TLC: R_f (V): 0.69; Anal. Calcd for C$_{27}$H$_{34}$N$_4$O$_6$ (**13**): C, 63.51; H, 6.71; N, 10.98. Found: C, 63.66; H, 6.79; N, 10.78. ^{1}H NMR (CDCl$_3$) δ (ppm): 1.44 (t, J = 6.92 Hz, 3H, OCH$_2$CH_3), 2.44–2.56 (m, 2H, Pp-CH$_2$), 2.63–2.65 (m, 2H, Pp-2,6-H$_a$), 2.81–2.87 (m, 2H, Pp-2,6-H$_e$), 3.10 (br s, 4H, Pp-3,5-H), 3.66–3.77 (m, 2H, N3-CH$_2$), 3.83 (s, 3H, OCH$_3$), 3.93 (s, 3H, OCH$_3$), 4.02–4.13 (m, 3H, OCH_2CH$_3$, CH–OH), 6.51–6.58 (m, 2H, Ph-3,5-H), 6.70 (s, 1H, Ph-CH=), 6.82–6.99 (m, 4H, PpPh-3,4,5,6-H), 7.26 (d, J = 8.72 Hz, 1H, Ph-6-H), 7.83 (s, 1H, N1H). IR KBr (cm^{-1}): 3420 (OH), 3246 (N1H), 3056 (=C–H), 1759 (C2=O), 1711 (C4=O), 1662 (ArCH=), 1607 (Ar). Compound **13** (0.20 g, 0.39 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **13a** (0.21 g, 0.36 mmol). Yield 92%, mp 235–241 °C; TLC: R_f (V): 0.57; Anal. Calcd for C$_{27}$H$_{34}$N$_4$O$_6$ × HCl × 2H$_2$O (**13a**): C, 55.62; H, 6.74; N, 9.61. Found: C, 55.87; H, 6.55; N, 9.57. ^{1}H NMR for **13a** (DMSO-d_6) δ (ppm): 1,34 (t, J = 6.92 Hz, 3H, OCH$_2$CH_3), 2.97–3.38 (m, 6H, Pp-CH$_2$, Pp-2,6-H), 3.40–3.65 (m, 6H, Pp-3,5-H, N3-CH$_2$), 3.80 (s, 3H, OCH$_3$), 3.84 (s, 3H, OCH$_3$), 4.00 (q, J = 6.92 Hz, 2H, OCH_2CH$_3$), 4.29–4.31 (m, 1H, CH–OH), 5.93 (br s, 1H, OH), 6.54–6.61 (m, 2H, Ph-3,5-H), 6.72 (s, 1H, Ph-CH=), 6.84–7.00 (m, 4H, PpPh-3,4,5,6-H), 7.59 (d, J = 8.72 Hz, 1H, Ph-6-H), 10.11 (br s, 1H, NH$^+$), 10.59 (s, 1H, N1H). IR KBr (cm^{-1}): 3414 (OH), 3232 (N1H), 2500 (NH$^+$), 1755 (C2=O), 1715 (C4=O), 1658 (ArCH=), 1608 (Ar).

4.1.2.10. Hydrochloride of (*Z*)-5-benzylidene-3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)imidazolidine-2,4-dione (14a). (*Z*)-5-Benzylidene-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione 32 (0.8 mmol, 0.20 g) and *N*-2-methoxyphenylpiperazine 39 (0.88 mmol, 0.14 g) were used. The irradiation: 450 W for 2 min, 600 W for 4 min (2 × 2 min) and 750 W for 19 min (2 × 2 min and 3 × 5 min). White crystals of pure compound 14 (300 mg, 0.69 mmol). Yield 86%, mp 188–190 °C; TLC: R_f (V): 0.58; Anal. Calcd; C$_{24}$H$_{28}$N$_4$O$_4$ (**14**): C, 66.04; H, 6.47; N, 12.84. Found: C, 66.24; H, 6.77; N, 12.90. ^{1}H NMR (CDCl$_3$) δ (ppm): 2.44–2.57 (m, 2H, Pp-CH$_2$), 2.62–2.65 (m, 2H, Pp-2,6-H$_a$), 2.82–2.87 (m, 2H, Pp-2,6-H$_e$), 3.07 (br s, 4H, Pp-3,5-H), 3.66–3.81 (m, 2H, N3-CH$_2$), 3.86 (s, 3H, OCH$_3$), 4.06–4.14 (m, 1H, CH–OH), 6.77 (s, 1H, Ph-CH=), 6.84–7.03 (m, 4H, PpPh-3,4,5,6-H), 7.33–7.47 (m, 5H, Ph-2,3,4,5,6-H), 8.17 (br s, 1H, N1H). IR KBr (cm^{-1}): 3457 (OH), 3320 (N1H), 3059 (=C–H), 1753 (C2=O), 1709 (C4=O), 1658 (ArCH=), 1593 (Ar). Compound **14** (0.20 g, 0.46 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **14a** (0.23 g, 0.46 mmol). Yield 99%, mp 232–236 °C; TLC: R_f (V): 0.53; Anal. Calcd for C$_{24}$H$_{28}$N$_4$O$_4$ × HCl × 1.4H$_2$O (**14a**): C, 57.86; H, 6.43; N, 11.25. Found: C, 57.74; H, 6.41; N, 11.26. ^{1}H NMR for **14a** (DMSO-d_6) δ (ppm): 3.00–3.39 (m, 6H, Pp-CH$_2$, Pp-2,6-H), 3.41–3.67 (m, 6H, Pp-3,5-H, N3-CH$_2$), 3.77 (s, 3H, OCH$_3$), 4.32–4.34 (m, 1H, CH–OH), 6.04 (br s, 1H, OH), 6.53 (s, 1H, Ph-CH=), 6.85–7.03 (m, 4H, PpPh-3,4,5,6-H), 7.31–7.44 (m, 3H, Ph-3,4,5-H), 7.64 (d, J = 7.18 Hz, 2H, Ph-2,6-H), 10.32 (br s, 1H, NH$^+$), 10.83 (s, 1H, N1H). IR KBr (cm^{-1}): 3358 (N1H), 3061 (=C–H), 2420 (NH$^+$), 1766 (C2=O), 1714 (C4=O), 1660 (ArCH=), 1614 (Ar).

4.1.2.11. Hydrochloride of (*Z*)-5-(2,3-dimethoxybenzylidene)-3-(2-hydroxy-3-(4-phenylpiperazin-1-yl)propyl)imidazolidine-2,4-dione (15a). (*Z*)-5-(2,3-Dimethoxybenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **35** (1 mmol, 0.30 g) and *N*-phenylpiperazine **38** (1.1 mmol, 0.18 g) were used. The irradiation: 450 W for 3 min, 600 W for 4 min (2 × 2 min) and 750 W for 15 min (3 × 5 min). White crystals of pure compound **15** (300 mg, 0.64 mmol). Yield 64.4%, mp 180–182 °C; TLC: R_f (V): 0.59; Anal. Calcd for C$_{25}$H$_{30}$N$_4$O$_5$ (**15**): C, 64.36; H, 6.48; N, 12.01. Found: C, 64.38; H, 6.72; N, 12.02. 1H NMR (CDCl$_3$) δ (ppm): 2.45–2.55 (m, 2H, Pp-CH$_2$), 2.58–2.65 (m, 2H, Pp-2,6-H$_a$), 2.77–2.84 (m, 2H, Pp-2,6-H$_e$), 3.18–3.24 (m, 4H, Pp-3,5-H), 3.59–3.78 (m, 2H, N3-CH$_2$), 3.80 (s, 3H, OCH$_3$), 3.91 (s, 3H, OCH$_3$), 4.05–4.12 (m, 1H, CH–OH), 6.65 (s, 1H, Ph-CH=), 6.83–6.97 (m, 5H, PpPh-2,4,6-H, Ph-4,5-H), 7.09–7.14 (m, 1H, Ph-6-H), 7.23–7.28 (m, 2H, PpPh-3,5-H), 8.22 (s, 1H, N1H). IR KBr (cm^{-1}): 3482 (OH), 3329 (N1H), 1762 (C2=O), 1710 (C4=O), 1660 (ArCH=), 1599 (Ar).Compound **15** (0.20 g, 0.43 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **15a** (0.23 g, 0.34 mmol). Yield 79%, mp 215–218 °C; TLC: R_f (V): 0.65; Anal. Calcd for C$_{24}$H$_{27}$N$_4$O$_4$Cl × HCl × 2.67H$_2$O (**15a**): C, 54.49; H, 6.65; N, 10.17. Found: C, 54.79; H, 6.62; N, 10.23. ^{1}H NMR for **15a** (DMSO-d_6) δ (ppm): 3.07–3.41 (m, 6H, Pp-CH$_2$, Pp-2,6-H), 3.43–3.68 (m, 6H, Pp-3,5-H, N3-CH$_2$), 3.74 (s, 3H, OCH$_3$), 3.81 (s, 3H, OCH$_3$), 4.32–4.34 (m, 1H, CH–OH), 5.91 (br s, 1H, OH), 6.69 (s, 1H, Ph-CH=), 6.84 (t, J = 7.18 Hz, 1H, PpPh-4-H), 6.97 (d, J = 7.95 Hz, 2H, PpPh-2,6-H), 7.04–7.13 (m, 2H, Ph-4,5-H), 7.21–7.29 (m, 3H, PpPh-3,5-H, Ph-6H), 10.33 (br s, 1H, NH$^+$), 10.72 (s, 1H, N1H). IR KBr (cm^{-1}): 3359 (N1H), 2582 (NH$^+$), 1765 (C2=O), 1714 (C4=O), 1665 (ArCH=), 1599 (Ar).

4.1.2.12. Hydrochloride of (*Z*)-5-(4-(dimethylamino)benzylidene)-3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)imidazolidine-2,4-dione (16a). (*Z*)-5-((4-Dimethylamino)benzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **34**

(1 mmol, 0.29 g) and *N*-2-methoxyphenylpiperazine **39** (1.1 mmol, 0.21 g) were used. The irradiation: 450 W for 3 min, 600 W for 4 min (2 × 2 min) and 750 W for 15 min (3 × 5 min). Yellow crystals of pure compound **16** (440 mg, 0.82 mmol). Yield 92%, mp 170–173 °C; TLC: R_f (V): 0.59; Anal. Calcd for $C_{26}H_{33}N_5O_4$ (**16**): C, 65.11; H, 6.94; N, 11.61. Found: C, 64.94; H, 7.09; N, 11.51. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.48–2.55 (m, 2H, Pp-CH_2), 2.62–2.65 (m, 2H, Pp-2,6-H_a), 2.81–2.86 (m, 2H, Pp-2,6-H_e), 3.03 (s, 6H, 4-N(CH_3)$_2$), 3.06 (br s, 4H, Pp-3,5-H), 3.68–3.81 (m, 2H, N3-CH_2), 3.85 (s, 3H, OCH_3), 4.07–4.12 (m, 1H, C*H*–OH), 6.70–6.72 (m, 3H, Ph-CH=, Ph-3,5-H), 6.84–7.02 (m, 4H, PpPh-3,4,5,6-H), 7.33 (d, *J* = 8.72 Hz, 2H, Ph-2,6-H), 7.99 (s, 1H, N1H). IR KBr (cm^{-1}): 3409 (OH), 3320 (N1H), 3058 (=C–H), 1742 (C2=O), 1692 (C4=O), 1643 (ArCH=), 1603 (Ar). Compound **16** (0.20 g, 0.42 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **16a** (0.23 g, 0.39 mmol). Yield 93%, mp 226–230 °C; TLC: R_f (V): 0.50; Anal. Calcd for $C_{26}H_{33}N_5O_4$ xHCl × $4.5H_2O$ (**16a**): C, 52.39; H, 7.10; N, 11.75 Found: C, 52.28 H, 6.83; N, 11.74. ^{1}H NMR for **16a** (DMSO-d_6) δ (ppm): 3.00 (s, 6H, 4-N(CH_3)$_2$), 3.06–3.33 (m, 6H, Pp-CH_2, Pp-2,6-H), 3.42–3.66 (m, 6H, Pp-3,5-H, N3-CH_2), 3.77 (s, 3H, OCH_3), 4.30–4.33 (m, 1H, C*H*–OH), 5.58 (br s, 1H, OH), 6.48 (s, 1H, Ph-CH=), 6.85–7.03 (m, 6H, PpPh-3,4,5,6-H, Ph-3,5-H), 7.60 (d, *J* = 8.82 Hz, 2H, Ph-2,6-H), 10.26 (br s, 1H, NH^+), 10.65 (s, 1H, N1H). IR KBr (cm^{-1}): 3418 (OH), 3238 (N1H), 3026 (=C–H), 2459 (NH^+), 1765 (C2=O), 1708 (C4=O), 1662 (ArCH=), 1612 (Ar).

4.1.2.13. Hydrochloride of (*Z*)-5-(4-chlorobenzylidene)-3-(2-hydroxy-3-(4-phenylpiperazin-1-yl)propyl)imidazolidine-2,4-dione (17a). (*Z*)-5-(4-Chlorobenzylidene)-3-(oxiran-2-ylmethyl) imidazolidine-2,4-dione **33** (1.0 mmol, 0.28 g) and *N*-phenylpiperazine **38** (1.1 mmol, 0.18 g) were used. The irradiation: 450 W for 3 min, 600 W for 4 min (2 × 2 min) and 750 W for 15 min (3 × 5 min). Bright-yellow crystals of pure compound **17** (250 mg, 0.57 mmol). Yield 57%, mp 230–233 °C; TLC: R_f (V): 0.73; Anal. Calcd for $C_{23}H_{25}N_4O_3Cl$ (**17**): C, 62.65; H, 5.71; N, 12.71. Found: C, 62.73; H, 6.03; N, 12.72. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.29–2.55 (m, 6H, Pp-CH_2, Pp-2,6-H), 2.99–3.05 (m, 4H, Pp-3,5-H), 3.44–3.56 (m, 2H, N3-CH_2),3.96–4.02 (m, 1H, C*H*–OH), 4.99 (d, *J* = 5.13 Hz, 1H, OH), 6.49 (s, 1H, Ph-CH=), 6.72 (t, *J* = 7.31 Hz, 1H, PpPh-4-H), 6.84 (d, *J* = 7.95 Hz, 2H, PpPh-2,6-H), 7.12–7.17 (m, 2H, PpPh-3,5-H), 7.40 (d, *J* = 8.46 Hz, 2H, Ph-3,5-H), 7.60 (d, *J* = 8.72 Hz, 2H, Ph-2,6-H), 10.77 (s, 1H, N1H). IR KBr (cm^{-1}): 3449 (OH), 3269 (N1H), 3066 (=C–H), 1769 (C2=O), 1714 (C4=O), 1660 (ArCH=), 1592 (Ar) Compound **17** (0.20 g, 0.45 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **17a** (0.12 g, 0.43 mmol). Yield 96%, mp 277–280 °C; TLC: R_f (V): 0.66; Anal. Calcd for $C_{25}H_{30}N_4O_4Cl$ × HCl × $1.75H_2O$ (**17a**): C, 54.28; H, 5.84; N, 11.01. Found: C, 54.24; H, 5.60; N, 10.86. ^{1}H NMR for **17a** (DMSO-d_6) δ (ppm): 3.08–3.38 (m, 6H, Pp-CH_2, Pp-2,6-H), 3.41–3.80 (m, 6H, Pp-3,5-H, N3-CH_2), 4.32–4.35 (m, 1H, C*H*–OH), 6.06 (br s, 1H, OH), 6.52 (s, 1H, Ph-CH=), 6.84 (t, *J* = 7.18 Hz, 1H, PpPh-4-H), 6.97 (d, *J* = 7.95 Hz, 2H, PpPh-2,6-H), 7.21–7.26 (m, 2H, PpPh-3,5-H), 7.45 (d, *J* = 8.72 Hz, 2H, Ph-3,5-H), 7.67 (d, *J* = 8.72 Hz, 2H, Ph-2,6-H), 10.43 (br s, 1H, NH^+), 10.90 (s, 1H, N1H). IR KBr (cm^{-1}): 3305 (N1H), 3062 (=C–H), 2445 (NH^+), 1772 (C2=O), 1714 (C4=O), 1659 (ArCH=), 1592 (Ar).

4.1.2.14. Hydrochloride of (*Z*)-5-(4-(dimethylamino)benzylidene)-3-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)-2-hydroxypropyl)imidazolidine-2,4-dione (18a). (*Z*)-5-((4-Dimethylamino) benzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **34** (1 mmol, 0.29 g) and *N*-2-ethoxyphenylpiperazine **40** (1.1 mmol, 0.23 g) were used. The irradiation: 450 W for 3 min, 600 W for 4 min (2 × 2 min) and 750 W for 15 min (3 × 5 min). Yellow crystals of pure compound **18** (0.35 g, 0.71 mmol). Yield 71%, mp 178–180 °C; TLC: R_f (V): 0.62; Anal. Calcd for $C_{27}H_{35}N_5O_4$ (**18**): C, 65.70; H, 6.15; N, 14.19. Found: C, 65.50; H, 6.27; N, 14.16. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.44 (t, *J* = 6.92 Hz, 3H, OCH_2CH_3), 2.49–2.55 (m, 2H, Pp-CH_2), 2.62–2.65 (m, 2H, Pp-2,6-H_a), 2.80–2.84 (m, 2H, Pp-2,6-H_e), 3.03 (s, 6H, 4-N(CH_3)$_2$), 3.09 (br s, 4H, Pp-3,5-H), 3.68–3.82 (m, 2H, N3-CH_2), 4.02–4.12 (m, 3H, OCH_2CH_3, C*H*–OH), 6.70–6.73 (m, 3H, Ph-CH=, Ph-3,5-H), 6.82–6.99 (m, 4-H, PpPh-3,4,5,6-H), 7.33 (d, *J* = 8.47 Hz, 2H, Ph-2,6-H), 8.07 (br s, 1H, N1H). IR KBr (cm^{-1}) 3408 (OH), 3324 (N1H), 1744 (C2=O), 1700 (C4=O), 1644 (ArCH=), 1602 (Ar). Compound **18** (0.20 g, 0.41 mmol) in dry MeOH (10 mL) with HCl concd (4 drops) was converted into white crystals of hydrochloride **18a** (0.24 g, 0.41 mmol). Yield 99%, mp 230–236 °C; TLC: R_f (V): 0.54; Anal. Calcd for $C_{27}H_{35}N_5O_4$ × HCl × $3.5H_2O$ (**18a**): C, 54.77; H, 7.15; N, 11.83. Found: C, 54.21; H, 7.03; N, 11.80. ^{1}H NMR for **18a** (DMSO-d_6) δ (ppm): 1.34 (t, *J* = 6.92 Hz, 3H, OCH_2CH_3), 3.00 (s, 6H, 4-N(CH_3)$_2$), 3.04–3.41 (m, 6H, Pp-CH_2, Pp-2,6-H), 3,43–3.67 (m, 6H, Pp-3,5-H, N3-CH_2), 4.00 (q, *J* = 6.92 Hz, 2H, OCH_2CH_3), 4.31–4.33 (m, 1H, C*H*–OH), 5.39 (br s, 1H, OH), 6.49 (s, 1H, Ph-CH=), 6.84–7.03 (m, 6H, PpPh-3,4,5,6-H, Ph-3,5-H), 7.60 (d, *J* = 8.72 Hz, 2H, Ph-2,6-H), 10.29 (br s, 1H, NH^+), 10.67 (s, 1H, N1H). IR KBr (cm^{-1}): 3420 (OH), 3256 (N1H), 3028 (=C–H), 2461 (NH^+), 1764 (C2=O), 1707 (C4=O), 1599 (Ar).

4.2. X-ray structure analysis

Crystal data for **7**: $C_{25}H_{29}N_4O_4Cl$, *M* = 484.97, monoclinic, space group *C*2/*c*, *a* = 43.9312(8) Å, *b* = 6.4095(1) Å, *c* = 19.4298(3) Å, β = 115.95(1)°, *V* = 4919.46(15) Å^3, *Z* = 8, D_x = 1.310 g cm^{-3}, *T* = 293 K, μ = 1.694 mm^{-1}, λ = 1.54178 Å, data/parameters = 4293/312, final R_1 = 0.0401.

Crystal data for ***10a***: $[C_{24}H_{27}N_4O_4Cl]^+$ Cl^-, *M* = 506.40, orthorombic, space group $Pca2_1$, *a* = 11.0513(2) Å, *b* = 6.2161(1) Å, *c* = 73.6494(10) Å, *V* = 5059.42(14)Å^3, *Z* = 8, D_x = 1.330 g cm^{-3}, *T* = 293 K, μ = 2.619 mm^{-1}, λ = 1.54178 Å, data/parameters = 8934/622; Flack *x* = 0.56(2), final R_1 = 0.068.

Crystals of **7** and **10a** were obtained by slow evaporation from their DMSO solutions. The measurements of the crystals were performed on a SMART diffractometer with graphite-monochromated CuKα radiation (λ = 1.54178 Å) at room temperature. The structures were solved by direct method and refined with SHELXTL.[29] E-maps provided positions for all non-H-atoms. The full-matrix least-squares refinement was carried out on F^2's using anisotropic temperature factors for all non-H-atoms. All C-bound H atoms were placed in idealized locations and refined using a riding model, with C–H = 0.93 Å and $U_{iso}(H) = 1.2U_{eq}(C)$.

Crystallographic data (excluding structural factors) for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre and allocated with the deposition numbers: CCDC 873235 & 873236 for compounds **7** and **10a,** respectively. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EW, UK (Fax: Int code + (1223)336-033; E-mail: deposit@ccdc.cam.ac.uk).

4.3. Molecular modelling methods

In the present work the racemic mixtures of the final compounds were obtained and tested pharmacologically, for this reason both enantiomers of described hydantoin derivatives were submitted to molecular modelling procedures. The 3D structures of **5a**–**18a** were built using Schrödinger Maestro molecular modelling environment[30] basing on the solved crystal structure of **7**. Basic piperazine nitrogens N4 in all structures were protonated and the charge of +1 was assigned. For each compound a conformational search was then performed using the Monte Carlo method (mixed MCMM/Low Mode Sampling) as implemented in

MacroModel 9.8[30] with OPLS-2005 (Optimized Potential for Liquid Simulations) force field and TNCG (Truncated Newton Conjugate Gradient) method of energy minimization. The conformational analysis was carried out for aqueous solutions with continuum solvation treatment (Generalised Born/Solvent Accessible, GB/SA). Found global minimum energy conformations of the ligands were superimposed by a least-squares method; carbon atoms of imidazole ring and the methylidene group were chosen as fitting points (Fig. 7, RMSD values in range 0.0001–0.0023). For the graphic presentation of superimposed lowest energy conformations PyMOL software[31] was used.

4.4. Pharmacology

4.4.1. Radioligand binding assays

The compounds were evaluated on their affinity for α_1-adrenergic receptors by determining for each compound its ability to displace [^{3}H]-prazosin from specific binding sites on 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). 240 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. Transfer solutions and adding reagents was performed on automated pipetting system epMotion 5070 (Eppendorf, Germany).

After incubation reaction mix was filtered immediately onto GF/B glass fiber filter mate presoaked 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.

4.5. Functional bioassays

Isolated rat aorta was used in order to test antagonistic activity of investigated compounds for α_1-adrenoceptors. The male Wistar rats weighing 200–350 g were anaethetised with thiopental, sodium (75 mg/kg ip) and the aorta was dissected and placed into Krebs–Henseleit solution and cleaned of surrounding fat tissue. The thoracic aorta was denuded of endothelium and cut into approximately 4-mm long rings. These 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 temperature 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 (3×10^{-9}–3×10^{-6} M) were obtained by the method of van Rossum.[32] Following the first phenylephrine curve, aorta rings were incubated with one of three to four concentration 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 analyzed 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 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 analyses were performed, and when the slope was not significantly different from unity, the pA_2 value was determined, but when the slope significantly different from unity, the affinity was estimated with the equation $pK_B = \log(\text{concentration ratio} - 1) - \log(\text{molar antagonist concentration})$, where concentration ratio is the ratio of equieffective agonist concentration in the absence and in the presence of the antagonist.[33]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.05.064.

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