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Brief Article

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Structure−Activity Relationships in 1,4-Benzodioxan-Related Compounds. 11.[1] Reversed Enantioselectivity of 1,4-Dioxane Derivatives in α_1-Adrenergic and 5-HT$_{1A}$ Receptor Binding Sites Recognition

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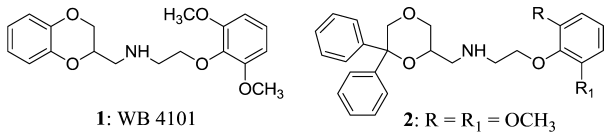
Supporting Information

ABSTRACT: 5-HT$_{1A}$ receptor and α_1-adrenoreceptor (α_1-AR) binding sites recognized by the 1,4-dioxanes **2**−**4** display reversed stereochemical requirements. (*S*)-**2** proved to be a potent 5-HT$_{1A}$ receptor agonist highly selective over α_1-AR subtypes. Chirality influenced the anticancer activity of **3** and **4** in human prostate cancer cells (PC-3): (*R*)-**4**, eutomer at the α_{1d}-AR subtype, was the most potent. The decreased effect of **4** and (*R*)-**4** in α_{1d}-AR silenced PC-3 cells confirmed that their anticancer activity was α_{1d}-AR-dependent.

α_1-Adrenoreceptors (α_1-ARs), belonging to G-protein-coupled receptor (GPCR) family A, are subdivided into three pharmacologically distinct subtypes ($\alpha_{1a/A}$, $\alpha_{1b/B}$, $\alpha_{1d/D}$), with upper and lower case subscript designating the native or recombinant receptors, respectively.[2] α_{1A}- and α_{1B}-AR subtypes play an important role in cardiac development and/or function as well as in blood pressure via vasoconstriction.[3] Moreover, the α_{1A}-AR subtype, dominant in the prostate, bladder neck, and urethra, together with the α_{1D}-AR subtype, located in the bladder or the spinal cord, mediates lower urinary tract symptoms (LUTS) caused by benign prostatic hyperplasia (BPH).[4] In addition to their smooth-muscle-relaxing effects, α_1-AR antagonists inhibit the primary tumor growth and progression to metastasis in human prostate cancer.[5] Like the α_1-ARs, 5-HT$_{1A}$ receptor, a member of the serotonin 5-HT$_1$ class, is a GPCR.[6] In the past decade, the 5-HT$_{1A}$ subtype has been the most studied as a major target for agents therapeutically useful not only in well-established areas such as depression and anxiety but also in recent perspectives such as neuroprotection,[6] cognition disorders,[7] and in pain relief therapy.[8] It is well documented that the transmembrane amino acid sequence of 5-HT$_{1A}$ receptor exhibits a notably high degree of homology to α_1-ARs (approximately 45%).[9] Therefore, several classes of compounds show high affinity for both receptor systems, poor specificity, and consequently, adverse effect problems. Among these classes, the benzodioxane derivatives are effective ligands of both receptor systems,[10,11] and the potent and non-subtype-selective α_1-antagonist WB 4101 (**1**) (Chart 1) is also an effective partial agonist of 5-HT$_{1A}$ receptor.[12]

Many efforts have been devoted to understand the structural requirements of ligands useful to differentiate the binding sites

Chart 1. Structures of WB 4101 (1) and 2−4



1: WB 4101

2: R = R$_1$ = OCH$_3$
3: R = H; R$_1$ = OCH$_3$
4: R = R$_1$ = H

of 5-HT$_{1A}$ receptor and α_1-ARs. We have recently demonstrated that the replacement of the quite planar 1,4-benzodioxane template of **1** with the less conformationally constrained 6,6-diphenyl-1,4-dioxane moiety[13] afforded **2** (Chart 1), which was able to discriminate between the two systems, maintaining the high 5-HT$_{1A}$ affinity of **1** but displaying markedly reduced affinities at all three α_1-AR subtypes. The elimination of one or both ortho-methoxy substituents of **2**, affording **3** and **4** (Chart 1), respectively, though slightly reducing intrinsic activity, was compatible with the maintenance of the high affinity for 5-HT$_{1A}$ receptor. Interestingly, such a modification conferred to these ligands the ability to potently and preferentially interact with the α_{1d}-AR subtype with respect to α_{1a}- and α_{1b}-AR subtypes. Compounds **2**−**4** behaved as antagonists at all three α_1-AR subtypes and as full or partial agonists at 5-HT$_{1A}$ receptor.[13] Tested in human PC-3 prostate cancer cells, they also showed antiproliferative and cytotoxic activity: **3** and especially **4** proved to be the most potent, probably owing to their high α_{1D}-AR antagonism.[13] Moreover, since serotonin induces proliferation in PC-3 cells,

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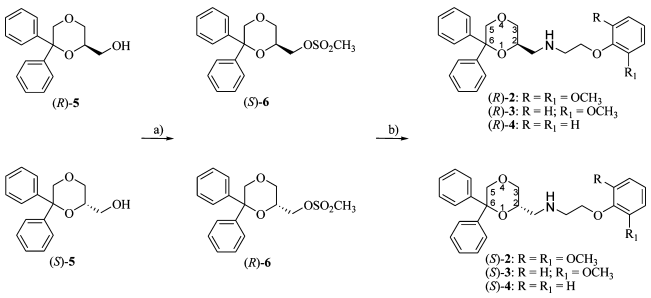
we demonstrated that the 5-HT$_{1A}$ antagonist (*S*)-WAY 100135 did not affect the antiproliferative and cytotoxic effects of **2**−**4**, indicating that their potent 5-HT$_{1A}$ agonist activity was not in contrast to their anticancer activity.[13] Considering that the interactions with α_1-ARs and 5-HT$_{1A}$ receptor are well-known to be highly stereospecific and stereochemistry can influence quantitatively and qualitatively the biological profile of ligands, the enantiomers of **2**−**4** were prepared and studied to investigate whether a definite configuration might enhance the discrimination between the two receptor systems. In addition, the study of enantioselectivity of chiral **2**−**4** might allow us to determine whether their anticancer activity is triggered by a specific or nonspecific mechanism of action. Therefore, the evaluation of the cytotoxic activity of the enantiomers of **2**−**4** in PC-3 prostate cancer cells and of **4** and its enantiomers in human PC-3 cells transfected with a control (siGLO) or silenced α_{1d}-AR gene (siα_{1d}-AR) might allow us to confirm the previously hypothesized involvement of α_{1d}-AR in this activity.[13,14]

RESULTS AND DISCUSSION

To prepare the novel enantiomers (*R*)- and (*S*)-**2**−**4**, alcohols (*R*)- and (*S*)-**5**[15] were treated with mesyl chloride in pyridine to yield (*S*)- and (*R*)-**6**, respectively, which were aminated with the suitable amines, 2-(2,6-dimethoxyphenoxy)ethanamine,[16] 2-(2-methoxyphenoxy)ethanamine, or 2-phenoxyethanamine (Scheme 1). Enantiomeric purity of amines (*R*)-(+)- and (*S*)-

Scheme 1[a]



[a]Reagents: (a) MsCl, pyridine; (b) 2-(2,6-dimethoxyphenoxy)-ethanamine or 2-(2-methoxyphenoxy)ethanamine or 2-phenoxyethanamine, $CH_3CH_2OCH_2CH_2OH$, Δ.

(−)-**2**−**4**, determined by HPLC and by ^{1}H NMR spectroscopy on addition of the chiral shift reagent (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(+)-MTPA] in comparison with the spectrum of racemic (±)-**2**−**4**, was found to be 100% for all the enantiomers with the exception of (*S*)-(−)-**4**, whose value is 98.7%. The HPLC chromatograms and ^{1}H NMR spectra of (±)-, (*R*)-(+)-, and (*S*)-(−)-**4** in the presence of (+)-MTPA are reported in Supporting Information.

The binding profiles of (*R*)- and (*S*)-**2**−**4** were evaluated using [^{3}H]prazosin and [^{3}H]8-hydroxy-2-(di-*n*-propylamino)-tetralin ([^{3}H]8-OH-DPAT) to label cloned human α_1-ARs expressed in Chinese hamster ovary (CHO) cells and 5-HT$_{1A}$ receptors expressed in HeLa cells, respectively. The potent α_{1d}-AR antagonist 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (BMY-7378) and the 5-HT$_{1A}$ receptor full agonist 8-OH-DPAT were used as reference compounds.[13] The affinity values, expressed as pK_i, are reported in Table 1 and compared with those of WB 4101 (**1**) and its enantiomers,[17] included for useful comparison. An analysis of data showed that the configuration of the stereocenter in position 2 of the 1,4-dioxane nucleus of **2**−**4** affected affinity and selectivity at α_1-ARs and 5-HT$_{1A}$ receptor.

For the interaction with the α_1-AR system, (*R*)-**2**, (*R*)-**3**, and (*R*)-**4**, showing pK_i values not significantly different from those of the corresponding racemic compounds, were the eutomers at the three α_1-AR subtypes. Considering that the enantiomeric purity of all the enantiomers is 100% with the exception of (*S*)-(−)-**4**, whose value is 98.7%, the eudismic ratios (ER) are >6, 8, and >13 for the first pair, 15, 19, and >18 for the second pair, and 3, 3, and >26 for the third pair. In contrast, in the case of WB 4101 (**1**) and many of its analogues,[17] (*S*)-enantiomers were significantly more potent than (*R*)-enantiomers at α_1-ARs. This observation seems to confirm that, as we previously hypothesized by structure−activity relationships and docking studies,[13] the 1,4-dioxanes **2**−**4** do not interact with the same α_1-AR binding sites recognized by **1**. Concerning 5-HT$_{1A}$ receptor, similar to **1** and unlike what was observed for the α_1-AR interaction, the higher affinity resided in the (*S*)-configured enantiomers. However, only in the pair of enantiomers (*S*)-**2**/(*R*)-**2**, in which the phenoxy group is 2,6-dimethoxy-substituted, (*S*)-**2** was significantly more affine than (*R*)-**2** and the eudismic ratio (ER = 81) assumed a high value. Interestingly, since the eutomers at 5-HT$_{1A}$ receptor were distomers at α_1-AR, a reversal of enantioselectivity was observed. Consequently, the selectivity ratio 5-HT$_{1A}$/α_1-AR considerably increased compared to the corresponding racemic compounds. In particular, (*S*)-**2** showed significantly high 5-HT$_{1A}$/α_1-AR subtype affinity ratios (5-HT$_{1A}$/α_{1a} > 1820; 5-HT$_{1A}$/α_{1b} = 3020; 5-HT$_{1A}$/α_{1d} = 1318), which were unusual because of the notably high degree of homology existing between these two receptor systems. This result suggests that the binding sites of the two receptor systems recognized by the 1,4-dioxane derivatives have different stereochemical requirements and the (*S*) configuration is a structural requirement crucial for the design of novel compounds able to selectively recognize the 5-HT$_{1A}$ receptor over α_1-ARs. Moreover, (*S*)-**2** might be a pharmacologically useful tool to fully activate the 5-HT$_{1A}$ receptor with reduced hypotensive side effects due to its low affinities for α_1-AR subtypes. The agonist efficacy, expressed as pD_2, of enantiomers (*R*)- and (*S*)-**2**−**4** toward the 5-HT$_{1A}$ receptor was assessed by determining the induced stimulation of [^{35}S]GTPγS binding in cell membranes from HeLa cells, using the full agonist 5-hydroxytryptamine (5-HT) as reference compound (Table 1).[13] Data showed that enantiomers (*S*)-**2**−**4** were significantly more potent than (*R*)-enantiomers (ER = 263, 31, and >23, respectively), with pD_2 values similar to those of the corresponding racemic compounds and significantly higher than those of the full agonists 8-OH-DPAT and 5-HT. Moreover, the configuration of the stereocenter in position 2 proved to be crucial for the potency but not for the intrinsic activity, both enantiomers of the three pairs behaving as full [(*R*)- and (*S*)-**2**] or partial agonists [(*R*)- and (*S*)-**3**; (*R*)- and (*S*)-**4**].

The in vitro cytotoxic activity of (*R*)- and (*S*)-**2**−**4** and **1** in human PC-3 cells, using 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-ylcarbonyl)piperazine (doxazosin)[18] as reference compound, was carried out using the sulforhodamine B (SRB) assay according to the National Cancer Institute protocol, as previously reported.[13] All compounds were active at low micromolar concentration (Table 2) and chirality affected the antiproliferative and cytotoxic activity of **3** and **4**, highlighting a specific mechanism

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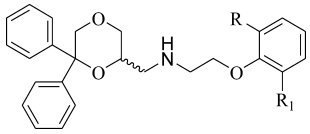
Table 1. Affinity Constants (pK_i) of 1−4 and Their Enantiomers, BMY-7378, and 8-OH-DPAT for Human Recombinant α_1-AR Subtypes and 5-HT_{1A} Receptor[a] and Agonist Efficacy (pD_2)[b] and Relative Efficacy (E_{max})[c] on 5-HT_{1A} Receptor in Comparison to BMY-7378, 8-OH-DPAT, and 5-HT



			pK_i, human cloned receptor				binding [^{35}S]GTPγS	
compd	R	R_1	α_{1a}	α_{1b}	α_{1d}	5-HT_{1A}	5-HT_{1A}, pD_2	E_{max}, %
(±)-**2**	OCH_3	OCH_3	6.47	6.49	7.18	8.85	8.28	106.3
(*R*)-**2**	OCH_3	OCH_3	6.78	6.71	7.25	7.35	5.88	119.5
(*S*)-**2**	OCH_3	OCH_3	<6	5.78	6.14	9.26	8.30	103.6
(±)-**3**	OCH_3	H	7.56	7.25	8.94	9.18	9.40	81.5
(*R*)-**3**	OCH_3	H	7.78	7.66	8.86	9.22	7.70	66.6
(*S*)-**3**	OCH_3	H	6.59	6.38	7.60	9.51	9.19	61.9
(±)-**4**	H	H	6.77	6.92	8.44	9.23	9.11	77.1
(*R*)-**4**	H	H	6.89	7.28	8.49	8.83	7.82	72.7
(*S*)-**4**	H	H	6.36	6.77	7.08	9.40	9.19	66.0
WB 4101 (**1**)			9.37	8.00	9.29	8.68		
(*R*)-**1**			7.95	7.14	7.98	7.39		
(*S*)-**1**			9.39	8.24	9.29	8.61		
BMY-7378			6.42	6.15	8.89	9.43	9.27	26.0
8-OH-DPAT			<6	<6	<6	8.47	7.60	100
5-HT							7.30	100

[a]Equilibrium dissociation constants (K_i) were derived from IC_{50} values using the Cheng−Prusoff equation.[20] Each experiment was performed in triplicate. K_i values were from two to three experiments, which agreed within ±20%. [b]pD_2 values are the negative logarithm of the agonist concentration required to obtain 50% of the maximal stimulation of [^{35}S]GTPγS binding and were calculated from two to three experiments, which agreed within ±20. [c]Maximal stimulation is expressed as a percentage of the maximal 5-HT response.

Table 2. Cytotoxic Activity of 2−4, Their Enantiomers, and WB 4101 (1) in Comparison to Doxazosin in PC-3 Cells and Cytotoxic Activity of 4 and Its Enantiomers in siGLO- and α_{1d}-AR-Silenced PC-3 Cells[a]

compd	PC-3 cell	GI_{50}, μM	TGI, μM	LC_{50}, μM
(±)-**2**	UT[b]	14.0 ± 0.7[c]	32.0 ± 1.3[c]	45.0 ± 1.2[c]
(*R*)-**2**	UT[b]	32.0 ± 1.3	58.0 ± 1.6	65.0 ± 1.6
(*S*)-**2**	UT[b]	16.0 ± 0.9	30.0 ± 0.6	37.0 ± 1.5
(±)-**3**	UT[b]	9.0 ± 0.8[c]	16.0 ± 0.9[c]	45.0 ± 1.4[c]
(*R*)-**3**	UT[b]	4.0 ± 0.3	9.0 ± 0.6	29.0 ± 0.8
(*S*)-**3**	UT[b]	21.0 ± 1.2	34.0 ± 1.5	67.0 ± 1.6
(±)-**4**	UT[b]	3.0 ± 0.2[c]	6.0 ± 0.7[c]	8.0 ± 0.5[c]
	siGLO	4.1 ± 0.8	7.1 ± 0.6	10.7 ± 0.5
	siα_{1d}-AR	23.6 ± 1.2	25.0 ± 0.8	39.8 ± 0.9
(*R*)-**4**	UT[b]	0.6 ± 0.07	2.1 ± 0.1	4.0 ± 0.2
	siGLO	0.9 ± 0.5	2.5 ± 0.4	5.8 ± 0.2
	siα_{1d}-AR	10.2 ± 0.6	27.1 ± 0.3	52.1 ± 1.1
(*S*)-**4**	UT[b]	19.1 ± 1.1	43.0 ± 0.9	>300
	siGLO	22.8 ± 0.4	41.7 ± 1.3	>300
	siα_{1d}-AR	20.7 ± 0.7	49.2 ± 0.9	>300
WB 4101 (**1**)	UT[b]	64.6 ± 0.4	199 ± 1.6	>200
doxazosin	UT[b]	26.9 ± 1.3	49.0 ± 2.5	75.8 ± 3.6

[a]Each quoted value represents the mean of quadruplicate determinations ± standard error (n = 5). Statistical analysis was performed by comparing the anticancer effect induced by enantiomers (*R*)- and (*S*)-**2**−**4** with respect to the corresponding racemic compounds and by (±)-**4**, (*R*)-**4**, and (*S*)-**4** in α_{1d}-AR-silenced with respect to siGLO-transfected PC-3 cells. [b]UT = untransfected. [c]This value is not significantly different from that previously reported.[13]

of action. Interestingly, the eutomers at the α_{1d}-AR subtype [(*R*)-**3** and (*R*)-**4**] were also efficient cytotoxic agents, with (*R*)-**4** exhibiting the highest potency. This observation supported the previously hypothesized relationship between the interaction with α_{1d}-AR subtype and anticancer activity.[13] To confirm such a hypothesis, cytotoxic activity of **4** and its enantiomers in human PC-3 cells transfected with siGLO or siα_{1d}-AR was evaluated. Silencing of α_{1d}-AR significantly reduced the anticancer activity of **4** and its enantiomer (*R*)-**4** (Table 2). In particular, for (*R*)-**4** GI_{50} increased from 0.6 to 10.2 μM (17-fold), TGI from 2.1 to 27.1 μM (12.9-fold), and LC_{50} from 4.0 to 52.1 μM (13-fold). No significant differences in cytotoxic activity were found comparing siGLO-transfected with untransfected cells. Moreover, silencing of α_{1d}-AR significantly inhibited (about 76%) the noradrenaline (−)-NA-induced proliferation compared to siGLO-transfected PC-3 control cells (Figure 1C in Supporting Information), whereas no changes were found by comparing siGLO-transfected and untransfected PC-3 prostate cancer cells. These results strongly confirm the hypothesis that the antiproliferative and cytotoxic effects of **4** and (*R*)-**4** are α_{1d}-AR-dependent. Moreover, it is known that antidepressant drugs can also be prescribed to prevent symptoms of depression in prostate cancer patients.[19] Therefore, the potentially beneficial antidepressant and antianxiety effects, due to the agonism at 5-HT_{1A} receptor, and the reduced hypotensive side effects, due to the low affinities for α_{1a} and α_{1b}-AR subtypes, make (*R*)-**4** a promising lead to be optimized for the development of α_{1d}-AR antagonists potentially useful in the treatment of human prostate cancer.

In conclusion, in the present study we demonstrated that the binding sites of 5-HT_{1A} receptor and α_1-ARs recognized by the 1,4-dioxanes **2**−**4** possessed reversed stereochemical requirements. Other novel derivatives and molecular modeling studies,

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objects of a forthcoming paper, might confirm and justify such an interesting result. (*S*)-**2** proved to be a potent 5-HT$_{1A}$ receptor full agonist highly selective over α_1-AR subtypes. (*R*)-**3** and (*R*)-**4**, eutomers at the α_{1d}-AR subtype, also displayed the best antiproliferative and cytotoxic effects. The critical role of chirality in the anticancer activity and the decreased antiproliferative and cytotoxic effects of **4** and (*R*)-**4** in α_{1d}-AR silenced PC-3 cells strongly confirmed the involvement of the α_{1d}-AR subtype in their anticancer properties.

EXPERIMENTAL SECTION

General. Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian EM-390 instrument. The microanalyses were performed by the microanalytical laboratory of our department. The elemental composition of the compounds agreed to within ±0.4% of the calculated value. Optical activity was measured at 20 °C with a Perkin-Elmer 241 polarimeter. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040−0.063 mm, Merck) by flash chromatography. Chemical names were generated using ChemDraw Ultra (CambridgeSoft, version 9.0). The enantiomeric purity, determined by HPLC, was found to be 100% for all the enantiomers with the exception of (*S*)-(−)-**4**, whose value is 98.7%. Instruments used were the following: a gradient capable separation system fitted with a UV or DAD detector; a Waters alliance 2695 HPLC instrument comprising a quaternary pump, Waters 2996 DAD, an automatic injector, a degassing system. Column was Daicel Chiralcel OJ-H 5 μm, 250 mm × 4.6 mm, part no. OJH0CE-FA025. Elution conditions were the following: solvent, MeOH for (±)-**3**, (*R*)-**3**, (*S*)-**3**, (±)-**4**, (*R*)-**4**, (*S*)-**4**; 90% MeOH/10% 2-PrOH for (±)-**2**, (*R*)-**2**, (*S*)-**2**; gradient table, isocratic 100% MeOH; duration, 35 min; flow, 0.6 mL/min; 18 °C; detection at λ = 205 nm. The purity of the novel compounds, determined by combustion and HPLC analysis, was >95%. The procedures for the synthesis of (*R*)-(+)- and (*S*)-(−)-**2** are reported below.

(*S*)-(6,6-Diphenyl-1,4-dioxan-2-yl)methyl Methanesulfonate [(*S*)-(+)-6]. Et_3N (1.0 mL, 7.4 mmol) and mesyl chloride (0.85 g, 7.4 mmol) were added to a stirred solution of (*R*)-(+)-**5**[15] (1.0 g, 3.7 mmol) in $CHCl_3$ (20 mL) at 0 °C. After 2 h at 0 °C, the mixture was washed with H_2O and dried over Na_2SO_4. After evaporation of the solvent the residue was purified by column chromatography, eluting with cyclohexane/EtOAc (9:1) to give (*S*)-(+)-**6** as an oil: 1.2 g (93% yield); $[\alpha]^{20}_D$ +119.6 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 3.10 (s, 3H, CH_3), 3.59−4.01 (m, 4H, CH_2O, cycle), 4.32 (m, 2H, cycle), 4.61 (d, 1H, 5-CH), 7.22−7.57 (m, 10H, ArH).

(*R*)-(6,6-Diphenyl-1,4-dioxan-2-yl)methyl Methanesulfonate [(*R*)-(−)-6]. This was prepared as described for (*S*)-(+)-**6** starting from (*S*)-(−)-**5**.[15] An oil was obtained: 96% yield; $[\alpha]^{20}_D$ −118.9 (*c* 1, $CHCl_3$). The ^{1}H NMR spectrum was identical to that of (*S*)-(+)-**6**.

(*R*)-2-(2,6-Dimethoxyphenoxy)-*N*-((6,6-diphenyl-1,4-dioxan-2-yl)methyl)ethanamine [(*R*)-(+)-2]. A solution of (*S*)-(+)-**6** (1.43 g, 4.1 mmol) and 2-(2,6-dimethoxyphenoxy)ethanamine[16] (4.0 g, 20.5 mmol) in 2-ethoxyethanol (25 mL) was heated to reflux for 4 h. Removal of the solvent under reduced pressure gave a residue, which was dissolved in water. The aqueous solution was basified with NaOH and extracted with $CHCl_3$. Removal of dried solvents gave a residue, which was purified by column chromatography, eluting with $CHCl_3$ to give (*R*)-(+)-**2** as an oil: 0.66 g (36% yield). The ^{1}H NMR spectrum was identical to that of the corresponding racemic compound.[13] The free base was transformed into the oxalate salt, which was crystallized from 2-PrOH; mp 151−153 °C; $[\alpha]^{20}_D$ +111.4 (*c* 1, MeOH). Anal. ($C_{27}H_{31}NO_5 \cdot H_2C_2O_4$) C, H, N.

(*S*)-2-(2,6-Dimethoxyphenoxy)-*N*-((6,6-diphenyl-1,4-dioxan-2-yl)methyl)ethanamine [(*S*)-(−)-2]. This was prepared as described for (*R*)-(+)-**2** starting from (*R*)-(−)-**6**: 25% yield. The ^{1}H NMR spectrum was identical to that of the corresponding racemic compound.[13] The free base was transformed into the oxalate salt, which was crystallized from 2-PrOH; mp 151−153 °C; $[\alpha]^{20}_D$ −109.2 (*c* 1, MeOH). Anal. ($C_{27}H_{31}NO_5 \cdot H_2C_2O_4 \cdot 0.5H_2O$) C, H, N.

ASSOCIATED CONTENT

Supporting Information

Elemental analysis results for enantiomers (*R*)- and (*S*)-**2**−**4**; experimental and spectroscopic details for (*R*)-(+)-**3**, (*S*)-(−)-**3**, (*R*)-(+)-**4**, and (*S*)-(−)-**4**; HPLC chromatograms and ^{1}H NMR spectra of (±)-, (*R*)-(+)-, and (*S*)-(−)-**4** in the presence of (+)-MTPA; experimental details of binding, cytotoxic assays, cell proliferation in siGLO- and siα_{1d}-AR-transfected PC-3 cells treated with (−)-NA (Figure 1C); quantitative real time PCR (qRT-PCR) and Western blot analysis demonstrating the silencing of α_{1d}-AR gene in PC-3 cells (Figure 1A,B). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

α_1-AR, α_1-adrenoreceptor; GPCR, G-protein-coupled receptor; LUTS, lower urinary tract symptoms; BPH, benign prostatic hyperplasia; siα_{1d}-AR, silenced α_{1d}-AR gene; CHO, Chinese hamster ovary; SRB, sulforhodamine B; GI, growth inhibition; TGI, total growth inhibition; LC, lethal concentration; K_i, inhibition or dissociation constant; ER, eudismic ratio; qRT-PCR, quantitative real time polymerase chain reaction; (−)-NA, noradrenaline

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