Synthesis and In Vitro Pharmacological Evaluation of Novel 2-Hydroxypropyl-4-arylpiperazine Derivatives as Serotoninergic Ligands

ARTICLE *in* ARCHIV DER PHARMAZIE · OCTOBER 2014

Impact Factor: 1.53 · DOI: 10.1002/ardp.201400174

Full Paper

Synthesis and *In Vitro* Pharmacological Evaluation of Novel 2-Hydroxypropyl-4-arylpiperazine Derivatives as Serotoninergic Ligands

Ferdinando Fiorino[1], Elisa Magli[1], Beatrice Severino[1], Angela Corvino[1], Antonio Ciano[1], Elisa Perissutti[1], Francesco Frecentese[1], Paola Massarelli[2], Cristina Nencini[2], Vincenzo Santagada[1], and Giuseppe Caliendo[1]

[1] Dipartimento di Farmacia, Università degli Studi di Napoli "Federico II", Napoli, Italy
[2] Dipartimento di Scienze Mediche, Chirurgiche e Neuroscienze, Università di Siena, Siena, Italy

This paper reports the synthesis of new norbornene and exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide derivatives and their binding to the 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors, in order to identify selective ligands for these 5-hydroxytryptamine (5-HT, serotonine) receptor subtypes. The combination of structural elements (heterocyclic nucleus, hydroxyalkyl chain, and 4-substituted piperazine) known to be critical for affinity to 5-HT_{1A} receptors and the proper selection of substituents led to compounds with high specificity and affinity toward serotoninergic receptors. The most active compounds were selected and further evaluated for their binding affinities to D_1, D_2 dopaminergic and α_1, α_2 adrenergic receptors. 4-[3-[4-(2-Furoyl)piperazin-1-yl]propoxy-2-ol]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione **3e** with $K_i = 5.04 \pm 0.227$ nM was the most active and selective derivative for the 5-HT_{2C} receptor with respect to other serotonin receptors, and the most selective derivative versus dopaminergic and adrenergic receptors.

Keywords: Arylpiperazine derivatives / *exo-N*-Hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide / 5-HT_{1A} receptor ligands / Norbornene nucleus / Serotonin

Received: May 6, 2014; Revised: June 18, 2014; Accepted: June 24, 2014

DOI 10.1002/ardp.201400174

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter in the central and peripheral nervous systems (CNS and PNS, respectively), implicated in numerous physiological and pathophysiological processes. The effects of 5-HT are mediated by a distinctive class of the G-protein coupled receptors (GPCRs) [1] except for 5-HT_3 receptor, which is a cation channel. There are six classes of GPCR 5-HT receptors: 5-HT_1, 5-HT_2, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 with additional subclasses amounting to 15 receptors. Serotonin receptors (5-HTRs) may be involved in impulsivity and alcoholism and in the different phases of sleep, sexual behavior, appetite control, thermoregulation, and cardiovascular function. In addition, it is well established that 5-HT plays a fundamental role in regulation of growth, differentiation, and gene expression. Moreover, serotonin is known to act as a growth factor on several types of non-tumoral and tumoral cells, and recently it was also related to oncogenes. Pharmacological manipulation of the 5-HT system is believed to have therapeutic potential, and therefore, it has been the subject of intense research [2].

In particular, 5-HT_{1A} receptor shows effects on a wide range of psychiatric disorders, and also it is involved in the proliferation of human tumor cells (PC3) and in human hormone refractory prostate cancer tissue [3]. The 5-HT_2 receptor family has three known subtypes, 5-HT_{2A}, 5-HT_{2B}, and

Correspondence: Dr. Ferdinando Fiorino, Dipartimento di Farmacia, Università degli Studi di Napoli "Federico II", Via D. Montesano, 49, 80131 Napoli, Italy.
E-mail: fefiorin@unina.it
Fax: +39 081 678649

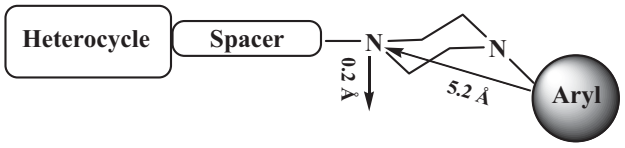


Figure 1. General structure of long-chain arylpiperazines (LCAPs) and pharmacophoric model of 5-HT_{1A} agonist.

5-HT_{2C}; activation of 5-HT_{2A} receptors stimulates the secretion of various hormones and influences neuronal plasticity; peripheral 5-HT_{2A} receptors mediate several processes such as vasoconstriction and platelet aggregation [4]. The 5-HT_{2C} receptor is involved in physiological functions such as locomotory activity, anxiogenesis, and neuro-endocrine functions, and is implicated in sexual dysfunction in males [5, 6]. Numerous chemicals are already known for their high affinity toward these receptors and, from a chemical point of view, they can be subdivided into different classes. Among them, *N*-1-substituted *N*-4-arylpiperazines (so-called "long-chain arylpiperazines" or LCAPs) have been extensively studied as a structural element useful in designing 5-HT_{1A} receptor ligands. The general formula of these compounds presents a 1-arylpiperazine moiety linked via an alkyl chain of variable length to a terminal fragment (imides, amides, alkyl, arylalkyl, or heteroarylalkyl derivatives and tetralines) [7]. The identification of new 5-HT_{1A} receptor agonists led to the definition of the generally accepted 5-HT_{1A} agonist pharmacophore, in which an aromatic ring and a coplanar basic nitrogen atom are two of the structural features required for ligand recognition [8].

The distance between the aromatic center and the basic nitrogen is optimal at 5.2 Å, with the amino group 0.2 Å above the plane of the aromatic moiety. Finally, the electron lone pair is almost perpendicular to the plane of the aromatic ring (Fig. 1) [7, 8].

However, a limitation of many 5-HT_{1A} receptor ligands for their potential use as drugs or pharmacological tools is their undesired high affinity for other receptors. The dopaminergic D_2 receptor and α_1-adrenoceptor are two examples of receptors for which several 5-HT_{1A} ligands show high affinity. In our laboratories, there has been an ongoing effort to develop more selective serotoninergic ligands in order to have novel pharmacological tools that could improve our knowledge of the signal transduction mechanism leading to compounds with high affinity and selectivity [9–18]. In continuation of our research program, we designed a new set of derivatives where the piperazine-N-alkyl moiety has been linked via a 2-hydroxy-propyl spacing unit to a norbornene and an exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide nucleus, respectively. The norbornene nucleus was already investigated by our group, in order to develop arylpiperazine derivatives with high affinity and selectivity for the 5-HT_{1A} receptor [14, 16].

In this study, the bicyclic nucleus (norbornene) together with the analog exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide is linked to some of the most studied 4-substituted piperazines (Fig. 2). Moreover, the choice of the 2-hydroxy-propyl spacing unit was done in order to further verify if this structural subunit could influence the pattern of ligand–receptor interaction as already described [19]. Furthermore, the hydroxyl group could serve as a handle to modify the pharmacokinetic properties of the molecules if needed. All the new compounds were tested for their affinity for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors. Moreover, the multireceptor profiles of promising derivatives were also evaluated in terms of binding affinities for dopaminergic D_1, D_2 and α_1, α_2 adrenoreceptors.

Results and discussion

Chemistry

The general strategy for the synthesis of the target compounds (Table 1) is summarized in Scheme 1. The general procedure is as follows: alkylation of the starting 4-X-substituted-piperazines with epichlorhydrin in absolute ethanol gave

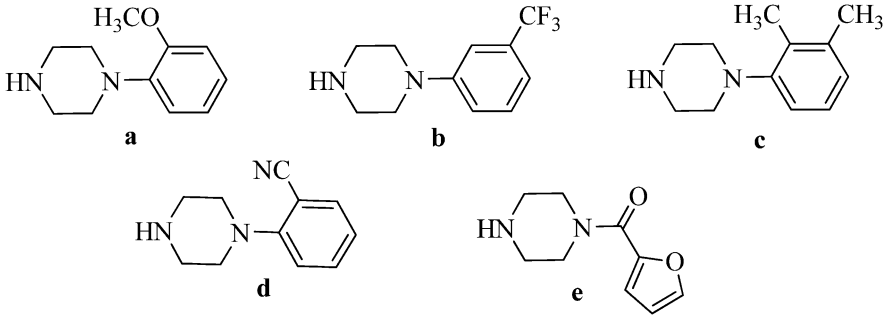
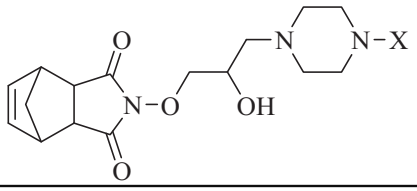
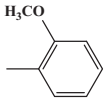
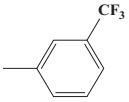
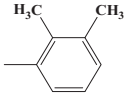
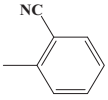
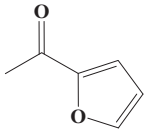


Figure 2. Arylpiperazines used for the synthesis of derivatives **3a–e** and **4a–e**.

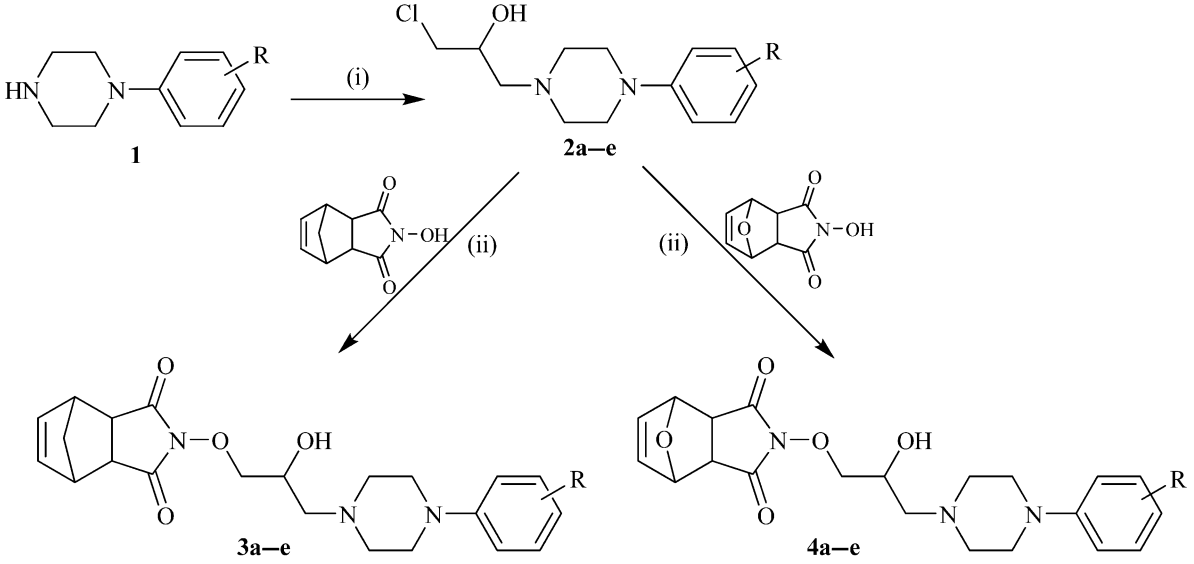
Table 1. Affinities of compounds **3a–e** for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors.



		Receptor affinity $K_i \pm$ SD (nM)		
Compd.[a)]	**X**	**5-HT_{1A} [^{3}H]-8-OH-DPAT**	**5-HT_{2A} [^{3}H]ketanserin**	**5-HT_{2C} [^{3}H]mesulergine**
3a		3.61 ± 1.094	$>10^4$	0.836 ± 0.702
3b		1.85 ± 1.479	2270 ± 0.707	111 ± 0.157
3c		3.58 ± 1.173	204 ± 0.203	$>10^4$
3d		43.1 ± 0.195	133 ± 0.126	$>10^4$
3e		1050 ± 0.231	$>10^4$	5.04 ± 0.227

For purpose of comparison, 8-OH-DPAT, ketanserine, and mesulergine bind 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors with values of 0.80, 0.85, and 1.90 nM, respectively, under these assay conditions.

[a)] All the final compounds were obtained and tested as racemic mixtures (*R*,*S*); $[\alpha]^{25}D = \pm 0.01°$ ($c = 0.01$, MeOH).



Scheme 1. Reagents and conditions: (i) Cl–CH_2–CH(O)CH_2, EtOH abs; (ii) NaOH pellets, EtOH abs.

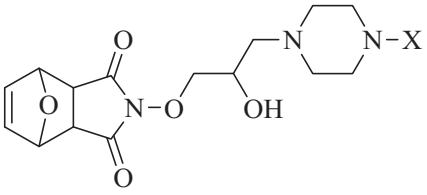
the corresponding 3-chloro-2-hydroxy-propyl-4-X-substituted-piperazines **2a–e**. The obtained intermediates were condensed with the desired heterocycle endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide or exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide, in presence of NaOH pellets in absolute ethanol, to give the final compounds **3a–e** and **4a–e**. Purification of each final product was carried out by chromatography on silica gel column and further by crystallization from the appropriate solvent. All new compounds gave satisfactory elemental analyses and were characterized by ^{1}H NMR and mass spectrometry (API 2000 Applied Biosystem). ^{1}H NMR, MS, and optical data for all final compounds were consistent with the proposed structures that have been obtained as racemic mixtures.

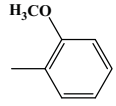
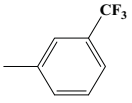
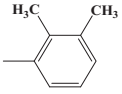
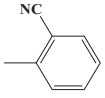
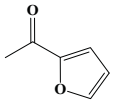
Pharmacological results

The 10 piperazine derivatives (**3a–e** and **4a–e**) were evaluated for their binding affinity and selectivity toward 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2C}$ receptors and some of the molecules showed affinity in the nanomolar range toward these receptors (Tables 1 and 2). The compounds showing the highest affinity toward serotonin receptors were selected and evaluated for their binding affinities for dopaminergic D_1, D_2 and α_1, α_2 adrenoreceptors (Table 3). Besides the outstanding 5-HT$_{1A}$ receptor affinity of compound **3b** ($K_i = 1.85$ nM), other interesting K_i values were those of compounds **3c** ($K_i = 3.58$ nM) and **4b** ($K_i = 3.33$ nM), while **3d**, **4c**, **4d**, and **4e** were less active with K_i values of 43.1, 509, 2080, and $>10^4$, respectively. Compound **3e** showed the most interesting affinity/selectivity profile toward 5-HT$_{2C}$ receptor ($K_i = 5.04$ nM), whereas compounds **3a** and **4a** presented a mixed 5-HT$_{1A}$/5-HT$_{2C}$ affinity ($K_i = 3.61/0.836$ nM and 1.40/0.861 nM, respectively).

Additionally, the affinity of the most active compounds (**3a**, **3b**, **3c**, **3e**, **4a**, and **4b**) on several other receptors (α_1 and α_2 adrenergic and D_1 and D_2 dopaminergic receptors) was examined in order to verify the selectivity of these compounds. Results are summarized in Table 3. All the compounds proved high selectivity against dopaminergic

Table 2. Affinities of compounds **4a–e** for 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.



		Receptor affinity $K_i \pm$ SD (nM)		
Compd.[a)]	**X**	**5-HT$_{1A}$ [^{3}H]-8-OH-DPAT**	**5-HT$_{2A}$ [^{3}H]ketanserin**	**5-HT$_{2C}$ [^{3}H]mesulergine**
4a		1.40 ± 1.091	$>10^4$	0.861 ± 0.064
4b		3.33 ± 1.105	1490 ± 0.138	$>10^4$
4c		509 ± 0.090	360 ± 0.181	$>10^4$
4d		2080 ± 0.107	No affinity	$>10^4$
4e		$>10^4$	$>10^4$	$>10^4$

For purpose of comparison, 8-OH-DPAT, ketanserine, and mesulergine bind 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2C}$ receptors with values of 0.80, 0.85, and 1.90 nM, respectively, under these assay conditions.

[a)] All the final compounds were obtained and tested as racemic mixtures (*R*,*S*); $[\alpha]^{25}D = \pm 0.01°$ ($c = 0.01$, MeOH).

Table 3. Affinities of compounds **3a**, **3b**, **3c**, **3e**, **4a**, and **4b** for D_1, D_2, α_1, and α_2 receptors.

	Receptor affinity $K_i \pm$ SD (nM)			
Compd.	**D_1 [^{3}H]SCH-23390**	**D_2 [^{3}H]spiperone**	**α_1 [^{3}H]prazosin**	**α_2 [^{3}H]yohimbine**
3a	$>10^4$	$>10^4$	311 ± 0.383	$>10^4$
3b	$>10^4$	$>10^4$	1920 ± 0.269	$>10^4$
3c	$>10^4$	$>10^4$	$>10^4$	264 ± 0.536
3e	$>10^4$	$>10^4$	$>10^4$	$>10^4$
4a	$>10^4$	$>10^4$	1050 ± 0.356	$>10^4$
4b	$>10^4$	70.4 ± 0.264	$>10^4$	621 ± 0.613

receptors with K_i values of above 10^4 nM except for compound **4b**, which exhibited K_i value of 70.4 nM on D_2 receptor. Regarding α_1 and α_2 adrenergic receptors, only compounds **3a** ($K_i = 311$ nM) and **3c** ($K_i = 264$ nM) showed quite moderate affinity towards α_1 and α_2 receptors, respectively; the good selectivity of the tested compounds is very interesting considering the high degree of homology existing between the considered receptors and demonstrate that these compounds possess a very good binding profile.

These results further support the choice of the norbornene and exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide as heterocyclic nuclei suitable for the preparation of serotoninergic ligands endowed with high affinity toward 5-HT_{1A} and 5-HT_{2C} receptors.

The difference in affinity observed between this new series of derivatives and the previously described norbornene series [14, 16] demonstrates that, besides the substituents on the N-4 of the piperazine moiety and the nature of heterocyclic nucleus, also alkyl chain represents a critical structural feature in determining 5-HT_{1A} receptor affinity and selectivity. Consequently, the new derivatives characterized by the same norbornene scaffold or by the analog exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide nucleus linked via 2-hydroxy-propyl spacing unit to some of the most studied 4-substituted piperazines (**3a**, **3b**, **4a**, and **4b**), exhibit a lower or higher affinity and selectivity for this receptor. Moreover, the derivatives **4a–e** differ from derivatives **3a–e** for an isosteric substitution of a methylene group with an oxygen atom that does not seem to represent a critical feature in determining differences in binding with 5-HTR_s, thus leading to two series of derivatives (**3a–e** and **4a–e**) characterized by a similar binding profile toward these receptors.

The influence of the 2-hydroxy-propyl spacer was already described for a series of arylpiperazine derivatives containing a hydantoin [20] or an imidazole nucleus [21]. This structural element associated to the appropriate substituents on phenylpiperazine ring and heterocyclic nucleus were particularly profitable not only for affinity and selectivity for 5-HT_{1A} but also toward 5-HT_{2A} and 5-HT_{2C} receptors. This effect could be correlated, as already proposed [20], to an intramolecular hydrogen bond involving the hydroxyl group and the N-1 of the piperazine moiety [22]. Consequently in our compounds, the distance between the hydrophobic heterocyclic scaffold and the basic center of the piperazine moiety is shorter and is in accordance to the Leppailleur's pharmacophore model for 5-HT_{1A} receptor [23]. The 4-[3-[4-(2-furoyl)piperazin-1-yl]propoxy-2-ol]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione **3e** with $K_i = 5.04$ nM was the most active and selective derivative for the 5-HT_{2C} receptor with respect to other serotoninergic, dopaminergic, and adrenergic receptors. This result is particularly interesting also because there are few evidences regarding the interaction of the LCAPs structures on the 5-HT_{2C} receptor. Only few piperazine analogs were disclosed as potent and selective 5-HT_{2C} agonists, but unfortunately no supporting *in vivo* data were reported [24]. Additionally, the mixed 5-HT_{1A}/5-HT_{2C} profile ($K_i = 3.61/0.836$ nM and 1.40/0.861, respectively) shown by compounds **3a** and **4a** is of particular interest and outlines a potential atypical antipsychotic profile for these derivatives. In summary, we have synthesized a new series of 4-substituted piperazines linked to a norbornene nucleus or to the analog exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide nucleus via a 2-hydroxy-propyl spacing unit. The binding data presented in this study have shed additional light on the influence of the LCAPs on the 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors affinity and selectivity. In accordance with reported data, also alkyl chain represents a critical structural feature in determining 5-HT_{1A} receptor affinity and selectivity. Consequently, comparing the here reported results with those obtained with analog derivatives containing a propyl chain between the norbornene nucleus and the piperazine ring [14, 16], the presence of a 2-hydroxy-propyl spacing unit is not always tolerated. In fact, the association of this structural feature to *o*-methoxyphenylpiperazine and *m*-trifluoromethylpiperazine (**3a** and **3b**) determined a loss of selectivity toward 5-HT_{1A} receptor. On the contrary, the presence of the 2-hydroxy-propyl group is beneficial in conjunction with 2,3-dimethylphenylpiperazine and 2-furoylpiperazine (**3c** and **3e**), if compared to the corresponding propyl derivatives [16], affording compounds

with a better affinity/selectivity profile toward 5-HT_{1A} and 5-HT_{2C}, respectively. Simultaneously, we have disclosed some interesting compounds as 5-HT_{2C} (**3e**) and mixed 5-HT_{1A}/5-HT_{2C} ligands (**3a** and **4a**), with a potential therapeutical profile as antiepileptic, anxiolytic, or atypical antipsychotic agents.

Experimental

Synthesis

General procedures

All reagents and substituted piperazines were commercial products purchased from Aldrich. Melting points, determined using a Buchi Melting Point B-540 instrument, are uncorrected and represent values obtained on recrystallized or chromatographically purified material. ^{1}H NMR spectra were recorded on Varian Mercury Plus 400 MHz instrument. Unless otherwise stated, all spectra were recorded in $CDCl_3$. Chemical shifts are reported in ppm. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), m (multiplet), q (quartet), qt (quintet), dd (doublet of doublet), bs (broad singlet), and mm (multiplet of multiplet). Mass spectra of the final products were performed on API 2000 Applied Biosystem mass spectrometer. Optical rotation (α) of the racemic mixture was evaluated by JASCO P-2000 optical activity polarimeter. Elemental analyses were carried out on a Carlo Erba model 1106; analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values. All reactions were followed by TLC, carried out on Merck silica gel 60 F_{254} plates with fluorescent indicator and the plates were visualized with UV light (254 nm). Preparative chromatographic purifications were performed using silica gel column (Kieselgel 60). Solutions were dried over Na_2SO_4 and concentrated with a Buchi R-114 rotary evaporator at low pressure.

*General procedure for the synthesis of 1-chloro-3-(4-substitued-arylpiperazin-1-yl)propan-2-ol (**2a–e**)*

To a solution of the appropriate 4-substituted arylpiperazine (**1**) (1 g, 0.005 mol) in absolute ethanol (35 mL), epichlorohydrin (0.462 g, 0.005) was added dropwise and the reaction mixture was stirred overnight at room temperature. After evaporation, the crude products were recrystallized from diethyl ether to give intermediates **2a–e** as solids (yield ranging between 55 and 71%). ^{1}H NMR spectra for all intermediates were consistent with the proposed structures.

*General procedure for the reaction of endo-N-hydroxy-5-norbornene-2,3-dicarboximide and exo-N-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide with derivatives **2a–e** (**3a–e**, **4a–e**)*

A solution of absolute ethanol (35 mL) and 0.2 g (0.05 mol) of sodium hydroxide was reacted with 1 g (0.005 mol) of commercially available endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide or exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide and 1.420 g (0.005 mol) of the appropriate 1-chloro-3-(4-substitued-arylpiperazin-1-yl)propan-2-ol (**2a–e**) at 70°C for 24 h. Afterwards, the mixture was cooled to room temperature, concentrated to dryness, and the residue diluted in water (40 mL). The solution was extracted several times with CH_2Cl_2. The combined organic layers were dried on anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2/methanol 9:1 v/v). The combined and evaporated product fractions were crystallized from diethyl ether yielding the desired products (**3a–e** and **4a–e**) as white solids.

The analytical data of **3a–e** and **4a–e** and the ^{1}H NMR spectrum of **3a** are provided online as Supporting Information.

*Synthesis of 4-[3-[4-(o-methoxyphenyl)piperazin-1-yl]-propoxy-2-ol]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3a**)*

From **2a** and endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide. Yield: 77%; m.p.: 124–125°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.52 (d, 1H, $J = 8.9$); 1.75 (d, 1H, $J = 8.9$); 2.45 (dd, 1H, CH_2–N, $J = 4.0$, $J = 8.8$); 2.55 (dd, 1H, CH_2–N, $J = 4.2$, $J = 8.1$); 2.66 (bs, 2H, CH_2 pip); 2.77 (bs, 2H, CH_2 pip); 3.07 (bs, 4H, $2CH_2$ pip); 3.21 (s, 2H); 3.43 (s, 2H); 3.85 (s, 3H, $-OCH_3$); 3.89–3.96 (m, 1H, CH–OH); 4.06 (dd, 2H, O–CH_2, $J = 3.0$, $J = 7.0$); 6.16 (s, 2H); 6.84–7.00 (mm, 4H, ArH). ESI-MS: 428.21 $[M+H]^+$; 450.0 $[M+Na]^+$ (Calcd: 427.49). Anal. ($C_{23}H_{29}N_3O_5$), C, H, N.

*Synthesis of 4-[3-[4-(3-trifluoromethylphenyl)piperazin-1-yl]propoxy-2-ol]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3b**)*

From **2b** and endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide. Yield: 83%; m.p.: 140–142°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.51 (d, 1H, $J = 8.9$); 1.75 (d, 1H, $J = 8.9$); 2.59 (m, 2H, CH_2–N); 2.72 (bs, 4H, $2CH_2$ pip); 2.78 (bs, 2H, CH_2 pip); 3.2 (bs, 2H, $2CH_2$ pip); 3.26 (s, 2H); 3.44 (s, 2H); 3.91–3.98 (m, 1H, CH–OH); 4.07 (dd, 2H, O–CH_2, $J = 3.0$, $J = 7.0$); 6.17 (s, 2H); 7.04–7.36 (mm, 4H, ArH). ESI-MS: 466.3 $[M+H]^+$; 488.20 $[M+Na]^+$ (Calcd: 465.47). Anal. ($C_{23}H_{26}F_3N_3O_4$), C, H, N.

*Synthesis of 4-[3-[4-(2,3-dimethylphenyl)piperazin-1-yl]-propoxy-2-ol]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3c**)*

From **2c** and endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide. Yield: 70%; m.p.: 100–101°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.50 (d, 1H, $J = 8.9$); 1.76 (d, 1H, $J = 8.9$); 2.20 (s, 3H, CH_3); 2.25 (s, 3H, CH_3); 2.48 (dd, 1H, CH_2–N, $J = 4.0$, $J = 8.8$); 2.55 (dd, 1H, CH_2–N, $J = 4.2$, $J = 8.1$); 2.63 (bs, 2H, CH_2 pip); 2.72 (bs, 2H, CH_2 pip); 2.88 (bs, 4H, $2CH_2$ pip); 3.22 (s, 2H); 3.44 (s, 2H); 3.91–3.94 (m, 1H, CH–OH); 4.07 (dd, 2H, O–CH_2, $J = 3.0$, $J = 7.0$); 6.17 (s, 2H); 6.88 (d, 2H, $J = 7.6$); 7.07 (t, 1H, $J = 7.6$). ESI-MS: 426.3 $[M+H]^+$ (Calcd: 425.52). Anal. ($C_{24}H_{31}N_3O_4$), C, H, N.

*Synthesis of 4-[3-[4-(2-cyanophenyl)piperazin-1-yl]-propoxy-2-ol]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3d**)*

From **2d** and endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide. Yield: 74%; m.p.: 161–163°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.50 (d, 1H, $J = 8.9$); 1.76 (d, 1H, $J = 8.9$); 2.45 (dd, 1H, CH_2–N, $J = 4.0$, $J = 8.8$); 2.56 (dd, 1H, CH_2–N, $J = 4.2$, $J = 8.1$); 2.69 (bs, 2H, CH_2 pip); 2.77 (bs, 2H, CH_2 pip); 2.80 (bs, 4H, $2CH_2$ pip); 3.21 (s, 2H); 3.43 (s, 2H); 3.89–3.92 (m, 1H, CH–OH); 4.07 (dd, 2H, O–CH_2, $J = 3.0$, $J = 7.0$); 6.16 (s, 2H); 6.98–7.02 (m, 2H); 7.45 (t, 1H, $J = 7.3$); 7.54

(d, 1H, $J = 7.3$). ESI-MS: 423.4 $[M+H]^+$ (Calcd: 422.48). Anal. ($C_{23}H_{26}N_4O_4$), C, H, N.

*Synthesis of 4-[3-[4-(2-furoyl)piperazin-1-yl]propoxy-2-ol]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3e**)*

From **2e** and endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide. Yield: 61%; m.p.: 168–170°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.57 (d, 1H, $J = 8.9$); 1.71 (d, 1H, $J = 8.9$); 2.46 (dd, 1H, CH_2–N, $J = 4.0$, $J = 8.8$); 2.51 (dd, 1H, CH_2–N, $J = 4.2$, $J = 8.1$); 2.63 (bs, 2H, CH_2 pip); 2.79 (bs, 2H, CH_2 pip); 3.29 (bs, 4H, $2CH_2$ pip); 3.22 (s, 2H); 3.44 (s, 2H); 3.99–4.03 (m, 1H, CH–OH); 4.2 (dd, 2H, O–CH_2, $J = 3.0$, $J = 7.0$); 6.13 (s, 2H); 6.46 (d, 1H, $J = 3.2$); 6.97 (d, 1H, $J = 3.2$); 7.46 (bs, 1H). ESI-MS: 416.3 $[M+H]^+$ (Calcd: 415.44). Anal. ($C_{21}H_{25}N_3O_6$), C, H, N.

*Synthesis of 4-[3-[4-(o-methoxyphenyl)piperazin-1-yl]-propoxy-2-ol]-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**4a**)*

From **2a** and exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide. Yield: 53%; m.p.: 128–130°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.68 (dd, 1H, CH_2–N, $J = 4.0$, $J = 8.8$); 2.71 (dd, 1H, CH_2–N, $J = 4.2$, $J = 8.1$); 2.79 (s, 2H); 2.84 (bs, 2H, CH_2 pip); 2.92 (bs, 2H, CH_2 pip); 3.16 (bs, 4H, $2CH_2$ pip); 3.85 (s, 3H, $-OCH_3$); 4.04–4.08 (m, 1H, CH–OH); 4.19 (d, 2H, O–CH_2, $J = 8.1$); 5.30 (d, 2H, $J = 4.8$); 6.51 (s, 2H); 6.85–7.03 (mm, 4H, ArH). ESI-MS: 430 $[M+H]^+$ (Calcd: 429.47). Anal. ($C_{22}H_{27}N_3O_6$), C, H, N.

*Synthesis of 4-[3-[4-(3-trifluoromethylphenyl)piperazin-1-yl]propoxy-2-ol]-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**4b**)*

From **2b** and exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide. Yield: 30%; m.p.: 116–118°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.65 (dd, 1H, CH_2–N, $J = 4.0$, $J = 8.8$); 2.69 (dd, 1H, CH_2–N, $J = 4.2$, $J = 8.1$); 2.79 (s, 2H); 2.84 (bs, 2H, CH_2 pip); 2.92 (bs, 2H, CH_2 pip); 3.28 (bs, 4H, $2CH_2$ pip); 4.04–4.06 (m, 1H, CH–OH); 4.19 (d, 2H, O–CH_2, $J = 8.1$); 5.29 (d, 2H, $J = 4.8$); 6.52 (s, 2H); 7.03–7.36 (mm, 4H, ArH). ESI-MS: 468.1 $[M+H]^+$ (Calcd: 467.44). Anal. ($C_{22}H_{24}F_3N_3O_5$), C, H, N.

*Synthesis of 4-[3-[4-(2,3-dimethylphenyl)piperazin-1-yl]-propoxy-2-ol]-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**4c**)*

From **2c** and exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide. Yield: 31%; m.p.: 103–105°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.20 (s, 3H, CH_3); 2.25 (s, 3H, CH_3); 2.51 (dd, 1H, CH_2–N, $J = 4.0$, $J = 8.8$); 2.57 (dd, 1H, CH_2–N, $J = 4.2$, $J = 8.1$); 2.62 (bs, 2H, CH_2 pip); 2.73 (bs, 2H, CH_2 pip); 2.79 (s, 2H); 2.81 (bs, 4H, $2CH_2$ pip); 4.01–4.06 (m, 1H, CH–OH); 4.21 (d, 2H, O–CH_2, $J = 8.1$); 5.29 (d, 2H, $J = 4.8$); 6.52 (s, 2H); 6.89 (d, 2H, $J = 7.6$); 7.05 (t, 1H, $J = 7.6$). ESI-MS: 428.3 $[M+H]^+$ (Calcd: 427.49). Anal. ($C_{23}H_{29}N_3O_5$), C, H, N.

*Synthesis of 4-[3-[4-(2-cyanophenyl)piperazin-1-yl]-propoxy-2-ol]-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**4d**)*

From **2d** and exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide. Yield: 24%; m.p.: 78–80°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.35 (dd, 1H, CH_2–N, $J = 4.0$, $J = 8.8$); 2.51 (dd, 1H, CH_2–N, $J = 4.2$, $J = 8.1$); 2.66 (bs, 2H, CH_2 pip); 2.73 (bs, 2H, CH_2 pip); 2.79 (s, 2H); 3.26 (bs, 4H, $2CH_2$ pip); 3.85–3.92 (m, 1H, CH–OH); 4.21 (d, 2H, O–CH_2, $J = 8.1$); 5.29 (d, 2H, $J = 4.8$); 6.51 (s, 2H); 6.99–7.01 (m, 2H); 7.47 (t, 1H, $J = 7.3$); 7.54 (d, 1H, $J = 7.3$). ESI-MS: 425.17 $[M+H]^+$ (Calcd: 424.45). Anal. ($C_{22}H_{24}N_4O_5$), C, H, N.

*Synthesis of 4-[3-[4-(2-furoyl)piperazin-1-yl]propoxy-2-ol]-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**4e**)*

From **2e** and exo-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide. Yield: 27%; m.p.: 93–95°C. ^{1}H NMR (400 MHz, $CDCl_3$) 2.46 (dd, 1H, CH_2–N, $J = 4.0$, $J = 8.8$); 2.52 (dd, 1H, CH_2–N, $J = 4.2$, $J = 8.1$); 2.61 (bs, 2H, CH_2 pip); 2.63 (bs, 2H, CH_2 pip); 2.79 (s, 2H); 3.79 (bs, 4H, $2CH_2$ pip); 3.99–4.03 (m, 1H, CH–OH); 4.20 (d, 2H, O–CH_2, $J = 8.2$); 5.28 (d, 2H, $J = 4.4$); 6.46 (bs, 1H); 6.52 (s, 2H); 6.97 (d, 1H, $J = 3.2$); 7.46 (bs, 1H). ESI-MS: 418.4 $[M+H]^+$ (Calcd: 417.41). Anal. ($C_{20}H_{23}N_3O_7$), C, H, N.

Pharmacology

General procedures

The newly synthesized compounds were tested for *in vitro* affinity for serotonin 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors by radioligand binding assays. The more active compounds on serotonin receptors have been selected and evaluated for their affinity for dopaminergic (D_1 and D_2) and adrenergic (α_1 and α_2) receptors. All the compounds were dissolved in ethanol or in 5% DMSO. The following specific radioligands and tissue sources were used: (a) serotonin 5-HT_{1A} receptor, [^{3}H]-8-OH-DPAT, and rat brain cortex; (b) serotonin 5-HT_{2A} receptor and [^{3}H]ketanserin, rat brain cortex; (c) serotonin 5-HT_{2C} receptor and [^{3}H]mesulergine, rat brain cortex; (d) dopamine D_1 receptor [^{3}H]SCH-23390 and rat striatum; (e) dopamine D_2 receptor [^{3}H]spiperone and rat striatum; (f) $\alpha_1$adrenergic receptor [^{3}H]prazosin and rat brain cortex; and (g) α_2 adrenergic receptor [^{3}H]yohimbine and rat brain cortex.

Non-specific binding was determined as described in the Experimental section, and specific binding as the difference between total and non-specific binding. Blank experiments were carried out to determine the effect of 5% DMSO on the binding and no effects were observed. Competition experiments were analyzed by the "Easy Fit" program [25] to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding (IC_{50}), with six concentrations of test compounds, each performed in triplicate. The IC_{50} values obtained were used to calculate apparent inhibition constants (K_i) by the method of Cheng and Prussoff [26], from the following equation: $K_i = IC_{50}/(1 + S/K_D)$ where S represents the concentration of the hot ligand used and K_D its receptor dissociation constant (K_D values, obtained by Scatchard analysis [27], were calculated for each labeled ligand: [^{3}H]-8-OH-DPAT 0.82 nM; [^{3}H]ketanserin hydrochloride 1.14 nM; [^{3}H]mesulergine 1.54 nM; [^{3}H]SCH-23390 0.30 nM; [^{3}H]spiperone 0.21 nM; [^{3}H]prazosin 0.57 nM; and [^{3}H]yohimbine 2.50 nM).

5-HT_{1A} binding assay

Radioligand binding assays were performed following a published procedure [28]. Cerebral cortex from male Sprague-Dawley rats (180–220 g) was homogenized in 20 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.7 at 22°C) with a Polytron PT10, Brinkmann Instruments (setting 5 for 15 s), and the homogenate was centrifuged at 50,000*g* for 10 min at 0°C. The resulting pellet was then resuspended in the same buffer, incubated for 10 min at 37°C, and centrifuged at 50,000*g* for 10 min. The final pellet was

resuspended in 80 volumes of the Tris-HCl buffer containing 10 μM pargyline, 4 mM $CaCl_2$, and 0.1% ascorbate. To each assay tube was added the following: 0.1 mL of the drug dilution (0.1 mL of distilled water if no competing drug was added), 0.1 mL of [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]-8-OH-DPAT) (170.0 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA) in the same buffer as above to achieve a final assay concentration of 1.0 nM, and 0.8 mL of resuspended membranes. The tubes were incubated for 30 min at 37°C, and the incubations were terminated by vacuum filtration through Whatman GF/B filters (Brandel Biomedical Research and Laboratories, Inc., Gaithersburg, MD, USA). The filters were washed twice with 5 mL of ice-cold Tris-HCl buffer, and the radioactivity bound to the filters was measured by liquid scintillation spectrometer (Packard TRI-CARB® 2000CA – Packard BioScience s.r.l, Pero, Milan, Italy). Specific [^{3}H]-8-OH-DPAT binding was defined as the difference between binding in the absence and presence of 5-HT (10 μM).

5-HT$_{2A}$ and 5-HT$_{2C}$ binding assays

Radioligand binding assays were performed as previously reported by Herndon et al. [29]. Briefly, frontal cortical regions of male Sprague-Dawley rats (180–220 g) were dissected on ice and homogenized (1:10 w/v) in ice-cold buffer solution (50 mM Tris-HCl, 0.5 mM EDTA, and 10 mM $MgCl_2$ at pH 7.4) with a Polytron PT10 (setting 5 for 15 s) and centrifuged at 3000*g* for 15 min. The pellet was resuspended in buffer (1:30 w/v), incubated at 37°C for 15 min and then centrifuged twice more at 3000*g* for 10 min (with resuspension between centrifugations). The final pellet was resuspended in buffer that also contained 0.1% ascorbate and 10^{-5} M pargyline.

Assays were performed in triplicate in a 2.0 mL volume containing 5 mg wet weight of tissue and 0.4 nM [^{3}H]ketanserin hydrochloride (88.0 Ci/mmol; Perkin Elmer Life Sciences) for 5-HT$_{2A}$ receptor assays, and 10 mg wet weight of tissue and 1 nM [^{3}H]mesulergine (87.0 Ci/mmol; Amersham Biosciences Europe GmbH) for 5-HT$_{2C}$ receptor assays. Cinanserin (1.0 μM) was used to define nonspecific binding in the 5-HT$_{2A}$ assay. In the 5-HT$_{2C}$ assays, mianserin (1.0 μM) was used to define nonspecific binding, and 100 nM spiperone was added to all tubes to block binding to 5-HT$_{2A}$ receptors. Tubes were incubated for 15 min at 37°C, filtered on Schleicher and Schuell (Keene, NH, USA) glass fiber filters presoaked in polyethylene imine, and washed with 10 mL of ice-cold buffer. Filters were counted at an efficiency of 50%.

D$_1$ dopaminergic binding assay

The binding assay for D_1 dopaminergic receptors was that described by Billard et al. [30]. Corpora striata were homogenized in 30 vol. (w/v) ice cold 50 mM Tris-HCl buffer (pH 7.7 at 25°C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50,000*g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice cold Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid, and 10 μM pargyline (pH 7.1 at 37°C). Each assay tube contained 50 μL [^{3}H]SCH-23390 (85.0 Ci/mmol; Perkin Elmer Life Sciences) to achieve a final concentration of 0.4 nM, and 900 μL resuspended membranes (3 mg fresh tissue). The tubes were incubated for 15 min at 37°C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]SCH-23390 binding was defined as the difference between binding in the absence or in the presence of 0.1 μM piflutixol.

D$_2$ dopaminergic binding assay

The procedure used in the radioligand binding assay was reported in detail by Creese et al. [31]. Corpora striata were homogenized in 30 vol. (w/v) ice cold 50 mM Tris-HCl buffer (pH 7.7 at 25°C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50,000*g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice cold Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid, and 10 μM pargyline (pH 7.1 at 37°C). Each assay tube contained 50 μL [^{3}H]spiperone (15.7 Ci/mmol; Perkin Elmer Life Sciences) to achieve a final concentration of 0.4 nM, and 900 μL resuspended membranes (3 mg fresh tissue). The tubes were incubated for 15 min at 37°C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]spiperone binding was defined as the difference between binding in the absence or in the presence of 1 μM (+)-butaclamol.

α$_1$ adrenergic binding assay

The procedure used in the radioligand binding assay has been reported in detail by Greengrass and Bremner [32]. Brain cortex was homogenized in 30 vol. (w/v) ice-cold 50 mM Tris-HCl buffer, (pH 7.2 at 25°C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50,000*g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris-HCl (pH 7.4 at 25°C). Each assay tube contained 50 μL drug solution, 50 μL [^{3}H]prazosin (80.5 Ci/mmol; Perkin Elmer Life Sciences) to achieve a final concentration of 0.4 nM, and 900 μL resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25°C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl, buffer (pH 7.2 at 25°C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]prazosin binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

α$_2$ adrenergic binding assay

The procedure used in the radioligand binding assay was reported in detail by Perry and U'Prichard [33]. Brain cortex was homogenized in 30 vol. (w/v) ice-cold 5 mM Tris-HCl, 5 mM EDTA buffer (pH 7.3 at 25°C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged three times for 10 min at 50,000*g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris-HCl, 0.5 mM EDTA (pH 7.5 at 25°C). Each assay tube contained 50 μL drug solution, 50 μL [^{3}H]-yohimbine (80.5 Ci/mmol; Perkin Elmer Life Sciences) to achieve a final concentration of 1 nM, and 900 μL resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25°C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl, 0.5 mM

EDTA buffer (pH 7.5 at 25°C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]-yohimbine binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

The NMR spectral data were provided by Centro di Ricerca Interdipartimentale di Analisi Strumentale, Università degli Studi di Napoli "Federico II". The assistance of the staff is gratefully appreciated.

The authors have declared no conflict of interest.

References

[1] R. Fredriksson, M. C. Lagerström, L. G. Lundin, H. B. Schiöth, *Mol. Pharmacol.* **2003**, *63* (6), 1256–1272.

[2] D. E. Nichols, C. D. Nichols, *Chem. Rev.* **2008**, *108*, 1614–1641.

[3] E. J. Siddiqui, M. Shabbir, D. P. Mikhailidis, C. S. Thompson, F. H. Mumtaz, *J. Urol.* **2006**, *176*, 1648–1653.

[4] D. Hoyer, J. P. Hannon, G. R. Martin, *Pharmacol. Biochem. Behav.* **2002**, *71*, 533–554.

[5] M. Bancila, D. Verge, O. Rampin, J. R. Backstrom, E. Sanders-Bush, K. E. McKenna, L. Marson, A. Calas, F. Giuliano, *Neuroscience* **1999**, *92* (4), 1523–1537.

[6] Y. Kimura, K. Hatanaka, Y. Naitou, K. Maeno, I. Shimada, A. Koakutsu, F. Wanibuchi, T. Yamaguchi, *Eur. J. Pharmacol.* **2004**, *483* (1), 37–43.

[7] M. L. López-Rodríguez, D. Ayala, B. Benhamú, M. J. Morcillo, A. Viso, *Curr. Med. Chem.* **2002**, *9*, 443–469.

[8] F. Fiorino, B. Severino, E. Magli, A. Ciano, G. Caliendo, V. Santagada, F. Frecentese, E. Perissutti, *J. Med. Chem.* **2014**, *57* (11), 4407–4426.

[9] G. Caliendo, G. Greco, P. Grieco, E. Novellino, E. Perissutti, V. Santagada, D. Barbarulo, E. Esposito, A. De Blasi, *Eur. J. Med. Chem.* **1996**, *31* (3), 207–213.

[10] G. Caliendo, F. Fiorino, E. Perissutti, B. Severino, D. Scolaro, S. Gessi, E. Cattabriga, P. A. Borea, V. Santagada, *Eur. J. Pharm. Sci.* **2002**, *16* (1–2), 15–28.

[11] G. Caliendo, F. Fiorino, P. Grieco, E. Perissutti, V. Santagada, S. Albrizio, L. Spadola, G. Bruni, M. R. Romeo, *Eur. J. Med. Chem.* **1999**, *34* (9), 719–727.

[12] G. Caliendo, F. Fiorino, P. Grieco, E. Perissutti, V. Santagada, B. Severino, G. Bruni, M. R. Romeo, *Bioorg. Med. Chem.* **2000**, *8*, 533–538.

[13] G. Caliendo, F. Fiorino, E. Perissutti, B. Severino, S. Gessi, E. Cattabriga, P. A. Borea, V. Santagada, *Eur. J. Med. Chem.* **2001**, *36* (11–12), 873–886.

[14] F. Fiorino, E. Perissutti, B. Severino, V. Santagada, D. Cirillo, S. Terracciano, P. Massarelli, G. Bruni, E. Collavoli, C. Renner, G. Caliendo, *J. Med. Chem.* **2005**, *48*, 5495–5503.

[15] F. Fiorino, B. Severino, F. De Angelis, E. Perissutti, F. Frecentese, P. Massarelli, G. Bruni, E. Collavoli, V. Santagada, G. Caliendo, *Arch. Pharm. (Weinheim)* **2008**, *341*, 20–27.

[16] F. Fiorino, B. Severino, F. De Angelis, E. Perissutti, E. Magli, F. Frecentese, A. Esposito, P. Massarelli, C. Nencini, B. Viti, V. Santagada, G. Caliendo, *Pharmazie* **2009**, *64* (9), 555–564.

[17] F. Fiorino, B. Severino, F. De Angelis, E. Perissutti, E. Magli, F. Frecentese, A. Esposito, P. Massarelli, C. Nencini, V. Santagada, G. Caliendo, *Bioorg. Med. Chem. Lett.* **2010**, *20* (9), 2978–2982.

[18] F. Fiorino, B. Severino, E. Magli, E. Perissutti, F. Frecentese, A. Esposito, G. M. Incisivo, A. Ciano, P. Massarelli, C. Nencini, V. Santagada, G. Caliendo, *Eur. J. Med. Chem.* **2012**, *47*, 520–529.

[19] J. Kossakowski, A. Bielenica, M. Struga, A. E. Kozil, *Med. Chem. Res.* **2008**, *17*, 507–514.

[20] J. Handzlik, E. Szyma ska, K. N dza, M. Kubacka, A. Siwek, S. Mogilski, J. Handzlik, B. Filipek, K. Kie -Kononowicz, *Bioorg. Med. Chem.* **2011**, *19*, 1349–1360.

[21] H. J. Seo, E. J. Park, M. J. Kim, S. Y. Kang, S. H. Lee, H. J. Kim, K. N. Lee, M. E. Jung, M. W. Lee, M. S. Kim, E. J. Son, W. K. Park, J. Kim, J. Lee, *J. Med. Chem.* **2011**, *54*, 6305–6318.

[22] J. Kossakowski, A. Bielenica, M. Struga, A. E. Koziol, *Med. Chem. Res.* **2008**, *17*, 507–514.

[23] A. Lepailleur, R. Bureau, M. Paillet-Loilier, F. Fabis, N. Saettel, S. Lemaître, F. Dauphin, J. C. Lancelot, S. Rault, *J. Chem. Inf. Model.* **2005**, *45*, 1075–1081.

[24] M. Isaac, *Curr. Top. Med. Chem.* **2005**, *5* (1), 59–67.

[25] EasyFit 1.4, **1989–1991**, Matteo Vaccari and Mario Negri Institute, Milan, Italy.

[26] Y. C. Cheng, W. H. Prusoff, *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

[27] G. Scatchard, *Ann. N. Y. Acad. Sci.* **1949**, *51*, 660–672.

[28] J. R. Schlegel, S. J. Peroutka, *Biochem. Pharmacol.* **1986**, *35*, 1943–1949.

[29] J. L. Herndon, A. Ismaiel, S. P. Ingher, M. Teitler, R. A. Glennon, *J. Med. Chem.* **1992**, *35*, 4903–4910.

[30] W. Billard, V. Ruperto, G. Crosby, L. C. Iorio, A. Barnett, *Life Sci.* **1984**, *35* (18), 1885–1893.

[31] I. Creese, R. Schneider, S. H. Snyder, *Eur. J. Pharmacol.* **1977**, *46* (4), 377–381.

[32] P. Greengrass, R. Bremner, *Eur. J. Pharmacol.* **1979**, *55* (3), 323–326.

[33] B. D. Perry, D. C. U'Prichard, *Eur. J. Pharmacol.* **1981**, *76* (4), 461–464.

