1,3-Dioxolane-Based Ligands as a Novel Class of α_1-Adrenoceptor Antagonists

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1,3-Dioxolane-based compounds (**2**−**14**) were synthesized, and the pharmacological profiles at α_1-adrenoceptor subtypes were assessed by functional experiments in isolated rat vas deferens (α_{1A}), spleen (α_{1B}), and aorta (α_{1D}). Compound **9**, with a pA_2 of 7.53, 7.36, and 8.65 at α_{1A}, α_{1B}, and α_{1D}, respectively, is the most potent antagonist of the series, while compound **10** with a pA_2 of 8.37 at α_{1D} subtype and selectivity ratios of 162 (α_{1D}/α_{1A}) and 324 (α_{1D}/α_{1B}) is the most selective. Binding assays in CHO cell membranes expressing human cloned α_1-adrenoceptor subtypes confirm the pharmacological profiles derived from functional experiments, although the selectivity values are somewhat lower. Therefore, it is concluded that 1,3-dioxolane-based ligands are a new class of α_1-adrenoceptor antagonists.

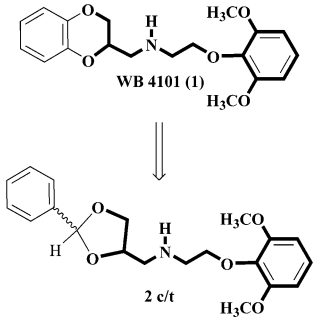
Introduction

The receptors for adrenaline and noradrenaline, designed as the adrenoceptors, have been studied for almost a century and have provided many targets for drug action. Before the advent of molecular biology, functional and binding assays were used initially to divide adrenoceptors into the major classes α and β[1] and then into α_1 and α_2[2,3] and β_1 and β_2.[4] Adrenoceptors were one of the first targets for the cloning technique developed in the 1980s. At present, nine human ones falling into three groups (α_1, α_2, and β) have been cloned. After some initial confusion,[5] there now appears to be a correlation between the recombinant adrenoceptors and those previously characterized in native tissues. Amino acid sequence analyses show 50% identity among the three members of each group and 30−40% identity between receptors in different groups. This is consistent with functional adrenoceptor pharmacology, which suggests much greater divergence among α_1-, α_2-, and β-adrenoceptors than among subtypes of either class.

As far as α_1-adrenoceptors are concerned, they can be divided into at least three subtypes, namely, α_{1A} (α_{1a}), α_{1B} (α_{1b}), and α_{1D} (α_{1d}), with upper and lower case subscripts being used to designate the native and recombinant receptors, respectively.[5−7]

This situation, although not definitive because emerging data seem to predict further subtyping, has given new impulse to medicinal chemists in their search of new and more selective ligands. As a result, several relatively selective ligands for α_1-adrenoceptor subtypes are now available.[8,9] The α_{1A} subtype, being the most prevalent subtype present in the prostate,[10,11] has received much attention as a potential target for symptomatic treatment of benign prostatic hyperplasia (BPH), and several uroselective agents have been revealed.[12]

Chart 1. Strategy for the Design of 1,3-Dioxolanes as α_1-Adrenoceptor Antagonists



In contrast, a potential therapeutic use for α_{1B} and α_{1D} subtype selective ligands has not been found yet. However, there is some evidence of a prominent role of the α_{1B} subtype in the regulation of blood pressure because α_{1B} knockout mice displayed a significantly reduced responsiveness to phenylephrine-induced increase in blood pressure.[13] There is also much interest in better understanding the role of the α_{1D} subtype. It has been shown that in the human bladder detrusor, α_{1d} mRNA is the predominant subtype,[14] and it has been postulated that the α_{1D} receptor blockade may ameliorate the irritative symptoms of BHP that result from involuntary contraction of the bladder smooth muscle.[15] Therefore, the discovery of selective agents is of paramount importance in order to define better their physiological role(s) and to discover new drug candidates.

Our group has recently undertaken a research project aimed at developing new and selective α_1-subtype antagonists using as a starting point WB 4101 {*N*-[2-(2,6-dimethoxyphenoxy)ethyl]-2,3-dihydro-1,4-benzodioxin-2-methanamine, **1**}, a prototype of the well-known class of α_1-adrenoceptor antagonists (benzodioxanes) whose chemical structure incorporates a 2,3-dihydro-1,4-benzodioxin-2-yl moiety as its main feature.[16]

Our first aim was to verify whether the 2,3-dihydro-1,4-benzodioxin-2-yl moiety could be replaced by the 2-phenyl-1,3-dioxolanyl structure, as in **2** (Chart 1). Structure modification of compound **2** has led to the

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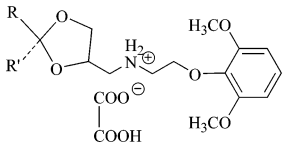
Table 1. Antagonist Potency, Expressed as pK_b (or pA_2) Values, and Selectivities of Compounds **1**, **2**−**4c/t**, **5**, **6**−**8c/t** at α_1-Adrenoceptors in Isolated Rat Prostatic Vas Deferens (α_{1A}), Spleen (α_{1B}), and Thoracic Aorta (α_{1D})



n	R	R'	pKbα_{1A}	pKbα_{1B}	pKbα_{1D}	α_{1D} / α_{1A}	α_{1D} / α_{1B}	α_{1A}/ α_{1B}
1			(9.36±0.04)	(8.21±0.02)	(8.60±0.02)	0.2	3	14
2c		H	6.61±0.21	5.70±0.09	6.44±0.05	0.7	6	8
2t	H		6.31±0.11	6.19±0.13	6.18±0.02	0.7	1	1
3c		H	5.36±0.25	5.77±0.16	5.13±0.03	0.6	0.2	0.4
3t	H		6.82±0.19	5.45±0.25	6.00±0.25	0.2	5	23
4c		CH_3	5.80±0.31	5.56±0.01	(6.89±0.09)	12	21	2
4t	CH_3		5.74±0.13	5.93±0.17	6.69±0.04	11	7	0.6
5			5.73±0.14	5.89±0.05	(6.93±0.28)	16	11	0.7
6c		H	5.60±0.11	5.51±0.15	7.15±0.12	36	44	12
6t	H		6.77±0.20	5.44±0.03	6.88±0.01	1	28	21
7c		H	<5	6.38±0.07	7.32±0.19	>209	9	<0.04
7t	H		5.69±0.13	6.38±0.20	7.07±0.38	24	5	0.2
8c		H	5.68±0.07	5.42±0.09	6.80±0.03	13	24	2
8t	H		5.79±0.21	5.12±0.10	6.65±0.17	7	33	5

discovery of a new class of α_1-adrenoceptor ligands. Here, we report on the syntheses and initial qualitative structure−activity relationship studies.

Chemistry

Compounds **2**−**14** were synthesized (Schemes 1−3) by standard procedures and characterized by ^{1}H nuclear magnetic resonance (NMR) and elemental analysis. The chloro derivatives **21**−**28**, the key intermediates obtained by reacting the appropriate aldehyde or ketone with 3-chloro-1,2-propanediol, were aminated with amines **18**−**20**. The diastereomeric mixtures were separated by flash chromatography at this stage, and the cis and trans stereochemistry was assigned by measurements of the nuclear Overhauser effect (NOE) between the hydrogen atom (or methyl group) at C_2 and the hydrogen atom at C_4. Methylation of the nitrogen atom, as in **41**, was achieved through the acylation reaction to give **40**, followed by reduction with $LiAlH_4$. Amines **18**−**20** were prepared by reacting chloroacetamide and the appropriate phenol followed by reduction with diborane.

Pharmacology

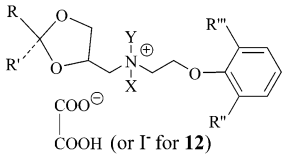
Receptor subtype selectivity of compounds **2**−**14** was determined at α_1-adrenoceptors on different isolated tissues using **1** and BMY-7378 as standard compounds. Blocking activity was assessed by antagonism of (−)-noradrenaline-induced contraction of rat prostatic vas deferens (α_{1A})[17] or thoracic aorta (α_{1D})[19] and by antagonism of (−)-phenylephrine-induced contraction of rat spleen (α_{1B}).[18]

The pharmacological profile was further evaluated by radioligand binding assay using [^{3}H]prazosin to label cloned human α_1-adrenoceptors expressed in CHO cells.[20]

Results and Discussion

The pharmacological results expressed as pK_b or pA_2, obtained on functional preparations such as prostatic vas deferens (α_{1A}), spleen (α_{1B}), and thoracic aorta (α_{1D}) of rat, are reported in Tables 1 and 2. All the compounds behave as α_1-adrenoceptor antagonists with varying degrees of potency and selectivity. Compounds **2c/t** (**c**,

Table 2. Antagonist Potency, Expressed as pK_b (or pA_2) Values, and Selectivities of Compounds **5**, **9**−**12**, **13c/t**, **14**, and BMY-7378 at α_1-Adrenoceptors in Isolated Rat Prostatic Vas Deferens (α_{1A}), Spleen (α_{1B}), and Thoracic Aorta (α_{1D})



n	R	R'	R''	R'''	X	Y	pKbα_{1A}	pKbα_{1B}	pKbα_{1D}	α_{1D}/α_{1A}	α_{1D}/α_{1B}	α_{1A}/α_{1B}
5	⬡	⬡	OCH_3	OCH_3	H	H	5.73±0.14	5.89±0.05	(6.93±0.28)	16	11	0.7
9	⬡	⬡	OCH_3	H	H	H	**(7.53±0.13)**	**(7.36±0.18)**	**(8.65±0.01)**	13	20	1
10	⬡	⬡	H	H	H	H	6.16±0.01	5.86±0.13	**(8.37±0.23)**	**162**	**324**	2
11	⬡	⬡	H	H	H	CH_3	5.06±0.02	5.31±0.02	6.06±0.09	10	6	0.6
12	⬡	⬡	H	H	CH_3	CH_3	<5	5.60±0.04	6.19±0.20	>15	4	<0.3
13c	⬡	H	H	H	H	H	5.44±0.12	6.30±0.12	7.09±0.13	45	6	0.1
13t	H	⬡	H	H	H	H	6.01±0.12	6.40±0.11	6.94±0.21	9	4	0.4
14	$(CH_2)_5$		H	H	H	H	5.27±0.18	6.20±0.12	6.54±0.09	19	2	0.1
BMY7378							(7.01±0.08)	(7.48±0.09)	(8.40±0.09)	25	8	0.3

cis; **t**, trans), regardless of the stereochemistry at positions 2 and 4 of the 1,3-dioxolane ring, are less active than the lead compound **1**, indicating that the 2-phenyl-1,3-dioxolanyl moiety as such is not a good replacement for the 2,3-dihydro-1,4-benzodioxin-2-yl structure. However, the affinity values found encouraged us to carry out further structure modification in an attempt to improve both affinity and selectivity.

Introduction of bulkier substituents at position 2 has a divergent effects on α_{1A} and α_{1D} subtypes. At α_{1A}, a decrease of affinity is observed (the exceptions being **3t** and **6t**), while at α_{1D} the affinity is increased (with the exception of **3c/t**). As a consequence, a significant selectivity for the latter subtype is observed. Particularly interesting are the results obtained by replacing the phenyl ring with an α-naphthyl to give compounds **7c/t**. In this case when the stereochemistry is cis (compound **7c**), although the affinity is not striking (pK_b = 7.32), the α_{1D} selectivity is relevant and higher by 2 orders of magnitude over the α_{1A}-subtype ($\alpha_{1D}/\alpha_{1A} \geq$ 209).

Owing to the presence of only one chiral center, which would avoid the time-consuming diastereomer separation, we chose compound **5** as a new starting point at which to modify the structure of the lateral chain carrying the basic moiety. Removing one of the two methoxy groups of the (2,6-dimethoxyphenoxy)ethyl portion of the molecule, affording compound **9**, caused 30- to 60-fold increase of affinity at the three receptor subtypes, while the selectivity for the α_{1D} subtype was virtually unchanged. When the second methoxy group was also removed, as in **10**, the affinity decreased by 23-fold and 32-fold, respectively, at the α_{1A} and α_{1B} subtypes, while at the α_{1D} subtype a small, not significant, variation was observed. As a result, the α_{1D} selectivity was therefore increased by up to more than 2 orders of magnitude (α_{1D}/α_{1A} = 162; α_{1D}/α_{1B} = 324). It appears that only one methoxy group, in the ortho position, is allowed for optimal interaction at the three receptor subtypes (**9** vs **5**), a second one being detrimental, most probably because of steric hindrance, to affinity. Furthermore, one methoxy group seems to contribute positively to the binding at α_{1A} and α_{1B} subtypes, while at the α_{1D} subtype it does not play any significant role.

Compound **10** has a basic center represented by the secondary amine that, once protonated at physiological pH, should constitute the first recognition step through a long-range electrostatic interaction. We synthesized compounds **11** and **12** in order to verify the importance of a secondary amine as the basic center. The results show that the secondary amine is important for affinity, since the tertiary amine and its quaternary derivative are less active than the secondary one.

Another important finding of this investigation is the role played by the diphenyl substitution. The results obtained with monophenyl derivatives **13c/t** and with the spiro derivative **14** show that both aromatic rings are important for affinity at α_{1A} and α_{1D} subtypes and selectivity, since the three derivatives are less active at the two receptor subtypes and slightly more active than compound **10** at the α_{1B} subtype.

Compounds **9** and **10** were also tested on cloned human α_1-adrenoceptor subtypes. The results, shown in Table 3, confirm that this new class of compounds potently binds to the α_1-adrenoceptors, although the

Table 3. Affinity Constants, Expressed as pK_i [a] Values, and Selectivities of Compounds **1**, **9**−**12**, and BMY-7378 for Human Recombinant α_1-Adrenoceptor Subtypes

compd	$pK_i(\alpha_{1a})$	$pK_i(\alpha_{1b})$	$pK_i(\alpha_{1d})$	α_{1d}/α_{1a}	α_{1d}/α_{1b}	α_{1a}/α_{1b}
1	9.37	8.0	9.29	0.8	20	23
9	7.61	7.34	8.03	3	7	2
10	7.43	7.20	7.94	3	6	2
11	6.08	6.59	<6	<0.9	<0.3	0.3
12	7.47	7.69	<6	<0.03	<0.02	0.6
BMY-7378	6.42	6.15	8.89	295	550	2

[a] pK_i values agreed to ±20%.

selectivity is not as large as that found in functional studies. Binding studies also confirm the detrimental effect of nitrogen methylation, since compound **11** shows affinity values lower than those of parent compound **10**. Surprisingly, the methyl iodide derivative of **11**, compound **12**, behaves differently with respect to functional studies. In fact, **12** either maintains affinity at the α_{1A} subtype or slightly increases it at the α_{1B} one, while at the α_{1D} subtype the affinity is practically lost. These discrepancies are not unusual and may be ascribed to several factors such as species variations and/or different bioavailability of compounds at the receptor level. Recently, other explanations have been taken into consideration.[16,21] Receptor dimerization or heterodimerization, which may occur in both natural and artificial systems, is one of these. In this case, the new entities formed do not always give a signal as a monomer or as dimers. Alternatively, the antagonist may not adhere perfectly to the concept of neutral antagonism but behaves as an inverse agonist that is system-dependent.

In conclusion, starting from WB 4101, we have discovered a new class of α_1-adrenoreceptor ligands bearing a 1,3-dioxolane structure. Adequate substituents may address the selectivity toward one subtype or the other. Compounds **9** and **10** are outstanding for their affinity and selectivity, with the latter being one of the most selective antagonists, at least in functional studies, for the α_{1D} subtype.

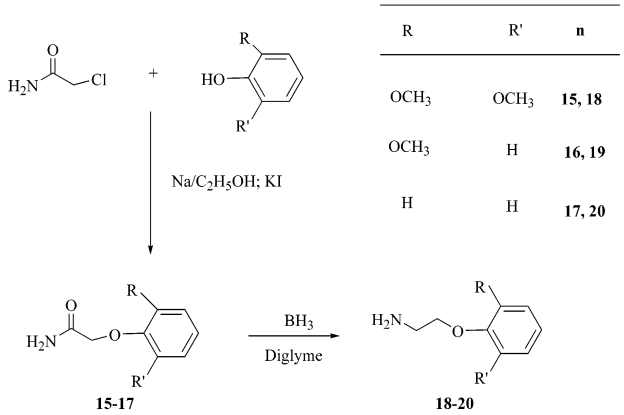
More extensive structure−activity relationship studies are in progress and will be reported in due course.

Experimental Section

Chemistry. The structural characterization was done with NMR and elemental analyses techniques (C, H, N elemental analyzer model 1106, Carlo Erba Instruments). Analyses indicated by the symbols were within ±0.4 of the theoretical values. Melting points were determined on a Büchi 510 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DPX 200 Avance working at 200.13 MHz and at a temperature of 300 K. Chemical shifts are reported as δ (ppm) relative to tetramethylsilane (s = singlet, brs = broad singlet, d = doublet, dd = double doublet, ddd = double double−doublet, t = triplet, m = multiplet, pseudot = pseudotriplet). Silica gel TLC plates (Merck, Kieselgel 60, F_{254}) were used to monitor the progression of the reactions. Chromatographic separations were performed on silica gel columns (Kieselgel 60, 0.040−0.063 mm, Merck) by flash chromatography. The names of compounds were generated by applying the PC software AUTONOM, version 2.1.

General Procedure for the Synthesis of Compounds 16 and 17 (Scheme 1). These compounds were prepared following the procedure already described for **15**.[22] To a solution of Na in ethanol was added, under mechanical stirring, guaiacol or phenol. The mixture was refluxed for 30 min. Then 2-chloroacetamide and a catalytic amount of KI were added and heating was maintained for 20−22 h. The solvent was evaporated under vacuum, and the residue was treated with a solution of 5% NaOH. Then it was extracted with $CHCl_3$ (3×). The organic layer was dried over Na_2SO_4 and the solvent was evaporated under vacuum to give the desired compound.

Scheme 1



2-(2-Methoxyphenoxy)acetamide (16): 18.13 g (100 mmol); yield 83%; mp 131−132 °C; ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3H), 4.57 (s, 2H), 5.95 (brs, 2H), 7.00 (m, 4H).

2-Phenoxyacetamide (17): 4.45 g (29 mmol); yield 56%; mp 90−92 °C; ^{1}H NMR ($CDCl_3$) δ 4.48 (s, 2H), 5.80 (brs, 1H), 6.55 (brs, 1H), 6.94 (m, 3H), 7.28 (m, 2H).

General Procedure for the Synthesis of Compounds 19 and 20 (Scheme 1). These compounds were prepared following the procedure already described for **18**.[22] To a solution of **16** or **17** in dry diglyme was added dropwise a solution of 2 M $BH_3S(CH_3)_2$ in dry THF while stirring at room temperature and under nitrogen atmosphere. The mixture was heated at 90 °C for 18 h. After the mixture was cooled, the excess reducing agent was destroyed by cautious addition of MeOH. After 2 h, the solution was acidified with concentrated HCl (pH 2) and then refluxed for an additional 2 h. The solvent was evaporated under vacuum, water was added, and the residue was washed with $CHCl_3$ (3×). The aqueous phase was basified with NaOH pellets and then extracted with $CHCl_3$ (3×). The organic layer was dried over Na_2SO_4, and the solvent was evaporated under vacuum to give the desired compound as oils.

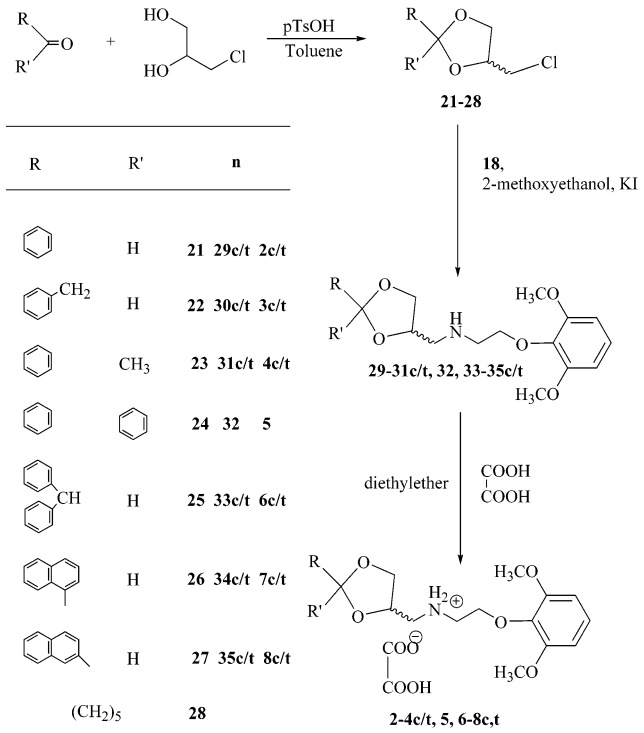
2-(2-Methoxyphenoxy)ethylamine (19): 4.47 g (27 mmol); yield 55%; ^{1}H NMR ($CDCl_3$) δ 1.59 (s, 2H), 3.14 (t, 2H), 3.90 (s, 3H), 4.08 (t, 2H), 6.95 (m, 4H).

2-Phenoxyethylamine (20): 2.35 g (17 mmol); yield 59%; ^{1}H NMR ($CDCl_3$) δ 1.59 (s, 2H), 3.03 (t, 2H), 3.94 (t, 2H), 6.90 (m, 3H), 7.25 (m, 2H).

General Procedure for the Synthesis of Compounds 21−28 (Scheme 2). To a solution of aldehyde or ketone in toluene was added an excess (1.5−3 equiv) of 3-chloro-1,2-propanediol and a catalytic amount of *p*-toluenesulfonic acid (pTsOH). The reaction mixture was then refluxed, using a Dean−Stark apparatus, for 4−5 h in the case of reactions with aldehydes and for 2−4 days in the case of those with ketones. The mixture was washed with 10% $NaHSO_3$ solution (3×) (in the case of aldehydes) or with H_2O (2×) (in the case of ketones) and then with a saturated solution of $NaHCO_3$ (3×) and brine (2×). The organic layer was dried over Na_2SO_4, and the solvent was evaporated under vacuum to give the desired compounds **21**−**28** as oils. Compounds **22** and **23** were purified by flash chromatography, eluting with cyclohexane/ethyl acetate, 95:5. With the exception of **24**, all the compounds were cis and trans mixtures and no attempts at diastereomeric separation were made at this stage.

4-Chloromethyl-2-phenyl[1,3]dioxolane (21): 9.70 g (49 mmol); yield 75%; ^{1}H NMR ($CDCl_3$) δ 3.57 (dd, 1H), 3.64 (dd, 1H), 3.70 (dd, 1H), 3.76 (dd, 1H), 3.96 (dd, 1H), 4.17 (d, 2H), 4.33 (dd, 1H), 4.49, 4.51 (m, m, 1H, 1H), 5.87, 6.03 (s, s, 1H, 1H), 7.46 (m, m, 5H, 5H).

Scheme 2[a]



[a] **c**: cis. **t**: trans. The cis and trans compounds were separated when indicated by c/t.

2-Benzyl-4-chloromethyl[1,3]dioxolane (22): 1.57 g (7.4 mmol); yield 89%; ^{1}H NMR ($CDCl_3$) δ 3.03 (m, 4H), 3.24, 3.46 (dd, dd, 1H, 1H), 3.52, 3.66 (dd, dd, 1H, 1H), 3.77, 4.18 (dd, dd, 1H, 1H), 3.94, 3.99 (ddd, dd, 1H, 1H), 4.32 (m, 2H), 5.19, 5.31 (t, t, 1H, 1H), 7.33 (m, 10H).

4-Chloromethyl-2-methyl-2-phenyl[1,3]dioxolane (23): 1.21 g (5.7 mmol); yield 14%; ^{1}H NMR ($CDCl_3$) δ 1.68, 1.72 (s, s, 6H), 3.18, 4.30 (dd, dd, 1H, 1H), 3.64 (m, 4H), 3.85, 3.98 (dd, dd, 1H, 1H), 4.27 (m, 1H), 4.48 (m, 1H), 7.37 (m, 6H), 7.50 (m, 4H).

4-Chloromethyl-2,2-diphenyl[1,3] dioxolane (24): 15.00 g (55 mmol); quantitative yield; ^{1}H NMR ($CDCl_3$) δ 3.50 (dd, 1H), 3.71 (dd, 1H), 4.04 (dd, 1H), 4.16 (dd, 1H), 4.46 (m, 1H), 7.34 (m, 6H), 7.53 (m, 4H).

2-Benzydryl-4-chloromethyl[1,3] dioxolane (25): 7.20 g (25 mmol); quantitative yield; ^{1}H NMR ($CDCl_3$) δ 2.80 (dd, 1H), 3.22 (dd, 1H), 3.60 (ddd, 2H), 3.80 (dd, 1H), 3.94 (ddd, 2H), 3.99 (dd, 1H), 4.17 (m, 1H), 4.27 (d, 1H), 4.29 (m, 1H), 4.32 (d, 1H), 5.62 (d, 1H), 5.78 (d, 1H), 7.33 (m, 20H).

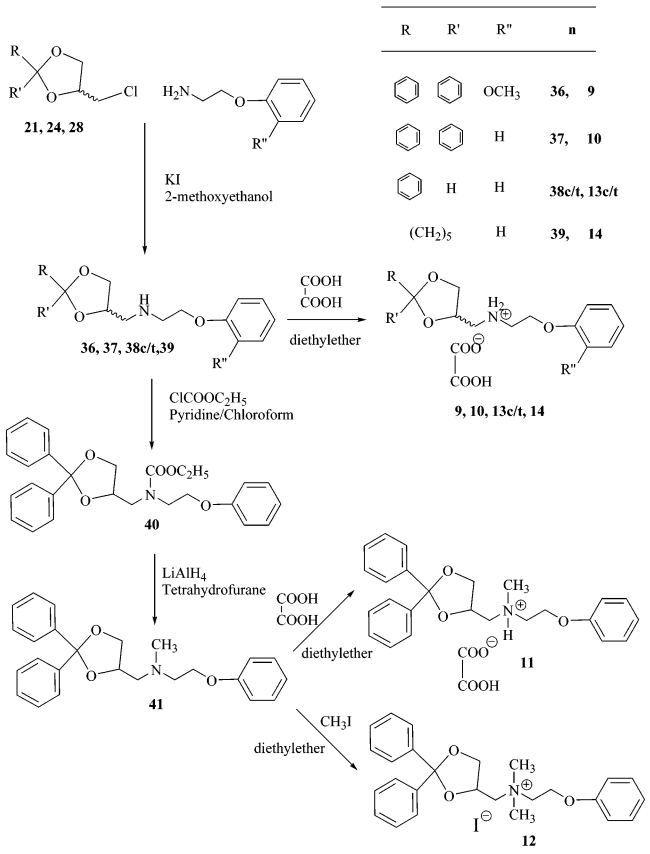
4-Chloromethyl-2-naphthalen-1-yl[1,3]dioxolane (26): 1.04 g (4.2 mmol); yield 65%; ^{1}H NMR ($CDCl_3$) δ 3.60 (dd, 1H), 3.79 (m, 3H), 4.12 (dd, 1H), 4.30 (m, 2H), 4.41 (dd, 1H), 4.62 (m, 2H), 6.57 (s, 1H), 6.71 (s, 1H), 7.57, 7.90 (m, m, 12H), 8.20 (m, 2H).

4-Chloromethyl-2-naphthalen-2-yl[1,3]dioxolane (27): 3.20 g (12.9 mmol); quantitative yield; ^{1}H NMR ($CDCl_3$) δ 3.71 (m, 4H), 4.04 (dd, 1H), 4.24 (d, 2H), 4.40 (dd, 1H), 4.56 (m, 2H), 6.05 (s, 1H), 6.22 (s, 1H), 7.58 (m, 6H), 7.95 (m, 8H).

(1,4-Dioxaspiro[4.5]dec-2-ylmethyl)(2-phenoxyethyl)-amine (28): 5.80 g (30 mmol); quantitative yield; ^{1}H NMR ($CDCl_3$) δ 1.40, 1.58 (m, m, 10H), 3.44 (dd, 1H), 3.58 (dd, 1H), 3.87 (dd, 1H), 4.10 (dd, 1H), 4.30 (m, 1H).

General Procedure for the Synthesis of Amines 29−31c/t, 32, 33−35c/t, 36, 37, 38c/t, and 39 and Their Oxalate Salts 2−4c/t, 5, 6−8c/t, 9, 10, 13c/t, 14 (Schemes 2 and 3). A solution of chloromethyl derivative (**21**−**28**) in 2-methoxyethanol with a large excess (5−10 equiv) of amine (**18**−**20**) and with a catalytic amount of KI was refluxed for 18−24 h. The solvent was evaporated under vacuum, $CHCl_3$ was added, and the residue was washed with a solution of 5% NaOH (3×) and then with brine (2×). The organic layer was dried over Na_2SO_4 and the solvent was evaporated under vacuum to give the desired amines as oils. Diastereomeric separations were accomplished by flash chromatography, eluting with cyclohexane/ethyl acetate, 20:80 → ethyl acetate, 100% → ethyl acetate/methanol, 80:20. In the case of amines **32**, **36**, **37**, and **39**, the residues were purified by flash chromatography, eluting with ethyl acetate, 100%.

Scheme 3



***cis*-[2-(2,6-Dimethoxyphenoxy)ethyl](2-phenyl[1,3]-dioxolan-4-ylmethyl)amine (29c):** 0.17 g (0.47 mmol); yield 11%; ^{1}H NMR ($CDCl_3$) δ 2.35 (brs, 1H), 2.96 (m, 4H), 3.80 (s, 6H), 3.91 (dd, 1H), 4.15 (m, 3H), 4.41 (m, 1H), 5.85 (s, 1H), 6.57 (d, 2H), 6.99 (dd, 1H), 7.38 (m, 3H), 7.53 (m, 2H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from methanol to give compound **2c**: mp 156 °C; ^{1}H NMR (DMSO) δ 3.30 (m, 4H), 3.78 (s, 6H), 3.98 (dd, 1H), 4.16 (m, 3H), 4.59 (m, 1H), 5.83 (s, 1H), 6.71 (d, 2H), 7.06 (dd, 1H), 7.47, 7.60 (m, m, 5H). Anal. ($C_{22}H_{27}NO_9$) C, H, N.

***trans*-[2-(2,6-Dimethoxyphenoxy)ethyl](2-phenyl[1,3]-dioxolan-4-ylmethyl)amine (29t):** 0.26 g (0.72 mmol); yield 17%; ^{1}H NMR ($CDCl_3$) δ 2.29 (brs, 1H), 2.86 (dd, 1H), 2.97 (m, 2H), 3.01 (dd, 1H), 3.81 (dd, 1H), 3.84 (s, 6H), 4.22 (m, 3H), 4.45 (m, 1H), 5.98 (s, 1H), 6.58 (d, 2H), 7.00 (dd, 1H), 7.39, 7.50 (m, m, 5H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from methanol to give compound **2t**: mp 165−168°C; ^{1}H NMR (DMSO) δ 3.30 (m, 4H), 3.78 (dd, 1H), 3.80 (s, 6H), 4.13 (t, 2H), 4.32 (dd, 1H), 4.64 (m, 1H), 5.98 (s, 1H), 6.72 (d, 2H), 7.07 (dd, 1H), 7.47 (m, 5H). Anal. ($C_{22}H_{27}NO_9$) C, H, N.

***cis*-(2-Benzyl[1,3]dioxolan-4-ylmethyl)[2-(2,6-dimethoxyphenoxy)ethyl]amine (30c):** 0.44 g (1.18 mmol); yield 26%; ^{1}H NMR ($CDCl_3$) δ 2.57 (brs, 1H), 2.72 (dd, 2H), 2.88 (m, 2H), 2.99 (ddd, 2H), 3.67 (dd, 1H), 3.82 (s, 6H), 3.92 (dd, 1H), 4.14 (m, 3H), 5.12 (t, 1H), 6.58 (d, 2H), 7.00 (dd, 1H), 7.24 (m, 5H).

The free amine was transformed into the corresponding oxalate salt**,** which is crystallized from methanol to give compound **3c**; mp 197°-200 °C; ^{1}H NMR (DMSO) δ 2.98 (m,

2H), 3.05 (m, 1H), 3.20 (m, 3H), 3.80 (s, 6H), 3.82 (dd, 1H), 3.95 (dd, 1H), 4.09 (m, 2H), 4.39 (m, 1H), 5.09 (t, 1H), 6.73 (d, 2H), 7.08 (dd, 1H), 7.26 (m, 5H). Anal. ($C_{23}H_{29}NO_9$) C, H, N.

***trans*-(2-Benzyl[1,3]dioxolan-4-ylmethyl)[2-(2,6-dimethoxyphenoxy)ethyl]amine (30t):** 0.42 g (1.13 mmol); yield 25%; ^{1}H NMR ($CDCl_3$) δ 2.03 (brs, 1H), 2.70 (dd, 1H), 2.92 (m, 5H), 3.58 (dd, 1H), 3.80 (s, 6H), 4.11 (m, 3H), 4.24 (m, 1H), 5.22 (t, 1H), 6.56 (d, 2H), 6.99 (dd, 1H), 7.26 (m, 5H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from ethanol to give compound **3t**; mp 164°-165 °C; ^{1}H NMR (DMSO) δ 2.93 (dd, 2H), 3.20 (m, 4H), 3.61 (dd, 1H), 3.78 (s, 6H), 4.08 (m, 2H), 4.15 (dd, 1H), 4.47 (m, 1H), 5.25 (t, 1H), 6.72 (d, 2H), 7.07 (dd, 1H), 7.27 (m, 5H). Anal. ($C_{23}H_{29}NO_9$) C, H, N.

***cis*-[2-(2,6-Dimethoxyphenoxy)ethyl](2-methyl-2-phenyl[1,3]dioxolan-4-ylmethyl)amine (31c):** 0.16 g (0.44 mmol); yield 9%; ^{1}H NMR ($CDCl_3$) δ 1.62 (s, 3H), 2.63 (m, 2H), 2.83 (pseudot, 2H), 3.48 (dd, 1H), 3.80 (s, 6H), 4.10 (m, 2H), 4.18 (dd, 1H), 4.39 (m, 1H), 6.55 (d, 2H), 6.97 (dd, 1H), 7.28 (m, 3H), 7.50 (m, 2H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from methanol to give compound **4c**: mp 157°-158 °C; ^{1}H NMR (DMSO) δ 1.61 (s, 3H), 2.99 (dd, 1H), 3.25 (m, 3H), 3.54 (dd, 1H), 3.76 (s, 6H), 4.09 (m, 2H), 4.32 (dd, 1H), 4.59 (m, 1H), 6.71 (d, 2H), 7.07 (dd, 1H), 7.34 (m, 3H), 7.53 (m, 2H). Anal. ($C_{23}H_{29}NO_9 \cdot 1H_2O$) C, H, N.

***trans*-[2-(2,6-Dimethoxyphenoxy)ethyl](2-methyl-2-phenyl[1,3]dioxolan-4-ylmethyl)amine (31t):** 0.71 g (1.9 mmol); yield 38%; ^{1}H NMR ($CDCl_3$) δ 1.70 (s, 3H), 2.44 (brs, 1H), 2.81 (dd, 1H), 2.94 (m, 3H), 3.75 (dd, 1H), 3.82 (m, 1H), 3.86 (s, 6H), 4.16 (m, 3H), 6.58 (d, 2H), 7.00 (dd, 1H), 7.32 (m, 3H), 7.51 (m, 2H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from ethanol to give compound **4t**: mp 166°-168 °C; ^{1}H NMR (DMSO) δ 1.66 (s, 3H), 3.30 (m, 4H), 3.80, 3.82 (m, s, 8H), 4.13 (m, 2H), 4.31 (m, 1H), 6.73 (d, 2H), 7.08 (dd, 1H), 7.41 (m, 5H). Anal. ($C_{23}H_{29}NO_9$) C, H, N.

[2-(2,6-Dimethoxyphenoxy)ethyl](2,2-diphenyl[1,3]-dioxolan-4-ylmethyl)amine (32): 1.24 g (2.85 mmol); yield 70%; ^{1}H NMR ($CDCl_3$) δ 2.22 (brs, 1H), 2.85 (dd, 1H), 2.96 (m, 2H), 3.01 (dd, 1H), 3.83 (s, 6H), 3.89 (dd, 1H), 4.16 (m, 3H), 4.40 (m, 1H), 6.59 (d, 2H), 7.02 (dd, 1H), 7.34 (m, 6H), 7.55 (m, 4H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from methanol to give compound **5**: mp 187−189 °C; ^{1}H NMR (DMSO) δ 3.26 (m, 4H), 3.76 (s, 6H), 3.88 (dd, 1H), 4.13 (m, 3H), 4.49 (m, 1H), 6.71 (d, 2H), 7.07 (dd, 1H), 7.42 (m, 10H). Anal. ($C_{28}H_{31}NO_9$) C, H, N.

***cis*-(2-Benzydryl[1,3]dioxolan-4-ylmethyl)[2-(2,6-dimethoxyphenoxy)ethyl]amine (33c):** 0.13 g (0.29 mmol); yield 12%; ^{1}H NMR ($CDCl_3$) δ 2.21 (brs, 1H), 2.56 (d, 2H), 2.84 (m, 2H), 3.56 (dd, 1H), 3.83 (s, 6H), 3.98 (dd, 1H), 4.12 (m, 3H), 4.30 (d, 1H), 5.63 (d, 1H), 6.60 (d, 2H), 7.03 (dd, 1H), 7.32 (m, 10H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from ethanol to give compound **6c**: mp 191−193°C; ^{1}H NMR (DMSO) δ 2.83 (dd, 1H), 3.11 (m, 3H), 3.73 (m, 1H), 3.76 (s, 6H), 4.01 (m, 3H), 4.26 (d, 1H), 4.42 (m, 1H), 5.66 (d, 1H), 6.72 (d, 2H), 7.08 (dd, 1H), 7.29 (m, 10H). Anal. ($C_{29}H_{33}NO_9$) C, H, N.

***trans*-(2-Benzydryl[1,3]dioxolan-4-ylmethyl)[2-(2,6-dimethoxyphenoxy)ethyl]amine (33t):** 0.20 g (0.45 mmol); yield 18%; ^{1}H NMR ($CDCl_3$) δ 2.28 (brs, 1H), 2.74 (dd, 1H), 2.90 (m, 3H), 3.67 (dd, 1H), 3.80 (s, 6H), 3.97 (dd, 1H), 4.13 (m, 3H), 4.24 (d, 1H), 5.74 (d, 1H), 6.59 (d, 2H), 7.02 (dd, 1H), 7.32 (m, 10H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from ethanol to give compound **6t**: mp 184−188°C; ^{1}H NMR (DMSO) δ 3.22 (m, 4H), 3.66 (dd, 1H), 3.75 (s, 6H), 4.05 (m, 3H), 4.24 (d, 1H), 4.39 (m, 1H), 5.82 (d, 1H), 6.71 (d, 2H), 7.07 (dd, 1H), 7.30 (m, 10H). Anal. ($C_{29}H_{33}NO_9$) C, H, N.

***cis*-[2-(2,6-Dimethoxyphenoxy)ethyl](2-naphthalen-1-yl[1,3]dioxolan-4-ylmethyl)amine (34c):** 0.24 g (0.59 mmol); yield 21%; ^{1}H NMR ($CDCl_3$) δ 2.05 (brs, 1H), 3.01 (m, 4H), 3.78 (s, 6H), 4.00 (dd, 1H), 4.16 (m, 2H), 4.30 (dd, 1H), 4.58 (m, 1H), 6.52 (s, 1H), 6.56 (d, 2H), 7.00 (dd, 1H), 7.52 (m, 3H), 7.88 (m, 3H), 8.26 (m, 1H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from ethanol to give compound **7c**: mp 154−157 °C; ^{1}H NMR (DMSO) δ 3.32 (m, 4H), 3.75 (s, 6H), 4.09 (m, 3H), 4.31 (m, 1H), 4.71 (m, 1H), 6.51 (s, 1H), 6.70 (d, 2H), 7.06 (dd, 1H), 7.55 (m, 3H), 7.86 (m, 1H), 8.00 (m, 2H), 8.22 (m, 1H). Anal. ($C_{26}H_{29}NO_9$) C, H, N.

***trans*-[2-(2,6-Dimethoxyphenoxy)ethyl](2-naphthalen-1-yl[1,3]dioxolan-4-ylmethyl)amine (34t):** 0.30 g (0.74 mmol); yield 26%; ^{1}H NMR ($CDCl_3$) δ 2.31 (brs, 1H), 3.03 (m, 4H), 3.84 (s, 6H), 3.96 (dd, 1H), 4.19 (m, 2H), 4.38 (dd, 1H), 4.57 (m, 1H), 6.59 (d, 2H), 6.66 (s, 1H), 7.02 (dd, 1H), 7.51 (m, 3H), 7.86 (m, 3H), 8.28 (m, 1H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from ethanol to give compound that is crystallized from methanol to give compound **7t**: mp 146−150°C; ^{1}H NMR (DMSO) δ 3.38 (m, 4H), 3.80 (s, 6H), 3.92 (dd, 1H), 4.15 (m, 2H), 4.39 (dd, 1H), 4.75 (m, 1H), 6.64 (s, 1H), 6.72 (d, 2H), 7.07 (dd, 1H), 7.57 (m, 3H), 7.75 (m, 1H), 8.00 (m, 2H), 8.27 (m, 1H). Anal. ($C_{26}H_{29}NO_9$) C, H, N.

***cis*-[2-(2,6-Dimethoxyphenoxy)ethyl](2-naphthalen-2-yl[1,3]dioxolan-4-ylmethyl)amine (35c):** 0.13 g (0.32 mmol); yield 12%; ^{1}H NMR ($CDCl_3$) δ 2.16 (brs, 1H), 3.01 (m, 4H), 3.80 (s, 6H), 3.98 (dd, 1H), 4.19 (m, 3H), 4.49 (m, 1H), 6.04 (s, 1H), 6.57 (d, 2H), 7.00 (dd, 1H), 7.52 (m, 2H), 7.65 (dd, 1H), 7.93 (m, 4H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from ethanol to give compound **8c**: mp 160−163°C; ^{1}H NMR (DMSO) δ 3.36 (m, 4H), 3.76 (s, 6H), 4.14 (m, 4H), 4.66 (m, 1H), 6.01 (s, 1H), 6.70 (d, 2H), 7.06 (dd, 1H), 7.62 (m, 3H), 8.00 (m, 4H). Anal. ($C_{26}H_{29}NO_9$) C, H, N.

***trans*-[2-(2,6-Dimethoxyphenoxy)ethyl](2-naphthalen-2-yl[1,3]dioxolan-4-ylmethyl)amine (35t):** 0.24 g (0.59 mmol); yield 21%; ^{1}H NMR ($CDCl_3$) δ 2.17 (brs, 1H), 2.91 (dd, 1H), 2.99 (m, 2H), 3.07 (dd, 1H), 3.86 (m, 6H), 3.90 (dd, 1H), 4.19 (m, 2H), 4.35 (dd, 1H), 4.54 (m, 1H), 6.17 (s, 1H), 6.60 (d, 2H), 7.02 (dd, 1H), 7.50 (m, 2H), 7.63 (dd, 1H), 7.92 (m, 4H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from ethanol to give compound **8t**: mp 176−178 °C; ^{1}H NMR (DMSO) δ 3.35 (m, 4H), 3.80 (s, 6H), 3.81 (dd, 1H), 4.16 (m, 2H), 4.38 (dd, 1H), 4.74 (m, 1H), 6.16 (s, 1H), 6.72 (d, 2H), 7.07 (dd, 1H), 7.60 (m, 3H), 7.99 (m, 4H). Anal. ($C_{26}H_{29}NO_9$) C, H, N.

(2,2-Diphenyl[1,3]dioxolan-4-ylmethyl)[2-(2-methoxyphenoxy)ethyl]amine (36): 0.44 g (1.09 mmol); yield 30%; ^{1}H NMR ($CDCl_3$) δ 2.99 (d, 2H), 3.15 (m, 2H), 3.85 (s, 3H), 3.93 (dd, 1H), 4.15 (dd, 1H), 4.19 (t, 2H), 4.53 (m, 1H), 6.96 (m, 4H), 7.32 (m, 6H), 7.54 (m, 4H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from methanol to give compound **9**: mp 189 °C; ^{1}H NMR (DMSO) δ 3.31 (m, 4H), 3.76 (s, 3H), 3.86 (dd, 1H), 4.12 (dd, 1H), 4.24 (pseudot, 2H), 4.48 (m, 1H), 7.00 (m, 4H), 7.41 (m, 10H). Anal. ($C_{27}H_{29}NO_8$) C, H, N.

(2,2-Diphenyl[1,3]dioxolan-4-ylmethyl)(2-phenoxyethyl)-amine (37): 1.45 g (3.87 mmol); yield 35%; ^{1}H NMR ($CDCl_3$) δ 1.67 (brs, 1H), 2.86 (ddd, 2H), 3.01 (t, 2H), 3.84 (dd, 1H), 4.04 (t, 2H), 4.09 (dd, 1H), 4.33 (m, 1H), 6.91 (m, 3H), 7.29 (m, 8H), 7.48 (m, 4H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from methanol to give compound **10**: mp 189−191 °C; ^{1}H NMR (DMSO) δ 3.29 (ddd, 2H), 3.49 (m, 2H), 3.95 (dd, 1H), 4.20 (dd, 1H), 4.34 (t, 2H), 4.58 (m, 1H), 7.09 (m, 3H), 7.49 (m, 12H). Anal. ($C_{26}H_{27}NO_7$) C, H, N.

***cis*-(2-Phenoxyethyl)(2-phenyl[1,3]dioxolan-4-ylmethyl)amine (38c):** 0.52 g (1.7 mmol); yield 35%; ^{1}H NMR ($CDCl_3$) δ 1.95 (brs, 1H), 2.98 (m, 2H), 3.10 (t, 2H), 3.93 (dd, 1H), 4.08 (t, 2H), 4.16 (dd, 1H), 4.44 (m, 1H), 5.86 (s, 1H), 6.95 (m, 3H), 7.36 (m, 5H), 7.53 (m, 2H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from methanol to give compound **13c**: mp 189−190 °C; ^{1}H NMR (DMSO) δ 3.24, 3.36 (m, m, 4H), 3.97 (dd, 1H), 4.13 (dd, 1H), 4.24 (m, 2H), 4.55 (m, 1H), 5.81 (s, 1H), 6.99 (m, 3H), 7.34, 7.47 (m, m, 7H). Anal. ($C_{20}H_{23}NO_7$) C, H, N.

***trans*-(2-Phenoxyethyl)(2-phenyl[1,3]dioxolan-4-ylmethyl)amine (38t):** 0.23 g (0.77 mmol); yield 15%; ^{1}H NMR ($CDCl_3$) δ 1.74 (brs, 1H), 2.96 (ddd, 2H), 3.12 (t, 2H), 3.81 (dd, 1H), 4.13 (t, 2H), 4.29 (dd, 1H), 4.45 (m, 1H), 5.99 (s, 1H), 6.96 (m, 3H), 7.35 (m, 5H), 7.50 (m, 2H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from methanol to give compound **13t**: mp 206−207 °C; ^{1}H NMR (DMSO) δ 3.27, 3.41 (m, m, 4H), 3.76 (dd, 1H), 4.30 (m, 3H), 4.63 (m, 1H), 5.97 (s, 1H), 7.00 (m, 3H), 7.34, 7.44 (m, m, 7H). Anal. ($C_{20}H_{23}NO_7$) C, H, N.

(1,4-Dioxaspiro[4.5]dec-2-ylmethyl)(2-phenoxyethyl)amine (39): 0.12 g (0.41 mmol); yield 15%; ^{1}H NMR ($CDCl_3$) δ 1.44, 1.64 (m, m, 10H), 2.85 (ddd, 2H), 3.08 (t, 2H), 3.72 (dd, 1H), 4.08 (dd, 1H), 4.11 (t, 2H), 4.29 (m, 1H), 6.97 (m, 3H), 7.31 (m, 2H).

The free amine was transformed into the corresponding oxalate salt, which is crystallized from methanol to give compound **14**: mp 222−223 °C; ^{1}H NMR (DMSO) δ 1.37, 1.57 (m, m, 10H), 3.10 (m, 2H), 3.37 (m, 2H), 3.72 (dd, 1H), 4.07 (dd, 1H), 4.25 (m, 2H), 4.39 (m, 1H), 7.00 (m, 3H), 7.34 (m, 2H). Anal. ($C_{19}H_{27}NO_7$) C, H, N.

(2,2-Diphenyl[1,3]dioxolan-4-ylmethyl)(2-phenoxyethyl)carbamic Acid Ethyl Ester (40). To a solution of **37** (3.6 mmol) in dry $CHCl_3$ (75 mL) and dry pyridine (3.6 mmol) was added dropwise at 0°C and under nitrogen a solution of ethyl chloroformate (4.3 mmol) in dry $CHCl_3$ (20 mL). The mixture was maintained at room temperature for 17 h and then washed with a solution of 2 N NaOH (75 mL) and then with water (100 mL). The organic layer was dried over Na_2SO_4, and the solvent was evaporated under vacuum. The residue was purified by flash chromatography, eluting with cyclohexane/ethyl acetate, 90:10, to give the desired compound **40**: 1.18 g (2.64 mmol); yield 73%; ^{1}H NMR ($CDCl_3$) δ 1.25 (m, 3H), 3.50 (m, 1H), 3.74, 3.85 (m, dd, 4H), 4.13 (m, 5H), 4.41 (m, 1H), 6.84 (m, 2H), 6.94 (m, 1H), 7.29 (m, 8H), 7.49 (m, 4H).

(2,2-Diphenyl[1,3]dioxolan-4-ylmethyl)methyl(2-phenoxyethyl)amine (41). To a suspension of $LiAlH_4$ (19.2 mmol) in dry THF (33 mL) was added dropwise a solution of compound **40** (2.4 mmol) in dry THF (50 mL). The mixture was refluxed for 24 h. After the mixture was cooled, water was added (0.22 mL), then a solution of 5 N NaOH (0.22 mL), and again water (1.1 mL). The solid was filtered off, and the liquid phase was dried over Na_2SO_4. The solvent was evaporated under vacuum to give the desired compound **41**: 0.75 g (1.93 mmol); yield 80%; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H), 2.75 (ddd, 2H), 2.91 (m, 2H), 3.81 (dd, 1H), 4.06 (t, 2H), 4.14 (dd, 1H), 4.38 (m, 1H), 6.92 (m, 3H), 7.27 (m, 8H), 7.52 (m, 4H).

The amine was then transformed into the corresponding oxalate salt **11**: mp 104−106 °C; ^{1}H NMR (DMSO) δ 2.62 (brs, 3H), 3.00, 3.16 (m, m, 4H), 3.73 (m, 1H), 4.08, 4.15 (m, m, 3H), 4.41 (m, 1H), 6.93 (m, 3H), 7.35 (m, 12H). Anal. ($C_{27}H_{29}NO_7$) C, H, N.

(2,2-Diphenyl[1,3]dioxolan-4-ylmethyl)dimethyl(2-phenoxyethyl)ammonium Iodide (12). To a solution of compound **41** (0.5 mmol) in dry diethyl ether (10 mL) was added CH_3I (5 mmol), and the mixture stood at room temperature for 24 h. Then it was stored for an additional 24 h at 5 °C. The obtained precipitate was washed with diethyl ether and the solid was dried to give the methyl iodide salt **12**: 0.069 g (0.13 mmol); yield 26%, mp 133−135 °C; ^{1}H NMR (DMSO) δ 3.27 (t, 6H), 3.74 (m, 3H), 3.92 (m, 2H), 4.18 (dd, 1H), 4.44 (m, 2H), 4.76 (m, 1H), 6.97 (m, 3H), 7.40 (m, 12H). Anal. ($C_{26}H_{30}NO_3I$) C, H, N.

Pharmacology. 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 of organ baths containing physiological salt solution kept at 37 °C and aerated with 5% CO_2−95% 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 3-fold 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 in which tissues did not receive any antagonist were run in order to check any variation in sensitivity.

Vas Deferens Prostatic Portion. This tissue was used to assess the antagonism toward α_{1A} adrenoceptors.[17] Prostatic portions of 2 cm length were mounted under 0.5 g of tension at 37 °C in tyrode solution of the following composition (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 the neuronal uptake of (−)-noradrenaline. 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 noradrenaline. After another washing and equilibration period of 60 min, a noradrenaline concentration−response curve was constructed (basal response). The antagonist was equilibrated for 30 min before construction of a new concentration−response curve to the agonist. (−)-Noradrenaline solutions contained 0.05% $Na_2S_2O_5$ to prevent oxidation.

Spleen. This tissue was used to assess the antagonism toward α_{1B} adrenoceptors.[18] The spleen was removed and bisected longitudinally into two strips, which were suspended in tissue baths containing Krebs solution of the following composition (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 of 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.[19] Thoracic aorta was cleaned from extraneous connective tissues and placed in Krebs solution of the following composition (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_2-EDTA, 0.01. Cocaine hydrochloride (0.1 μM) and propranolol hydrochloride (4 μM) were added to prevent the neuronal uptake of (−)-noradrenaline and to block β-adrenoceptors, respectively. Two helicoidal strips (15 mm × 3 mm) were cut from each aorta, beginning from the end that is most proximal to the heart. The endothelium was removed by rubbing with filter paper. The absence of acetylcholine (100 μM) induced relaxation in preparations contracted with (−)-noradrenaline (1 μM) was taken as an indicator that the vessels were denuded successfully. Vascular strips were then tied with surgical thread and suspended in a jacketed tissue bath containing tyrode solution. Strip contractions were measured isometrically. After at least a 2 h equilibration period under an optimal tension of 1 g, cumulative (−)-noradrenaline concentration−response curves were recorded at 1 h intervals, the first two being discarded and the third one taken as control. The antagonist was equilibrated with the tissue for 30 min before the generation of the fourth cumulative concentration−response curve to (−)-noradrenaline. (−)-Norad-

renaline solutions contained 0.05% K_2EDTA in 0.9% NaCl to prevent oxidation.

Radiolingand Binding Assay. Binding to cloned human α_1-adrenoceptor subtype was performed in membranes from CHO (Chinese hamster ovary) cells transfected by electrophoration with DNA expressing the gene encoding each α_1-adrenoceptor subtype.[20] Cloning and stable expression of the human α_1-adrenoceptor gene was performed as previously described. CHO cell membranes (30 μg of protein) were incubated in 50 mM Tris-HCl, pH 7.4, with 0.1−0.4 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 (1−10 pM). Nonspecific binding was determined in the presence of 10 μM phentolamine. Incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through 0.2% poly(ethylenimine)-pretreated Whatman GF/B or Schleicher & Schuell GF52 filters.

Data Analysis. 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. pA_2 values were calculated according to Arunlakshana and Schild[23] from the dose ratios at EC_{50} values of the agonists calculated at three different antagonist concentrations. Each concentration was tested at least four times, and Schild plots were constrained to a slope of −1 as required by theory.[24] pK_b values were calculated according to van Rossum[25] at one or two concentrations. Binding data were analyzed using the nonlinear curve-fitting program Allfit.[26] Scatchard plots were linear for all preparations. The pseudo-Hill coefficients (n^H) were not significantly different from unity ($p > 0.05$). Equilibrium dissociation constants (K_i) were derived from the Cheng−Prusoff equation[27] $K_i = IC_{50}/(1 + L/K_d)$, where L and K_d are the concentration and the equilibrium dissociation constant of the radioligand. p*K*i values are the mean of two to three separate experiments performed in triplicate, which agreed to ±20%.

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