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Original article

SAR-studies on the importance of aromatic ring topologies in search for selective 5-HT$_7$ receptor ligands among phenylpiperazine hydantoin derivatives

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ARTICLE INFO

Article history:
Received 28 August 2013
Received in revised form
5 January 2014
Accepted 7 January 2014
Available online 18 March 2014

Keywords:
Phenylpiperazines
Hydantoins
Mitsunobu reaction
5-HT$_{1A}$
5-HT$_{3A}$
5-HT$_6$
5-HT$_7$
Alpha1-adrenoceptor

ABSTRACT

The current study is focused on newly developed phenylpiperazine derivatives of aromatic methylhydantoin differing in mutual positions of methyl and phenyl moieties. The new compounds were synthesized using Bucherer–Bergs reaction, two-phase alkylation, Mitsunobu reaction and/or an alkylation under microwave irradiation. The compounds developed were assessed on their affinity for serotoninergic receptors 5-HT$_{1A}$, 5-HT$_6$, 5-HT$_7$ and α_1-ARs in radioligand binding assays. Selected compounds were tested on their inhibitory effect at human 5-HT$_{3A}$ expressed in *Xenopus* Oocytes as well as on their activity at α_1-adrenoceptor subtypes in functional and electrophysiological bioassays, respectively. Most of investigated compounds exhibited affinities for α_1-ARs, 5-HT$_{1A}$, 5-HT$_7$ ($K_i \sim 0.8$–353 nM) significantly higher than those for 5-HT$_6$ receptors. Very weak inhibitory effect at 5-HT$_{3A}$ accompanied with high activity at α_{1D}-AR subtypes were observed for selected representative compounds. Among the current series, particularly 5-(4-fluorophenyl)-3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl) propyl)-5-methylimidazolidine-2,4-dione hydrochloride (**25a**) displayed the highest 5-HT$_7$ affinity with $K_i = 3$ nM and selectivity with 40–3600 fold towards 5-HT$_{1A}$, 5-HT$_6$, and α_1-ARs.

1. Introduction

Arylpiperazine partial structure is a very popular chemical class present in many biologically active compounds including drugs of therapeutic implication and compounds under countless stages of pharmacological screening. The aromatic area in combination with positive ionizable nitrogen of piperazine meets the structural requirements of binding pockets found in various protein targets that play important physiological roles in mammal tissues. The latest lines of evidence indicated their anticancer properties [1], antituberculosis efficacy [2], antiarrhythmic and/or antihypertensive action [3]. Their ability to combat cancer or/and bacterial multidrug resistance [4–7] as well as their action on G-protein coupled receptors (GPCRs) including adenosine [8], dopaminergic [9], serotonin [10,11] and adrenergic [12] receptors have been demonstrated. Although arylpiperazine derived ligands are particularly widespread for serotonin receptor 5-HT$_{1A}$ (**1**), 5-HT$_7$ (**2**) [11,13–15] and all α_1-adrenoceptors subtypes (α_{1A}) (**3a**), (α_{1B}) (**3b**) and (α_{1D}) (**3c**) [16–19], they similarly occur in the case of 5-HT$_6$ (**4**) [20] and the ionotropic serotonin receptors 5-HT$_3$ [21] (**5**, Fig. 1). Especially, the role of arylpiperazine moiety in modulating the interactions with receptors like 5-HT$_{1A}$, 5-HT$_7$ [11,23,24] or α_1-adrenergic receptors [17,18,22] is underlined by several pharmacophore models (Fig. 2) that were established on the basis of large number of compounds evaluated in radioligand binding assays. The latter models have been and are still useful in the search for potent and selective ligands for variety of

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http://dx.doi.org/10.1016/j.ejmech.2014.01.065
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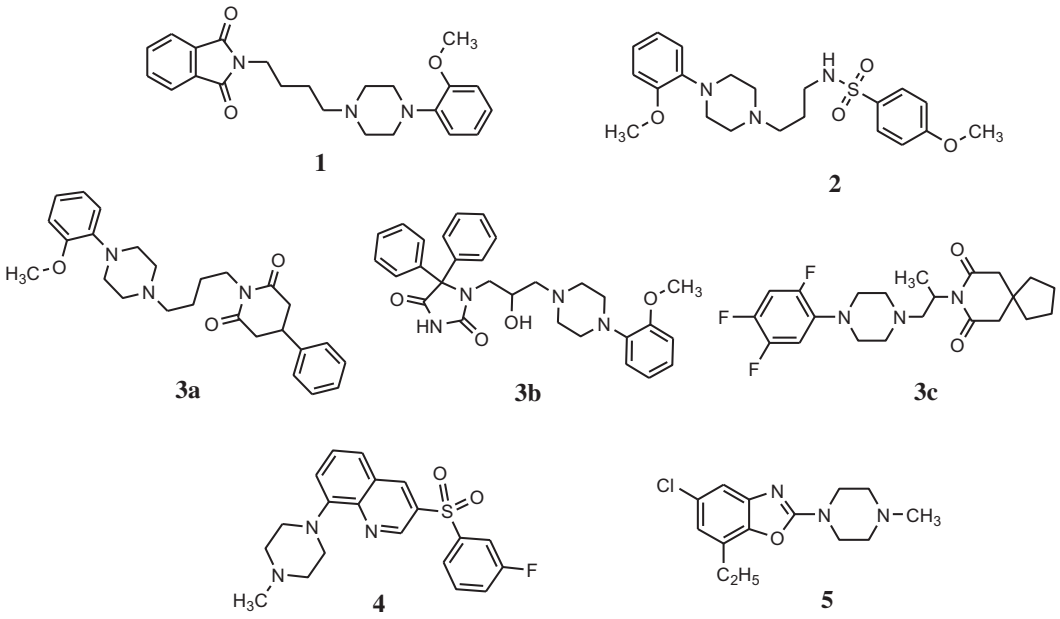


Fig. 1. Selective arylpiperazine ligands for serotonin receptors 5-HT_{1A} (**1**) [13], 5-HT_7 (**2**) [15], α_1-adrenoceptor subtypes α_{1A} (**3a**) [18], α_{1B} (**3b**) [19] α_{1D} (**3c**) [16,17], 5-HT_6 (**4**) [20] and the ionotropic serotonin receptors 5-HT_3 (**5**) [21].

receptors, particularly, because any experimental 3D-structures of these GPCRs have not yet been identified. Among these protein targets, a special attention is given to 5-HT_7 ones as they are the most recently identified serotonin receptor subtypes and their role in controlling various CNS functions is still increasing based on pharmacological studies that have been performed for the last decades [11]. Recent pharmacological results indicated the important modulating role of 5-HT_7 receptors in anxiety, circadian rhythm, depression, epilepsy, learning, memory, locomotion, migraine, pain, schizophrenia, sleep, substance abuse, and thermoregulation processes [11]. Considering the aforementioned roles, the search for effective and selective 5-HT_7 receptor agents provides a new hope for future therapy of a variety of CNS diseases. Arylpiperazine class is one of the most promising groups in the search of highly potent ligands for 5-HT_7, while their selectivity remains rather problematic due to high probability of interactions with other protein targets [1–12]. Thus, studies on chemical modifications of arylpiperazines targeting an improved selectivity profile towards 5-HT_7 receptors are an important and interesting field of current medicinal chemistry research.

Our previous studies were focused on phenylpiperazine derivatives of phenytoin, which displayed various affinities at both, α_1-drenergic and 5-HT_{1A} receptors [3,19,25,26] Among those compounds, the 3-methylhydantoin derivative **6a** (Table 1) displayed comparable and moderate affinities for both of the considered GPCRs [25] as well as for 5-HT_7 receptor, identified within later assays, almost identical as that for 5-HT_{1A}. Pharmacophore models (Fig. 2) indicated that number and relative position of aromatic/hydrophobic moieties, hydrogen bond acceptors, and positive ionizable center are responsible for interactions with the considered GPCRs and they also seem to be accountable for the receptors discrimination. Our previous studies for hydantoin derivatives have shown that, in addition to these pharmacophoric features, a role of substituent at 3-position of hydantoin was important for selective interactions with the GPCRs.

Based on previous observations, compound **6a** was selected as a lead structure for further chemical modifications to design new hydantoin-phenylpiperazine derivatives with higher affinity and enhanced selectivity profile towards 5-HT_7 receptors in respect to other competitive GPCRs, including 5-HT_{1A}, 5-HT_{3A}, 5-HT_6 or α_1-adrenoceptors. Our current investigations focus on four steps of modifications of compound **6a** (A-D, Table 1), which were designed to gradually increase the structural divergence in the field of relative locations of important structural fragments within the lead compound. Synthesis of new compounds, radioligand binding assays and functional bioassays were carried out, as well as structure–activity relationship analyses were established.

2. Results and discussion

2.1. Synthesis

Syntheses of the final products **6a**–**9a** and certain intermediates (**27, 30**–**32** and **41**) were described elsewhere [3,4,23–25]. Phenylpiperazine derivatives of hydantoin **10a**–**25a** were obtained within four parallel synthesis routes according to Schemes 1 and 2. The phenylpiperazinepentyl derivative of 5,5-diphenylhydantoin **10** was synthesized within three-steps of alkylation [24] starting from the methylation process at 3-position of 5,5-diphenylhydantoin **26** (Scheme 1a). An introduction of bromopentyl substituent at 1-position (compound **28**) was performed by two-phase alkylation in acetone with K_2CO_3 and TEBA using long-term stirring at room temperature. Contrary to its 3- or 4-carbons analogues [3], **28** did not precipitate during simple crystallization procedure with alcohols. Pure precipitate of **28** was achievable by double column chromatography separation and crystallization with ethanol supported by diethyl ether and *n*-hexane. The pure alkylating agent **28** was used for *N*-alkylation of commercial phenylpiperazine to afford compound **10**. The process was performed by reflux in microwave reactor "Plazmatronika" in two-phase basic conditions. A special 60-min irradiation program was elaborated on the basis of TLC control of the reaction progress. The established program was used for syntheses of compounds **11**–**13**, as well.

Compounds **11**–**13** were obtained within four-step syntheses (Scheme 1b), starting from Bucherer–Bergs condensation [25] that allowed the synthesis of racemic 5-methyl-5-phenylhydantoin,

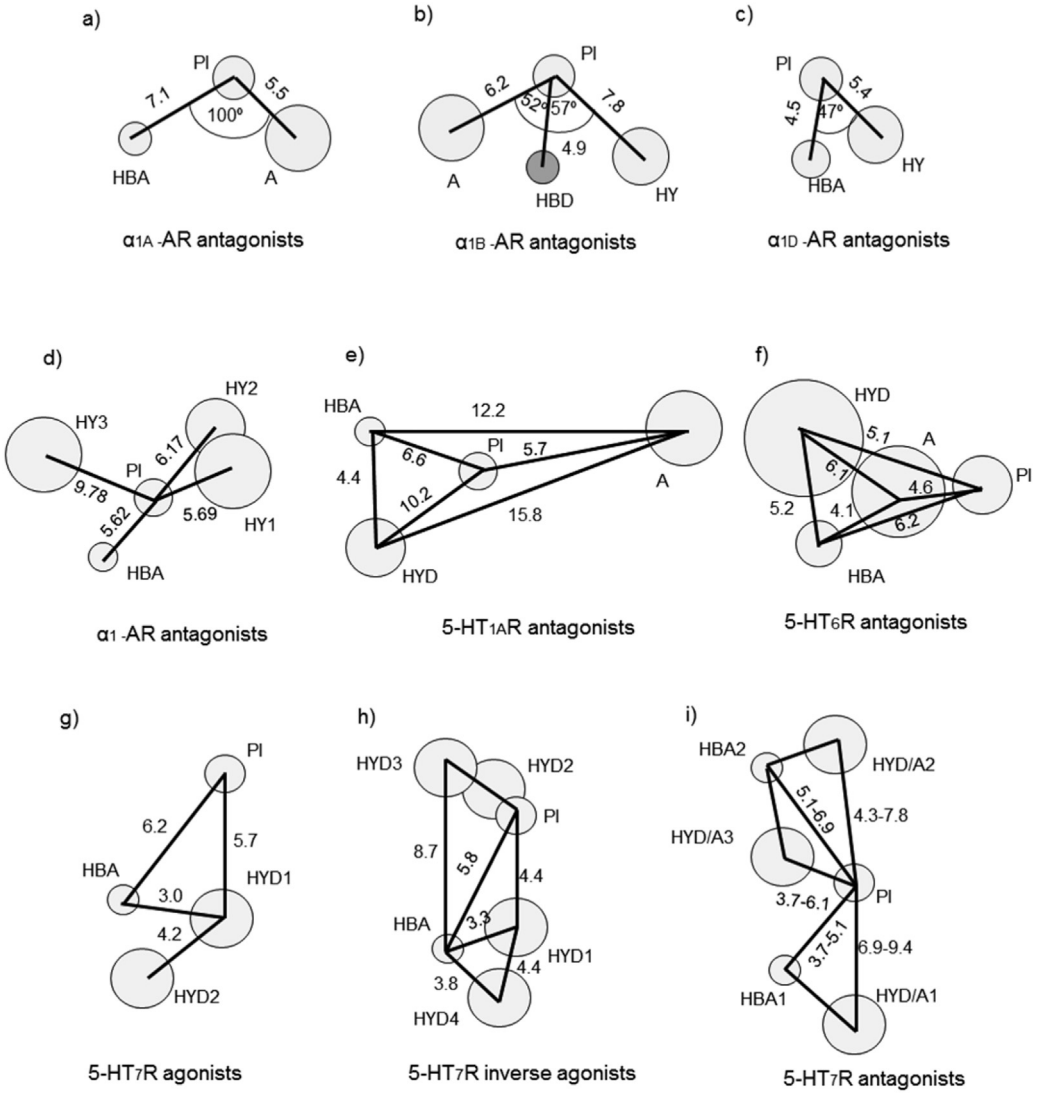


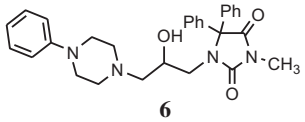
Fig. 2. Pharmacophore features elaborated for α_1-adrenrgic- or serotonin receptors agents: (a–c) pharmacophore features for α_1-AR antagonists postulated by Bremner's group in respect to subtype-selectivity [16]; (d) the Barbaro's model of phenylpiperazine derived α_1-AR antagonists [22]; (e) the Lepailleur's model for 5-HT_{1A} receptor antagonists [24]; (f) the pharmacophore features for 5-HT_6 receptor antagonists elaborated by Lopèz-Rodriguez et al. [23]; (g–i) the 5-HT_7 agents pharmacophore features for (g) agonists and (h) inverse agonists, described by Vermeulen et al., (i) the model of antagonists elaborated by Bojarski's research group [11]. A- an aromatic fragment, HY or HYD- hydrophobic areas, HBA-hydrogen bond acceptors, HBD-hydrogen bond donors, PI-positive ionizable nitrogen. All distances are expressed in [Å].

followed by alkylation at 1- and 3-position according to the previously described methods [4]. The bromopentyl derivative of 3,5-dimethyl-5-phenylhydantoin (**32)** was used as an alkylating agent for the microwave-aided two-phase alkylation of suitable phenylpiperazines to give compounds **11**–**13**. Compounds **10-13** were obtained as free bases by column chromatography separations.

The synthetic route of phenylpiperazine 5,5-dimethylhydantoin derivatives **14**–**24** was carried out according to Scheme 2a. Starting from commercially available 5,5-dimethylhydantoin (**33**), alkylation at 3-position was achieved by the use of suitable (un) substituted benzyl bromide or chlorides to afford compounds **34**–**36**. In the following step, the 3-benzyl derivatives **34**–**36** were converted into corresponding alkylating agents (**37**–**39**) by two-phase bromoalkylation with 1,5-dibromopentane under similar conditions to those of used to achieve compounds **28** and **32**. As no precipitate of compounds **37**–**39** ensued, the compounds were purified through column chromatography, and the resulted pure glass-residues (**37**–**39**) were used for reactions with suitable commercially available phenylpiperazines to give compounds **14**–**24**. Compounds **20**–**24** were obtained as free bases. However, compounds **14**–**19** that did not freely precipitate from ethanol solutions, were achieved through direct saturations of the solutions with gaseous HCl to give precipitates of their hydrochlorides **14a**–**19a**. The phenylpiperazine derivatives **10**–**13**, **20**, **21**, **23**, and **24** were converted into their corresponding hydrochlorides (**10a**–**13a**, **20a, 21a**, **23a** and **24a**) by saturation of their solutions in absolute ethanol with gaseous HCl.

Compound **25** representing reversal position of the phenylpiperazinealkyl substituent (at 3-position of hydantoin) was synthesized within three steps (Scheme 2b). Starting from Bucherer–Bergs cyclic condensation of 1-(4-fluorophenyl)ethanone (**40**), followed by oxiranmethyl introduction at 3-position of 5-(4-fluorophenyl)-5-methylhydantoin, the product 5-(4-fluorophenyl)-5-methyl-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (**42**) was obtained. As described earlier [4,24], the introduction of oxiranmethyl at 3-position was much more complicated than that at 1-position as the 3-oxiranmethyl derivative **42** was very sensitive to water and ethanol resulting in undesirable ring openings of the

Table 1
Structure of the tested compounds (**6a**–**25a**).



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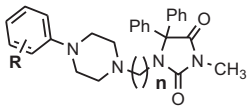
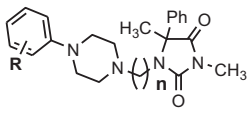
(Lead)



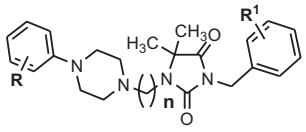
7-10

Group A



11-13

Group B



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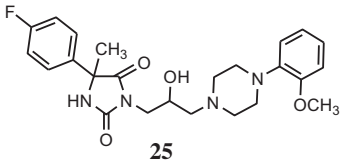
Group C



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Group D

Comp.	Group	R	R^1	*n*	α_1-AR K_i (nM) [^{3}H]-prazosin	5-HT_{1A} K_i (nM) [^{3}H]-8-OH-DPAT	5-HT_{3A}% inhibition at [30 mM] conc.	5-HT_6 K_i (nM) [^{3}H]-LSD	5-HT_7 K_i (nM) [^{3}H]-5-CT
6a					542.3 ± 19.4	324 ± 9	–	20 410 ± 2037	353 ± 19
7a	A	2-OCH_3	–	3	412.9 ± 21	6.5 ± 0.5	–	29 070 ± 3061	210 ± 11
8a	A	2-OCH_3	–	4	4.7 ± 1.5	0.8 ± 0.1	–	3567 ± 251	16 ± 1
9a	A	2-OC_2H_5	–	3	2600 ± 0.1	5.5 ± 0.6	–	4139 ± 513	95 ± 7
10a	A	H	–	5	6.7 ± 0.1	13.2 ± 1.2	36.86 ± 6.65	3071 ± 152	77 ± 6
11a	B	H	–	5	7.3 ± 0.8	135 ± 11	–	9600 ± 715	–
12a	B	2-F	–	5	11.3 ± 0.1	124 ± 9	–	25 520 ± 2824	157 ± 15
13a	B	2- OCH_3	–	5	42.3 ± 1.4	23.3 ± 2.1	19.75 ± 1.76	14 650 ± 1495	34 ± 2
14a	C	H	H	5	68.0 ± 6.4	79 ± 4	14.28 ± 1.22	4368 ± 522	70 ± 5
15a	C	2-OCH_3	H	5	42.6 ± 1.0	26 ± 2	–	8611 ± 627	72 ± 4
16a	C	3-OCH_3	H	5	97.2 ± 1.4	45 ± 3	–	2926 ± 134	122 ± 7
17a	C	2-F	H	5	71.0 ± 2.3	76 ± 3	–	2506 ± 283	135 ± 9
18a	C	4-F	H	5	68.4 ± 1.8	201 ± 16	–	1945 ± 191	45 ± 2
19a	C	2,4-diF	H	5	71.5 ± 2.5	235 ± 20	–	7469 ± 820	172 ± 8
20a	C	2,4-diF	4-F	5	38.8 ± 2.6	299 ± 17	–	8104 ± 367	123 ± 9
21a	C	4-F	4-F	5	36.8 ± 3.7	305 ± 13	17.22 ± 0.98	1762 ± 153	46 ± 3
22a	C	2,3-diCl	2,4-diCl	5	968.8 ± 82.6	88 ± 7	13.75 ± 1.29	395 ± 29	31 ± 2
23a	C	3,4-diCl	2,4-diCl	5	673.0 ± 63.0	301 ± 23	–	314 ± 17	78 ± 4
24a	C	4-Cl	2,4-diCl	5	758.5 ± 30.5	2178 ± 194	–	2879 ± 182	456 ± 23
25a	D	–	–		181.1 ± 12.2	121 ± 7	16.56 ± 1.29	10 790 ± 847	3 ± 0.2
Phentolamine	–	–	–	–	10.3 ± 0.6	–	–	–	–
Buspirone	–	–	–	–	–	22 ± 2	–	–	–
Olanzapine	–	–	–	–	–	–	–	7.0 ± 0.5	–
Clozapine	–	–	–	–	–	–	–	–	25 ± 3
LY278584							96.85 ± 2.13		

oxiran moiety. After several probes, the compound **42** was obtained by the application of Mitsunobu reaction in dry THF.

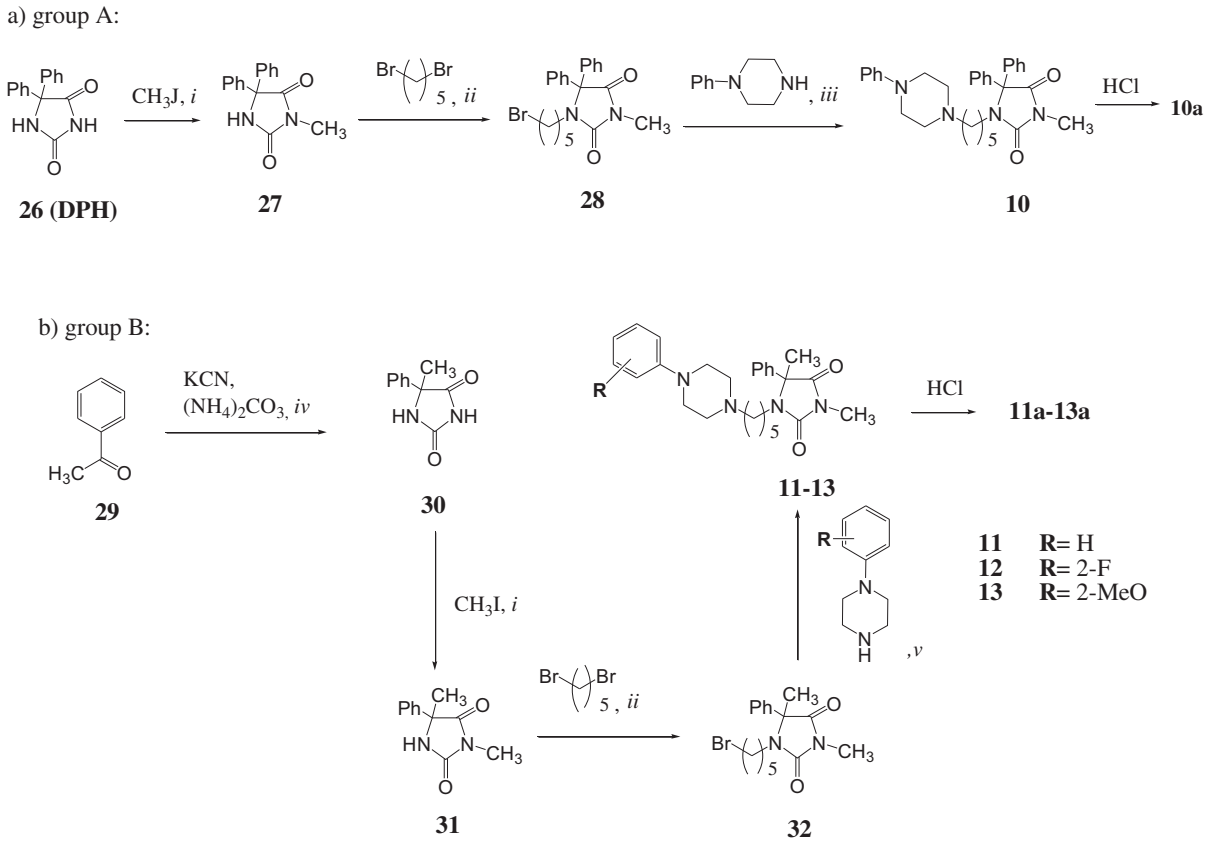
In the last step of the synthesis route, 5-(4-fluorophenyl)-5-methyl-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (**42**) was used as an alkylating agent for *N*-alkylation of 2-methoxyphenylpiperazine in solvent-free conditions under microwave irradiation [4,24]. The pure compound **25** was obtained as hydrochloride salt (**25a**) by column chromatography purification and saturation of the pure fractions in ethanol solution using gaseous HCl.

2.2. Pharmacology

2.2.1. Radioligand binding studies

Compounds **6a**–**25a** were examined *in vitro* for their affinity on α_1-adrenoceptors and serotonin receptors 5-HT_{1A}, 5-HT_6, 5-HT_7 in radioligand binding assays. The results expressed by K_i values (nM) are shown in Table 1. The affinity for α_1-adrenoceptors was tested on rat cerebral cortex by the using of [^{3}H]-prazosin as a specific radioligand. In the case of serotonin receptors, the studies were performed on human recombinant receptors expressed in HEK293 cells using [^{3}H]-8-OH-DPAT, [^{3}H]-LSD and [^{3}H]-5-CT for 5-HT_{1A}, 5-HT_6 and 5-HT_7 assays, respectively.

Most of the tested compounds **6a**–**25a** displayed moderate to high affinities for α_1-adrenoceptors, 5-HT_{1A} and 5-HT_7, whereas their affinities for 5-HT_6 were significantly lower (Table 1) with $K_{i(5-HT_6)}$ values in micromolar range. The highest activity and selectivity at α_1-AR was observed for compounds **10a**–**12a** with $K_i < 15$ nM. Certain selectivity towards α_1-AR was comprehended for compounds **19a**–**21a**, however their activities were lower (30 nM $< K_i <$ 80 nM). Furthermore, compound **8a** was the most active one when considering α_1-adrenoceptors and 5-HT_{1A} receptor with distinct selectivity profile towards serotonin receptor ($K_i = 0.8$ nM). In general, the most potent 5-HT_{1A} agents (**7a**–**10a**) were found in group A (Table 1) with noticeable selectivity in the case of compounds **7a**–**9a**. Moreover, a slight selectivity profile towards 5-HT_{1A} was also observed for compounds **15a** and **16a**, whereas tested population displayed significant submicromolar affinity for 5-HT_7 receptors (3 nM $< K_i <$ 456 nM). In particular, compound **25a** showed the highest affinity and selectivity towards 5-HT_7, in respect to the rest of considered GPCRs (sel 5-HT_7/α_1-AR = 60; sel 5-HT_7/5-HT_{1A} = 40; sel 5-HT_7/5-HT_6 = 3597). Some selectivity towards 5-HT_7 was also observed (Table 1) for the potent agents **17a**, **22a** and **23a** ($K_{i5-HT_7} < 80$ nM) as well as for a moderate one, **24a** ($K_{i5-HT_7} = 456$ nM). Radioligand binding assays at 5-HT_6 receptors resulted only in selecting two compounds with



Scheme 1. The synthesis route for compounds of group A and B (**10**–**13a**). *i* – EtONa, reflux; *ii* – acetone, TEBA, K_2CO_3, rt; *iii* – acetone, TEBA, K_2CO_3, reflux; *iv* – Bucherer–Bergs reaction, EtOH 50%, 55 °C; *v* – acetone, TEBA, K_2CO_3, mv irradiation.

submicromolar affinities (**22a** and **23a**). Both compounds, however, displayed higher potency at 5-HT_{1A} and 5-HT_7 than that at 5-HT_6 receptors (Table 1).

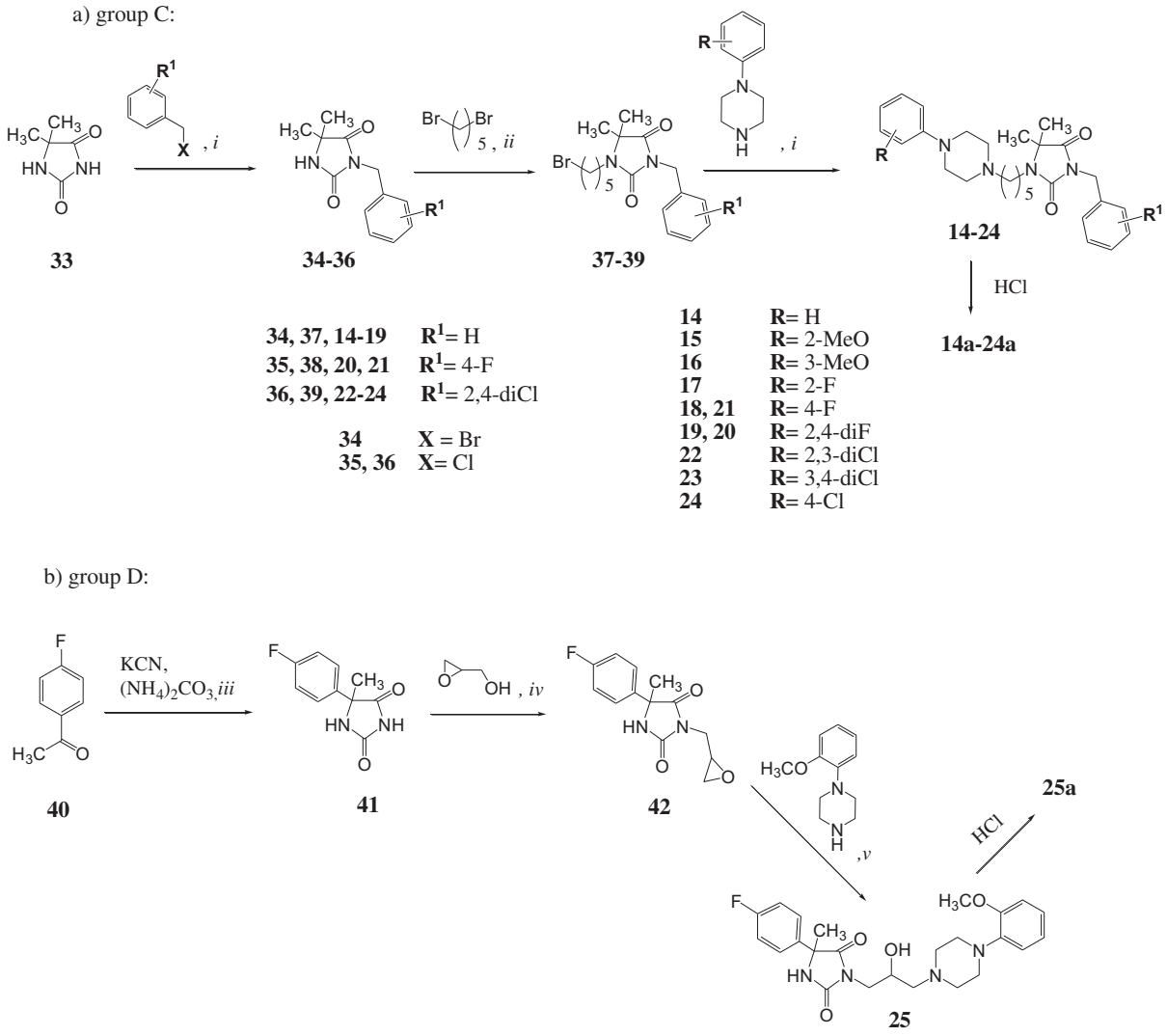
2.2.2. Functional bioassays

2.2.2.1. The α_1-adrenoceptor subtypes activity. The two most selective α_1-adrenoceptor compounds (**11a** and **12a**) were chosen for further investigation, and their selectivity at different α_1-adrenoceptor subtypes was assessed in functional experiments. In the functional bioassays, tail artery for α_{1A} [27,28], mouse spleen for α_{1B} [29] and rat aorta for subtype α_{1D} [29] were used. On the tissues applied, the antagonism exerted by **11a** and **12a** was competitive thus enabled the calculation of functional affinities (pA_2 values). The results are shown in Fig. 3 (**11a**), Fig 4 (**12a**) and Table 2. Our results indicated that **11a** and **12a** are potent antagonists at all three receptor subtypes with pA_2 values ranging from 7.312 to 8.521. Compound **11a** showed high antagonistic properties for α_{1D}-adrenoceptors ($pA_2 = 8.136$) and its ability to block α_{1A}- and α_{1B}-adrenoceptors was approximately six fold lower. Importantly, compound **12a** displayed similar antagonistic affinity for α_{1B}- and α_{1D}-adrenoceptors ($pA_2 = 8.142$ and 8.521 respectively), however, lower antagonistic properties for α_{1A}-subtype ($pA_2 = 7.316$). Moreover, both compounds (**11a**, **12a**) were not found to be selective, since the differences in potency were not high enough to consider them as selective antagonists for α_1-adrenoceptor subtypes.

2.2.2.2. Studies on inhibitory effect at human 5-HT_{3A}. In this study, the profile of compounds **10a**, **13a**, **14a**, **21a**, **22a**, and **25a** representing the chemical groups A-D was further investigated in order to better characterize their biochemical and molecular effects. Expression of specific human 5-hydroxytryptamine type 5-HT_{3A} receptors (*h*5-HT_{3A}) receptor subunit was constructed in *Xenopus laevis* oocytes, and electrophysiological method was utilized to evaluate the efficacy of the positive modulation of 5-HT_{3A}-evoked chloride currents by derivatives **10a**, **13a**, **14a**, **21a**, **22a**, and **25a** in comparison with that of 0.1 μM LY278584, a specific antagonist of 5-HT_{3A} receptors (Fig. 5, Table 1). To this end, the agonist 5-HT (10 μM) activated fast inward currents only in oocytes injected with cRNA transcribed from cloned cDNA encoding human 5-HT_{3A} receptors (data not shown, $n = 12$). Currents activated by 1 μM 5-HT were completely inhibited by 0.1 μM **LY278584**, a specific antagonist of 5-HT_3 receptor, further indicating that the 5-HT induced current responses were mediated by the 5-HT_3 receptor-ion channel complex ($n = 7$). 5-HT (1 μM)-evoked currents recorded by two-electrode voltage clamp technique, were reversibly inhibited by tested compounds **10a**, **13a**, **14a**, **21a**, **22a**, and **25a** at a dose of 30 mM with percent values in the range of 13.75–36.86% ($n = 5$) (Fig. 5, Table 1).

2.3. Structure activity relationship

The lead structure **6a** is a member of phenylpiperazine phenytoin family incorporating derivatives with 2-hydroxypropyl linker at 1-position of hydantoin skeleton. This family was well defined in the field of structural properties which have been postulated based on our previously performed studies focusing on crystallographic analyses and molecular modeling [19,25,26]. Therefore, moderate as well as almost equal activity of compound **6a** at all three investigated receptors, α_1-AR, 5-HT_{1A} and 5-HT_7, can



Scheme 2. The synthesis route for compounds of group C and D (**14a**–**25a**). *i* – acetone, TEBA, K_2CO_3, reflux; *ii* – acetone, TEBA, K_2CO_3, rt; *iii* – Bucherer–Bergs reaction, EtOH 50%, 55 °C; *iv* – Mitsunobu reaction, DEAD, TPP, dry THF, 0 °C; *v* – microwave irradiation, solvent-free condition.

be explained on the basis of pharmacophore models suitable for these receptors (Fig 2). According to our previous studies [25], the phenylpiperazine phenytoin derivatives include all structural features required by pharmacophore models of both α_1-AR and 5-HT_{1A} antagonists, however, some differences from spatial colocations of ideal antagonists moieties were observed in both cases. When considering previous results [25] compound **6a** can be suggested as a perfect agreement with pharmacophore model of α_1-adrenoceptor antagonist in the case of PI-HY1 distance (Fig. 2d), some agreement in PI-HBA distances (5.11–5.15 A in the case of phenylpiperazine phenytoin derivatives, and 5.62 in the pharmacophore model of Barbaro) and too short distance between piperazine protonable nitrogen (PI) and the third hydrophobic area (HY3) matched by phenyl ring(s) at hydantoin's 5-position [25,26]. In particular, the presence of two hydrophobic rings at 5-position of hydantoin, which can represent pharmacophoric feature HY3, was expected as a factor that limits antagonistic interaction with α_1-adrenoceptor, resulting only in average submicromolar affinities for α_1-AR of those phenylpiperazine compounds [25,26]. In the case of compound **6a**, lack of hydrophobic substituents at *o*- or *m*-position of phenylpiperazine phenyl ring, which fit in the HY2 pharmacophoric feature, can be responsible for only mean affinity observed in the radioligand binding assay (K_i = 542.3 nM, Table 1), too. When considering pharmacophore model of 5-HT_{1A}-antagonist (Fig. 2e), the previous studies on phenylpiperazine phenytoin derivatives [25] indicated an agreement in the distance PI-A but the rest of distances (A-HYD, A-HBA, PI-HYD and PI-HBA) were slightly too short as compared to those of ideal antagonists. These small spatial disagreements are highly probable to be a reason behind moderate affinity observed for **6a** at 5-HT_{1A}. The current studies demonstrated that affinity of the lead structure (**6a**) for 5-HT_7 receptors is also in submicromolar range. Although the radioligand binding assay did not explore the way of interaction with receptor, some differences can be observed between compound **6a** and each 5-HT_7 pharmacophore model, including model of agonist, inverse agonist and antagonist (Fig. 2g–i). As the highest distance-tolerance is described for pharmacophore features of the 5-HT_7 antagonist, it is suggested that compound **6a** can fit in this model with much better agreement than those for agonists and inverse agonists. Nevertheless, its affinity for 5-HT_7 is not very high (K_i = 353 nM) and almost identical as that for 5-HT_{1A} (Table 1). The analysis of structural properties of compound **6a** based on pharmacophore models suitable for the considered GPCRs gave some suggestions in the field of activity and selectivity modulation. However, the

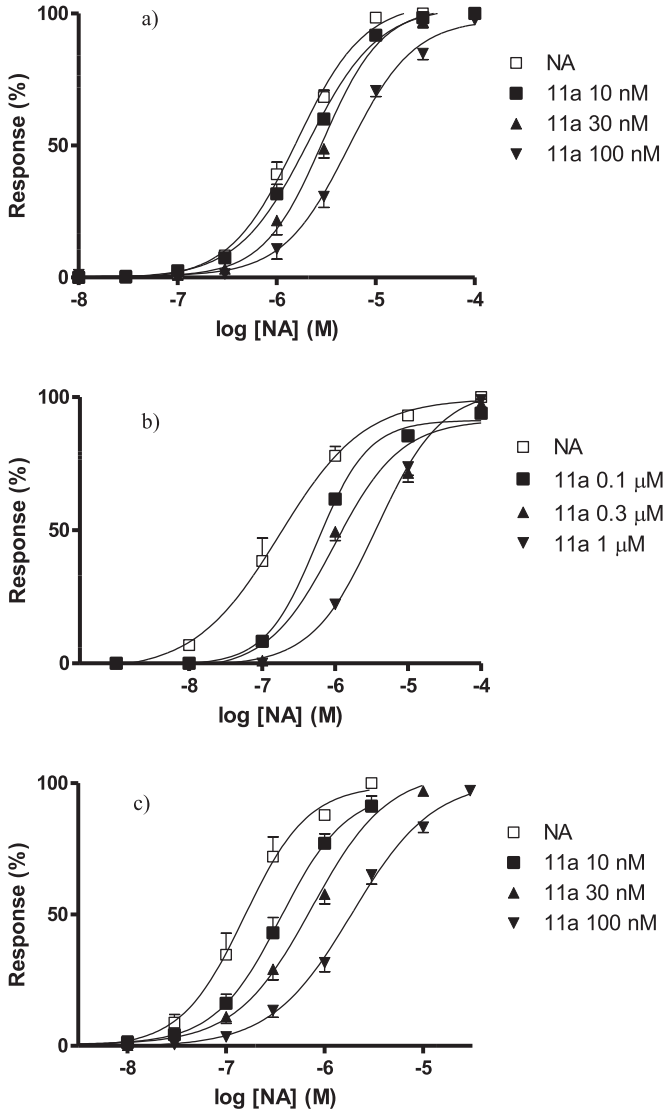


Fig. 3. Effect of compound **11a** on α_1-adrenoceptors. Concentration-response curves to noradrenaline (NA) in the absence (□) or presence of increasing concentrations of **11a** (filled symbols). a) rat tail artery (α_{1A}-adrenoceptors); b) mouse spleen (α_{1B}-adrenoceptors); c) rat aorta (α_{1D}-adrenoceptors). Results are expressed as percentage of the maximal response to NA in the first concentration–response curve. Each point represents the mean ± SEM (n = 4–7).

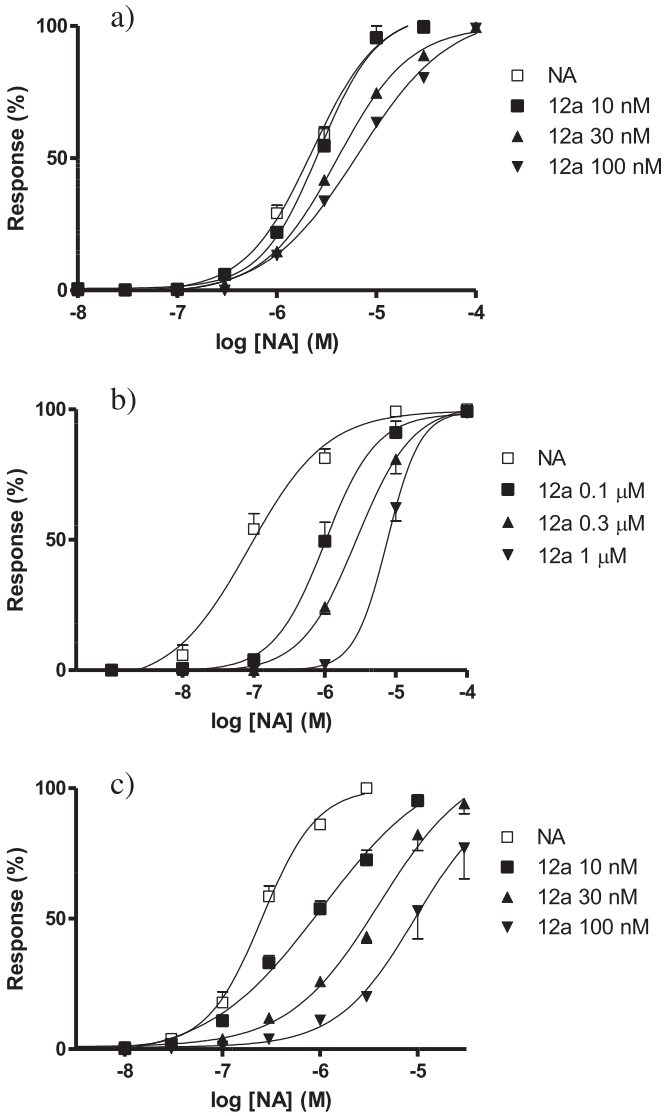


Fig. 4. Effect of compound **12a** on α_1-adrenoceptors. Concentration-response curves to noradrenaline (NA) in the absence (□) or presence of increasing concentrations of **12a** (filled symbols). a) rat tail artery (α_{1A}-adrenoceptors); b) mouse spleen (α_{1B}-adrenoceptors); c) rat aorta (α_{1D}-adrenoceptors). Results are expressed as percentage of the maximal response to NA in the first concentration–response curve. Each point represents the mean ± SEM (n = 4).

presented models show mutual commonalities and therefore are not sufficient to be followed in search to design agents of selective action towards one of the receptor subtypes.

In this context, four modifications (A–D, Table 1) of the lead were designed and performed to gradually expand structural divergence from the starting compound (**6a**), and on this way to improve the compounds discrimination between the considered GPCRs with special accent on 5-HT$_7$ receptors.

In consensus with suggestions resulting from pharmacophore models as well as from our previous SAR-analyses [19,25,26], certain prolongation of distances between pharmacophoric fragments seemed to be profitable to gain biological activity at target. Thus, the first modifications (A, Table 1) were focused on 2-hydroxypropyl linker, which was deprived of OH-substituents to increase flexibility and prolonged from tri- to pentamethylene length. These intramolecular changes enabled to accommodate distances between pharmacophoric fragments (PI-HY3, PI-HBA, PI-HYD). In compounds **7a**–**9a**, alkoxyl substituents at *o*-position of phenylpiperazine were introduced to facilitate further interactions with the receptors. Our results indicated that such modifications were particularly enhancing affinity and selectivity at 5-HT$_{1A}$.

Table 2
Functional affinities of test compounds **11a** and **12a** at α_{1A}-AR in rat tail artery, at α_{1B}-AR in mouse spleen and at α_{1D}-AR in rat aorta. Antagonist potency of test compounds expressed as pA_2 ± SEM.

Agonist: NA compd	α_{1A}-AR rat tail artery pA_2 ± SEM (slope ± SEM)	α_{1B}-AR mouse spleen pA_2 ± SEM (slope ± SEM)	α_{1D}-AR rat aorta pA_2 ± SEM (slope ± SEM)
11a	7.413 ± 0.011 (0.90 ± 0.01)	7.312 ± 0.167 (0.97 ± 0.08)	8.136 ± 0.013 (0.93 ± 0.01)
12a	7.316 ± 0.116 (1.02 ± 0.12)	8.142 ± 0.050 (0.91 ± 0.01)	8.521 ± 0.200 (1.06 ± 0.09)

pA_2 values were obtained from the linear regression of Schild plot.
Each value was the mean ± SEM of 4–7 experimental results.

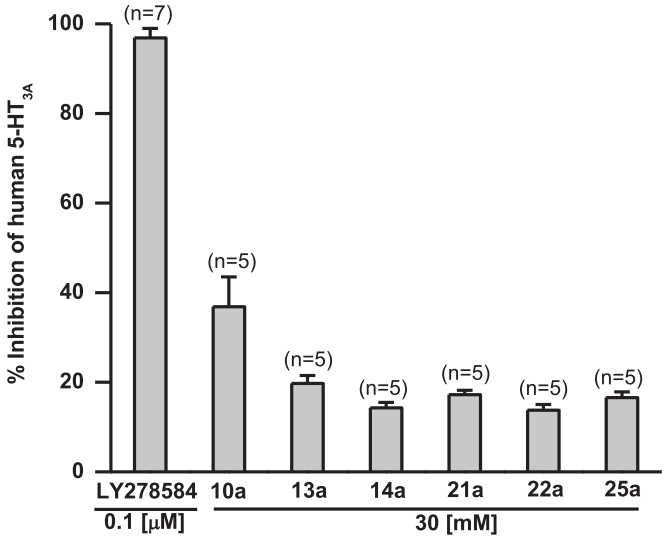


Fig. 5. Inhibition of **10a**, **13a**, **14a**, **21a**, **22a**, and **25a** in comparison to standard 5-HT_{3A} receptor antagonist **LY278584**. Results are expressed as percentage inhibition of 5-HT-induced currents in *Xenopus laevis* oocyte expressing human 5-HT_{3A} receptors. Each value represents the mean ± SEM (n: number of oocytes tested). **LY278584**: 1-Methyl-*N*-(8-methyl-8-azabicyclo[3.2.1]-oct-3-yl)-1*H*-indazole-3-carboxamide, a specific antagonist for 5-HT_3 receptors.

Among the derivatives designed, the obtained results clearly showed that 2-alkoxyphenylpiperazine derivatives having unbranched 3–5-carbon linker were more effective at 5-HT_{1A} receptors. Moreover, compound (**8a**) as a derivative of 2-methoxyphenylpiperazine with butyl linker demonstrated subnanomolar affinities for 5-HT_{1A}, whereas compound (**9a**) as a derivative of 2-ethoxyphenylpiperazine with propyl linker displayed the highest selectivity towards 5-HT_{1A} compared to α_1-AR (~473) and a significant effect (~17) at 5-HT_7 receptors (Table 1). Furthermore, most members of modifications A were significantly more potent than lead compound **6a** at both 5-HT_7 and α_1-adrenergic receptors, indicating a superiority of the unbranched linkers over the 2-hydroxypropyl ones. However, the modifications of lead compound **6a** were not sufficient to provide selectivity for 5-HT_7, thus greater structural changes starting from the lead structure were necessary. Therefore, further modifications (B and C, Table 1) were focused on the number and colocation of aromatic- and methyl groups at 3- and 5-position of hydantoin.

Within the modifications B, compounds having C_5-linker were under consideration, in which one of the 5,5-diphenyl rings was replaced with the methyl moiety (**11a**–**13a**). Analysis of the obtained results showed that these changes were distinctly favorable for interactions with α_1-adrenoceptors. Although the 2-methoxyphenyl derivative was found to be not selective at α_1/5-HT_{1A}/and 5-HT_7 receptors_selective, the unsubstituted- (**11a**) and 2-fluorophenylpiperazine (**12a**) derivatives demonstrated the highest α_1-AR selectivity when compared to the rest of other modifications (A, C and D, Table 1). The affinities for α_1-AR of all three compounds **11a**–**13a** were found to be significant, with K_i values in nanomolar ranges (Table 1). The most active and selective α_1-AR agents **11a** and **12a**, investigated on their activities at α_1-adrenoceptor subtypes, displayed antagonistic effect at α_1-AR subtype with the highest potency for the α_{1D}-AR in both cases. These results are in good agreement with conclusions evolving from our previous pharmacophore-based studies on 5,5-diphenylhydantoin derivatives, in which the bulky 5,5-diphenyl aromatic area did not fit well in HY3 pharmacophoric features. The removal of one phenyl ring with a concomitant increase of distance between hydantoin and piperazine, performed by introduction of C_5-linker, approached compounds (**11a**–**13a**) that meet the structural requirements proposed by Barbaro et al. (Fig. 2d) for α_1-antagonist model [22]. Compounds of modifications B contain structural moieties that can well fit into pharmacophore features of both, α_{1A} and α_{1D}, adrenoceptor subtypes antagonists (Fig. 2a,c) [16]. In the case of α_{1B}, the hydrogen bond donor area (HBD) is missing in the chemical structures of **11a**–**13a** (Fig. 2b, Table 1), however, both tested compounds (**11a** and **12a**) demonstrated significant antagonistic properties at the α_{1B} subtype during the functional bioassays. These results clearly suggest that pharmacophore models of Bremner [16] are not sufficient to characterize the group of 5-phenylhydantoin derivatives, especially those with antagonistic properties at α_{1B}-ARs.

Modifications C gave a series of eleven compounds with C_5-linker (**14a**–**24a**), in which an aromatic area was shifted from 5-position into the benzyl substituent at 3-position of hydantoin skeleton. The activities at target GPCRs were modulated by number and position of small hydrophobic substituents at aromatic rings on both, *N*1- and *N*3-position of hydantoin skeleton sides. The modifications C were applied to increase selectivity at 5-HT_7 and to decrease those at both α_1-AR and 5-HT_{1A} receptors. The expected results were only obtained by highly lipophilic compounds bearing 2,4-dichlorobenzyl substituents at 3-position of hydantoin and chloride substituent(s) at the phenylpiperazine phenyl ring (**22a**–**24a**). The optimal 5-HT_7 activity and selectivity with respect to α_1-AR (K_{i5-HT_7} = 31 nM, sel α_1 = ~31, $sel_{5-HT_{1A}}$ = ~3) was observed for 2,3-dichlorophenylpiperazine derivative **22a**, whereas a slightly higher 5-HT_7 selectivity with respect to 5-HT_{1A} was observed by 3,4-dichlorophenylpiperazine derivative **23a** (K_{i5-HT_7} = 78 nM, sel α_1 = ~9, $sel_{5-HT_{1A}}$ = ~4). Interestingly, compounds with benzyl- or 4-fluorobenzyl substituents (**14a**–**21a**) displayed significant affinities for α_1-AR (35 nM < K_i < 100 nM) even in case of halogen substituents at *p*-position of phenylpiperazine, which was evaluated as a disadvantageous factor by different previous pharmacophore-based studies [3,12,22]. Our current results clearly indicate the importance of 4-fluorobenzyl moiety for interactions with α_1-AR as the *p*-substituted phenylpiperazine hydantoin derivatives of 4-fluorobenzyl (**20a** and **21a**) were more potent at α_1-AR than the *o*-methoxyphenylpiperazine derivative with unsubstituted benzyl ring (**15a**). A comparison of the 4-fluorobenzylhydantoin derivatives with their respective benzyl ones (2,4-difluorophenylpiperazine-**20a** and **19a**, 4-fluorophenylpiperazine derivatives **21a** and **18a**) confirmed higher activities for 4-fluorobenzyl analogues observed in both cases of the fluoropiperazines. Moreover, the results obtained in the 5-HT_{1A} radioligand binding assays evidently indicated that substituents at benzyl ring are not as much important as types and positions of those substituents at phenylpiperazine phenyl ring. Thus, *o*- or/and *m*-substituents at phenylpiperazine (**15a**–**17a** and **22a**) triggered significantly higher activity than those of compounds with substituents at *p*-position of phenylpiperazine phenyl ring (**18a**–**21a**, **23a** and **24a**). The modifications C resulted in selection of few compounds with stronger actions at 5-HT_7 receptors than those on 5-HT_{1A} or α_1-AR, however, their selectivity profile was not remarkable when considering 5-HT_{1A} and 5-HT_7 receptors.

In this context, we decided to depart from further systematic modifications and perform more drastic changes within lead (**6a**) structures, including introduction of 4-fluorophenyl- and methyl moieties at 5-position and an exchange of the location of 2-hydroxypropylphenylpiperazine substituent from 1-position into 3-position of hydantoin with simultaneous introduction of 2-methoxy function at phenylpiperazine phenyl ring (compound **25a**, Table 1). These significant changes enabled us to improve the expected pharmacological properties, since compound **25a** was much more potent at 5-HT_7 receptors among the tested hydantoin

compounds (**6a**–**24a**), displaying K_i value in the nanomolar range (3 nM) and a significant selectivity in respect to the rest of investigated GPCRs (sel α_1 ~60, $sel_{5-HT_{1A}}$ ~40, sel_{5-HT_6} ~3597).

In general, the investigated series of hydantoin phenylpiperazine derivatives did not show any potent interactions with 5-HT_6 receptors. It can be explained based on pharmacophore models (Fig. 2g) which postulate more compact 5-HT_6-antagonist structures with short distances between aromatic, bulky hydrophobic, and positive ionizable areas. The central position is postulated for aromatic fragment, and an extended (terminated) one for a positive ionizable fragment (PI) which can be well matched by *N*-methyl- (**4**, Fig. 1), however, not by *N*-phenylpiperazine derivatives. Interestingly, the most hydrophobic compounds with four chloride substituents at aromatic rings (**22a** and **23a)** demonstrated moderate affinities for 5-HT_6 receptors with K_i values in submicromolar range (Table 1). The rest of series was found to be weakly- or almost inactive (1792 nM < K_i < 30 000 nM).

The representative members of each modification way (A–D) were tested on their pharmacological properties at 5-HT_{3A} receptors. The results observed clearly show that tested compounds displayed low inhibitory activities in the range of 13.75–36.86%. Among tested representative derivatives, phenylpiperazinepentyl derivative **10a** was found to be the most active one with an inhibitory value of 36.86% at 5-HT_{3A} receptors (Table 1), however, even this activity was much lower than that of standard antagonist **LY278584** (Fig. 5).

3. Conclusion

The performed studies allowed to obtain a series of active GPCRs agents among phenylpiperazine derivatives of hydantoin with various collocation of chemical moieties, important for interaction with α_1-adrenergic and serotonin receptors. Although the 5-HT_7 receptors are the main pharmacological target of the current work, the series of hydantoin derivatives were also evaluated on their affinities for α_1-adrenergic-, serotonin 5-HT_{1A}, and 5-HT_6 receptors in the radio-ligand binding assays, and selected representative structures were further investigated on their action on 5-HT_{3A} or α_1-adrenergic subtypes, α_{1A}, α_{1B} and α_{1D}, in functional bioassays. Results of the assays showed that hydantoin derivatives tend to strongly interact with α_1-AR, 5-HT_{1A} and 5-HT_7 receptors, whereas their actions on 5-HT_{3A} or 5-HT_6 were found to be very weak.

The pharmacophore-based SAR-analysis clearly indicated that compounds with phenylpiperazine-alkyl substituent at 1-position of 3-methylhydantoin: (1) are particularly active at 5-HT_{1A} in case of phenytoin-like compounds with unbranched alkyl linker; (2) are particularly active at α_1-AR in case of the 5-methyl-5-phenylhydantoin compounds with pentyl linker. Regarding 5,5-dimethyl-3-(halogen)benzylhydantoin with pentyl linker at 1-position: (3) arylpiperazine derivatives of 3-(4-fluorobenzyl) hydantoin are evidently the most potent at α_1-AR, and (4) 3-(2,4-dichlorobenzyl)hydantoin derivatives are selective towards 5-HT_7 when chloride substituent is placed at *p*-position of the phenylpiperazine moiety.

The optimal potency and selectivity at 5-HT_7 was achieved by the compound (**25a**) through the following modifications: (i) reversing phenylpiperazine-alkyl substituent of the lead (**6a**) hydantoin ring from 1- to 3-position, (ii) removal of one phenyl ring from 5-position, and (iii) introducing of lipophilic substituents at both aromatic rings. Since the most promising 5-HT_7 receptor agent (**25a**) was found as an orphan's member of its group (modifications D), it is difficult to recognize which one of the three modifications (i–iii) was crucial for the increasing activity of the lead compound **6a**. Thus, the current question needs further SAR-studies, in which compound **25a** seems to be a good lead structure for further chemical modifications in search for potent and selective 5-HT_7 receptors antagonists among phenylpiperazine derivatives of hydantoin class.

4. Experimental

4.1. Chemistry

^{1}H NMR spectra were recorded on a Varian Mercury VX 300 MHz PFG instrument (Varian Inc., Palo Alto, CA, USA) in DMSO-d_6 at ambient temperature using the solvent signal as an internal standard. IR spectra were recorded on a Jasco FT/IR-410 apparatus using KBr pellets and are reported in cm^{-1}. Thin-layer chromatography was performed on pre-coated Merck silica gel 60 F_{254} aluminum sheets, the used solvent systems were (I) toluene/acetone 40:3; (II) $CHCl_2$/acetone 10:1; (III) toluene/acetone 10:1. (IV) toluene/acetone/methanol 15:5:1. Melting points were determined using Mel-Temp II apparatus and are uncorrected. The mass for compounds **10**–**25a** were obtained on Waters ACQUITY™ TQD system with the TQ Detector (Waters, Milford, USA). The ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 50 mm column was used (Waters, Milford, USA). Elemental analyses (C, H, N) were measured on Elemental Vario-EL III instrument and are within ±0.4% of the theoretical values unless stated otherwise. Syntheses under microwave irradiation were performed in household microwave oven Samsung M1618 or in microwave reactor "Plazmatronika". Syntheses of compounds **6**–**9**, **27**, **30**–**32** and **41** are described elsewhere [25,26].

4.1.1. Synthesis of 1-(5-bromopentyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione (*28*)

A mixture of 3-methyl-5,5-diphenylhydantoin **27** (15 mmol, 4.00 g), TEBA (2 mmol, 0.45 g) and potassium carbonate (44 mmol, 6 g) in acetone (30 mL) was stirred under reflux for 30 min, then 1,5-dibromopropane (20 mmol, 4.60 g) in acetone (15 mL) was added. The mixture was stirred at room temperature for 90 h, according to a progress controlled by TLC (I). Then, inorganic precipitate was filtered off, the mother liquor was evaporated. The residue was purified by double chromatography columns (II) crystallized using ethanol with diethyl ether and *n*-hexane to give white crystals of **28** (2.74 g, 6.60 mmol). Yield 44%, mp 58–59 °C; TLC: R_f (I): 0.60. Anal. Calcd for $C_{21}H_{23}BrN_2O_2$: C, 60.73; H, 5.58; N, 6.74. Found: C, 60.77; H, 5.56; N, 6.69. ^{1}H NMR δ (ppm): 0.70 (qu, J = 7.40 Hz, 2H, N1–CH_2–CH_2–CH_2), 0.96 (qu, J = 7.40 Hz, 2H, N1–CH_2–CH_2), 1.41 (qu, J = 7.40 Hz, 2H, Br–CH_2–CH_2), 2.96 (s, 3H, N–CH_3), 3.23–3.29 ($t_{def.}$, 4H, N1–CH_2, Br–CH_2), 7.19–7.24 (m, 4H, 2× Ph-3,5-H), 7.40–7.44 (m, 6H, 2× Ph-2,4,6-H).

4.1.2. General procedure for synthesis of 3-benzyl derivatives of 5,5-dimethylhydantoin (*34*–*36*)

5,5-Dimethylhydantoin **33** (50–100 mmol) was stirred under reflux with TEBA (1.5–3 g), K_2CO_3 (20–40 g) in acetone (200–500 mL) for 30 min. An appropriate benzyl chloride or bromide (50–90 mmol) in acetone (40–100 mL) was added. Then, the mixture was boiled under reflux for 5–6 h and left at room temperature for 16 h. The precipitate was filtrated off. A pure product was obtained from the filtrate by two methods A or B.

Method A: the pure product was precipitated from the filtrate with water.
Method B: the filtrate was evaporated. The obtained residue was dissolved by boiling in ethanol. The pure product was precipitated from the ethanol solution with water.

*4.1.2.1. 3-Benzyl-5,5-dimethylimidazolidine-2,4-dione (**34**).* 5,5-Dimethylhydantoin **33** (12.80 g, 100 mmol), K_2CO_3 (40.0 g),

TEBA (3.00 g) in acetone (500 mL) and benzyl bromide (17.00 g, 99 mmol) in acetone (100 mL) were refluxed for 5 h, purified by method A to give white crystals of product **34** (16.0 g, 73 mmol). Yield 73%, mp 86–87 °C; TLC: R_f (III): 0.19. Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 66.12; H, 6.49; N, 12.88. ^{1}H NMR δ (ppm): 1.28 (s, 6H, 2 × CH_3), 4.50 (s, 2H, CH_2-Ph), 7.18–7.35 (m, 5H, Ph), 8.37 (br. s, 1H, NH).

*4.1.2.2. 3-(4-Fluorobenzyl)-5,5-dimethylimidazolidine-2,4-dione (**35**).* 5,5-Dimethylhydantoin **33** (6.40 g, 50 mmol), K_2CO_3 (20.00 g), TEBA (1.50 g) in acetone (200 mL) and 4-fluorobenzyl chloride (7.23 g, 50 mmol) in acetone (40 mL) were refluxed for 3 h, purified by method B to give white crystals of product **35** (8.70 g, 37 mmol). Yield 74%, mp 74–75 °C; TLC: R_f (III): 0.20. Anal. Calcd for $C_{12}H_{13}FN_2O_2$: C, 61.01; H, 5.55; N, 11.86. Found: C, 60.98; H, 5.62; N, 11.80. ^{1}H NMR δ (ppm): 1.27 (s, 6H, 2 × CH_3), 4.48 (s, 2H, CH_2-Ph), 7.12–7.16 ($d_{def.}$, 2H, Ph-2,6-H), 7.23–7.24 ($d_{def.}$, 2H, Ph-3,5-H), 8.37 (br. s, 1H, NH).

*4.1.2.3. 3-(2,4-Dichlorobenzyl)-5,5-dimethylimidazolidine-2,4-dione (**36**).* 5,5-Dimethylhydantoin **33** (6.40 g, 50 mmol), K_2CO_3 (20.00 g), TEBA (1.50 g) in acetone (200 mL) and 2,4-dichlorobenzyl chloride (79.77 g, 50 mmol) in acetone (40 mL) were refluxed for 3.5 h, purified by method A to give white crystals of product **36** (12.46 g, 43 mmol). Yield 87%, mp 134–135 °C; TLC: R_f (III): 0.23. Anal. Calcd for $C_{12}H_{12}Cl_2N_2O_2$: C, 50.19; H, 4.21; N, 9.76. Found: C, 50.38; H, 4.32; N, 9.69. ^{1}H NMR δ (ppm): 1.31 (s, 6H, 2 × CH_3), 4.55 (s, 2H, CH_2-Ph), 7.12 (d, J = 8.20 Hz, 1H, Ph-6-H), 7.38–7.40 ($d_{def.}$, 1H, Ph-5-H), 7.63 (s, 1H, Ph-3-H), 8.46 (s, 1H, NH).

*4.1.3. General procedure for synthesis of 1-bromopentyl derivatives of 3-benzyl-5,5-dimethylhydantoins (**37**–**39**)*

A suitable 3-benzyl-5,5-dimethylohydantoin **34**–**36** (20–60 mmol), TEBA (0.60–1.80 g), K_2CO_3 (8.00–24.00 g) was stirred in acetone (40–120 mL) for 30 min. A solution of 1,5-dibromopentane (26–95 mmol) in acetone (20–60 ml) was added. The mixture was stirred at room temperature for 72–90 h. The precipitate was filtrated off. The filtrate was evaporated. The residue was dissolved in methylene chloride (100 mL), washed with NaOH 2% (2 × 100 mL) and water (2 × 100 mL) and dried with anhydrous Na_2SO_4 for 18 h. After separation from the drying agent, the solution was concentrated and purified by column chromatography (II).

*4.1.3.1. 3-Benzyl-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione (**37**).* 3-Benzyl-5,5-dimethylimidazolidine-2,4-dione **34** (13.10 g, 60 mmol), K_2CO_3 (24.00 g), TEBA (1.80 g) in acetone (120 ml) and 1,5-dibromopentane (21.94 g, 95 mmol) in acetone (60 ml) were stirred for 90 h to give product **37** in form of bright glass-oil (13.0 g, 35 mmol). Yield 59%; TLC: R_f (III): 0.53. Anal. Calcd for $C_{17}H_{23}BrN_2O_2$: C, 55.59; H, 6.31; N, 7.63. Found: C, 55.20; H, 6.02; N, 7.39. ^{1}H NMR δ (ppm): 1.32 (s, 6H, 2 × CH_3), 1.35–1.43 (m, 2H, CH_2–CH_2–CH_2–N1), 1.45–1.59 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 1.76–1.85 ($qu_{def.}$, 2H, CH_2–CH_2–Br), 3.21 (t, J = 7.57 Hz, 2H, CH_2–N1), 3.49–3.55 (m, 2H, CH_2–Br), 4.54 (s, 2H, CH_2-Ph), 7.18–7.35 (m, 5H, Ph).

*4.1.3.2. 1-(5-Bromopentyl)-3-(4-fluorobenzyl)-5,5-dimethylimidazolidine-2,4-dione (**38**).* 3-(4-Fluorobenzyl)-5,5-dimethylimidazolidine-2,4-dione **35** (4.72 g, 20 mmol), K_2CO_3 (8.00 g), TEBA (0.60 g) in acetone (40 ml) and 1,5-dibromopentane (5.98 g, 26 mmol) in acetone (20 ml) were stirred for 72 h to give product **38** in form of white glass-oil (4.98 g, 13 mmol). Yield 65%; TLC: R_f (III): 0.38. Anal. Calcd for $C_{17}H_{22}BrFN_2O_2$: C, 53.00; H, 5.76; N, 7.27. Found: C, 52.77; H, 5.49; N, 7.08. ^{1}H NMR δ (ppm): 1.31 (s, 6H, 2 × CH_3), 1.31–1.40 (m, 2H, CH_2–CH_2–CH_2–N1), 1.50–1.56 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 1.76–1.82 ($qu_{def.}$, 2H, CH_2–CH_2–Br), 3.20 (t, J = 7.44 Hz, 2H, CH_2–N1), 3.49 (t, J = 6.67 Hz, 2H, CH_2–Br), 4.52 (s, 2H, CH_2-Ph), 7.11–7.18 ($d_{def.}$, 2H, Ph-2,6-H), 7.23–7.28 ($d_{def.}$, 2H, Ph-3,5-H).

*4.1.3.3. 1-(5-Bromopentyl)-3-(2,4-dichlorobenzyl)-5,5-dimethylimidazolidine-2,4-dione (**39**).* 3-(2,4-Dichlorobenzyl)-5,5-dimethylimidazolidine-2,4-dione **36** (8.61 g, 30 mmol), K_2CO_3 (12.00 g), TEBA (0.90 g) in acetone (120 ml) and 1,5-dibromopentane (17.94 g, 78 mmol) in acetone (60 ml) were stirred for 72 h to give product **39** in form of yellow glass-oil (4.98 g, 13,6 mmol). Yield 45%; TLC: R_f (III): 0.58. Anal. Calcd for $C_{17}H_{21}BrCl_2N_2O_2$: C, 46.81; H, 4.85; N, 6.42. Found: C, 46.50; H, 4.54; N, 6.11. ^{1}H NMR δ (ppm): 1.35 (s, 6H, 2 × CH_3), 1.35–1.43 (m, 2H, CH_2–CH_2–CH_2–N1), 1.52 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 1.77 ($qu_{def.}$, 2H, CH_2–CH_2–Br), 3.22 (t, J = 7.60 Hz, 2H, CH_2–N1), 3.49 (t, J = 6.70 Hz, 2H, CH_2–Br), 4.60 (s, 2H, CH_2-Ph), 7.14 (d, J = 8.20 Hz, Ph-6-H), 7.38–7.44 ($d_{def.}$, 1H, Ph-5-H), 7.62 (d, J = 2.30 Hz, 1H, Ph-3-H).

*4.1.4. General procedure for synthesis of phenylpiperazine hydantoin derivatives (**10a**–**13a**)*

Commercially available phenylpiperazine (5 mmol), K_2CO_3 (2.00 g) and acetone (16 mL) were stirred and refluxed for 15 min in special round flask "Plazmatronika". Then, correspondingly substituted bromopentyl hydantoin derivative **28** or **32** (5.50 mmol) in acetone (16 mL) was added. The mixture was placed in microwave reactor and stirred under reflux using the following irradiation program: time heating 10 min, maximal power 20%, high temperature 58 °C, and low temperature 45 °C. The program was repeated 6 times under TLC control (IV). Then, the solution was stirred overnight. The inorganic precipitate was separated by filtration. The filtrate was evaporated and purified by the chromatography column (II) giving pure compound **10**–**13** in basic forms. Compound **10**–**13** (0.50 g) was dissolved in ethanol (10 mL). To the solution 3-5 drops of concentrated HCl was added to give precipitate of desirable compound (**10**). As no precipitate was generated (**11**–**13**), the solution was evaporated, the residue was dried under vacuum at the presence of $CaCl_2$, and crystallized with dry EtOH and 3-6 drops of acetone to give the desirable product (**11a**–**13a**) in the hydrochloride form.

*4.1.4.1. 3-Methyl-5,5-diphenyl-1-(5-(4-phenylpiperazin-1-yl)pentyl) imidazolidine-2,4-dione hydrochloride (**10a**).* 1-Phenylpiperazine (0.81 g) and 1-(5-bromopentyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione **28** (2.28 g) were used to give white crystals compound **10** (0.79 g, 1.59 mmol). Yield 32%; mp 148–149 °C; TLC: R_f (IV): 0.69. MW 496.64. Monoisotopic Mass 496.28, $[M + H]^+$ 497.41. Anal. Calcd for $C_{31}H_{36}N_4O_2$: C, 74.97; H, 7.31; N, 11.28. Found: C, 75.04; H, 7.44; N, 11.19. ^{1}H NMR for **10** δ (ppm): 0.64–0.79 ($qu_{def.}$, 2H, Pp–C_2H_4–CH_2–C_2H_4-hyd), 0.90–0.98 ($qu_{def.}$, Pp–CH_2–CH_2–C_3H_6-hyd), 1.12–1.15 (m, 4H, Pp–C_3H_6–CH_2–CH_2-hyd), 2.07–2.10 (m, 2H, Pp-CH_2), 2.46 (br.s, 4H, Pp-2,6-H), 2.97 (s, 3H, 3-CH_3), 3.06 (br.s, 4H, Pp-3,5-H), 3.25 (t, J = 7.70 Hz, 2H, CH_2–N1-hyd), 6.73 (t, J = 7.18 Hz, 1H, PpPh-4-H), 6.88 (d, J = 7.94 Hz, 2H, PpPh-2,6-H), 7.16–7.23 (m, 6H, Ph-2,4,6-H), 7.42–7.44 (m, 6H, PpPh-3,5-H, Ph-3,5-H). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 24.3, 25.8, 26.1, 27.8, 41.7, 48.8, 58.1, 75.2, 115.7, 119.9, 128.6, 129.2, 129.3, 137.6, 151.8, 155.9, 173.1.

White powder of compound **10a.** Yield 90%; mp 236–237 °C; TLC: R_f (IV): 0.69. Anal. Calcd for $C_{31}H_{37}ClN_4O_2$: C, 69.84; H, 7.00; N, 10.51. Found: C, 69.93; H, 7.11; N, 10.39. ^{1}H NMR for **10** δ (ppm): 0.75–0.79 ($qu_{def.}$, 2H, Pp–C_2H_4–CH_2–C_2H_4-hyd), 0.92–0.97 ($qu_{def.}$, Pp–CH_2–CH_2-C_3H_6-hyd), 1.39 (br. s, 4H, Pp–C_3H_6–CH_2–CH_2-hyd), 2.85 (br. s, 2H, Pp-CH_2), 2.98 (s, 3H, 3-CH_3), 3.06–3.09 (br.s, 4H, Pp-3,5-H), 3.25 (m, 2H, CH_2–N1-hyd), 3.39–3.45 (br.s, 2H, Pp-2,6-Ha),

3.74–3.78 (br.s, 2H, Pp-2,6-Hb), 6.82 (t, $J = 7.18$ Hz, 1H, PpPh-4-H), 6.95 (d, $J = 7.94$ Hz, 2H, PpPh-2,6-H), 7.21–7.27 (m, 6H, Ph-2,4,6-H), 7.42–7.64 (m, 6H, PpPh-3,5-H, Ph-3,5-H), 10.28 (br. s, 1H, NH^+).

*4.1.4.2. 3,5-Dimethyl-5-phenyl-1-(5-(4-phenylpiperazin-1-yl)pentyl) imidazolidine-2,4-dione (**11**).* 1-Phenylpiperazine (0.81 g) and 1-(5-bromopentyl)-3,5-dimethyl-5-phenylimidazolidine-2,4-dione **32** (1.94 g) were used to give white crystals of compound **11** (0.15 g, 0.35 mmol). Yield 7%; mp 164–165 °C; TLC: R_f (IV): 0.72. MW 434.57. Monoisotopic Mass 434.27, $[M + H]^+$ 435.39. Anal. Calcd for $C_{26}H_{34}N_4O_2$: C, 71.86; H, 7.89; N, 12.89. Found: C, 71.64; H, 7.80; N, 12.78. ^{1}H NMR δ (ppm): 1.13–1.17 ($qu_{def.}$, 2H, Pp–C_2H_4–CH_2–C_2H_4-hyd), 1.20–1.42 ($qu_{def.}$, 4H, CH_2–CH_2–CH_2–CH_2–CH_2), 1.77 (s,3H, 5-CH_3), 2.34 (br.s, 2H, Pp-CH_2), 2.58 (br.s, 4H, Pp-2,6-H), 2.86–2.96 (m, 2H, CH_2–N1-hyd), 2.91 (s, 3H, 3-CH_3), 3.13 (br.s, 4H, Pp-3,5-H), 6.74 (t, $J = 7.18$ Hz, 1H, PpPh-4-H), 6.89 (d, $J = 7.95$ Hz, 2H, PpPh-2,6-H), 7.16 (t, $J = 7.18$ Hz, 2H, PpPh-3,5-H), 7.27–7.43 (m, 5H, 5-Ph).

*4.1.4.3. 1-(5-(4-(2-Fluorophenyl)piperazin-1-yl)pentyl)-3,5-dimethyl-5-phenylimidazolidine-2,4-dione (**12**).* 1-(2-Fluorophenyl) piperazine (0.90 g) and 1-(5-bromopentyl)-3,5-dimethyl-5-phenylimidazolidine-2,4-dione **32** (1.94 g) were used to give white crystals of compound **12** (0.50 g, 1.11 mmol). Yield 22%; mp 178–179 °C; TLC: R_f (IV): 0.56. MW 452.56. Monoisotopic Mass 452.26, $[M + H]^+$ 453.40. Anal. Calcd for $C_{26}H_{33}FN_4O_2$: C, 69.00; H, 7.35; N, 12.38. Found: C, 68.94.64; H, 7.42; N, 12.17. ^{1}H NMR δ (ppm): 1.12–1.17 ($qu_{def.}$, 2H, Pp–C_2H_4–CH_2–C_2H_4-hyd), 1.20–1.55 (m, 4H, CH_2–CH_2–CH_2–CH_2–CH_2), 1.77 (s, 3H, 5-CH_3), 2.36 (br.s, 2H, Pp-CH_2), 2.62 (br.s, 4H, Pp-2,6-H), 2.86–3.15 (m, 2H, CH_2–N1-hyd), 2.91 (s, 3H, 3-CH_3), 3.02 (br.s, 4H, Pp-3,5-H), 6.91–7.13 (m, 4H, PpPh), 7.27–7.43 (m, 5H, 5-Ph). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 20.4, 22.9, 23.9, 25.2, 28.6, 47.3, 51.1, 55.7, 67.2, 119.9, 125.5, 126.7, 129.0, 129.3, 137.9, 156.1, 175.1.

*4.1.4.4. 1-(5-(4-(2-Methoxyphenyl)piperazin-1-yl)pentyl)-3,5-dimethyl-5-phenylimidazolidine-2,4-dione (**13**).* 1-(2-Methoxyphenyl) piperazine (0.96 g) and 1-(5-bromopentyl)-3,5-dimethyl-5-phenylimidazolidine-2,4-dione **32** (1.94 g) were used to give white crystals of compound **13** (0.90 g, 1.94 mmol). Yield 39%; mp 136–137 °C; TLC: R_f (IV): 0.62. MW 464.60. Monoisotopic Mass 464.28, $[M + H]^+$ 465.44. Anal. Calcd for $C_{27}H_{36}N_4O_3$: C, 69.80; H, 7.81; N, 12.06. Found: C, 69.75.64; H, 7.79; N, 11.95. ^{1}H NMR δ (ppm): 1.12–1.18 ($qu_{def.}$, 2H, Pp–C_2H_4–CH_2–C_2H_4-hyd), 1.20–1.39 (qu def, 4H, CH_2–CH_2–CH_2–CH_2–CH_2), 1.77 (s, 3H, 5-CH_3), 2.39 (br.s, 2H, Pp-CH_2), 2.64 (br.s, 4H, Pp-2,6-H), 2.83–3.15 (m, 6H, CH_2–N1-hyd, Pp-3,5-H), 2.91 (s, 3H, 3-CH_3), 3.75 (s, 3H, OCH_3), 6.84–6.97(m, 4H, PpPh), 7.28–7.44 (m, 5H, 5-Ph).

*4.1.5. General procedure for synthesis of hydrochlorides of phenylpiperazine hydantoin derivatives (**14a**–**24a**)*

A suitable commercial phenylpiperazine (2.00–5.10 mmol), K_2CO_3 (0.9–2.00 g) and acetone (7–16 mL) were stirred and refluxed for 30 min. Then, correspondingly substituted bromopentyl 3-benzyl-5,5-hydantoin derivatives **37**–**39** (2.20–5.70 mmol) in acetone (9–20 mL) were added and the mixture was refluxed for 6 h, left at room temperature overnight and separated from the inorganic precipitate by filtration. The solvent was evaporated from the filtrate. The pure product (**14a**–**24a**) was obtained from the residue using method C or D.

Method C. The residue after evaporation was crystallized with ethanol to give precipitate of products in the basic form (**20**–**24**). A compound in the basic form (500–900 mg) was dissolved in EtOH (10–15 mL) and saturated with gaseous HCl to give hydrochlorides of the desirable product (**20**, **21**, **23** and **24**). As method C*, the method C was performed for basic form only (**22**).

Method D. The residue after evaporation was dissolved in EtOH and stored at 4 °C for 1–2 days. As no crystal appeared, the solution was saturated with gaseous HCl, left at 4 °C for 24 h to give crystals of desirable products in hydrochloride forms (**14a**–**19a**, **22a**).

*4.1.5.1. 3-Benzyl-5,5-dimethyl-1-(5-(4-phenylpiperazin-1-yl)pentyl) imidazolidine-2,4-dione hydrochloride (**14a**).* **Method D.** 1-Phenylpiperazine (2.50 mmol, 0.41 g) K_2CO_3 (1.0 g) in acetone (10 ml) and 1-benzyl-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione **37** (2.70 mmol, 1.00 g) in acetone (10 mL) were used to give white crystals of compound **14a** (0.50 g, 1.00 mmol). Yield 38%; mp 206–207 °C; TLC: R_f (IV): 0.31. MW 448.60. Monoisotopic Mass 448.28, $[M + H]^+$ 449.42. Anal. Calcd for $C_{27}H_{37}ClN_4O_2$: C, 66.86; H, 7.69; N, 11.55. Found: C, 66.64; H, 7.71; N, 11.50. ^{1}H NMR δ (ppm): 1.22 (s, 2H, CH_2–CH_2–CH_2–N1), 1.34 (s, 6H, 2 × CH_3), 1.56 ($qu_{def.}$, 2H, CH_2–CH_2–Br), 1.74 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 3.06 (d, $J = 7.69$ Hz, 4H, Pp-3,5-H), 3.23 (t, $J = 7.44$ Hz, 2H, CH_2–N1), 3.52 (d, $J = 5.39$ Hz, 2H, CH_2–Br), 3.79 (s, 4H, Pp-2,6-H), 4.54 (s, 2H, CH_2-Ph), 6.82 (t, $J = 7.36$ Hz, 1H, PpPh-4-H), 6.97 (d, $J = 8.72$ Hz, 2H, PpPh-2,6-H), 7.19–7.40(m, 7H, Ph, PpPh-3,5-H), 10.40 (br. s, 1H, NH^+). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 23.1, 23.9, 29.0, 40.7, 41.7, 45.8, 50.8, 55.5, 62.0, 116.5, 118.4, 127.5, 127.8, 129.1, 129.6, 132.1, 137.2, 149.9, 155.1, 176.6. IR (cm^{-1}): 3067, 3036 (C–H(Ar)), 2981, 2933 (C–H(Aliph)), 2414 (NH^+), 1765 (C2=O), 1703 (C4=O), 1598 (C–C(Ar)).

*4.1.5.2. 3-Benzyl-1-(5-(4-(2-methoxyphenyl)piperazin-1-yl)pentyl)-5,5-dimethylimidazolidine-2,4-dione hydrochloride (**15a**).* **Method D.** 1-(2-Methoxyphenyl)piperazine (3.50 mmol, 0.67 g) K_2CO_3 (1.4 g) in acetone (12 mL) and 1-benzyl-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione **37** (5.00 mmol, 1.84 g) in acetone (10 mL) were used to give white crystals of compound **15a** (0.89 g, 1.70 mmol). Yield 49%; mp 225–227 °C; TLC: R_f (IV): 0.21. Initial LC/MS purity 93%, $t_R = 4.77$. MW 478.63. Monoisotopic Mass 478.29, $[M + H]^+$ 479.45. Anal. Calcd for $C_{28}H_{39}ClN_4O_3$: C, 65.29; H, 7.63; N, 10.88. Found: C, 65.27; H, 7.77; N, 10.72. ^{1}H NMR δ (ppm): 1.28 (s, 2H, CH_2–CH_2–CH_2–N1), 1.34 (s, 6H, 2 × CH_3), 1.57 ($qu_{def.}$, 2H, CH_2–CH_2–Br), 1.71 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 2.96–3.14 (m, 4H, Pp-3,5-H), 3.23 (t, $J = 7.44$ Hz, 2H, CH_2–N1), 3.35 (s, 2H, CH_2–Br), 3.43 (s, 4H, Pp-2,6-H), 3.77 (s, 3H, CH_3O), 4.54 (s, 2H, CH_2-Ph), 6.88–7.01 (m, 4H, Ph-3,5-H, PpPh-2,6-H), 7.19–7.35 (m, 5H, Ph-2,4,6-H, PpPh-3,5-H), 10.24 (br. s, 1H, NH^+). IR (cm^{-1}): 3034, 3016 (C–H(Ar)), 2985, 2964, 2938 (C–H(Aliph)), 2383 (NH^+), 1763 (C2=O), 1698 (C4=O), 1608 (C–C(Ar)).

*4.1.5.3. 3-Benzyl-1-(5-(4-(3-methoxyphenyl)piperazin-1-yl)pentyl)-5,5-dimethylimidazolidine-2,4-dione hydrochloride (**16a**).* **Method D.** 1-(3-Methoxyphenyl)piperazine (3.50 mmol, 0.67 g) K_2CO_3 (1.4 g) in acetone (12 mL) and 1-benzyl-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione **37** (5.00 mmol, 1.84 g) in acetone (18 mL) were used to give white crystals of compound **16a** (1.44 g, 2.8 mmol). Yield 80%; mp 198–199 °C; TLC: R_f (IV): 0.31. MW 478.63. Monoisotopic Mass 478.29, $[M + H]^+$ 479.53. Anal. Calcd for $C_{28}H_{39}ClN_4O_3$: C, 65.29; H, 7.63; N, 10.88. Found: C, 65.19; H, 7.65; N, 10.69. ^{1}H NMR δ (ppm): 1.22–1.34 (m, 2H, CH_2–CH_2–CH_2–N1), 1.34 (s, 6H, 2 × CH_3), 1.54 ($qu_{def.}$, 2H, CH_2–CH_2–Br), 1.73 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 3.04 (m, 4H,Pp-3,5-H), 3.23 (t, $J = 7.57$ Hz, 2H, CH_2–N1), 3.48 (d, $J = 9.75$ Hz, 2H, CH_2–Br), 3.71 (s, 4H, Pp-2,6-H), 3.82 (s, 3H, CH_3–O), 4.54 (s, CH_2-Ph), 6.41–6.57 (m, 3H, PpPh-2,4,6-H), 7.11–7.37 (m, 6H, Ph, PpPh-5-H), 10.70 (b.s, 1H,

NH^+). IR (cm^{-1}): 3055, 3033 (C–H(Ar)), 2981, 2933 (C–H(Aliph)), 2413 (NH^+), 1763 (C2=O), 1704 (C4=O), 1614 (C–C(Ar)).

*4.1.5.4. 3-Benzyl-1-(5-(4-(2-fluorophenyl)piperazin-1-yl)pentyl)-5,5-dimethylimidazolidine-2,4-dione hydrochloride (**17a**).* **Method D.** 1-(2-Fluorophenyl)piperazine (3.50 mmol, 0.63 g) K_2CO_3 (1.4 g) in acetone (12 mL) and 1-benzyl-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione **37** (5.00 mmol, 1.84 g) in acetone (18 mL) were used to give white crystals of compound **17a** (0.78 g, 1.60 mmol). Yield 44%; mp 140–141 °C; TLC: R_f (IV): 0.32. MW 466.59. Monoisotopic Mass 466.27, $[M + H]^+$ 467.43. Anal. Calcd for $C_{27}H_{36}ClFN_4O_3$: C, 64.46; H, 7.21; N, 11.14. Found: C, 64.40; H, 7.31; N, 10.96. ^{1}H NMR δ (ppm): 1.29 (s, 2H, CH_2–CH_2–CH_2–N1), 1.34 (s, 6H, 2 × CH_3), 1.57 ($qu_{def.}$, 2H, CH_2–CH_2–Br), 1.71 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 3.08 (d, J = 7.98 Hz, 4H, Pp-3,5-H), 3.23 (t, J = 7.57 Hz, 2H, CH_2–N1), 3.45 (d, J = 9.49 Hz, 2H, CH_2–Br), 3.54 (s, 4H, Pp-2,6-H), 4.54 (s, 2H, CH_2-Ph), 7.02–7.36 (m, 9H, Ar), 10.45 (b.s, 1H, NH^+). IR (cm^{-1}): 3036 (C–H(Ar)), 2988, 2977, 2934 (C–H(Aliph)), 2357 (NH^+), 1765 (C2=O), 1702 (C4=O), 1602 (C–C(Ar)).

*4.1.5.5. 3-Benzyl-1-(5-(4-(4-fluorophenyl)piperazin-1-yl)pentyl)-5,5-dimethylimidazolidine-2,4-dione hydrochloride (**18a**).* **Method D.** 1-(4-Fluorophenyl)piperazine (3.50 mmol, 0.63 g) K_2CO_3 (1.4 g) in acetone (12 mL) and 1-benzyl-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione **37** (5.00 mmol, 1.84 g) in acetone (18 mL) were used to give white crystals of compound **18a** (0.50 g, 1.00 mmol). Yield 28%; mp 174–175 °C; TLC: R_f (IV): 0.29. MW 466.59. Monoisotopic Mass 466.27, $[M + H]^+$ 467.43. Anal. Calcd for $C_{27}H_{36}ClFN_4O_3$: C, 64.46; H, 7.21; N, 11.14. Found: C, 64.34; H, 7.17; N, 11.05. ^{1}H NMR δ (ppm): 1.28 (s, 2H, CH_2–CH_2–CH_2–N1), 1.34 (s, 6H, 2 × CH_3), 1.57 ($qu_{def.}$, 2H, CH_2–CH_2–Br), 1.72 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 3.05 (d, J = 8.72 Hz, 4H, Pp-3,5-H), 3.23 (t, J = 7.57 Hz, 2H, CH_2–N1), 3.51 (d, J = 6.41 Hz, 2H, CH_2–Br), 3.68 (d, J = 8.46 Hz, 3H, Pp-2,6-H_b), 3.86 (s, 1H, Pp-2.6-H_a), 4.54 (s, 2H, CH_2-Ph), 6.98–7.12 (m, 4H, Ph-3,5-H, PpPh-2,6-H), 7.19–7.36 (m, 5H, Ph-2,4,6-H, PpPh-3,5-H), 10.62 (br. s, 1H, NH^+). IR (cm^{-1}): 3064, 3037 (C–H(Ar)), 2981, 2933 (C–H(Aliph)), 2420 (NH^+), 1767 (C2=O), 1703 (C4=O), 1607 (C–C(Ar)).

*4.1.5.6. 3-Benzyl-1-(5-(4-(2,4-difluorophenyl)piperazin-1-yl)pentyl)-5,5-dimethylimidazolidine-2,4-dione hydrochloride (**19a**).* **Method D.** 1-(2,4-Difluorophenyl)piperazine (3.50 mmol, 0.69 g) K_2CO_3 (1.4 g) in acetone (12 mL) and 1-benzyl-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione **37** (5.00 mmol, 1.84 g) in acetone (18 mL) were used to give white crystals of compound **19a** (0.43 g, 0.80 mmol). Yield 24%; mp 147–148 °C; TLC: R_f (IV): 0.33. MW 484.58. Monoisotopic Mass 484.26, $[M + H]^+$ 485.45. Anal. Calcd for $C_{27}H_{36}ClF_2N_4O_3$: C, 62.24; H, 6.77; N, 10.75. Found: C, 62.17; H, 6.81; N, 10.59. ^{1}H NMR δ (ppm): 1.34 (s, 2H, CH_2–CH_2–CH_2–N1), 1.34 (s, 6H, 2 × CH_3), 1.57 ($qu_{def.}$, 2H, CH_2–CH_2–Br), 1.72 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 3.08 (d, J = 7.18 Hz, 4H, Pp-3,5-H), 3.23 (t, J = 7.56 Hz, 2H, CH_2–N1), 3.36 (d, J = 8.46 Hz, 2H, CH_2–Br), 3.51 (d, J = 5.64 Hz, 3H, Pp-2,6-H_b), 3.64 (s, 1H, Pp-2,6-H_a), 4.54 (s, 2H, CH_2-Ph), 7.01–7.19 (m, 1H, PpPh-6-H), 7.20–7.36 (m, 7H, PpPh-3,5-H, Ph), 10.64 (s, 1H, NH^+). IR (cm^{-1}): 3047 (C–H(Ar)), 2988, 2978, 2959, 2934 (C–H(Aliph)), 2377, 2356 (NH^+), 1766 (C2=O), 1702 (C4=O), 1620 (C–C(Ar)).

*4.1.5.7. 3-(4-Fluorobenzyl)-1-(5-(4-(2,4-difluorophenyl)piperazin-1-yl)pentyl)-5,5-dimethylimidazolidine-2,4-dione hydrochloride (**20a**).* **Method C.** 1-(2,4-Difluorophenyl)piperazine (5.10 mmol, 1.00 g) K_2CO_3 (2.0 g) in acetone (16 mL) and 3-(4-fluorobenzyl)-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione **38** (5.70 mmol, 2.20 g) in acetone (20 mL) were used to give white crystals of compound **20** (0.76 g, 1.50 mmol). Yield 30%; mp 155–156 °C; TLC: R_f (IV): 0.43. MW 502.57. Monoisotopic Mass 502.27, $[M + H]^+$ 503.40. Anal. Calcd for $C_{27}H_{33}F_3N_4O_2$: C, 64.53; H, 6.62; N, 11.15. Found: C, 64.44; H, 6.83; N, 11.07. ^{1}H NMR for **20** δ (ppm): 1.27 (s, 2H, CH_2–CH_2–CH_2–N1), 1,31 (s, 6H, 2 × CH_3), 1.40 ($qu_{def.}$, 2H, CH_2–CH_2-Pp), 1.51–1.61 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 2.26 (t, J = 7.18 Hz, 2H, CH_2-Pp), 2.47 (s, 4H, Pp-2,6-H), 2.90 (t, J = 4.75 Hz, 4H, Pp-3,5-H), 3.20–3.30 (m, 2H, CH_2–N1), 4.51 (s, 2H, CH_2-Ph), 6.95–7.07 (m, 2H, Ph-2,6-H), 7.11–7.20 (m, 3H, PpPh-3,5-H, PpPh-6-H), 7.23–7.28 ($d_{def.}$, 2H, Ph-3,5-H).

White crystals of compound **20a**. Yield 37%; mp 163–164 °C; TLC: R_f (IV): 0.43. Anal. Calcd for $C_{27}H_{34}ClF_3N_4O_2$: C, 60.16; H, 6.36; N, 10.39. Found: C, 59.88; H, 6.40; N, 10.17. ^{1}H NMR for **20a** δ (ppm): 1.28–1.31 (m, 2H, CH_2–CH_2–CH_2–N1), 1,32 (s, 6H, 2 × CH_3), 1.53–1.61 ($qu_{def.}$, 2H, CH_2–CH_2-Pp), 1.62–1.75 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 3.07–3.27 (m, 6H, CH_2-Pp, Pp-3,5-H, CH_2–N1), 3.36–3.38 ($d_{def.}$, 2H, Pp-2,6-H_a), 3.49–3.53 ($d_{def.}$, 2H, Pp-2,6-H_b), 4.52 (s, 2H, CH_2-Ph), 6.99–7.28 (m, 7H, Ph), 10.91 (br. s, 1H, NH^+). IR (cm^{-1}): 3050 (C–H(Ar)), 2988, 2978, 2957, 2934 (C–H(Aliph)), 2382, 2359 (NH^+), 1765 (C2=O), 1704 (C4=O), 1620, 1608 (C–C(Ar)).

*4.1.5.8. 3-(4-Fluorobenzyl)-1-(5-(4-(4-fluorophenyl)piperazin-1-yl)pentyl)-5,5-dimethylimidazolidine-2,4-dione hydrochloride (**21a**).* **Method C.** 1-(4-Fluorophenyl)piperazine (4.5 mmol, 0.81 g) K_2CO_3 (1.6 g) in acetone (14 mL) and 3-(4-fluorobenzyl)-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione **38** (4.90 mmol, 1.90 g) in acetone (18 mL) were used to give white crystals of compound **21** (1.55 g, 2.1 mmol). Yield 46%; mp 113–114 °C; TLC: R_f (IV): 0.44. MW 484.58. Monoisotopic Mass 484.26, $[M + H]^+$ 485.45. Anal. Calcd for $C_{27}H_{34}F_2N_4O_2$: C, 66.92; H, 7.07; N, 11.56. Found: C, 66.68; H, 6.93; N, 11.48. ^{1}H NMR for **21** δ (ppm): 1.27 ($t_{def.}$, 2H, CH_2–CH_2–CH_2–N1), 1.31 (s, 6H, 2 × CH_3), 1.41 ($qu_{def.}$, 2H, CH_2–CH_2-Pp), 1.51 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 2.25 (t, J = 7.18 Hz, 2H, CH_2-Pp), 2.43 ($t_{def.}$, 4H, Pp-2,6-H), 3.00 (t, J = 4.87 Hz, 4H, Pp-3,5-H), 3.20 (t, J = 7.70 Hz, 2H, CH_2–N1), 4.51 (s, 2H, CH_2-Ph), 6.88 ($d_{def.}$, 2H, PpPh-3,5-H), 6.91 ($d_{def.}$, 2H, PpPh-2,6-H), 7.11 ($d_{def.}$, 2H, Ph-3,5-H), 7.22 ($d_{def.}$, 2H, Ph-2,6-H).

White crystals of compound **21a**. Yield 47%; mp 175 °C; TLC: R_f (IV): 0.44. Anal. Calcd for $C_{27}H_{35}ClF_2N_4O_2 \times H_2O$: C, 60.16; H, 6.92; N, 10.39. Found: C, 59.96; H, 7.04; N, 10.32. ^{1}H NMR for **21a** δ (ppm): 1.28–1.33 (m, 2H, CH_2–CH_2–CH_2–N1), 1,32 (s, 6H, 2 × CH_3), 1.53–1.61 ($qu_{def.}$, 2H, CH_2–CH_2-Pp), 1.73–1.78 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 3.07–3.15 ($qu_{def.}$, 6H, CH_2-Pp, Pp-3,5-H), 3.22 (t, J = 7.40 Hz, 2H, CH_2–N1), 3.50–3.53 ($d_{def.}$, 2H, Pp-2,6-H_a), 3.67–3.70 ($d_{def.}$, 2H, Pp-2,6-H_b), 4.52 (s, 2H, CH_2-Ph), 6.98–7.28 (m, 8H, Ph), 10.91 (br. s, 1H, NH^+). IR (cm^{-1}): 3057 (C–H(Ar)), 2982, 2948, 2933 (C–H(Aliph)), 2440 (NH^+), 1765 (C2=O), 1704 (C4=O), 1608 (C–C(Ar)). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 23.0, 24.0, 29.0, 46.5, 50.8, 55.5, 62.0, 115.7, 115.8, 116.0, 118.3, 118.4, 129.7, 129.8, 133.4, 146.8, 155.0, 176.6.

*4.1.5.9. 3-(2,4-Dichlorobenzyl)-1-(5-(4-(2,3-dichlorophenyl)piperazin-1-yl)pentyl)-5,5-dimethylimidazolidine-2,4-dione (**22**).* **Method C.*** 1-(2,3-dichlorophenyl)piperazine hydrochloride (4.0 mmol, 1.07 g) K_2CO_3 (2.2 g) in acetone (19 mL) and 3-(2,4-dichlorobenzyl)-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione **39** (4.90 mmol, 1.90 g) in acetone (18 mL) were used to give white crystals of compound **22** (1.30 g, 2.1 mmol). Yield 52%; mp 91–92 °C; TLC: R_f (IV): 0.53. MW 586.38. Monoisotopic Mass 586.13, $[M + H]^+$ 587.29. Anal. Calcd for $C_{27}H_{32}Cl_4N_4O_2$: C, 55.30; H, 5.50; N, 9.55. Found: C, 55.49; H, 5.60; N, 9.44. ^{1}H NMR for **22** δ (ppm): 1.01 ($qu_{def.}$, 2H, CH_2–CH_2–CH_2–N1), 1.35 (s, 6H, 2 × CH_3), 1.41 ($qu_{def.}$, 2H, CH_2–CH_2-Pp), 1.52 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 2.28 (t, J = 7.18 Hz, 2H, CH_2-Pp), 2.47 (s, 4H, Pp-2,6-H), 2.95 (s, 4H, Pp-3,5-H), 3.22 (t, J = 7.70 Hz, 2H, CH_2–N1), 4.59 (s, 2H, CH_2-Ph), 7.01–7.16 (m, 2H, PpPh-4,6-H), 7.26–7.29 (m, 2H, Ph-6-H, PpPh-5-H), 7.35 ($d_{def.}$, 1H, Ph-5-H), 7.62 (s, 1H, Ph-3-H). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 23.1, 24.7, 26.3, 29.5, 51.4, 53.3, 58.1, 62.1, 119.9, 124.7, 126.4, 128.1, 128.9, 129.4, 130.3, 133.1, 133.1, 133.3, 151.7, 154.7, 176.5. IR (cm^{-1}): 3075, 3045 (C–

H(Ar)), 2960, 2936 (C–H(Aliph)), 1758 (C2=O), 1705 (C4=O), 1578 (C–C(Ar)).

*4.1.5.10. 3-(2,4-Dichlorobenzyl)-1-(5-(4-(3,4-dichlorophenyl)piperazin-1-yl)pentyl)-5,5-dimethylimidazolidine-2,4-dione hydrochloride (**23a**).* **Method C.** 1-(3,4-dichlorophenyl)piperazine (2.0 mmol, 0.54 g) K_2CO_3 (1.0 g) in acetone (8 mL) and 3-(2,4-dichlorobenzyl)-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione **39** (2.2 mmol, 0.96 g) in acetone (9 mL) were used to give white crystals of compound **23** (0.61 g, 1.0 mmol). Yield 52%; mp 140 °C; TLC: R_f (IV): 0.60. MW 586.38. Monoisotopic Mass 586.13, $[M + H]^+$ 587.29. Anal. Calcd for $C_{27}H_{32}Cl_4N_4O_2$: C, 55.30; H, 5.50; N, 9.55. Found: C, 55.36; H, 5.57; N, 9.37. ^{1}H NMR for **23** δ (ppm): 1.01 ($qu_{def.}$, 2H, CH_2–CH_2–CH_2–N1), 1.35 (s, 6H, 2 × CH_3), 1.41 ($qu_{def.}$, 2H, CH_2–CH_2-Pp), 1.52 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 2.24 (t, J = 7.18 Hz, 2H, CH_2-Pp), 2.41 (t, J = 4.75 Hz, 4H, Pp-2,6-H), 3.11 (t, J = 4.75 Hz, 4H, Pp-3,5-H), 3.21 ($t_{def.}$, 2H, CH_2–N1), 4.59 (s, 2H, CH_2-Ph), 6.88 ($d_{def.}$, 1H, PpPh-2-H), 7.08 ($d_{def.}$, 2H, PpPh-6-H, PpPh-5-H), 7.35 ($d_{def.}$, 2H, Ph-5,6-H), 7.62 (s, 1H, Ph-3-H).

White crystals of compound **23a**. Yield 59%; mp 117 °C; TLC: R_f (IV): 0.60. Anal. Calcd for $C_{27}H_{33}Cl_5N_4O_2 \times 1/3C_2H_5OH$: C, 52.07; H, 5.53; N, 8.78. Found: C, 51.91; H, 5.27; N, 8.89. ^{1}H NMR for **23a** δ (ppm): 1.29–1.35 (m, 2H, CH_2–CH_2–CH_2–N1), 1.36 (s, 6H, 2 × CH_3), 1.57–1.59 ($qu_{def.}$, 2H, CH_2–CH_2-Pp), 1.71 (br. s, 2H, CH_2–CH_2–N1), 3.08–3.15 ($qu_{def.}$, 6H, CH_2-Pp, Pp-3,5-H), 3.20–3.25 (m, 2H, CH_2–N1), 3.41–3.62 (m, 2H, Pp-2,6-H_a), 3.86 (br. s, 2H, Pp-2,6-H_b), 4.60 (s, 2H, CH_2-Ph), 6.97–7.01 (m, 1H, PpPh-2-H), 7.13–7.17 (m, 1H, PpPh-6-H), 7.20–7.23 (m, 1H, PpPh-5-H), 7.37–7.45 (m, 2H, Ph-5,6-H), 6.62–7.64 (m, 1H, Ph-3-H), 10.20 (br. s, 1H, NH^+). IR (cm^{-1}): 3074 (C–H(Ar)), 2959, 2936 (C–H(Aliph)), 2595 (NH^+), 1769 (C2=O), 1704 (C4=O), 1593 (C–C(Ar)).

*4.1.5.11. 3-(2,4-Dichlorobenzyl)-1-(5-(4-(4-chlorophenyl)piperazin-1-yl)pentyl)-5,5-dimethylimidazolidine-2,4-dione hydrochloride (**24a**).* **Method C.** 1-(4-chlorophenyl)piperazine (2.0 mmol, 0.40 g) K_2CO_3 (0.90 g) in acetone (7 mL) and 3-(2,4-dichlorobenzyl)-1-(5-bromopentyl)-5,5-dimethylimidazolidine-2,4-dione **39** (2.2 mmol, 0.96 g) in acetone (9 mL) were used to give white crystals of basic compound **24** (0.78 g, 1.4 mmol). Yield 71%; mp 156 °C; TLC: R_f (IV): 0.53. MW 551.94. Monoisotopic Mass 551.17, $[M + H]^+$ 553.32. Anal. Calcd for $C_{27}H_{33}Cl_3N_4O_2$: C, 58.75; H, 6.03; N, 10.15. Found: C, 58.56; H, 5.97; N, 10.04. ^{1}H NMR for **24** δ (ppm): 1.28 ($qu_{def.}$, 2H, CH_2–CH_2–CH_2–N1), 1.35 (s, 6H, 2 × CH_3), 1.41 ($qu_{def.}$, 2H, CH_2–CH_2-Pp), 1.46 ($qu_{def.}$, 2H, CH_2–CH_2–N1), 2.25 (t, J = 6.82 Hz, 2H, CH_2-Pp), 2.43 ($t_{def.}$, 4H, Pp-2,6-H), 3.06 (t, J = 4.87 Hz, 4H, Pp-3,5-H), 3.21 (t, J = 7.44 Hz, 2H, CH_2–N1), 4.59 (s, 2H, CH_2-Ph), 6.89 ($d_{def.}$, 2H, PpPh-2,6-H), 7.13 ($d_{def.}$, 3H, PpPh-3,5-H, Ph-6-H), 7.37 ($d_{def.}$, 1H, Ph-5-H), 7.62 ($d_{def.}$, 1H, Ph-3-H).

White crystals of compound **24a**. Yield 53%; mp 71 °C; TLC: R_f (IV): 0.60. Anal. Calcd for $C_{27}H_{34}Cl_4N_4O_3$: C, 55.11; H, 5.82; N, 9.52. Found: C, 55.01; H, 6.11; N, 9.67. ^{1}H NMR for **21a** δ (ppm): 1.29–1.31 (m, 2H, CH_2–CH_2–CH_2–N1), 1.34 (s, 6H, 2 × CH_3), 1.56–1.61 ($qu_{def.}$, 2H, CH_2–CH_2-Pp), 2.47–2.50 (m, 2H, CH_2–CH_2–N1), 3.07–3.27 (m, 6H, CH_2-Pp, Pp-3,5-H, CH_2–N1), 3.31–3.35 (m, 4H, Pp-2,6-H), 4.59 (s, 2H, CH_2-Ph), 6.97–7.00 ($d_{def.}$, 1H, Ph-6-H) 7.13–7.16 ($d_{def.}$, 2H, PpPh-2,6-H), 7.25–7.28 ($d_{def.}$, 1H, Ph-5-H) 7.38–7.40 ($d_{def.}$, 2H, PpPh-3,5-H), 7.62 (s, 1H, Ph-3-H), 8.92 (br. s, 1H, NH^+). IR (cm^{-1}): 3073, 3055 (C–H(Ar)), 2958, 2934 (C–H(Alif)), 2489 (NH^+), 1773 (C2=O), 1703 (C4=O), 1588 (C–C(Ar)).

*4.1.6. Synthesis of 5-(4-fluorophenyl)-5-methyl-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (**42**)*

5-(4-Fluorophenyl)-5-methylhydantoin **41** (30 mmol, 6.24 g), oxiran-2-ylmethanol (30 mmol, 2 ml) and TPP (30 mmol, 7.86 g) in anhydrous THF (30 mL) were stirred in the hermetic closed flask-bottom on ice-bathroom at 0 °C. When the ingredients were totally dissolved, DEAD (30 mmol, 5.22 g) in THF (10 mL) were added dropwise for 45 min. The mixture was stirred at room temperature for 90 h under TLC control. The solvent was evaporated. The residue was treated with diethyl ether (200 mL). The precipitate was filtrated of, and the filtrate was condensed to give compound **42** (7.39 g, 28 mmol) in glue-mass form. Yield 93%; TLC: R_f (IV): 0.54. Anal. Calcd for $C_{13}H_{13}FN_2O_3$: C, 59.09; H, 4.96; N, 10.60. Found: C, 59.45; H, 5.05; N, 10.31. ^{1}H NMR for **42** δ (ppm): 1.66 (s, 3H, -CH_3), 2.38–2.46 (m, 1H, -CH_2-O-3-H_a), 2.65–2.69 (m,1H, -CH_2-O-3-H_b), 3.06–3.11 ($qu_{def.}$, 1H, CH–O), 3.53–3.55 ($q_{def.}$, 2H, N3–CH_2), 7.19–7.26 (m, 2H, Ph-2,6-H), 7.47–7.52 (m, 2H, Ph-3,5-H), 8.99 (br. s, 1H, NH).

*4.1.7. Synthesis of 5-(4-fluorophenyl)-3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-5-methylimidazolidine-2,4-dione hydrochloride (**25a**)*

5-(4-Fluorophenyl)-5-methyl-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione **42** (4.0 mmol, 1.1 g) and 2-methoxyphenylpiperazine (3.00 mmol, 0.58 g) were dissolved in methylene chloride (5 mL). The solvent was evaporated. The residue was irradiated in household-microwave oven using the following program of irradiation: 300 W (3 min), 450 W (2 × 3 min), 600 W (2 × 1 min). The obtained glue-residue was purified with chromatography column (CH_2Cl_2/aceton/MeOH). The fractions containing pure product **25** were collected and evaporated. The residue was dissolved in 99.8% EtOH (15 mL) and saturated with gaseous HCl to give white precipitate of **25a** (0.40 g, 0.81 mmol). Yield 27%; mp 235 °C; TLC: R_f (IV): 0.31. MW 456.51. Monoisotopic Mass 456.22, $[M + H]^+$ 457.39. Anal. Calcd for $C_{24}H_{30}ClFN_4O_4$: C, 58.47; H, 6.13; N, 11.37. Found: C, 58.54; H, 5.99; N, 11.29. ^{1}H NMR for **25a** δ (ppm): 1.7 (br. s, 3H, CH_3), 2.93–3.09 (m, 4H, Pp- CH_2, Pp-2,6-Ha), 3.20–3.24 (d, 4H, Pp-2,6-Hb, 2H, N_3–CH_2), 3.40 (d, J = 7.18 Hz, 4H, Pp-3,5-H), 3.49 (s, 1H, CH–OH), 3.77 (s, 3H, O–CH_3), 4.20 (br. s, 1H, OH), 6.87–7.03 (m, 4H, PpPh-3,4,5,6-H), 7.20–7.25 (m, 2H, Ph-2,6-H), 7.50–7.55 (m, 2H, Ph-3,5-H), 8.99 (br. s, 1H, N_1H), 9.79 (s, 1H, NH^+). IR (cm^{-1}): 1608.34 (C=C; Ar), 1715.37 (C=O (4)), 1772.26 (C=O (2)), 2400.94 (NH+), 2982.37 (CH; Aliph), 3007.44 (CH; Ar), 3314.07 (OH).

4.2. Pharmacology

4.2.1. Radioligand binding assays

4.2.1.1. The α_1-adrenoceptor binding assay. 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 of solutions and adding of reagents were 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.

4.2.1.2. The serotonin receptors binding assays

4.2.1.2.1. Cell culture and preparation of cell membranes. HEK293 cells with stable expression of human serotonin 5-HT_{1A}R, 5-HT_6R, 5-HT_7R were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were grown in Dulbeco's Modifier Eagle Medium containing 10% dialyzed fetal bovine serum and 500 μg/ml G418 sulfate. For membranes preparations, cells were subcultured in 10 cm diameter dishes, grown to 90% confluence, washed twice with prewarmed to 37 °C phosphate buffered saline (PBS), and were pelleted by centrifugation (200 g) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparations pellets were stored at −80 °C.

4.2.1.2.2. Radioligand binding assays. Cell pellets are thawed and homogenized in 10 volumes of assay buffer using an UltraTurrax tissue homogenizer and centrifuged twice at 35 000 g for 20 min at 4 °C, with incubation for 15 min at 37 °C in between. The composition of the assay buffers is as follows: for 5-HT_{1A}R: 50 mM Tris–HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate; for 5-HT_6R: 50 mMTris–HCl, 0.5 mM EDTA and 4 mM $MgCl_2$, for 5-HT_7R: 50 mMTris–HCl, 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate. All assays were incubated in a total volume of 200 μl in 96-well microtitre plates for 1 h at 37 °C except for 5-HT_{1A}R which were incubated at room temperature for 1 h. The process of equilibration is terminated by rapid filtration through Unifilter plates with a 96-well cell harvester and radioactivity retained on the filters was quantified on a MicroBeta plate reader. For displacement studies the assay samples contained as radioligands: 1.5 nM [^{3}H]-8-OH-DPAT (187 Ci/mmol) for 5-HT_{1A}R; 2 nM [^{3}H]-LSD (85.2 Ci/mmol) for 5-HT_6R; 0.6 nM [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT_7R. Non-specific binding is defined with 10 μM of 5-HT in 5-HT_{1A} and 5-HT_7 binding experiments, whereas 10 μM methiothepin was used in 5-HT_6 assay. Each compound was tested in triplicate at 7 concentrations (10^{-10}–10^{-4} M). The inhibition constants (K_i) were calculated from the Cheng–Prusoff equation [30]. Results were expressed as means of at least two separate experiments.

4.2.2. Functional bioassays

The experiments were carried out on male Wistar rats (Krf:(WI), (WU), 180–250 g), and male Albino Swiss mice (CD-1, 18–25 g). Animals were housed in plastic cages in room at a constant temperature of 20 ± 2 °C with natural light–dark cycles. They had free access to standard pellet diet and water. Treatment of laboratory animals in the present study was in full 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: (±)-noradrenaline hydrochloride (Sigma–Aldrich, Germany), (±)-propranolol hydrochloride (Sigma–Aldrich, Germany), acetylcholine hydrochloride (Sigma–Aldrich, Germany), chloroethylclonidine (CEC, Sigma–Aldrich, Germany), yohimbine hydrochloride (Sigma–Aldrich, Germany), Thiopental sodium (Biochemie Gmbh, Vienna). Other reagents were of analytical grade from local sources.

4.2.2.1. The activity at α_{1A}-adrenoceptors in rat tail artery. The male Wistar rats were anaesthetized with thiopental sodium (75 mg/kg ip), and the middle part of the ventral caudal artery was removed, cleaned of surrounding tissue, denuded of endothelium by gentle rubbing and cut into approximately 4 mm long rings. Arterial rings were horizontally suspended between two stainless steel hooks (diameter 0.15 mm). One hook 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), coupled to a MP100 analyser (BIOPAC Systems, Inc., COMMAT Ltd., Turkey) and processed by AcqKnowledge software (BIOPAC). The arterial rings were incubated in 30 ml chambers filled with a Krebs–Henseleit solution (NaCl 119 mM, KCl 4.7 mM, $CaCl_2$ 1.9 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, glucose 11 mM, EDTA 0.05 mM) at 37 °C and pH 7.4 with constant oxygenation (O_2/CO_2, 19:1) and subjected to a 0.75 g initial optimal tension. Caudal arterial rings were incubated with preferentially α_{1B}-adrenoceptor alkylating agent chloroethylclonidine (CEC; 3 μM), then 30 min later chloroethylclonidine was thoroughly washed off. During an equilibration period of 100 min tissues were stimulated four times with noradrenaline (NA 1 μM) followed by washout until the contractile response had become constant. Two cumulative concentration–response curves to noradrenaline were determined on each arterial ring at an interval of 60 min in the absence and presence of antagonist. Tissues were incubated with antagonists for 30 min. The experiments were conducted in the continuous presence of yohimbine (0.1 μM) and propranolol (1 μM) to block α_2- and β-adrenoceptors respectively.

4.2.2.2. The activity at α_{1B}-adrenoceptors in mouse spleen. Male mice were killed by cervical dislocation. The spleen was removed and cut longitudinally into two strips, which were set up in 30 ml organ bath under a resting tension of 0.8 g for the recording of isometric contractile responses in a Krebs–Henseleit solution of the above composition at 37 °C and pH 7.4 with constant oxygenation (O_2/CO_2, 19:1). During an equilibration period of 100 min tissues were stimulated with noradrenaline (10 μM) followed by washout until the contractile response had become constant. Two cumulative concentration–response curves to noradrenaline were determined on each tissue at an interval of 60 min in the absence and presence of antagonist. Tissues were incubated with antagonists for 30 min. The experiments were conducted in the continuous presence of propranolol (1 μM) to block β-adrenoceptors.

4.2.2.3. The activity at α_{1D}-adrenoceptors in rat thoracic aorta. The male Wistar rats were anaesthetized with thiopental sodium (75 mg/kg ip) and the aorta was isolated, denuded of endothelium, cut, mounted and incubated as described in a method described in 4.2.2.1. The aorta rings were stretched and maintained at optimal tension of 2 g and allowed to equilibrate for 3 h. During an equilibration period the preparations were stimulated three times with NA (0.3 μM). Two cumulative concentration–response curves to noradrenaline were determined on each arterial ring at interval of 60 min in the absence and presence of antagonist. Tissues were incubated with antagonists for 30 min. The experiments were conducted in the continuous presence of yohimbine (0.1 μM) and propranolol (1 μM) to block α_2- and β-adrenoceptors.

Concentration-response curves were analyzed using GraphPad Prism 5.0 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 noradrenaline effect (Emax = 100%), reached in the concentration–response curves obtained before incubation with the tested compounds. Data are the means ± SEM of at least 4 separate experiments. Schild analysis was performed, and the pA_2 value was determined.

4.2.3. Inhibitory effect at human 5-HT_{3A} expressed in Xenopus Oocytes

Mature female *X. laevis* frogs were purchased from Xenopus I (Ann Arbor, MI). They were housed in dechlorinated tap water at

18 °C under a 12:12-h light/dark cycle and fed beef liver at least twice a week. Clusters of oocytes were removed surgically under tricaine (Sigma–Aldrich, St. Louis, MO) anesthesia (0.15%), and individual oocytes were manually dissected away in a solution containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.8 mM $MgSO_4$, and 10 mM HEPES, pH 7.5. Dissected oocytes were stored 2–7 days in modified Barth's solution containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.3 mM $Ca(NO_3)_2$, 0.9 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 10 mM HEPES, pH 7.5, supplemented with 2 mM sodium pyruvate, 10 000 IU/l penicillin, 10 mg/l streptomycin, 50 mg/l gentamicin, and 0.5 mM theophylline. Oocytes were placed in a 0.2-ml recording chamber and superfused at a constant rate of 3–5 ml/min. The bathing solution consisted of 95 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, and 5 mM HEPES, pH 7.5. The amount of 5-HT_{3A} receptor cRNA injected into oocytes varied from 1 to 30 ng, as indicated. However, the injection volume of diethylpyrocarbonate-treated distilled water was kept at 30 nl throughout the experiments. The cells were impaled with two standard glass microelectrodes filled with 3 M KCl (1–3 MΩ). The oocytes were routinely voltage-clamped at a holding potential of −70 mV using a GeneClamp-500B amplifier (Molecular Devices, Sunnyvale, CA). Current responses were digitized by A/D converter and analyzed using pClamp 8 (Molecular Devices) run on an IBM PC or directly recorded on a 2400 rectilinear pen recorder (Gould Instrument Systems Inc., Cleveland, OH). Current-voltage curves were generated by holding each membrane potential in a series for 50–60 s, followed by a return to −70 mV for 5 min. Oocyte capacitance was measured by a paired ramp method described previously [31]. In brief, voltage ramps were used to elicit constant capacitive current, I_{cap}, and the charge associated with this current was calculated by the integration of I_{cap}. Ramps had slopes of 2 V/s and durations of 20 ms and started at a holding potential of 90 mV. A series of 10 paired ramps was delivered at 1-s intervals and averaged traces were used for charge calculations. In each oocyte, the averages of five to six measurements were used to obtain values for membrane capacitance (C_m). Currents for I_{cap} recordings were filtered at 20 kHz and sampled at 50 kHz. Current density was calculated by normalizing the average of three consecutive control currents to the oocyte capacitance. Compounds were applied by addition to the superfusate. All chemicals used in preparing the solutions were from Sigma–Aldrich. Pertussis toxin (PTX), BAPTA, actinomycin D (ActD), 5-HT, and MDL72222 [tropanyl 3,5-dichlorobenzoate] were purchased from Tocris Bioscience (Ellisville, MO). Procedures for the injections of PTX (50 nl; 50 μg/ml) or BAPTA (50 nl; 200 mM) were performed as described previously [32]. Injections were performed 1 h before recordings using oil-driven ultramicrosyringe pumps (Micro4; WPI, Sarasota, FL). Stock solutions of the standard antagonist **LY278584** (Sigma–Aldrich, St. Louis, MO) and test compounds **10**, **13**, **14**, **20**, **21**, **25** were prepared in dimethyl sulfoxide at a concentration of 30 mM. Dimethyl sulfoxide alone did not affect 5-HT_{3A} receptor function when added at concentrations as high as 0.2% (v/v), a concentration 2 times greater than the most concentrated application of the agents used. Electrophysiological recordings from oocytes were conducted 3–4 days after cRNA injections, and both control and treatment (PTX and BAPTA) groups were recorded on the same days.

4.2.3.1. Synthesis of cRNA. The cDNA clone of the human 5-HT_{3A} subunits was purchased from OriGen Technologies, Inc. (Rockville, MD). cRNA were synthesized *in vitro* using a mMessage mMachine RNA transcription kit (Ambion, Austin, TX). The quality and size of synthesized cRNA was confirmed by denatured RNA agarose gels.

4.2.3.2. Data analysis. For each test compound, six inhibitory values were obtained in each oocyte, and three oocytes of diverse batches were used. The average inhibitory values were calculated as mean ± S.E.M. Statistical significance was analyzed using ANOVA or Student's *t* test.

Acknowledgments

Authors thank students: Sara Borromeo, Paula Idzik and Małgorzata Frączek for their participation in the synthesis works. The work was partly supported by K/ZDS/003323 and K/ZDS/001915. Authors: J. Handzlik, A. J. Bojarski, G. Satała, K. Kucwaj, K. Kieć-Kononowicz participate in the European COST Action CM1207 (GLISTEN).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.065.

References

[1] Y.B. Lee, Y.D. Gong, D.J. Kim, C.H. Ahn, J.Y. Kong, N.S. Kang, Synthesis, anticancer activity and pharmacokinetic analysis of 1-[(substituted 2-alkoxyquinoxalin-3-yl)aminocarbonyl]-4-(hetero)arylpiperazine derivatives, Bioorganic & Medicinal Chemistry 20 (2012) 1303–1309.
[2] A. Blaser, B.D. Palmer, H.S. Sutherland, I. Kmentova, S.G. Franzblau, B. Wan, Y. Wang, Z. Ma, A.M. Thompson, W.A. Denny, Structure-activity relationships for amide-, carbamate- and urea-linked analogues of the tuberculosis drug (6S)-2-nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5H-imidazo [2,1-b][1,3]oxazine, Journal of Medicinal Chemistry 55 (2012) 312–326.
[3] J. Handzlik, M. Bajda, M. Zygmunt, D. Maciąg, M. Dybała, M. Bednarski, B. Filipek, B. Malawska, K. Kieć-Kononowicz, Antiarrhythmic properties of phenylpiperazine derivatives of phenytoin with α_1-adrenoceptor affinities, Bioorganic & Medicinal Chemistry 20 (2012) 2290–2303.
[4] J. Handzlik, E. Szymańska, J. Chevalier, E. Otrębska, K. Kieć-Kononowicz, J.-M. Pagès, S. Alibert, Amine-alkyl derivatives of hydantoin: new tool to combat resistant bacteria, European Journal of Medicinal Chemistry 46 (2011) 5807–5816.
[5] G. Spengler, M. Evaristo, J. Handzlik, J. Serly, J. Molnár, M. Viveiros, K. Kieć-Kononowicz, L. Amaral, Biological activity of hydantoin derivatives on p-glycoprotein (ABCB1) of mouse lymphoma cells, Anticancer Research 30 (2010) 4867–4871.
[6] G. Spengler, J. Handzlik, I. Ocsovszki, M. Viveiros, K. Kieć-Kononowicz, J. Molnar, L. Amaral, Modulation of multidrug efflux pump activity by new hydantoin derivatives on colon adenocarcinoma cells without inducing apoptosis, Anticancer Research 31 (2011) 3285–3288.
[7] J. Handzlik, E. Szymańska, S. Alibert, J. Chevalier, E. Otrębska, E. Pękala, J.-M. Pagès, K. Kieć-Kononowicz, Search for new tools to combat gram-negative resistant bacteria among amine derivatives of 5-arylidenehydantoin, Bioorganic & Medicinal Chemistry 21 (2013) 135–145.
[8] R. Romagnoli, P.G. Baraldi, M.D. Carrion, C.L. Cara, O. Cruz-Lopez, M.K. Salvador, D. Preti, M.A. Tabrizi, A.R. Moorman, F. Vincenzi, P.A. Borea, K. Varani, Synthesis and biological evaluation of 2-amino-3-(4-chlorobenzoyl)-4-[(4-arylpiperazin-1-yl)methyl]-5-substituted-thiophenes. effect of the 5-modification on allosteric enhancer activity at the A1 adenosine receptor, Journal of Medicinal Chemistry 55 (2012) 7719–7735.
[9] D. Ignjatović, D. Vojnović Milutinović, A. Nikolić-Kokić, M. Slavić, D. Andrić, M. Tomić, S. Kostić-Rajačić, The mechanisms responsible for neuroprotective capacity of arylpiperazine dopaminergic ligands against cell death induced by sodium nitroprusside, European Journal of Pharmacology 683 (2012) 93–100.
[10] P. Zajdel, K. Marciniec, A. Maślankiewicz, G. Satała, B. Duszyńska, A.J. Bojarski, A. Partyka, M. Jastrzębska-Więsek, D. Wróbel, A. Wesołowska, M. Pawłowski, Quinoline- and isoquinoline-sulfonamide derivatives of LCAP as potent CNS multi-receptor – 5-HT1A/5-HT2A/5-HT7 and D2/D3/D4 – agents: the synthesis and pharmacological evaluation, Bioorganic & Medicinal Chemistry 20 (2012) 1545–1556.
[11] M. Leopoldo, E. Lacivita, F. Berardi, R. Perrone, P.B. Hedlund, Serotonin 5-HT_7 receptor agents: structure-activity relationships and potential therapeutic applications in central nervous system disorders, Pharmacology & Therapeutics 129 (2011) 120–148.
[12] J. Handzlik, E. Szymańska, R. Wójcik, A. Dela, M. Jastrzębska-Więsek, J. Karolak-Wojciechowska, A. Fruziński, A. Siwek, B. Filipek, K. Kieć-Kononowicz, Synthesis and SAR-study for novel arylpiperazine derivatives of 5-arylidenehydantoin with α_1-adrenoceptor antagonistic properties, Bioorganic & Medicinal Chemistry 20 (2012) 4245–4257.

[13] M.L. López-Rodríguez, D. Ayala, B. Benhamú, M.J. Morcillo, A. Viso, Arylpiperazine derivatives acting at 5-HT(1A) receptors, Current Medicinal Chemistry 9 (2002) 443–469.
[14] P. Zajdel, K. Marciniec, A. Maślankiewicz, M.H. Paluchowska, G. Satała, A. Partyka, M. Jastrzębska-Więsek, D. Wróbel, A. Wesołowska, B. Duszyńska, A.J. Bojarski, M. Pawłowski, Arene- and quinoline-sulfonamides as novel 5-HT_7 receptor ligands, Bioorganic & Medicinal Chemistry 19 (2011) 6750–6759.
[15] J. Yoon, E.A. Yoo, J.-Y. Kim, A.N. Pae, H. Rhim, W.-K. Park, J.Y. Kong, H.-Y. Park Choo, Preparation of piperazine derivatives as 5-HT_7 receptor antagonists, Bioorganic & Medicinal Chemistry 16 (2008) 5405–5412.
[16] J.H. Bremner, B. Coban, R. Griffith, K.M. Groenewoud, B.F. Yates, Ligand design for alpha1 adrenoceptor subtype selective antagonists, Bioorganic & Medicinal Chemistry 8 (2000) 201–214.
[17] L. Du, M. Li, Modeling the interactions between alpha(1)-adrenergic receptors and their antagonists, Current Computer-Aided Drug Design 6 (2010) 165–178.
[18] G. Romeo, L. Materia, M.N. Modica, V. Pittalà, L. Salerno, M.A. Siracusa, F. Manetti, M. Botta, K.P. Minneman, Novel 4-phenylpiperidine-2,6-dione derivatives. Ligands for α_1-adrenoceptor subtypes, European Journal of Medicinal Chemistry 46 (2011) 2676–2690.
[19] J. Handzlik, H.H. Pertz, T. Görnemann, S. Jähnichen, K. Kieć-Kononowicz, Search for influence of spatial properties on affinity at α_1-adrenoceptor subtypes for phenylpiperazine derivatives of phenytoin, Bioorganic & Medicinal Chemistry Letters 20 (2010) 6152–6156.
[20] F. Liu, V.J. Majo, J. Prabhakaran, M.S. Millak, J.J. Mann, R.V. Parsey, J.S.D. Kumar, Synthesis and in vivo evaluation of [*O*-methyl-^{11}C] *N*-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzenesulfonamide as an imaging probe for 5-HT_6 receptors, Bioorganic & Medicinal Chemistry 19 (2011) 5255–5259.
[21] S. Yoshida, T. Watanabe, Y. Sato, Regulatory molecules for the 5-HT3 receptor ion channel gating system, Bioorganic & Medicinal Chemistry 15 (2007) 3515–3523.
[22] R. Barbaro, L. Betti, M. Botta, F. Corelli, G. Giannaccini, L. Maccari, F. Manetti, G. Strappaghetti, S. Corsano, Synthesis, biological evaluation, and pharmacophore generation of new pyridazinone derivatives with affinity toward alpha(1)- and alpha(2)-adrenoceptors, Journal of Medicinal Chemistry 44 (2001) 2118–2132.
[23] A.J. Bojarski, Pharmacophore models for metabotropic 5-HT receptor ligands, Current Topics in Medicinal Chemistry 6 (2006) 2005–2026.
[24] A. Lepailleur, R. Bureau, M. Paillet-Loilier, F. Fabis, N. Saettel, S. Lemaitre, F. Dauphin, A. Lesnard, J.-Ch Lancelot, S. Rault, Molecular modeling studies focused on 5-HT7 versus 5-HT1A selectivity. Discovery of novel phenylpyrrole derivatives with high affinity for 5-HT7 receptors, Journal of Chemical Information and Modeling 45 (2005) 1075–1081.
[25] J. Handzlik, E. Szymańska, K. Nędza, M. Kubacka, A. Siwek, S. Mogilski, J. Handzlik, B. Filipek, K. Kieć-Kononowicz, Pharmacophore models based studies on the affinity and selectivity toward 5-HT_{1A} with reference to α_1-adrenergic receptors among arylpiperazine derivatives of phenytoin, Bioorganic & Medicinal Chemistry 19 (2011) 1349–1360.
[26] J. Handzlik, D. Maciąg, M. Kubacka, S. Mogilski, B. Filipek, K. Stadnicka, K. Kieć-Kononowicz, Synthesis, α_1-adrenoceptor antagonist activity and SAR-study for novel arylpiperazine derivatives of phenytoin, Bioorganic & Medicinal Chemistry 16 (2008) 5982–5998.
[27] W.G. Lachnit, A.M. Tran, D.E. Clarke, A.P. Ford, Pharmacological characterization of an alpha 1A-adrenoceptor mediating contractile responses to noradrenaline in isolated caudal artery of rat, British Journal of Pharmacology 120 (1997) 819–826.
[28] S. Jähnichen, M. Eltze, H.H. Pertz, Evidence that alpha(1B)-adrenoceptors are involved in noradrenaline-induced contractions of rat tail artery, European Journal of Pharmacology 488 (2004) 157–167.
[29] M. Eltze, R. Boer, M.C. Michel, P. Hein, R. Testa, W.R. Ulrich, N. Kolassa, K.H. Sanders, In vitro and in vivo uroselectivity of B8805-033, an antagonist with high affinity at prostatic alpha1A- vs. alpha1B- and alpha1D-adrenoceptors, Naunyn-Schmiedeberg's Archives of Pharmacology 363 (2001) 649–662.
[30] Y. Cheng, W. Prusoff, Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction, Biochemical Pharmacology 22 (1973) 3099–3108.
[31] M. Oz, L. Zhang, M. Morales, Endogenous cannabinoid, anandamide, acts as a noncompetitive inhibitor on 5-HT_3 receptor-mediated responses in Xenopus oocytes, Synapse 46 (2002) 150–156.
[32] M. Oz, M.T. Melia, N.M. Soldatov, D.R. Abernethy, M. Morad, Functional coupling of human L-type Ca^{2+} channels and angiotensin AT1A receptors coexpressed in xenopus laevis oocytes: involvement of the carboxyl-terminal Ca^{2+} sensors, Molecular Pharmacology 54 (1998) 1106–1112.