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1,3-Dioxolane-Based Ligands as Rigid Analogues of Naftopidil: Structure–Affinity/Activity Relationships at α_1 and 5-HT_{1A} Receptors

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*Conformational restriction of naftopidil proved to be compatible with binding at α_1 adrenoceptor subtypes and 5-HT receptor 1A (5-HT_{1A}), and led to the discovery of a new class of ligands with a 1,3-dioxolane (1,3-oxathiolane, 1,3-dithiolane) structure. Compound **7** shows the highest affinity toward α_{1a} and α_{1d} adrenoceptor subtypes ($pK_i\alpha_{1a}=9.58$, $pK_i\alpha_{1d}=9.09$) and selectivity over 5-HT_{1A} receptors (α_{1a}/5-$HT_{1A}=100$, α_{1d}/5-$HT_{1A}=26$). In functional experiments it behaves as a potent competitive α_{1a} and α_{1d} adrenoceptor antagonist ($pK_b\alpha_{1A}=8.24$, $pK_b\alpha_{1D}=8.14$), whereas at 5-HT_{1A} receptors it is a potent partial agonist ($pD_2=8.30$). Compounds **8** and **10** display high affinity ($pK_i=8.29$ and 8.26, respectively) and selectivity for 5-HT_{1A} (5-HT_{1A}/$\alpha_1=18$ and 10). In functional experiments at the 5-HT_{1A} receptor, compound **8** appears to be neutral antagonist ($pK_b=7.29$), whereas compound **10** is a partial agonist ($pD_2=6.27$). Therefore, 1,3-dioxolane-based ligands are a versatile class of compounds useful for the development of more selective ligands for one (α_1) or the other (5-HT_{1A}) receptor system.*

Introduction

The α_1 adrenergic receptors (α_1 adrenoceptors) are a family of G-protein-coupled receptors (GPCRs) and can be divided into at least three subtypes: α_{1A} (α_{1a}), α_{1B} (α_{1b}), and α_{1D} (α_{1d}), with upper- and lower-case subscript designating the native or recombinant receptors, respectively.[1–3] As emerging data seem to predict further subtyping, this situation, although not definitive, has given new impetus to medicinal chemists in their search for new and more selective ligands. As a result, several relatively selective ligands for α_1 adrenoceptors are now available.[4,5]

The serotonin (5-HT) receptors, like the α_1 adrenoceptors, are also GPCRs, and the members of this class have a number of characteristic amino acid patterns in common. In particular, the transmembrane amino acid sequence of the 5-HT_{1A} subtype has a notably high degree of homology (~45% identity) to α_1 adrenoceptors.[6] As a consequence, a great number of α_1 adrenoceptor ligands show high affinity and poor selectivity.

The α_{1A} subtype, which is the most prevalent subtype in the prostate,[7,8] has received much attention as a potential target for symptomatic treatment of benign prostatic hyperplasia (BPH), and several uroselective agents have been revealed.[9] In contrast, potential therapeutic use for α_{1B} and α_{1D} subtype-selective ligands has not been found yet. However, there is some evidence that the α_{1B} subtype plays a prominent role in the regulation of blood pressure, as α_{1B} knockout mice are significantly less responsive to phenylephrine-induced blood pressure increases.[10] There is also much interest in getting a better understanding of the role of the α_{1D} subtype. It has been shown that in the human bladder detrusor, α_{1d} mRNA is the predominant subtype,[11] and it was postulated that the α_{1D} receptor blockade may ameliorate the irritative symptoms of BHP that result from involuntary contraction of the bladder smooth muscle.[12]

Naftopidil (**1**) is an α_1 adrenoceptor antagonist that belongs to the phenylpiperazine derivatives. It acts more selectively on the lower urinary tract than on the blood vessels, and it has been marketed in Japan since 1999 for the treatment of symptomatic BPH. It shows high affinity for the α_{1A} and α_{1D} subtypes, with predominant affinity for the latter.[13]

The 5-HT_{1A} receptor is the most studied subtype of the 5-HT receptors, and has been a major target for neurological research and drug development. Agonists and partial agonists of 5-HT_{1A} have been proven effective in treating anxiety and depression.[14–18] In addition to therapeutic applications in the field of psychiatry, more recent preclinical studies have suggested that 5-HT_{1A} receptor agonists also have pronounced neuroprotective properties.[19] Potential therapeutic applications for 5-HT_{1A} receptor antagonists have been evaluated, for example, in cognition disorders.[20]

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Our research group recently reported a new class of α_1 adrenoceptor ligands bearing a 1,3-dioxolane structure. Compounds **2** and **3** are outstanding in their affinity and selectivity; the former is selective for α_{1A} and α_{1D} over the α_{1B} subtype, and the latter is one of the most selective antagonists, at least in functional studies, for the α_{1D} subtype.[21]

A comparison of compound **1** and compounds **2** (and **3**) prompted us to design a hybrid structure, shown at right in Figure 1, which can be considered a rigid analogue of **1**. Compound **1** itself, a known α_1 adrenoceptor antagonist, also binds to 5-HT_{1A} receptors.[22] Therefore, the designed structures were synthesized and tested on both receptor systems with the dual aim of studying the effect of structure rigidification of **1** on affinity and selectivity, the ultimate goal being to discover new selective ligands for one receptor system or the other.

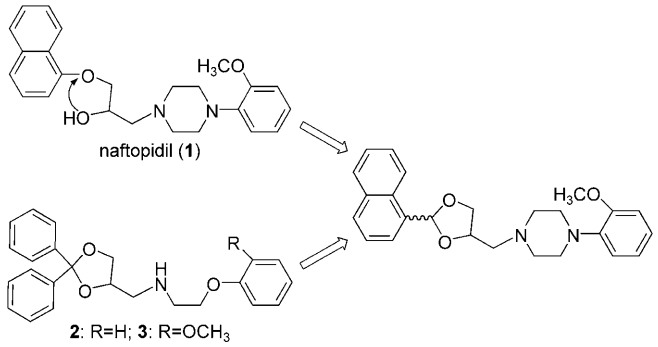
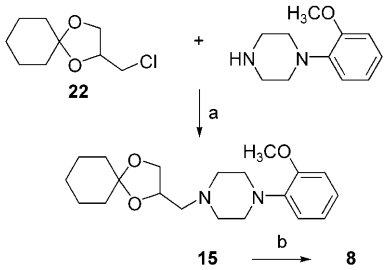


Figure 1. The constrained structure formed as a hybrid of naftopidil and compound **3**.

Results and Discussion

Chemistry

Compounds **4**–**10** were synthesized by standard procedures and characterized by ^{1}H NMR and elemental analysis. The chloro derivatives **18**–**24**, obtained as previously reported,[21,23] were aminated with 1-(2-methoxyphenyl)piperazine to obtain the final amines **11**–**17** (Scheme 1 and Scheme 2). The diastereomeric mixtures were separated by flash chromatography at this stage, and the *cis*/*trans* stereochemistry was assigned by nuclear Overhauser effect (nOe) measurements between the hydrogen atoms at C2 and C4.



Scheme 2. *Reagents and conditions*: a) KI, 2-methoxyethanol, reflux, 17 h; b) $C_2O_4H_2$, Et_2O.

Pharmacology

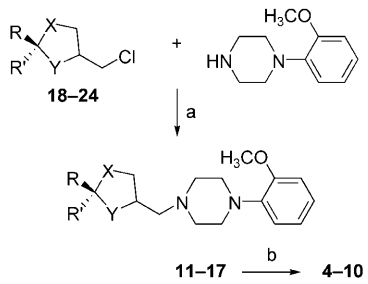
The pharmacological profile of compounds **1** and **4**–**10**, and BMY-3748, 8-OH-DPTA, and WAY100635 as reference compounds, was evaluated by radioligand binding assays using [^{3}H]prazosin to label cloned human α_1 adrenoceptors expressed in CHO cells,[24] and [^{3}H]8-OH-DPAT to label cloned human 5-HT_{1A} receptors expressed in HeLa cells.[25]

Functional α_1 adrenoceptor subtype selectivity was also determined on various isolated tissue types. Blocking activity was assessed by antagonism of (−)-norepinephrine-induced contraction of rat prostatic vas deferens (α_{1A})[26] or thoracic aorta (α_{1D})[27] and by antagonism of (−)-phenylephrine-induced contraction of rat spleen (α_{1B}).[28]

Functional characterization of some selected compounds (**6*t***, **7**, **8**, and **10**) at the 5-HT_{1A} receptor was performed according to methods of Stanton and Beer,[29] using [^{35}S]GTPγS binding, in cell membranes from HeLa cells transfected with the human cloned 5-HT_{1A} receptor. Stimulation of [^{35}S]GTPγS binding was expressed as the percent increase in binding above basal value; maximal stimulation observed with serotonin was established as 100%.

Structure–affinity relationships

Table 1 lists the affinity constants (p*K*$_i$) obtained in radioligand binding assays. The results show that compound **1** binds human cloned α_1 adrenoceptors with a preferential and equal affinity for α_{1a} (8.23) and α_{1d} (8.24) subtypes. It also binds to



X	Y	R	R'	oxalate salts	amines	chloro derivatives
O	O	naphthyl-1-yl	H	**4*c***	**11*c***	**18*c***
O	O	H	naphthyl-1-yl	**4*t***	**11*t***	**18*t***
O	O	naphthyl-2-yl	H	**5*c***	**12*c***	**19*c***
O	O	H	naphthyl-2-yl	**5*t***	**12*t***	**19*t***
O	O	Ph	H	**6*c***	**13*c***	**20*c***
O	O	H	Ph	**6*t***	**13*t***	**20*t***
O	O	Ph	Ph	**7**	**14**	**21**
S	O	Ph	Ph	**9**	**16**	**23**
S	S	Ph	Ph	**10**	**17**	**24**

Scheme 1. *Reagents and conditions*: a) KI, 2-methoxyethanol, reflux, 18–25 h; b) $C_2O_4H_2$, Et_2O.

Table 1. Affinity constants and selectivities of test and reference compounds for human recombinant α_1 adrenoceptor subtypes and 5-HT_{1A} receptors.

Compd	$pK_i\alpha_{1a}$[a]	$pK_i\alpha_{1b}$[a]	$pK_i\alpha_{1d}$[a]	pK_i 5-HT_{1A}[a]	α_{1d}/α_{1a}[b]	α_{1d}/α_{1b}[b]	α_{1a}/α_{1b}[b]	5-HT_{1A}/α_1[b]
1	8.23	7.59	8.24	7.28	1	5	5	0.1
4*c*	7.44	7.03	7.72	7.52	1	5	3	0.6
4*t*	8.10	--	8.43	7.63	2	--	--	0.2
5*c*	7.82	6.94	7.84	7.31	1	8	8	0.3
5*t*	7.46	6.99	7.54	8.75	1	4	3	16
6*c*	7.54	7.06	8.02	7.73	3	9	3	0.5
6*t*	8.57	6.36	7.42	8.22	0.1	12	162	0.5
7	9.58	8.17	9.09	7.64	0.3	8	26	0.01
8	7.04	6.90	<6	8.29	<0.1	<0.1	1	18
9	8.69	8.05	8.40	8.18	0.6	2	4	0.3
10	7.24	<6	6.64	8.26	0.3	<4	>17	10
BMY-7378	6.42	6.15	8.89	8.90	295	550	2	1
WAY10063	7.73	7.18	8.34	9.48	4	14	3	14

[a] K_i values were derived from the Cheng–Prusoff equation[31] at one or two concentrations and agreed within 10%. [b] Antilog of the difference between the pK_i values for α_{1a}, α_{1b}, α_{1d} adrenoceptors and the 5-HT_{1A} receptor.

the 5-HT_{1A} receptor with a pK_i value of 7.28, and is therefore 10-fold more selective for α_{1a} and α_{1d} adrenoceptor subtypes over 5-HT_{1A}.

Rigidification of the diol moiety as in **4** gives rise to the *cis* and *trans* diastereomers (denoted ***c*** and ***t***). At α_{1a} and α_{1d} adrenoceptor subtypes, the stereochemistry appears to play a role, although to a limited extent, with the affinity of the *cis* isomer roughly five- to sixfold lower than that of the *trans* isomer, which in turn has the same affinity as that of **1**. At the 5-HT_{1A} receptor, both isomers display the same affinity, which is similar to that of **1**. Therefore, conformational restriction of **1** is compatible with binding at the two receptor systems. Conversely, with the β-naphthyl derivative **5**, a regioisomer of **4**, the stereochemistry is important at the 5-HT_{1A} receptor, as the affinity of the *trans* isomer is 27-fold higher than that of the *cis* isomer; at α_1 adrenoceptor subtypes they show very similar affinities, lower than that of **1**. As a consequence, compound **5*t*** shows a reversal of selectivity (5-$HT_{1A}/\alpha_{1a}=16$) relative to **1** (5-$HT_{1A}/\alpha_{1a}=0.1$).

The monophenyl derivatives **6*c*,*t*** were designed in order to verify the role of naphthyl group at position 2 of the 1,3-dioxolane ring. In this case as well, the affinity of the *trans* isomer for the 5-HT_{1A} receptor is higher than that of the *cis* isomer, whereas at α_1 adrenoceptor subtypes, a divergent effect is observed. In fact, the *trans* isomer shows the highest affinity at the α_{1a} subtype ($pK_i=8.57$), while the *cis* isomer binds preferentially to the α_{1d} subtype ($pK_i=8.02$). Therefore, replacement of the naphthyl group with a phenyl ring maintains affinity, but selectivity is negatively affected.

In terms of diastereoselectivity, if any, the *trans* form is preferred at α_{1a} and 5-HT_{1A} receptors, while for α_{1b} and α_{1d} subtypes, the preferred form is *trans* in the case of β-naphthyl derivatives **4*c*,*t*** and *cis* in the case of phenyl derivative **6*c*,*t***. These results indicate much more similarity between 5-HT_{1A} and α_{1a} than 5-HT_{1A} and the other two α_1 subtypes (α_{1b} and α_{1d}). The spiro derivative **8** shows that even the phenyl ring is not essential for 5-HT_{1A} binding, as its affinity ($pK_i=8.29$) is very similar to that of the phenyl derivative **6*t*** ($pK_i=8.22$). At α_1 adrenoceptors, the lack of an aromatic system seems to be detrimental for the α_{1d} subtypes, for which a decrease of >100-fold is observed, whereas for α_{1a} and α_{1b} subtypes, the affinities are retained.

The insertion of a second phenyl ring at position 2, to give compound **7**, increases the affinity at α_1 adrenoceptor subtypes of at least 10-fold, while at the 5-HT_{1A} receptor it is somewhat decreased. Compound **7** displays the highest affinities of the series at α_1 adrenoceptor subtypes, with selectivity ratios of 100 (α_{1a}/5-HT_{1A}) and 26 (α_{1d}/5-HT_{1A}).

Isosteric substitution of the oxygen atoms of the 1,3-dioxolane ring with sulfur atoms was also studied. Replacement of one (compound **9**) or two (compound **10**) oxygen atoms elicits a progressive decrease in affinity at α_1 adrenoceptor subtypes, whereas an increase at the 5-HT_{1A} receptor, although limited, is observed, therefore resulting in a significant reversal of selectivity toward the 5-HT_{1A} receptor, with compound **10** showing a 5-HT_{1A}/α_{1a} selectivity ratio of 10. It seems that a sulfur-containing ring favors binding to the 5-HT_{1A} receptor, however, a greater number of derivatives is needed to confirm this hypothesis.

Structure–activity relationships

Antagonist potencies at α_1 adrenoceptor subtypes are listed in Table 2. With a few exceptions, the activity trend parallels the affinities observed in binding studies, and the selectivity values, generally toward the α_{1D} subtype, are larger. These discrepancies are not unusual and may be ascribed to several factors, as previously reported.[13]

It can be seen that **1** is a potent ($pK_b=8.21$) and selective α_{1D} antagonist ($\alpha_{1D}/\alpha_{1A}=20$, $\alpha_{1A}/\alpha_{1B}=37$). Its rigid 1,3-dioxolane analogue **4** and regioisomer **5**, in both diastereomeric forms, show similar potency and selectivity, indicating that the conformational restriction imposed on **1** to obtain **4** (and **5**) is compatible with antagonist activities, thus confirming the findings obtained in the binding study. Compound **7** is also a

Table 2. Antagonist potency and selectivities of test and reference compounds at α_1 adrenoceptors in isolated rat prostatic vas deferens (α_{1A}), spleen (α_{1B}), and thoracic aorta (α_{1D}).

Compd	$pK_b\,\alpha_{1A}$[a]	$pK_b\,\alpha_{1B}$[a]	$pK_b\,\alpha_{1D}$[a]	α_{1D}/α_{1A}[b]	α_{1D}/α_{1B}[b]	α_{1A}/α_{1B}[b]
1	6.92	6.64	8.21	20	37	2
4*c*	7.10	6.61	7.86	6	18	3
4*t*	6.79	6.60	8.11	21	32	1.5
5*c*	6.52	6.49	8.16	44	47	1
5*t*	5.99	6.30	7.54	35	17	0.5
6*c*	6.86	6.99	7.80	9	7	0.7
6*t*	7.93	6.81	7.55	0.4	6	13
7	8.24	6.69	8.14	0.8	28	36
8	6.78	6.72	7.88	13	15	1
9	7.35	6.21	7.33	1	13	12
10	6.04	6.79	7.52	30	5	0.2
BMY-7378	7.01	7.48	8.40	25	8	0.3

[a] Potency values were calculated according to van Rossum[32] at one or two concentrations and agreed within 2%. [b] Antilog of the difference between the pK_b values for α_{1A}, α_{1B}, and α_{1D} adrenoceptors.

potent antagonist with similar potency at α_{1A} ($pK_b = 8.24$) and α_{1D} ($pK_b = 8.21$) with selectivity ratios of 28 (α_{1A}/α_{1B}) and 36 (α_{1D}/α_{1B}). This pharmacological profile at α_1 adrenoceptors appears to be the ideal one for a better drug to treat BPH, as it combines two activities: α_{1A} antagonism (smooth muscle relaxant) and α_{1D} antagonism (amelioration of irritative symptoms) (see the Introduction).

Compounds **8** and **10** behave as α_{1D} subtype antagonists ($pK_b = 7.88$ and 7.52) with the following selectivity ratios: $\alpha_{1D}/\alpha_{1A} = 13$ and $\alpha_{1D}/\alpha_{1B} = 15$ for compound **8**, and $\alpha_{1D}/\alpha_{1A} = 30$ and $\alpha_{1D}/\alpha_{1B} = 5$ for compound **10**.

Functional characterization at the 5-HT_{1A} receptor was performed for selected compounds (**6*t***, **7**, **8**, and **10**). The results are reported in Table 3. In stimulation experiments, compounds

Table 3. Agonist potency (pD_2), relative effectiveness (E_{max}), and antagonist potency (pK_b) in the agonist-induced [^{35}S]GTPγS-binding assay at the human 5-HT_{1A} receptor.

Compd	pD_2	E_{max}[a]	pK_b
6*t*	n.a.[b]	--	7.22
7	8.30	16	--
8	n.a.[b]	--	7.29
10	6.27	41	--
BMY-7378	9.27	26	--
8-OH-DPTA	7.83	100	--
WAY100635	n.a.[b]	--	9.33

[a] Maximal stimulation expressed as a percentage of the maximal 5-HT response. [b] Neutral antagonist.

7 and **10** increase the binding of [^{35}S]GTPγS with pD_2 values of 8.30 and 6.27, and E_{max} values of 16 and 41, respectively, defining the two compounds as partial agonists. In contrast, compounds **6*t*** and **8** do not affect the binding of [^{35}S]GTPγS, therefore behaving as neutral antagonists (n.a.). They were then studied in the antagonist experiments designed to evaluate their potency. Both **6*t*** and **8** behave as competitive antagonists with moderate pK_b values of 7.22 and 7.29, respectively.

Conclusions

We show herein that conformational restriction of naftopidil (**1**) is compatible with binding at α_1 adrenoceptors as well as the 5-HT_{1A} receptor, leading to the discovery of a new class of ligands bearing a 1,3-dioxolane structure. Adequate structural modifications address the selectivity toward one or the other receptor system and within α_1 adrenoceptor subtypes.

Compound **7** shows the highest affinity and selectivity toward α_{1A} and α_{1D} adrenoceptor subtypes over 5-HT_{1A} receptor. Compound **8**, instead, displays high affinity and selectivity for the 5-HT_{1A} receptor over α_1 adrenoceptors. Furthermore, whereas at α_1 adrenoceptor subtypes all compounds behave as competitive antagonists, they show either partial agonist or antagonist activity toward the 5-HT_{1A} receptor. Therefore, 1,3-dioxolane-based ligands are a versatile class of compounds that represent a new starting point for developing more selective ligands for one (α_1) or the other (5-HT_{1A}) receptor system. Research along this line is in progress and will be disclosed in due course.

Experimental Section

Chemistry

Structural characterization was carried out with NMR spectroscopy and elemental analysis. ^{1}H NMR spectra were recorded with a Bruker DPX 200 Avance spectrometer at 200.13 MHz and 300 K. Chemical shifts (δ) are reported relative to tetramethylsilane (TMS) as internal standard. Elemental analysis was performed with an Elemental Analyzer 1106 (Carlo Erba Instruments, Italy). Analysis data are reported within ±0.4% of theoretical values. Melting points were measured with a Gallenkamp melting point apparatus and are uncorrected. Reaction progress was monitored by TLC (silica gel 60, F_{254}, Merck). Separations were performed on silica gel columns (silica gel 60, 230–400 mesh, Merck) by flash chromatography. Preparative TLC was carried out on silica gel glass plates (silica gel 60, F_{254}, 2 mm, 20×20 cm, Merck). Compound names were generated with AUTONOM software, version 2.1.

General procedure for the synthesis of amines 11–17 and their oxalate salts 4–10 (Scheme 1). A solution of the chloroalkyl derivatives **18–24** (0.5–1.3 g) in 2-methoxyethanol (1 g per 100 mL) with a large excess (5 equiv) of 1-(2-methoxyphenyl)piperazine and a catalytic amount of KI was held at reflux for 17–25 h. The solvent was evaporated under vacuum. $CHCl_3$ was added, and the residue was washed with a solution of NaOH (5%, 3×) and then with brine (2×). The organic layer was dried over Na_2SO_4, and the solvent was evaporated under vacuum. Diastereomeric separation of the *cis* and *trans* isomers and purification of all amines, obtained as oily residues, were carried out by flash chromatography eluting with *c*Hex/EtOAc 60:40 (**11** and **12**), 70:30 (**13**), 90:10→70:30 (**14**), 60:40 (**15**), 80:20 (**16**), and 90:10 (**17**). In cases of *cis*/*trans* isomer

separation, preparative TLC was also performed. All the amines were then transformed into the corresponding oxalate salts and crystallized as reported below.

1-(2-methoxyphenyl)-4-(2-*cis*-naphthalen-1-yl[1,3]dioxolan-4-ylmethyl)piperazine (11*c*): yield 0.39 g (24%); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.80 (m, 6H), 3.12 (m, 4H), 3.86 (s, 3H), 3.92 (dd, J = 7.8, 6.7 Hz, 1H), 4.28 (dd, J = 7.8, 6.8 Hz, 1H), 4.59 (m, 1H), 6.49 (s, 1H), 6.91 (m, 4H), 7.50 (m, 3H), 7.85 (m, 3H), 8.22 ppm (m, 1H). The corresponding oxalate salt **4*c*** was crystallized from EtOH: yield 0.04 g (46%); mp: 100–102 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 3.00 (m, 10H), 3.75 (s, 3H), 3.85 (m, 1H), 4.24 (m, 1H), 4.65 (m, 1H), 6.42 (s, 1H), 6.90 (m, 4H), 7.55 (m, 3H), 7.77 (m, 1H), 7.96 (m, 2H), 8.20 ppm (m, 1H); Anal. calcd for $C_{27}H_{30}N_2O_7 \cdot 1.5\,H_2O$: C 60.33, H 6.19, N 5.21, found: C 60.54, H 5.79, N 4.99.

1-(2-methoxyphenyl)-4-(2-*trans*-naphthalen-1-yl[1,3]dioxolan-4-ylmethyl)piperazine (11*t*): yield 0.21 g (13%), ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.79 (m, 6H), 3.14 (m, 4H), 3.86 (dd, J = 8.1, 6.3 Hz, 1H), 3.87 (s, 3H), 4.38 (dd, J = 8.1, 6.4 Hz, 1H), 4.58 (m, 1H), 6.62 (s, 1H), 6.96 (m, 4H), 7.52 (m, 3H), 7.78 (m, 1H), 7.87 (m, 2H), 8.25 ppm (m, 1H). The corresponding oxalate salt **4*t*** was crystallized from EtOH: yield 0.03 g (35%); mp: 150°-151 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 3.08 (m, 10H), 3.80 (s, 3H), 3.81 (m, 1H), 4.40 (m, 1H), 4.66 (m, 1H), 6.55 (s, 1H), 6.94 (m, 4H), 7.56 (m, 3H), 7.75 (m, 1H), 7.99 (m, 2H), 8.24 ppm (m, 1H); Anal. calcd for $C_{27}H_{30}N_2O_7 \cdot H_2O$: C 63.27, H 6.29, N 5.47, found: C 63.38, H 6.04, N 5.32.

1-(2-methoxyphenyl)-4-(2-*cis*-naphthalen-2-yl[1,3]dioxolan-4-ylmethyl)piperazine (12*c*): yield 0.35 g (17%), ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.83 (m, 6H), 3.16 (m, 4H), 3.89 (s, 3H), 3.92 (dd, J = 7.8, 6.6 Hz, 1H), 4.25 (dd, J = 7.8, 6.8 Hz, 1H), 4.56 (m, 1H), 6.04 (s, 1H), 6.98 (m, 4H), 7.53 (m, 2H), 7.65 (m, 1H), 7.92 ppm (m, 4H). The corresponding oxalate salt **5*c*** was crystallized from MeOH: yield 0.04 g (46%); mp: 108–110 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 3.12 (m, 10H), 3.75 (s, 3H), 3.83 (m, 1H), 4.17 (m, 1H), 4.56 (m, 1H), 5.94 (s, 1H), 6.92 (m, 4H), 7.58 (m, 3H), 7.96 ppm (m, 4H); Anal. calcd for $C_{27}H_{30}N_2O_7 \cdot 0.5\,H_2O$: C 64.39, H 6.21, N 5.57, found: C 64.67, H 6.06, N 5.20.

1-(2-methoxyphenyl)-4-(2-*trans*-naphthalen-2-yl[1,3]dioxolan-4-ylmethyl)piperazine (12*t*): yield 0.30 g (14%), ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.81 (m, 6H), 3.17 (m, 4H), 3.81 (dd, J = 8.0, 7.4 Hz, 1H), 3.90 (s, 3H), 4.39 (dd, J = 8.0, 6.6 Hz, 1H), 4.58 (m, 1H), 6.17 (s, 1H), 6.98 (m, 4H), 7.52 (m, 2H), 7.63 (m, 1H), 7.92 ppm (m, 4H). The corresponding oxalate salt **5*t*** was crystallized from EtOH: yield 0.05 g (38%); mp: 168–170 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 3.07 (m, 10H), 3.70 (m, 1H), 3.76 (s, 3H), 4.34 (m, 1H), 4.63 (m, 1H), 6.06 (s, 1H), 6.90 (m, 4H), 7.54 (m, 3H), 7.95 ppm (m, 4H); Anal. calcd for $C_{27}H_{30}N_2O_7$: C 65.56, H 6.12, N 5.67, found: C 65.58, H 6.10, N 5.79.

1-(2-methoxyphenyl)-4-(2-*cis*-phenyl[1,3]dioxolan-4-ylmethyl)piperazine (13*c*): yield 0.45 g (26%), ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.80 (m, 6H), 3.14 (m, 4H), 3.87 (dd, J = 7.8, 6.6 Hz, 1H), 3.90 (s, 3H), 4.19 (dd, J = 7.8, 6.8 Hz, 1H), 4.49 (m, 1H), 5.87 (s, 1H), 6.98 (m, 4H), 7.42 (m, 3H), 7.53 ppm (m, 2H). The corresponding oxalate salt **6*c*** was crystallized from MeOH: yield 0.13 g (83%); mp: 174–176 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 3.06 (m, 10H), 3.76 (s, 3H), 3.79 (m, 1H), 4.11 (m, 1H), 4.54 (m, 1H), 5.77 (s, 1H), 6.91 (m, 4H), 7.43 ppm (m, 5H); Anal. calcd for $C_{23}H_{28}N_2O_7$: C 62.14, H 6.35, N 6.30, found: C 62.39, H 6.40, N 6.25.

1-(2-methoxyphenyl)-4-(2-*trans*-phenyl[1,3]dioxolan-4-ylmethyl)piperazine (13*t*): yield 0.40 g (23%), ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.78 (m, 6H), 3.16 (m, 4H), 3.76 (dd, J = 8.0, 7.0 Hz, 1H), 3.90 (s, 3H), 4.33 (dd, J = 8.0, 6.0 Hz, 1H), 4.52 (m, 1H), 6.00 (s, 1H), 6.98 (m, 4H), 7.41 (m, 3H), 7.51 ppm (m, 2H). The corresponding oxalate salt **6*t*** was crystallized from MeOH: yield 0.04 g (54%); mp: 175–177 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 2.87 (m, 6H), 3.04 (m, 4H), 3.63 (m, 1H), 3.77 (s, 3H), 4.26 (m, 1H), 4.49 (m, 1H), 5.87 (s, 1H), 6.90 (m, 4H), 7.39 ppm (m, 5H); Anal. calcd for $C_{23}H_{28}N_2O_7$: C 62.14, H 6.35, N 6.30, found: C 62.26, H 6.37, N 6.31.

1-(2,2-diphenyl[1,3]dioxolan-4-ylmethyl)-4-(2-methoxyphenyl)piperazine (14): yield 0.70 g (45%), ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.71 (m, 6H), 3.09 (m, 4H), 3.80 (dd, J = 7.9, 7.0 Hz, 1H), 3.86 (s, 3H), 4.16 (dd, J = 7.9, 6.7 Hz, 1H), 4.42 (m, 1H), 6.94 (m, 4H), 7.32 (m, 6H), 7.51 ppm (m, 4H). The corresponding oxalate salt **7** was crystallized from MeOH: yield 0.17 g (43%); mp: 194–197 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 3.15 (m, 10H), 3.86 (m, 1H), 3.89 (s, 3H), 4.25 (m, 1H), 4.57 (m, 1H), 7.04 (m, 4H), 7.51 ppm (m, 10H); Anal. calcd for $C_{29}H_{32}N_2O_7$: C 66.89, H 6.20, N 5.38, found: C 67.02, H 6.21, N 5.72.

1-(1,4-dioxaspiro[4,5]dec-2-ylmethyl)-4-(2-methoxyphenyl)piperazine (15): yield 0.45 (49%), ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.44 (m, 2H), 1.65 (m, 8H), 2.63 (ddd, J = 13.0, 6.1, 5.3 Hz, 2H), 2.77 (m, 4H), 3.13 (m, 4H), 3.67 (dd, J = 8.0, 7.1 Hz, 1H), 3.90 (s, 3H), 4.12 (dd, J = 8.0, 6.2 Hz, 1H), 4.35 (m, 1H), 6.97 ppm (m, 4H). The corresponding oxalate salt **8** was crystallized from EtOH: yield 0.09 g (48%); mp: 148–150 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 1.38 (m, 2H), 1.57 (m, 8H), 3.02 (m, 10H), 3.62 (m, 1H), 3.80 (s, 3H), 4.08 (m, 1H), 4.41 (m, 1H), 6.95 ppm (m, 4H); Anal. calcd for $C_{22}H_{32}N_2O_7$: C 60.52, H 7.39, N 6.42, found: C 60.60, H 7.43, N 6.02.

1-(2,2-diphenyl[1,3]oxathiolan-5-ylmethyl)-4-(2-methoxyphenyl)-piperazine (16): yield 0.47 g (87%), ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.82 (m, 6H), 3.13 (m, 4H), 3.23 (ddd, J = 10.3, 8.1, 6.1 Hz, 2H), 3.90 (s, 3H), 4.41 (m, 1H), 6.98 (m, 4H), 7.35 (m, 8H), 7.68 ppm (m, 2H). The corresponding oxalate salt **9** was crystallized from MeOH: yield 0.38 g (64%); mp: 200–204 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 3.03 (m, 11H), 3.27 (m, 1H), 3.78 (s, 3H), 4.39 (m, 1H), 6.93 (m, 4H), 7.32 (m, 8H), 7.58 ppm (m, 2H); Anal. calcd for $C_{29}H_{32}N_2O_6S$: C 64.91, H 6.01, N 5.22, found: C 65.19, H 5.99, N 5.57.

1-(2,2-diphenyl[1,3]dithiolan-4-ylmethyl)-4-(2-methoxyphenyl)piperazine (17): yield 0.21 g (55%), ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.76 (m, 6H), 3.08 (m, 4H), 3.32 (ddd, J = 10.0, 8.0, 6.0 Hz, 2H), 3.83 (s, 3H), 4.10 (m, 1H), 6.92 (m, 4H), 7.28 (m, 6H), 7.60 (m, 2H), 7.70 ppm (m, 2H). The corresponding oxalate salt **10** was crystallized from EtOH: yield 0.14 g (67%); mp: 195–200 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 2.82 (m, 4H), 3.02 (m, 6H), 3.30 (m, 2H), 3.75 (s, 3H), 4.29 (m, 1H), 6.90 (m, 4H), 7.30 (m, 6H), 7.48 (m, 2H), 7.58 ppm (m, 2H); Anal. calcd for $C_{29}H_{32}N_2O_5S_2$: C 63.02, H 5.84, N 5.07, found: C 63.35, H 5.90, N 5.06.

Biological assays

Radioligand binding assay at human recombinant 5-HT$_{1A}$ receptors and α_1 adrenoceptor subtypes: A human cell line (HeLa) stably transfected with genomic clone G-21 coding for the human 5-HT$_{1A}$ serotoninergic receptor was used. Cells were grown as monolayers in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and gentamicin (100 mg mL^{-1}) under 5% CO_2 at 37 °C. Cells were detached from the growth flask at 95% confluence by a cell scraper and were lysed in ice-cold Tris (5 mM) and EDTA buffer (5 mM, pH 7.4). Homogenates were centrifuged for 20 min at 40000 *g*, and pellets were resuspended in a small volume of ice-cold Tris/EDTA buffer (above) and immediately frozen and stored at −70 °C until use. On the day of experiment, cell membranes were resuspended in binding buffer (50 mM Tris,

2.5 mM $MgCl_2$, and 10 mM pargiline, pH 7.4). Membranes were incubated in a final volume of 1 mL for 30 min at 30 °C with 1 nM [^{3}H]8-OH-DPAT, in the absence or presence of various concentrations of the competing drugs (1 pM to 10 mM); each experimental condition was performed in triplicate. Nonspecific binding was determined in the presence of 10 mM 5-HT. Binding to recombinant human α_1 adrenoceptor subtypes was performed in membranes from Chinese hamster ovary (CHO) cells transfected by electroporation with DNA expressing the gene encoding each α_1 adrenoceptor subtype. Cloning and stable expression of the human α_1 adrenoceptor genes were performed as described.[24] CHO cell membranes were incubated in 50 mM Tris (pH 7.4) with 0.2 nM [^{3}H]prazosin, in a final volume of 1.02 mL for 30 min at 25 °C, in the absence or presence of competing drugs (1 pM to 10 mM). Nonspecific binding was determined in the presence of 10 mM phentolamine. The incubation was stopped by addition of ice-cold Tris buffer and rapid filtration through 0.2% polyethyleneimine-pretreated Whatman GF/B or Schleicher & Schuell GF52 filters.

[^{35}S]GTPγS binding: The effects of the various compounds tested on [^{35}S]GTPγS binding in HeLa cells expressing the recombinant human 5-HT_{1A} receptor were evaluated according to the method of Stanton and Beer[32] with minor modifications.

Stimulation experiments: Cell membranes were resuspended in buffer containing 20 mM HEPES, 3 mM $MgSO_4$, and 120 mM NaCl (pH 7.4). The membranes were incubated with 30 mM GDP and various concentrations (from 0.1 nM to 100 mM) of test drugs or 5-HT (reference curve) for 20 min at 30 °C in a final volume of 0.5 mL. Samples were transferred to ice, [^{35}S]GTPγS (200 pM) was added, and samples were incubated for another 30 min at 30 °C.

Antagonism experiments: Cell membranes were resuspended in buffer containing 20 mM HEPES, 3 mM $MgSO_4$, and 120 mM NaCl (pH 7.4). The membranes were incubated with 30 mM GDP and various concentrations of 5-HT (from 0.1 nM to 100 mM) in the absence or presence of antagonist, or with 30 mM GDP and various concentrations (from 0.1 nM to 100 mM) of test drugs in the presence of 5-HT (1 mM) for 20 min at 30 °C in a final volume of 0.5 mL. Samples were transferred to ice, [^{35}S]GTPγS (200 pM) was added, and samples were incubated for a further 30 min at 30 °C. The pre-incubation with both agonist and antagonist, before initiating the [^{35}S]GTPγS binding, ensures that agonist and antagonist are at equilibrium.

For both procedures, nonspecific binding was determined in the presence of 10 mM GTPγS. Incubation was stopped by the addition of ice-cold HEPES buffer and rapid filtration on Schleicher & Schuell GF52 filters using a Brandel cell harvester. The filters were washed with ice-cold buffer, and the radioactivity retained on the filters was determined by liquid scintillation counting at 90% efficiency.

Functional antagonism in isolated tissues: Male Wistar rats (275–300 g) were killed by cervical dislocation, and the organs required were isolated, freed from adhering connective tissues, and set up rapidly under a suitable resting tension in 20 mL organ baths containing a physiological salt solution kept at 37 °C and aerated with 5% CO_2 and 95% O_2 at pH 7.4. Concentration–response curves were constructed by cumulative addition of agonist. The concentration of agonist in the organ bath was increased approximately threefold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Contractions were recorded by means of a force displacement transducer connected to the Mac Lab system PowerLab/800 and to a polygraph channel recorder (Gemini). In addition, parallel experiments were carried out in which tissues did not receive any antagonist in order to check any variation in sensitivity. All animal testing was carried out according to European Community Council Directive 86/609/EEC (November 1986).

Vas deferens prostatic portion: This tissue was used to assess the antagonism toward α_{1A} adrenoceptors.[26] Prostatic portions of 2 cm length were mounted under 0.5 g tension at 37 °C in tyrode solution of the following composition (in mM): NaCl, 130; KCl, 1; $CaCl_2$, 1.8; $MgCl_2$, 0.89; NaH_2PO_4, 0.42; $NaHCO_3$, 25; glucose, 5.6. Cocaine hydrochloride (0.1 μM) was added to the tyrode to prevent neuronal uptake of (−)-norepinephrine. The preparations were equilibrated for 60 min with washing every 15 min. After the equilibration period, tissues were primed twice by addition of 10 μM norepinephrine. After another washing and equilibration period of 60 min, a norepinephrine concentration–response curve was constructed (basal response). The antagonist was allowed to equilibrate for 30 min before constructing a new concentration–response curve to the agonist. (−)-Norepinephrine solutions contained 0.05% $Na_2S_2O_5$ to prevent oxidation.

Spleen: This tissue was used to assess the antagonism toward α_{1B} adrenoceptors.[27] The spleen was removed and bisected longitudinally into two strips, which were suspended in tissue baths containing Krebs solution of the following composition (in mM): NaCl, 120; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.5; KH_2PO_4, 1.2; $NaHCO_3$, 20; glucose, 11; K_2EDTA, 0.01. Propranolol hydrochloride (4 μM) was added to block β adrenoceptors. The spleen strips were placed under 1 g resting tension and equilibrated for 2 h. The cumulative concentration–response curves to phenylephrine were measured isometrically and obtained at 30 min intervals, the first one being discarded and the second taken as a control. The antagonist was allowed to equilibrate for 30 min before constructing a new concentration–response curve to the agonist.

Aorta: This tissue was used to assess the antagonism toward α_{1D} adrenoceptors.[28] Thoracic aorta was cleaned from extraneous connective tissues and placed in Krebs solution of the following composition (in mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 1.9; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.7; K_2EDTA, 0.01. Cocaine hydrochloride (0.1 μM) and propranolol hydrochloride (4 μM) were added to prevent the neuronal uptake of (−)-norepinephrine and to block β adrenoceptors, respectively. Two helical strips (15 mm × 3 mm) were cut from each aorta beginning from the end most proximal to the heart. The endothelium was removed by rubbing with filter paper: the absence of acetylcholine (100 μM)-induced relaxation to preparations contracted with (−)-norepinephrine (1 μM) was taken as an indicator that the vessels were successfully denuded. Vascular strips were then tied with surgical thread and suspended in a jacketed tissue bath containing tyrode solution. Strip contractions were measured isometrically. After an equilibration period of at least 2 h under an optimal tension of 1 g, cumulative (−)-norepinephrine concentration–response curves were recorded at 1 h intervals, the first two being discarded and the third one taken as control. The antagonist was allowed to equilibrate with the tissue for 30 min before the generation of the fourth cumulative concentration–response curve to (−)-norepinephrine. (−)-Norepinephrine solutions contained 0.05% K_2EDTA in 0.9% NaCl to prevent oxidation.

Data analysis: Binding data were analyzed using the nonlinear curve-fitting program Allfit.[30] Scatchard plots were linear in all preparations. None of the pseudo-Hill coefficients (n_H) were significantly different from unity ($p > 0.05$). Equilibrium dissociation constants (K_i) were derived from the Cheng–Prusoff equation:[31] $K_i = IC_{50}/(L/K_d)$, where L and K_d are the concentration and the equilibrium dissociation constant of the radioligand. pK_i values are the

mean of 2–3 separate experiments performed in triplicate. Stimulation of [^{35}S]GTPγS binding induced by the compounds tested was expressed as the percent increase in binding above basal value, with the maximal stimulation observed with 5-HT taken as 100%. The concentration–response curves of the agonistic activity were analyzed by Allfit as reported above. The maximum percentage of stimulation of [^{35}S]GTPγS binding (E_{max}) achieved for each drug, and the concentration required to obtain 50% of E_{max} ($pD_2 = -\log_{10}[EC_{50}$ value]), were evaluated. In functional studies, responses were expressed as a percentage of the maximal contraction observed in the agonist concentration–response curves, taken as a control, which were analyzed by pharmacological computer programs. pK_b values were calculated according to van Rossum[32] at one or two concentrations.

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