Bioorganic & Medicinal Chemistry Letters 20 (2010) 6152–6156

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Search for influence of spatial properties on affinity at α_1-adrenoceptor subtypes for phenylpiperazine derivatives of phenytoin

Jadwiga Handzlik [a], Heinz H. Pertz [b], Tilo Görnemann [b], Sven Jähnichen [b], Katarzyna Kieć-Kononowicz [a,*]

[a] Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Medyczna 9, PL 30-688 Kraków, Poland
[b] Institute of Pharmacy, Free University of Berlin, Königin-Luise Str. 2+4, 14195 Berlin, Germany

ARTICLE INFO

Article history:
Received 28 May 2010
Revised 23 July 2010
Accepted 25 July 2010
Available online 30 July 2010

Keywords:
Arylpiperazine derivatives
Phenytoin derivatives
α_1-Adrenoceptor subtypes

ABSTRACT

A series of phenylpiperazine derivatives of phenytoin was evaluated for their affinity at α_1-adrenoceptor subtypes in functional bioassays (rat tail artery: α_{1A} and/or α_{1B}; guinea pig spleen: α_{1B}; rat aorta: α_{1D}). The most potent compounds at α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors, **11**, **18** and **8**, showed affinities in the submicromolar range. The role of a hydrogen bond donor group for affinity and selectivity at α_{1B}-adrenoceptors, postulated by Bremner's pharmacophore model, was confirmed by functional and molecular modelling studies.

The α_1-adrenergic receptors (α_1-AR), belong to the G-protein coupled receptors that mediate actions in the sympathetic nervous system through the binding of the catecholamines, adrenaline and noradrenaline. Three α_1-AR subtypes have been cloned and are designed as α_{1A}, α_{1B} and α_{1D}.[1] Lines of evidence[2–10] have indicated therapeutic ability of α_1-adrenoceptor ligands that corresponds to various physiological effects mediated by these receptors. Although the early discovered α_1-AR antagonists are used in the treatment of hypertension, current works are focused on new therapeutic potency of α_1-adrenoceptors agents.[2] Some studies[4] described the role of α_1-adrenoceptor subtypes in arrhythmia. Indeed, α_{1A}-ARs are involved in the incidence of ventricular fibrillation in rat isolated hearts, whereas α_{1B}-AR subtypes may play a cardioprotective role.[4] In contrast, the role of the α_{1D}-AR in precipitating ischemic arrhythmia is not significant because of the low expression of this subtype in rat cardiomyocytes.[5] These results indicate that selective α_1-AR antagonists may be beneficial in the treatment of ischemic arrhythmia.

Recently, a number of promising subtype-selective α_1-adrenoceptor ligands has been described.[2,6–9] Structures of some subtype-selective α_1-adrenoceptor antagonists (Fig. 1) indicate that an arylpiperazine fragment appears in all, α_{1A}-, α_{1B} and α_{1D}-agents. The arylpiperazine derivative 5-methylurapidil (**1**) as well as the non-arylpiperazine KMD-3213 (**2**) were described as potent and selective α_{1A}-AR antagonists.[6,7] A constrained piperazine fragment appears in (+)-cyclazosin (**3**), the α_{1B}-selective antagonist,[8] whereas a non-piperazine α_{1B}-antagonist, spiperone (**4**), is also known as an antiserotonergic drug. Phenylpiperazine moieties are observed in many α_{1D}-AR antagonists, including WAY-100635 (**5**) and SNAP-8719 (**6**).[7]

On the other hand, the number of α_1-adrenoceptor ligands, with different subtypes-selectivity, including compounds **2**, **4** and **6**, was used by Bremner et al.[7] in a ligand-based drug design method to create three models of pharmacophores (Fig. 2), adequate for each, A-, B- or D-, α_1-AR subtype. To elaborate the pharmacophore models, Bremner et al. used 42 structures of compounds with described α_1-adrenoceptor subtypes affinities, estimated in binding or functional assays by the use of human- or animal α_1-ARs, expressed in various species.[7] In the case of α_{1A}-AR antagonists, distances of aromatic centre (A) and hydrogen bond acceptor (HBA) from positive ionisable moiety (P) as well as a planar angle created by HBA–P–A are spatial factors crucial for activity (Fig. 2a). The presence of a hydrogen bond donor (HBD) group and an additional hydrophobic moiety (H) together with P- and A-moieties are important for compounds with antagonist properties at the α_{1B}-AR (Fig. 2b). Pharmacophore model for α_{1D}-AR antagonists contains HBA- and H-moieties placed in similar distances from positive ionisable centre (Fig. 2c). The distances and angles, determined by Bremner et al.[7] for ideal α_{1A}-, α_{1B}- or α_{1D}-adrenoceptor antagonists, are shown in Figure 3.

In our previous work,[10,11] syntheses and pharmacological properties of new groups of hydantoin arylpiperazine derivatives were described including their affinities for α_1-adrenoceptors, evaluated in radioligand binding assays using rat brain cortex membranes,[10] as well as antiarrhythmic properties tested in vivo in rats.[11] A series of most active compounds **7–18** (Table 1) has been selected for further studies.

* Corresponding author. Tel.: +48 012 620 55 80; fax: +48 012 620 55 96.
E-mail address: mfkonono@cyf-kr.edu.pl (K. Kieć-Kononowicz).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.101

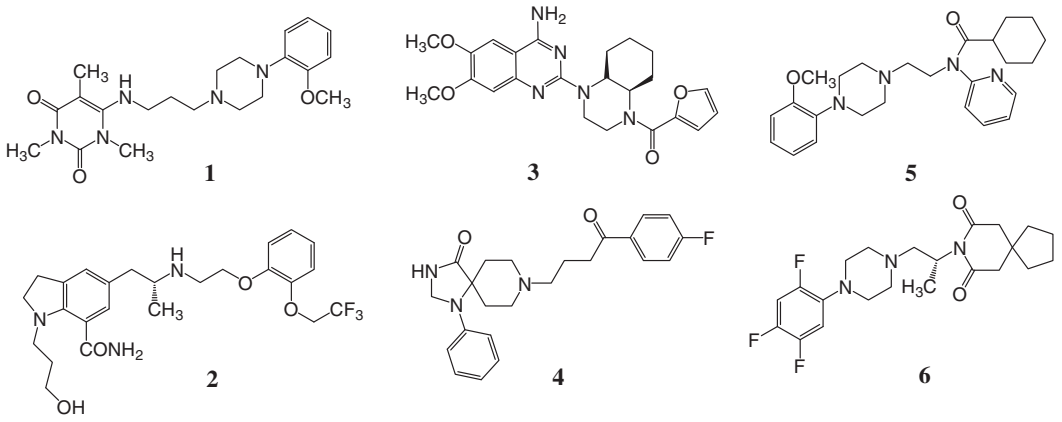


Figure 1. Selective ligands for α_1-adrenoceptor subtypes; α_{1A}-adrenoceptors selective antagonists: **1** (5-methylurapidil), **2** (KMD-3213); α_{1B}-adrenoceptors selective antagonists: **3** (cyclazosin), **4** (spiperone); α_{1D}-adrenoceptors selective antagonists: **5** (WAY-100635), **6** (SNAP-8719).

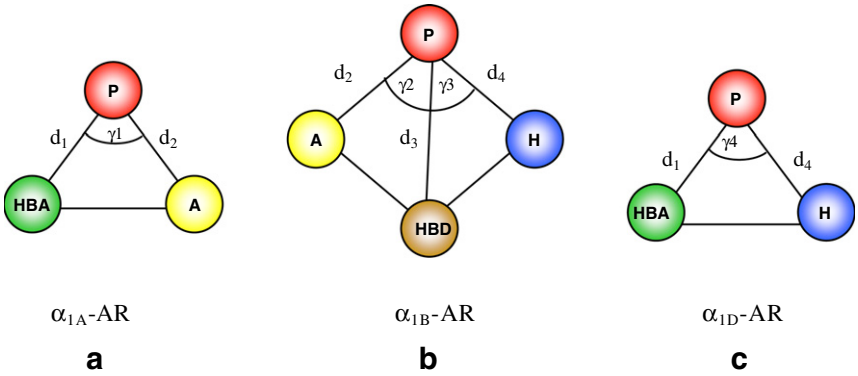


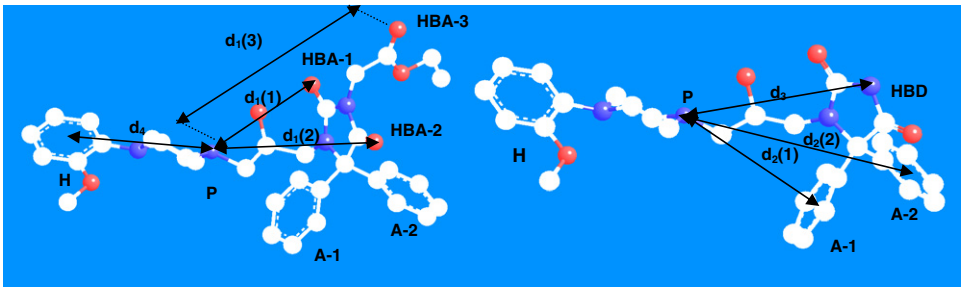
Figure 2. Pharmacophore models of subtype-selective α_1-AR antagonists elaborated by Bremner et al.[7] for α_{1A}- (a), α_{1B}-(b) and α_{1D}-(c) AR, respectively. P (red), protonated nitrogen atom; HBA (green), hydrogen bond acceptor; A (yellow) aromatic system; H (blue), hydrophobic group, HBD (brown) hydrogen bond donor. Receptor activities and subtypes-selectivity are determined by selected distances and angles value. Spatial descriptors, crucial for interaction with the receptor subtype: distances P–HBA (d_1) and P–A (d_2), an angle HBA–P–A (γ_1) for α_{1A}-AR; distances P–A (d_2), P–HBD (d_3) and P–H (d_4), angles A–P–HBD (γ_2) and HBD–P–H (γ_3) for α_{1B}-AR; distances P–HBA (d_1) and P–H (d_4), an angle HBA–P–H (γ_4) for α_{1D}-AR.

Relating to our radioligand binding studies, it has been shown by other groups that the rat brain cortex is not endowed with a unique population of α_1-AR subtypes; α_{1A}- and α_{1B}-ARs have been identified in this tissue but not α_{1D}-ARs.[12] Therefore, the present work is focused on an evaluation of compounds **7–18** for their affinity at the three α_1-AR subtypes, A, B or D, in functional bioassays. A further aim of the study is an analysis of geometrical features of the phenylpiperazine hydantoin derivatives **7–18**, responsible for their α_1-adrenoceptor subtypes-selectivity, based on Bremner's pharmacophore model.[7]

Compounds **7–18** were assessed for their antagonist affinity at α_1-adrenoceptor subtypes by inhibition of noradrenaline-induced contractions.[13] α_{1A}-ARs mediate the contractile response to noradrenaline in rat tail artery.[14] We could recently show that the rat tail artery is also endowed with contractile α_{1B}-ARs and that each component of contraction is separable by use of α_{1B}-AR alkylation and selective α_{1A}-AR blockade, respectively.[13] In addition, we used the guinea pig spleen as a bioassay for the α_{1B}-AR, because functional α_{1B}-ARs are also highly expressed in this tissue.[15] It should be mentioned that affinities for subtype-selective antagonists at α_{1B}-ARs of guinea pig spleen significantly correlated with rat liver α_{1B}-AR binding sites.[16] Moreover, subtype-selective antagonists showed similar affinities at guinea pig and rat α_{1B}-ARs.[16] Therefore, species differences appear not play a role in our studies on α_{1B}-ARs using rat and guinea pig tissue. The isolated rat thoracic aorta represents a well-established bioassay for α_{1D}-ARs.[17] In each case, two cumulative concentration–response curves to noradrenaline (NA) were determined on each tissue at an interval of 60 min in the absence and presence of antagonist. The full details for the functional bioassays method have been described in the Supplementary data. Affinities of compounds **7–18** for α_1-AR subtypes are summarised in Table 2; the blocking properties of selected compounds against noradrenaline are shown in Figure 4.

Compound **11**, 1-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)-2-hydroxypropyl)-3-methyl-5,5-diphenylimidazolidine-2,4-dione, showed the highest affinity at the α_{1A}-AR subtype (apparent $pA_2 = 7.59$), whereas methyl 2-(3-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-2,5-dioxo-4,4-diphenylimidazolidin-1-yl)propionate (**8**), an ester derivative, was the most potent at α_{1D}-AR (Table 2). In the case of α_{1B}-adrenoceptor, 1-(3-(4-(2-ethoxyphenyl) piperazin-1-yl)-2-hydroxypropyl)-5,5-diphenylimidazolidine-2,4-dione hydrochloride (**18**), a compound with free NH group at position 3, was the most active one (Table 2).

Generally, the phenylpiperazine derivatives of hydantoin **7–18** represent three groups of activities. The first group (**8–10**, **13–15**) contains compounds with higher activities at α_{1A}- and α_{1D}-adrenoceptor subtypes and significantly lower potency at α_{1B}-subtypes. Compounds **7**, **11** and **12** belong to the second group with the highest activity at α_{1A}-AR and significantly lower potency at both α_{1B}- and α_{1D}-subtypes. The both groups (**7–15**) possess alkyl- or ester substituent at N3-position of hydantoin. The third group, compounds with free NH-moiety in position 3 of hydantoin



Cpd	d1[Å] (P-HBA)	d2[Å] (P-A)	d3[Å] (P-HBD)	d4[Å] (P-H)	γ1[°] (A -P -HBA)	γ2[°] (A-P-HBD)	γ3[°] (H-P- HBD)	γ4[°] (H -P -HBA)
*α_{1A}	**7.1**	**5.5**	-	-	**100**	-	-	-
*α_{1B}	-	**6.2**	**4.9**	**7.8**	-	**52**	**57**	-
*α_{1D}	**4.5**	-	-	**5.4**	-	-	-	**47**
7	5.15 (1)	5.88(1)	-	5.68		-	-	44 (1)
	8.11 (2)	8.05(2)			122 (3)			
	8.13 (3)							
8	5.13 (1)	5.53(1)	-	5.68		-	-	44.3 (1)
	8.21 (2)	8.05(2)			138 (3)			
	9.61 (3)							
9	5.11 (1)	6.10(1)	-	5.68	139 (1)	-	-	43.9 (1)
	8.22 (2)	7.01(2)						
10	5.13 (1)	6.08(1)	-	5.68	140 (1)	-	-	43.8 (1)
	8.22 (2)	7.95(2)						
11	5.12 (1)	6.09(1)	-	5.68	139 (1)	-	-	43.6 (1)
	8.22 (2)	8.08(2)						
12	5.14 (1)	6.05(1)	-	5.68		-	-	43.9 (1)
	8.18 (2)	8.05(2)			124 (3)			
	8.58 (3)							
13	5.13 (1)	6.04(1)	-	5.68	124 (3)	-	-	44 (1)
	8.21 (2)	8.05(2)						
	8.61 (3)							
14	5.12 (1)	6.2(1)	-	5.69		-	-	44.2 (1)
	8.25 (2)	8.02(2)			123 (3)			
	8.23 (3)							
15	5.14 (1)	5.9(1)	-	5.69		-	-	44.5 (1)
	8.21 (2)	8.02(2)			124 (3)			
	8.13 (3)							
16	5.11 (1)	6.14(1)	7.4	5.69	138 (1)	41.8	145.8	43.6 (1)
	8.26 (2)	8.06(2)						
17	5.11 (1)	6.06(1)	7.4	5.69	139 (1)	42.3	146.4	43.6 (1)
	8.26 (2)	8.06(2)						
18	5.13 (1)	6.09(1)	7.39	5.69	140 (1)	42.8	146.7	43.6 (1)
	8.23 (2)	8.07(2)						

*Parameters of pharmacophore models

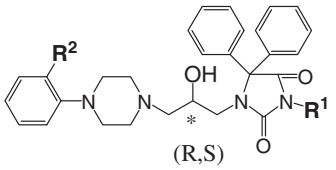
Figure 3. Structural parameters of compounds **7**–**18** in the comparison with Bremner's pharmacophore models[7] for α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor antagonists, respectively. Distances and angles, according to Figure 2, were evaluated based on molecular modelling calculation. Numbers in parenthesis indicate alternative HBA or A, according to those of compound **7** (shown in the pictures).

(**16–18**), displayed the highest activity at α_{1B}-AR subtypes (Table 2).

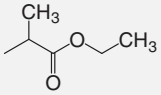
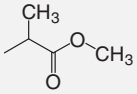
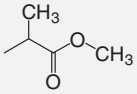
Molecular modelling was performed to search for 3D-structure properties of arylpiperazine derivatives (**7–18**) responsible for discrimination between the α_1-AR subtypes, A, B and D, based on three pharmacophore models of Bremner et al.[7] The calculations were carried out using *Schrödinger Maestro* molecular modelling environment.[18] The 3D molecule structures of compounds **7, 8** and **10–18** were built based on the crystal structure of **9**.[10] A conformational search was performed using the Monte Carlo method (MCMM) as implemented in MacroModel 9.7.[19] The geometry of the lowest energy structures obtained from the conformational analysis was finally optimised using density functional theory with Becke three-parameter B3LYP functional.[20,21] The 6-31G(d,p) basis set was applied. The DFT calculations were carried out using the GAUSSIAN 03 suite of programs.[22] The lowest-energy conformer for each compound was elected as representative for real 3D-structure, and its geometrical parameters were measured. Results of calculated spatial properties in comparison with Bremner's pharmacophore model are shown in Figure 3.

Concerning spatial properties of α_{1A}-adrenoceptor antagonist, distances d_1 (P–HBA), d_2 (P–A) and a planar angle γ_1 (A–P–HBA) were under consideration (Figs. 2 and 3). The hydantoin derivatives **7–18** possess two (**9–11**, **16–18**) or three (**7**, **8**, **12–15**) hydrogen bond acceptors (HBA-1, HBA-2, HBA-3) and two additional aromatic fragments (A-1, A-2). The distances d_1, possible for the compounds **7–18**, show low accordance with that for the ideal α_{1A}-antagonist described by Bremner et al.[7] (7.1 Å). Considering HBA at 2-position of hydantoin (HBA-1), the distance $d_1(1)$ is significantly lower (5.11–5.15 Å) than that of pharmacophore model. In the case of HBA on O4, described as $d_1(2)$, or on ester moieties, described as $d_1(3)$, the distances are longer than 8 Å for all tested compounds (Fig. 3). Distances between positive ionisable centre and one of the two aromatic rings, d_2 (P–A), show good agreements

Table 1
Structure of arylpiperazine derivatives of hydantoin **7–18** and their binding affinities (pK_i values) at α_1-adrenoceptors in rat brain cortex membranes. All compounds were obtained and tested in racemic form



x HCl

Compd	R^1	R^2	α_1-AR[a] pK_i
7	$-CH_2COOC_2H_5$	OCH_3	6.80
8		OCH_3	6.87
9	$-CH_3$	F	6.25
10	$-CH_3$	OCH_3	6.79
11	$-CH_3$	OC_2H_5	6.92
12	$-CH_2COOCH_3$	H	6.38
13	$-CH_2COOCH_3$	OCH_3	6.70
14		OCH_3	6.98
15		OC_2H_5	6.78
16	H	H	6.13
17	H	OCH_3	7.78
18	H	OC_2H_5	7.17

[a] pK_i calculated using K_i values described earlier.[10]

Table 2
Antagonist affinities (apparent pA_2 values) of phenylpiperazine derivatives **7–15** at α_{1A}-ARs in rat tail artery, α_{1B}-ARs in guinea pig spleen and α_{1D}-ARs in rat aorta (RA)

Compd	Affinity at α_1-AR subtypes; apparent pA_2[a]					
	α_{1A}[b]		α_{1B}		α_{1D}	
7	6.59 ± 0.07	4	5.71 ± 0.07	4	5.87 ± 0.05	4
8	7.19 ± 0.05	4	5.96 ± 0.08	4	7.15 ± 0.03	8
9	6.05 ± 0.04	4	5.74 ± 0.08	4	6.61 ± 0.13	4
10	6.80 ± 0.04	4	6.02 ± 0.04	4	6.85 ± 0.06	4
11	7.59 ± 0.06	4	6.07 ± 0.15	4	6.71 ± 0.02	6
12	5.86 ± 0.06	4	5.44 ± 0.10	4	5.36 ± 0.1	5
13	6.65 ± 0.06	4	5.90 ± 0.09	4	6.53 ± 0.02	4
14	6.81 ± 0.06	4	6.07 ± 0.11	4	6.46 ± 0.1	6
15	6.80 ± 0.09	4	6.03 ± 0.09	4	6.57 ± 0.11	4
16	5.98 ± 0.02	4	6.34 ± 0.10[c]	4	5.84 ± 0.06	4
17	6.87 ± 0.02	4	7.27 ± 0.07[c]	4	6.95 ± 0.05	4
18	7.01 ± 0.04	4	7.34 ± 0.04[c]	4	6.86 ± 0.09	4

[a] Calculated from the equation $pA_2 = -\log [B] + \log (r - 1)$. [B] is the molar concentration of the antagonist and r the ratio of agonist EC_{50} measured in the presence and absence of antagonist. The EC_{50} values were determined at the midpoint of each concentration–response curve.

[b] Affinities in rat tail artery following selective α_{1A}-AR protection with B8805-033 (3 μM) and alkylation of α_{1B}-ARs with chloroethylclonidine (100 μM).

[c] Affinities in rat tail artery in the continuous presence of the selective α_{1A}-AR antagonist B8805-033 (3 μM).

with that of ideal α_{1A}-adrenoceptor antagonist (5.5 Å) if the closer aromatic ring of hydantoin (A-1) is considered (5.53–6.20 Å). The best accordance is observed for compound **8** that displayed affinity at α_{1A}-subtype in the highest range (pA_2 = 7.19). In the whole population **7–18**, the distance d_2(1) is slightly longer (0.5–10%) than that of pharmacophore model. According to the Bremner's model,

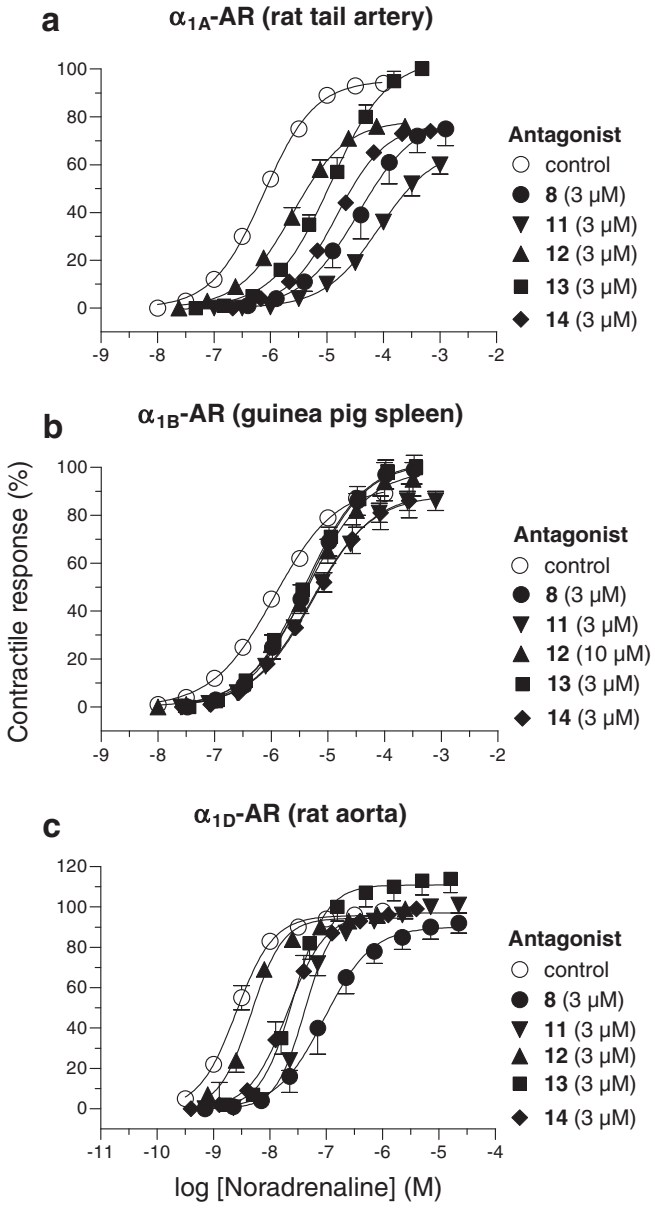


Figure 4. Contractile responses to noradrenaline (NA) in the absence and presence of compounds **8**, **11**, **12**, **13** and **14** in native tissues expressing α_{1A}-, α_{1B}- and α_{1D}-ARs. The data are means ± SEM (vertical bars) from 4–8 separate experiments.

an angle γ_1 should be in the range of 100° for an ideal α_{1A}-adrenoceptor antagonist. Considering several variants of the angles γ_1 in the group of phenylpiperazine derivatives **7–18**, obtuse angles, corresponding to that of Bremner's model, were found for closer aromatic ring (A-1) and HBA in ester part (**7**, **8**, **12–15**) or for HBA-1 on hydantoin O2-position (**9–11**, **16–18**). In both cases, the angle γ_1 values are significantly higher (22–40%) than that of an ideal α_{1A}-adrenoceptor antagonist. In the case of pharmacophore model for an ideal α_{1D}-adrenoceptor antagonist, distances d_1 and d_4 (P–H) as well as an angle γ_4 are limiting spatial factors. The three parameters are in close similarity for all compounds **7–18** which display slightly longer distances d_1 (12–13%) and d_2 (5%) than those of the α_{1D}-pharmacophore model and almost identical acute angle γ_4 (44–45°) as that of the Bremner's model (47°) in case of HBA-1 (Fig. 3). Despite of these results, the activities of compounds **7–18** (Table 2) at α_{1D}-adrenoceptors distinctly differed in values of pA_2 (pA_2 = 5.36–7.15). Bremner's model for an ideal α_{1B}-adrenoceptor antagonist is focused on five factors (Fig. 3), including

distances d_2–d_4 and angles γ_2 (A–P–HBD) and γ_3 (H–P–HBD). These factors can be detected only in compounds **16–18**. Compounds **7–15** are lack of hydrogen bond donor (HBD) at the *N*3-hydantoin fragment, a crucial feature for α_{1B}-affinity and selectivity.[7] Results of our functional bioassays confirmed this hypothesis of Bremner's pharmacophore model. Compounds with HBD at free NH-group (**16–18**) displayed distinct selectivity for α_{1B}-adrenoceptor subtype (Table 2) while the rest of compounds (**7–15**) showed the lowest activity at α_{1B}-AR comparing to both, α_{1A}- and α_{1D}-adrenoceptors (Table 2). Nevertheless, values of the additional factors (d_3, γ_2, γ_3), appearing in compounds **16–18**, are in a weak accordance with those of Bremner's α_{1B}-pharmacophore model. The distance d_3 (7.4 Å) is much longer than that of an ideal α_{1B}-antagonist (4.9 Å). The angles γ_2 and γ_3 were described by Bremner et al.[7] as acute angles, in a comparable range of 52° and 57°, respectively. In the case of compounds **16–18**, the angle γ_2 is slightly lower (42–43°) while the γ_3 one is in the huge divergence (146–147°). During the analysis of factors common for the whole population **7–18** (d_2 and d_4), it can be observed a good coherency with the pharmacophore model for d_2-values (5.88–6.20 Å) of **7–18** which differs from the ideal distance (6.20 Å) in 0–10%, respectively (Fig. 3). The highest accordance has been observed in the case of compound **14** that was a most active at α_{1B}-AR (Table 2) among compounds without HBD-moiety (**7–15**). The distances d_4 (P–H), calculated for all population **7–18**, are much lower (5.7 Å) than that of the ideal α_{1B}-antagonist described by Bremner et al.[7] These quantitative differences in the distances and angles in comparison to pharmacophore features might be responsible for some decrease of activities of compounds **7–18** comparing to subnanomolar activity of reference antagonists (Fig. 1).

In conclusion, results from functional bioassays, performed for phenylpiperazine derivatives of hydantoin **7–18**, confirmed the submicromolar range of affinities of the compounds for α_1-adrenoceptors obtained in radioligand binding assays. SAR-studies indicated the influence of *N*3-hydantoin substituent on α_1-adrenoceptor subtype-selectivity. Compounds with *N*3-alkyl- or *N*3-ester end had much higher activities at α_{1A}- and/or α_{1D}-adrenoceptors than those at α_{1B}-adrenoceptors, whereas compounds with an unsubstituted fragment N3–H, creating a hydrogen bond donor, were, distinctly, the most active at α_{1B}-adrenoceptor subtypes. Pharmacophore based molecular modelling studies indicated that the results have been in a good qualitative agreement with Bremner's pharmacophore model elaborated for each α_1-adrenoceptor subtype, A, B and D. Especially, the role of a hydrogen bond donor for the affinity and selectivity at α_{1B}-adrenoceptors, postulated by Bremner et al.,[7] was distinctly confirmed by the present study. Some quantitative divergence from ideal distances and angles, postulated in Bremner's pharmacophore model, was observed that could be considered as factors limiting receptor affinity and selectivity in the presented group of phenylpiperazine hydantoin derivatives.

Acknowledgement

The authors would like to thank Dr. Ewa Szymańska and Dr. Jarosław Handzlik for help in the performance of molecular modelling. The works were partly supported by grant K/ZDS/000727. Computing resources from Academic Computer Centre CYFRONET AGH (Grants MNiSW/SGI3700/UJ/078/2008) are gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.101.

References and notes

1. (a) Bylund, D. B.; Eikenberg, D. C.; Hieble, J. P.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Molinoff, P. B.; Ruffolo, R. R., Jr. *Pharmacol. Rev.* **1994**, *46*, 121; (b) Docherty, J. R. *Eur. J. Pharmacol.* **1998**, *361*, 1; (c) Guimarães, S.; Moura, D. *Pharmacol. Rev.* **2001**, *53*, 319; (d) Alexander, S. P.; Mathie, A.; Peters, J. A. *Br. J. Pharmacol.* **2009**, *158*, S1.
2. Rosini, M.; Bolognesi, M. L.; Giardinà, D.; Minarini, A.; Tumiatti, V.; Melchiorre, C. *Curr. Top. Med. Chem.* **2007**, *7*, 147.
3. (a) Kulig, K.; Malawska, B. *Curr. Med. Chem.* **2006**, *13*, 3395; (b) Michel, M. C.; Vrydag, W. *Br. J. Pharmacol.* **2006**, *147*, S88.
4. (a) Woodcock, E. A. *Clin. Exp. Pharm. Phys* **2007**, *34*, 884; (b) Tölg, R.; Schreieck, J.; Kurz, T.; Katus, H. A.; Richardt, G. *Basic Res. Cardiol.* **2001**, *96*, 68; (c) Tölg, R.; Kurz, T.; Ungerer, M.; Schreieck, J.; Görge, B.; Richardt, G. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1997**, *356*, 62.
5. Mier, K.; Kemkem, D.; Katus, H.; Richardt, G.; Kurz, T. *Cardiovasc. Res.* **2002**, *54*, 133.
6. Ishiguro, M.; Futabayashi, Y.; Ohnuki, T.; Ahmed, M.; Muramatsu, I.; Nagatomo, T. *Life Sci.* **2002**, *71*, 2531.
7. (a) Bremner, J. B.; Coban, B.; Griffith, R.; Groenewoud, K. M.; Yates, B. F. *Bioorg. Med. Chem.* **2000**, *8*, 201; (b) Bremner, J. B.; Coban, B.; Griffith, R. *J. Comput. Aided. Mol. Des.* **1996**, *10*, 545.
8. (a) Giardina, D.; Crucianelli, M.; Romanelli, R.; Leonardi, A.; Poggesi, E.; Melchiorre, C. *J. Med. Chem.* **1996**, *39*, 4602; (b) Giardina, D.; Sagratini, G.; Grelli, M.; Gulini, U.; Melchiorre, C.; Antolini, L. *Farmaco* **2004**, *59*, 965.
9. (a) Manetti, F.; Corelli, F.; Strappaghetti, G.; Botta, M. *Curr. Med. Chem.* **2002**, *9*, 1303; (b) Romeo, G.; Materia, L.; Marucci, G.; Modica, M.; Pittalá, V.; Salerno, L.; Siracusa, M. A.; Buccioni, M.; Angeli, P.; Minneman, K. P. *Bioorg. Med. Chem. Lett.* **2007**, *16*, 6200; (c) Siracusa, M. A.; Salerno, L.; Modica, M. N.; Pittala, V.; Romeo, G.; Amato, M. E.; Nowak, M.; Bojarski, A. J.; Mereghetti, I.; Cagnotto, A.; Mennini, T. *J. Med. Chem.* **2008**, *51*, 4529.
10. Handzlik, J.; Maciąg, D.; Kubacka, M.; Mogilski, S.; Filipek, B.; Stadnicka, K.; Kieć-Kononowicz, K. *Bioorg. Med. Chem.* **2008**, *16*, 5982.
11. Dyląg, T.; Zygmunt, M.; Maciąg, D.; Handzlik, J.; Bednarski, M.; Filipek, B.; Kieć-Kononowicz, K. *Eur. J. Med. Chem.* **2004**, *39*, 1013.
12. (a) Morrow, A. L.; Creese, I. *Mol. Pharmacol.* **1986**, *29*, 321; (b) Hanft, G.; Gross, G. *Br. J. Pharmacol.* **1989**, *97*, 691; (c) Sallés, J.; Badia, A. *Eur. J. Pharmacol.* **1994**, *15*, 301; (d) Yang, M.; Verfürth, F.; Büscher, R.; Michel, M. C. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1997**, *355*, 438; (e) Morishima, S.; Suzuki, F.; Yoshiki, H.; Md Anisuzzaman, A. S.; Sathi, Z. S.; Tanaka, T.; Muramatsu, I. *Br. J. Pharmacol.* **2008**, *153*, 1485.
13. Jähnichen, S.; Eltze, M.; Pertz, H. H. *Eur. J. Pharmacol.* **2004**, *488*, 157.
14. Lachnit, W. G.; Tran, A. M.; Clarke, D. E.; Ford, A. P. D. W. *Br. J. Pharmacol.* **1997**, *120*, 819.
15. Eltze, M. *Eur. J. Pharmacol.* **1994**, *260*, 211.
16. (a) Eltze, M. *Eur. J. Pharmacol.* **1996**, *295*, 69; (b) Burt, R. P.; Chapple, C. R.; Marschall, I. *Br. J. Pharmacol.* **1995**, *115*, 467.
17. Van der Graaf, P. H.; Shankle, N. P.; Black, J. W. *Br. J. Pharmacol.* **1996**, *118*, 299.
18. Schrödinger Suite 2009: Maestro 9.0, Schrödinger, LLC, New York, NY, 2009.
19. Schrödinger Suite 2009: MacroModel 9.7, Schrödinger, LLC, New York, NY, 2009.
20. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
21. Stevens, P. J.; Devlin, J. F.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623.
22. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. GAUSSIAN 03, Revision E.01; Gaussian: Wallingford CT, 2004.