Synthesis, Absolute Configuration, and Biological Profile of the Enantiomers of *trans*-[2-(2,6-Dimethoxyphenoxy)ethyl][(3-*p*-tolyl-2,3-dihydro-1,4-benzodioxin-2-yl)methyl]amine (Mephendioxan), a Potent Competitive α_{1A}-Adrenoreceptor Antagonist

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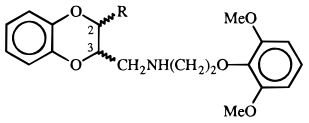
Received January 22, 1996[⊗]

The enantiomers of *trans*-[2-(2,6-dimethoxyphenoxy)ethyl][(3-*p*-tolyl-2,3-dihydro-1,4-benzodioxin-2-yl)methyl]amine (mephendioxan, **2**) were synthesized from the chiral *trans*-3-*p*-tolyl-2,3-dihydro-1,4-benzodioxin-2-carboxylic acids [(+)-**3** and (−)-**3**] which in turn were obtained through the resolution of the racemic acid with (*R*)- and (*S*)-α-methylbenzylamine. Comparison of CD spectra of the enantiomers of **2** with that of (2*S*,3*S*)-3-methyl-2-phenyl-1,4-benzodioxane allowed the assignment of the 2*S*,3*S* configuration to the (−)-enantiomer of **2** and of the 2*R*,3*R* configuration to the other enantiomer. The binding profile of the enantiomers of **2** was assessed at α_1, α_2, D_2, and 5-HT_{1A} receptors, in comparison to WB 4101 (**1**), 5-methylurapidil, and (+)-niguldipine. In addition, the two enantiomers were investigated at native and cloned α_1-adrenoreceptor subtypes. (−)-**2** was 10−30 times as potent as the (+)-enantiomer at α_1-adrenoreceptor subtypes in both functional and binding assays. It was 36-fold selective for the α_{1A}- versus α_{1B}-adrenoreceptor and 60- and 20-fold selective in binding to the α_{1a}-adrenoreceptor relative to α_{1b} and α_{1d} subtypes, respectively. Furthermore, the enantiomer (−)-**2** displayed selectivities of 12000-, 2500-, and 250-fold in binding to α_{1a}-adrenoreceptors relative to α_2-adrenoreceptors and 5-HT_{1A} and D_2 receptors. These results indicate that (−)-**2** may be a valuable tool in the characterization of α_1-adrenoreceptor subtypes.

Introduction

α_1-Adrenoreceptors are not a homogeneous family as clearly evidenced by pharmacological tools and molecular biological techniques.[1] Two native α_1-adrenoreceptor subtypes, α_{1A} and α_{1B}, have been formerly characterized by functional and binding assays. The α_{1A} subtype has high affinity for antagonists such as WB 4101, 5-methylurapidil, and (+)-niguldipine and is insensitive to inactivation by chloroethylclonidine (CEC). The α_{1B} subtype displays lower affinity for the above antagonists, but is preferentially inactivated by the alkylating agent CEC.[1] However, very recently it has been demonstrated that the α_1-adrenoreceptor mediating contraction upon activation in isolated rat aorta may belong to the α_{1D} subtype.[2] Cloning studies have confirmed the existence of at least three distinct α_1-adrenoreceptors, which are now designated as α_{1a}, α_{1b}, and α_{1d} subtypes.[3−5] The recombinant α_{1a}-adrenoreceptor (formerly designated as α_{1c})[4] corresponds to the native α_{1A}-adrenoreceptor, the recombinant α_{1b} to the native α_{1B}, and the α_{1d} (formerly designated as $\alpha_{1a/d}$ in some publications) to the native α_{1D}-adrenoreceptor recently characterized in rat aorta. Thus, α_1-adrenoreceptors are now classified as α_{1A} (α_{1a}), α_{1B} (α_{1b}), and α_{1D} (α_{1d}), with upper and lower case subscripts being used to designate native or recombinant receptor, respectively.[6,7]



1 (WB 4101): R = H
2 (Mephendioxan): R = 4-$CH_3C_6H_4$ (trans)

Our research group has previously been involved in designing new α_1-adrenoreceptor antagonists structurally related to WB 4101 {[2-(2,6-dimethoxyphenoxy)ethyl][(2,3-dihydro-1,4-benzodioxin-2-yl)methyl]amine, **1**} and in studying structure−affinity and structure−selectivity relationships with the goal of developing high-affinity, site-selective ligands for subtypes of the α_1-adrenoreceptor.[8−11] Among a variety of structural modifications performed on **1**, we have demonstrated that the insertion of a substituent at the 3-position having a trans relationship with the 2-side chain markedly affects the affinity for α_2-adrenoreceptors whereas that for α_1-adrenoreceptors is only slightly decreased.[12] The overall result of that structural modification was a significant improvement in selectivity toward rat vas deferens α_1-adrenoreceptors compared to **1**. Thus, it is evident that a 3-substituent (trans) may have a crucial role in the modulation of selectivity for α_1-adrenoreceptor subtypes.

Among the analogues of **1** bearing a 3-substituent the *p*-tolyl derivative mephendioxan (**2**) resulted in the most potent and selective antagonist for the rat vas deferens α_1-adrenoreceptor subtype. Since the enantiomers of **1** have different affinity for α_1-adrenoreceptors,[13,14] we

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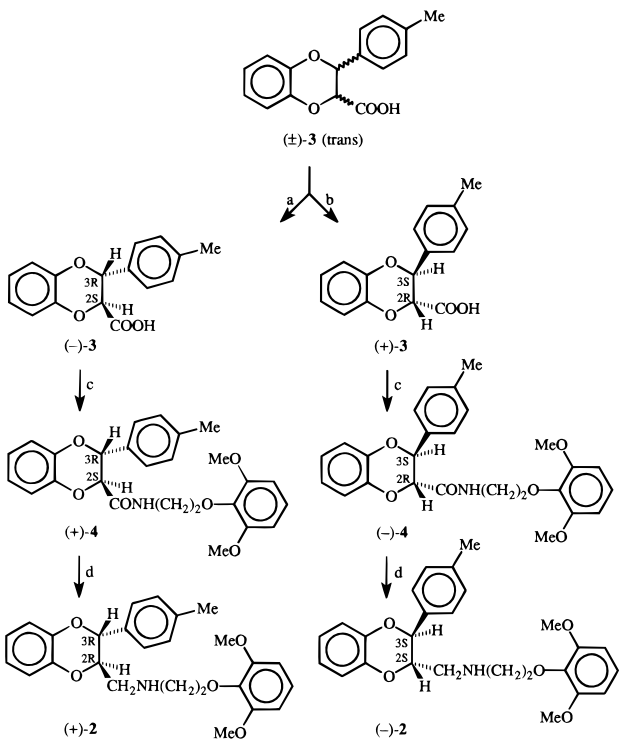
[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1996.

S0022-2623(96)00069-6 CCC: $12.00

Scheme 1[a]



[a] (a) (*S*)-(−)-α-methylbenzylamine, 95% EtOH, room temperature, 30 h; (b) (*R*)-(+)-α-methylbenzylamine, 95% EtOH, room temperature, 30 h; (c) EtOCOCl, Et_3N, $CHCl_3$, 30 min, 0 °C, then 2-(2,6-dimethoxyphenoxy)ethylamine, room temperature, 12 h, silica gel (petroleum ether−EtOAc, 75:25); (d) BH_3·Me_2S, diglyme, 120 °C, 12 h, then MeOH, room temperature, 5 h, HCl, 120 °C, 4 h, silica gel ($CHCl_3$−EtOAc, 95:5).

thought it of interest to investigate whether the enantiomers of **2**, that have an additional chiral center might be able to discriminate among α_1-adrenoreceptor subtypes.

We report here the synthesis of the two enantiomers of **2** together with their biological profile assessed by functional and binding assays. The absolute configuration of the enantiomers of **2** was assigned by correlating their CD spectra with that of (2*S*,3*S*)-3-methyl-2-phenyl-1,4-benzodioxanes.[15]

Chemistry

All the compounds were characterized by ^{1}H NMR, IR, and elemental analyses. Efforts to resolve racemic **2** by fractional crystallization of the dibenzoyl *l*-tartrate and *d*-tartrate salts were unsuccessful. Therefore, acid (±)-**3**[12] was resolved with (*R*)-(+)- and (*S*)-(−)-α-methylbenzylamine. The two enantiomeric salts were converted into the free acids (−)-**3** and (+)-**3**, followed by amidation with 2-(2,6-dimethoxyphenoxy)ethylamine.[16] Intermediate amides (+)-**4** and (−)-**4** were reduced with borane−methyl sulfide complex to (+)-**2** and (−)-**2** (Scheme 1). Enantiomeric purity, determined by ^{1}H NMR spectroscopy in comparison to the spectrum of racemic **2** and on addition of the chiral shift reagent (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(+)-MTPA],[17] was found to be >98% (detection limit) for both enantiomers. The spectrum of racemic **2** (as the free base) in the presence of (+)-MTPA showed a double singlet signal at δ 3.82 ppm for the methoxy protons of the 2,6-methoxyphenoxy group whereas only a singlet

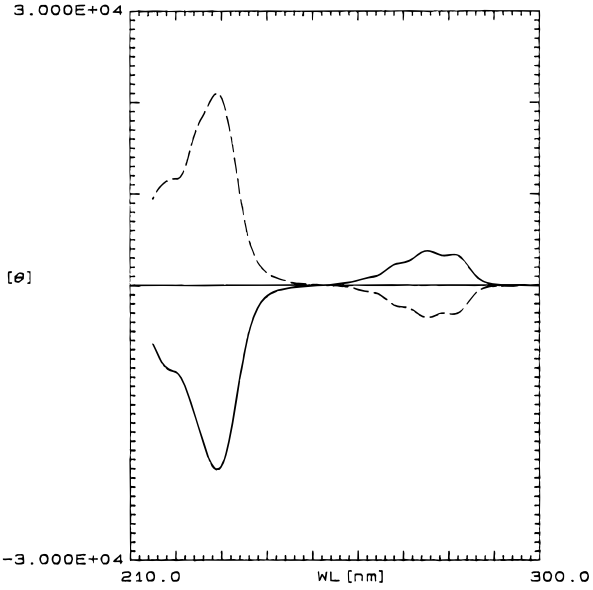
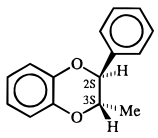


Figure 1. CD spectra of enantiomers of **2** (oxalate salts: *c* 0.015, MeOH): (−)-2 (− − −) and (+)-**2** (—).

was observed for (+)-**2** and (−)-**2** (as the free bases) at δ 3.81 and 3.83 ppm, respectively.

Knowledge of the absolute configuration of (2*S*,3*S*)-3-methyl-2-phenyl-1,4-benzodioxane[15] (**5**) allows a determination of the stereochemistry of the two enantiomers of **2**. The CD spectra for the enantiomers (as



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oxalate salts) of mephendioxan (**2**) are shown in Figure 1. They show a short-wavelength maximum at ca. 229 nm, $[\theta] \approx 20\,000$ and two nearly overlapping long-wavelength maxima at ca. 275.5 and 281 nm, $[\theta] \approx 3000$, of opposite sign from the short-wavelength transition. The (−)-enantiomer of **2** showed negative Cotton effects in the 275−281 nm region and a positive transition in the 229 nm region. The (+)-enantiomer of **2** displayed a CD spectrum which was the mirror image of that of the other enantiomer, suggesting that the two enantiomers have similar optical purity (>98%). The (−)-enantiomer of **2** and **5**[15] have almost identical CD spectra, which suggests that the (−)-enantiomer of **2** has the same absolute configuration (2*S*,3*S*) of the reference compound **5**. It derives that (+)-**2** has a 2*R*,3*R* configuration. Since the two enantiomers of **2** showed high enantiomeric purity, it reasonably follows that, unless there was an unlike complete inversion of configuration in the course of reactions outlined in Scheme 1, (−)-**3** and (+)-**3** have a 2*S*,3*R* and 2*R*,3*S* configuration, respectively, and, consequently, (+)-**4** and (−)-**4** have a 2*S*,3*R* and 2*R*,3*S* configuration, respectively.

Biology

Functional Studies. The pharmacological profile of the enantiomers of **2** was evaluated at α_1- and α_2-adrenoreceptors on isolated rat vas deferens tissues. To allow comparison of the results, we used the same techniques and statistical evaluation of the bioassays

as for other 1,4-benzodioxan-related compounds.[12] WB 4101 (**1**) was used as the standard compound. α_1-Adrenoreceptor blocking activity was assessed by antagonism of (−)-norepinephrine-induced contractions of the epididymal portion, while α_2-adrenoreceptor blocking activity was determined by antagonism of the clonidine-induced depression of the twitch responses of the field-stimulated prostatic portion of rat vas deferens. The potencies of the drugs were expressed as pA_2 values,[18,19] or, in the case of compounds with low potency, as $-\log K_B$ values.[20]

Binding Experiments. Receptor subtype selectivity of the enantiomers of **2** was further determined by employing receptor binding assays. [^{3}H]Prazosin was used to label α_1-adrenoreceptors binding sites of rat cerebral cortex homogenates as well as α_{1A}- and α_{1B}-adrenoreceptor subtypes of CEC pretreated rat hippocampus and liver membranes. In addition, competition assays were performed using [^{3}H]prazosin, and membranes were prepared from COS-7 cells expressing bovine α_{1a}-, hamster α_{1b}-, or rat α_{1d}-adrenoreceptor subtypes. Finally, [^{3}H]rauwolscine, [^{3}H]-8-OH-DPAT, and [^{3}H]spiperone were used to label α_2-adrenoreceptors in rat cerebral cortex, 5-HT$_{1A}$ receptors in rat hippocampus, and D_2 receptors in rat striatum, respectively. Binding affinities were expressed as pK_i values derived using the Cheng−Prusoff equation.[21] WB 4101 (**1**), 5-methylurapidil, and (+)-niguldipine were used as the reference compounds.

Results and Discussion

The characterization of α_1-adrenoreceptor subtypes has been difficult due to the lack of selective ligands. Availability of α_1-adrenoreceptor selective antagonists is of paramount importance not only for receptor characterization and classification but also for potential therapeutic implications such as the treatment of benign prostatic hyperplasia, cardiac arrhythmia and hypertension.[22] However, the design of receptor subtype selective ligands is inherently difficult and remains a formidable challenge to medicinal chemists due to the high homology among receptors. For example, α-adrenoreceptor subtypes share about 45% identity with serotoninergic and dopaminergic receptors.[23] As a consequence, α_1-adrenoreceptor antagonists which are currently classified as subtype selective have also significant affinity for other receptor systems. WB 4101 (**1**) is selective for α_{1A}-adrenoreceptors versus α_{1B}- and α_{1d}-adrenoreceptors but have also high affinity for α_2-adrenoreceptors and 5-HT$_{1A}$ receptors.

We have already demonstrated that the insertion of a 3-substituent in **1** affording mephendioxan (**2**) and related compounds decreases significantly the functional affinity for α_2-adrenoreceptors.[12] This finding has been confirmed by present investigation in both functional and binding assays (Tables 1 and 2). Furthermore, in comparison to **1**, compound **2** has 100-fold lower affinity for the 5-HT$_{1A}$ receptor.

The affinity profile of (+)-**2** and (−)-**2** is shown in Tables 1 and 2. Clearly, (−)-mephendioxan [(−)-**2**] was 10−30-fold more potent than the other enantiomer toward α_1-adrenoreceptor subtypes in both functional and binding assays. The observed stereoselectivity of the enantiomers of **2** is similar to that reported for the enantiomers of **1**.[13] Thus a 2*S*,3*S* configuration assigned to (−)-**2** is consistent with a 2*S* configuration for the most active enantiomer of **1** as one would expect if related compounds act on the same receptor site.

Table 1. α_1- and α_2-Adrenoreceptor pA_2 Values in the Isolated Rat Vas Deferens[a]

no.[b]	α_1 pA_2 against norepinephrine	α_2 -logK_B against clonidine	α_1/α_2[c] selectivity ratio
1	9.13 ± 0.05	6.29 ± 0.07[d]	692
2	8.67 ± 0.02	<5[e]	>4700
(+)-**2**	7.59 ± 0.10	<5[e]	>390
(−)-**2**	8.92 ± 0.09	<5[e]	>8300

[a] pA_2 and $-\log K_B$ values, plus or minus standard error of estimate, were calculated according to Arunlakshana and Schild[18,19] and van Rossum,[20] respectively. [b] With the exception of **1** that was a hydrochloride salt, all other compounds were oxalates. [c] The α_1/α_2 selectivity ratio is the antilog of the difference between pA_2 and $-\log K_B$ values at α_1- and α_2-adrenoreceptors, respectively. [d] Calculated at only one concentration (10 μM). [e] Inactive up to 10 μM.

An analysis of the binding affinities at native and cloned α_1-adrenoreceptor subtypes reveals that (−)-**2** is 36-fold selective for α_{1A}- versus α_{1B}-adrenoreceptors. Comparable results were obtained for 5-methylurapidil and (+)-niguldipine that exhibited selectivities of 47- and 60-fold, respectively. Compound (−)-**2** bound to cloned α_1-adrenoreceptors with pK_i values of 9.46 (α_{1a}), 7.68 (α_{1b}), and 8.18 (α_{1d}) and exhibited selectivities of 60- and 20-fold in binding to the α_{1a}-adrenoreceptors relative to α_{1b}- and α_{1d}-adrenoreceptors, respectively. In parallel experiments, 5-methylurapidil and (+)-niguldipine displayed selectivities of 520- and 66-fold, respectively, for the α_{1a}-adrenoreceptor relative to the α_{1b} subtype, and selectivities of 87- and 210-fold, respectively, for the α_{1a} versus the α_{1d} subtype. Clearly, the enantiomer (−)-**2** has an affinity profile at both native and cloned α_1-adrenoreceptor subtypes qualitatively similar to that of 5-methylurapidil and (+)-niguldipine which are classified as α_{1A}-adrenoreceptor selective antagonists. However, 5-methylurapidil and (+)-niguldipine have also additional biological properties such as high affinity for 5-HT$_{1A}$ receptors and potent calcium channel blocking activity, respectively, which limit their utility as pharmacological tools in receptor characterization. Interestingly, (−)-mephendioxan [(−)-**2**] was also 12000-, 2500-, and 250-fold selective in binding to α_{1a}-adrenoreceptors relative to α_2-adrenoreceptors and 5-HT$_{1A}$ and D_2 receptors, respectively. On these bases, (−)-**2** can be considered as a lead α_{1A}-selective antagonist having a similar profile but a structure very different from the recently reported analogue of (+)-niguldipine.[24]

In conclusion, the present investigation has demonstrated that the insertion of a trans aryl substituent at the 3-position of **1** affording **2** increases affinity and selectivity for α_{1A}-adrenoreceptors while significantly decreasing the affinity for α_2-adrenoreceptors and 5-HT$_{1A}$ and D_2 receptors in comparison to the prototype **1**. Furthermore, it has been shown that the affinity and selectivity reside predominantly in the enantiomer (−)-mephendioxan [(−)-**2**] which may represent not only a valuable tool for the characterization of α_1-adrenoreceptor subtypes but also a lead compound for the development of selective α_{1A}-adrenoreceptor antagonists for the treatment of benign prostatic hypertrophy.[22]

Table 2. Affinity Constants (K_i) of the Enantiomers of Mephendioxan (**2**) for Native and Cloned α_1-Adrenoreceptor Subtypes, Native α_2-Adrenoreceptor, and 5-HT_{1A} and D_2-Receptors in Comparison to Reference Compounds[a]

	K_i (nM), native receptors (rat)						K_i (nM), cloned receptors		
no.	cerebral cortex, α_1	hippocampus + 10 μM CEC, α_{1A}	liver, α_{1B}	cerebral cortex, α_2	hippocampus, 5-HT_{1A}	striatum, D_2	bovine brain, α_{1a}	hamster smooth muscle, α_{1b}	rat brain, α_{1d}
1	8.72 ± 2.02	1.29 ± 0.39	25.64 ± 4.86	14.72 ± 1.16	7.26 ± 2.40	122.8± 25.7	0.62 ± 0.16	57.34 ± 14.25	6.26 ± 1.18
(±)-**2**	17.03 ± 2.45	5.32 ± 0.67	102.9 ± 8.1	3501 ± 874	606.2 ± 52.1	82.34 ± 8.65	0.91 ± 0.21	51.38 ± 4.31	11.76 ± 2.43
(+)-**2**	121.2 ± 10.4	40.33 ± 4.11	774.3 ± 77.6	2142 ± 667	1262 ± 372	827.3 ± 5.2	8.34 ± 3.12	195.2 ± 36.4	191.1 ± 50.9
(−)-**2**	9.97 ± 0.92	1.76 ± 0.53	62.61 ± 2.38	4293 ± 1502	888.9 ± 379.0	86.48 ± 11.01	0.35 ± 0.18	21.08 ± 3.20	6.57 ± 0.87
MET[b]	28.25 ± 3.84	4.66 ± 1.82	220.2 ± 20.3	432.4 ± 65.1	1.20 ± 0.37	935.1 ± 115.6	2.02 ± 0.53	1058 ± 183	175.1 ± 21.3
NIG[c]	27.80 ± 7.40	6.38 ± 1.29	379.9 ± 41.0	1611 ± 235	> 10000	294.9 ± 56.6	0.38 ± 0.06	25.01 ± 1.67	82.41 ± 11.95

[a] Values are the mean ± SE of at least three separate experiments performed in triplicate. The pseudo-Hill coefficients (nH) were not significantly different from unity ($p > 0.05$) with the exception of rat cerebral cortex, where a heterogeneous α_1-adrenoreceptor population is present. Equilibrium dissociation constants (K_i) were derived using the Cheng−Prusoff equation.[21] Scatchard plots were linear or almost linear in all preparation tested. The affinity estimates were derived from displacement of [^{3}H]prazosin from α_1-adrenoreceptors, [^{3}H]rauwolscine from α_2-adrenoreceptors, [^{3}H]spiperone from D_2 receptors, and [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetraline from 5-HT_{1A} receptors. [b] MET, 5-methylurapidil. [c] NIG, (+)-niguldipine.

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and ^{1}H NMR spectra were recorded on Perkin-Elmer 297 and Varian VXR 300 instruments, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), or m (multiplet). Although the IR spectra data are not included (because of the lack of unusual features), they were obtained for all compounds reported and were consistent with the assigned structures. Optical activity was measured at 20 °C with a Perkin-Elmer 241 polarimeter. Circular dichroism (CD) spectra were measured at room temperature with a JASCO J710 spectropolarimeter using a 0.1 cm path length cell. Data were handled with a mathematic noise reduction routine (JASCO software). The elemental compositions of the compounds agreed to within ±0.4% of the calculated value. When the elemental analysis is not included, crude compounds were used in the next step without further purification. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040−0.063 mm, Merck) by flash chromatography. Petroleum ether refers to the fraction with a boiling point of 40−60 °C. The term "dried" refers to the use of anhydrous sodium sulfate.

Resolution of (±)-*trans*-3-*p*-Tolyl-2,3-dihydro-1,4-benzodioxin-2-carboxylic Acid (3). Racemic **3**[12] (4.1 g, 15.0 mmol) in 95% EtOH (65 mL) was treated with a solution of (*S*)-(−)-α-methylbenzylamine (1.82 g, 15.0 mmol) in 95% EtOH (77 mL) and left at room temperature for 30 h. The white crystals were crystallized twice from 95% EtOH: 1.4 g; mp 223.5−225.5 °C; $[\alpha]_D$ −4.71° (*c* 1, MeOH).

The salt was dissolved in water (50 mL), and the ice-cooled solution was made basic with 2 N NaOH (50 mL). The resulting mixture was extracted with ether (3 × 30 mL) to recover the amine and then acidified with cold 2 N HCl and extracted with $CHCl_3$ (3 × 30 mL). Removal of dried solvent gave (2*S*,3*R*)-(−)-3-*p*-tolyl-2,3-dihydro-1,4-benzodioxin-2-carboxylic acid [(−)-**3**]: 0.85 g; mp 169−172 °C; $[\alpha]_D$ −1.81° (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3, CH_3C$_6$H$_4$), 4.81 (d, J = 6.0 Hz, 1, 2-H), 5.24 (d, J = 6.0 Hz, 1, 3-H), 6.82−7.30 (m, 8, ArH), 8.50 (br s, 1, COOH). Anal. ($C_{16}H_{14}O_4$) C, H.

The acid recovered from the mother liquors by a similar alkaline treatment (3.25 g, 12.0 mmol) was dissolved in 95% EtOH (39 mL) and treated with a solution of (*R*)-(+)-α-methylbenzylamine (1.5 g, 12.0 mmol) in 95% EtOH (55 mL). The solution was left at room temperature for 30 h and the white crystals were crystallized twice from 95% EtOH: 1.1 g; mp 223.5−224.5 °C; $[\alpha]_D$ +5.48° (c 1, MeOH).

This salt was treated as described for the other enantiomer to give (2*R*,3*S*)-(+)-3-*p*-tolyl-2,3-dihydro-1,4-benzodioxin-2-carboxylic acid [(+)-**3**]: 0.7 g; mp 169−172 °C; $[\alpha]_D$ +1.64° (*c* 1, MeOH); the ^{1}H NMR spectrum was identical to that of (−)-**3**. Anal. ($C_{16}H_{14}O_4$) C, H.

(2*S*,3*R*)-(+)-3-*p*-Tolyl-2,3-dihydro-1,4-benzodioxin-2-carboxylic Acid [2-(2,6-Dimethoxyphenoxy)ethyl]amide [(+)-4]. Ethyl chlorocarbonate (0.34 g, 3.03 mmol) was added dropwise to a stirred and cooled (0 °C) solution of (−)-**3** (0.82 g, 3.03 mmol) and Et_3N (0.31 g, 3.03 mmol) in chloroform (20 mL), followed after 30 min by the addition of a solution of 2-(2,6-dimethoxyphenoxy)ethylamine[16] (0.59 g, 3.03 mmol) in chloroform (10 mL). The resulting reaction mixture was stirred overnight at room temperature and then washed with 2 N HCl, 2 N NaOH, and finally with water. Removal of dried solvent gave a solid which was purified by column chromatography. Eluting with petroleum ether−EtOAc (75:25) afforded (+)-**4**: 1.0 g (74% yield); mp 120−122 °C; $[\alpha]_D$ +3.81° (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3, CH_3C$_6$H$_4$), 3.50 (m, 2, NCH_2), 3.85 (s, 6, OCH_3), 4.02 (t, 2, CH_2O), 4.58 (d, J = 6.7 Hz, 1, 2-H), 5.07 (d, J = 6.7 Hz, 1, 3-H), 6.58−7.25 (m, 11, ArH), 7.52 (br t, 1, NH exchangeable with D_2O).

(2*R*,3*S*)-(−)-3-*p*-Tolyl-2,3-dihydro-1,4-benzodioxin-2-carboxylic Acid [2-(2,6-Dimethoxyphenoxy)ethyl]amide [(−)-4]. This was obtained from (+)-**3** following the procedure described for the enantiomer (+)-**4** and purified by column chromatography: 1.1 g (81% yield); mp 120−122 °C; $[\alpha]_D$ −3.96° (*c* 1, MeOH). The ^{1}H NMR spectrum was identical to that of (+)-**4**.

(2*R*,3*R*)-(+)-[2-(2,6-Dimethoxyphenoxy)ethyl][(3-*p*-tolyl-2,3-dihydro-1,4-benzodioxin-2-yl)methyl]amine Oxalate Emihydrate [(+)-2]. A solution of 10 M BH_3·CH_3SCH_3 (0.2 mL) in dry diglyme (1 mL) was added dropwise at room temperature to a solution of (+)-**4** (1.0 g, 2.2 mmol) in dry diglyme (40 mL) with stirring under a stream of dry nitrogen with exclusion of moisture. When the addition was completed, the reaction mixture was heated at 120 °C for 12 h. After cooling at 0 °C, excess borane was destroyed by cautious dropwise addition of MeOH (5 mL). The resulting mixture was left to stand for 5 h at room temperature, treated with HCl gas for 10 min, and then heated at 120 °C 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 pellets and extracted with chloroform (4 × 30 mL). Removal of dried solvent gave a residue which was purified by column chromatography. Eluting with $CHCl_3$−EtOAc (95:5) afforded (+)-**2** as the free base: ^{1}H NMR ($CDCl_3$) δ 2.25 (br s, 1, NH, exchangeable with D_2O), 2.38 (s, 3, CH_3C$_6$H$_4$), 2.70−2.84 (m, 4, CH_2NCH_2), 3.85 (s, 6, OCH_3), 4.10 (t, 2, CH_2O), 4.20 (m, 1, 2-H), 4.96 (d, J = 7.91 Hz, 1, 3-H), 6.56−7.35 (m, 11, ArH); enantiomeric purity > 98% (detection limit), determined with (+)-MTPA as the chiral shift reagent.

(+)-**2** free base was transformed into the oxalate salt by treating an ether solution with oxalic acid. It was recrystallized from 2-PrOH: 0.64 g (55% yield); $[\alpha]_D$ +22.04° (*c* 1, MeOH), CD (*c* 0.015, MeOH) $[\theta]_{281}$ +3369, $[\theta]_{275.5}$ +3768, $[\theta]_{229}$ −20140. The melting point was indefinite; fusion started at 147 °C and was complete at 157−158 °C. Anal. ($C_{26}H_{29}NO_5$·$H_2C_2O_4$·0.5H_2O) C, H, N.

(2*S*,3*S*)-(−)-[2-(2,6-Dimethoxyphenoxy)ethyl][(3-*p*-tolyl-2,3-dihydro-1,4-benzodioxin-2-yl)methyl]amine Oxalate Hydrate [(−)-2]. This was obtained as free base from (−)-**4**

as described for the enantiomer (+)-**2** and purified by column chromatography. The ^{1}H NMR spectrum exhibited the same resonances as that of the other enantiomer; enantiomeric purity was >98% (detection limit), determined with (+)-MTPA as the chiral shift reagent.

(−)-**2** free base was transformed into the oxalate salt by treating an ether solution with oxalic acid. It was recrystallized from 2-PrOH: 0.76 g (60% yield); $[\alpha]_D$ −23.23° (*c* 1, MeOH), CD (*c* 0.015, MeOH) $[\theta]_{281}$ −3173, $[\theta]_{275.5}$ −3566, $[\theta]_{229}$ +21000. The mp was indefinite; fusion started at 96 °C and was complete at 154−155 °C. Anal. ($C_{26}H_{29}NO_5 \cdot H_2C_2O_4 \cdot H_2O$) C, H, N.

Biology. Functional Antagonism in Isolated Rat Vas Deferens. Male albino rats (175−200 g) were killed by a sharp blow on the head, and both vasa deferentia were isolated, freed from adhering connective tissue, and transversely bisected. Functional experiments at α_1- and α_2-adrenoreceptors were carried out in the epididymal and prostatic portions of the vas deferens using (−)-norepinephrine and clonidine as the agonists, respectively, as previously described.[12]

Radioligand Binding Assays. Male rats (200−300 g, Charles River, Italy) were killed, and their hippocampus, striatum, cerebral cortex, and liver were dissected, frozen on dry ice, and then stored at −70 °C until used.

Native Receptors. Binding studies at α_1- and α_2-adrenoceptors and 5-HT_{1A} and D_2 receptors were carried out in rat cerebral cortex (α_1 and α_2), hippocampus (5-HT_{1A}), and striatum (D_2) membranes as previously described.[25] The respective radioligands were [^{3}H]prazosin (specific activity 79.2 Ci/mmol), [^{3}H]rauwolscine (specific activity 80.5 Ci/mmol), [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT) (specific activity 162.9 Ci/mmol), and [^{3}H]spiperone (specific activity 17.7 Ci/mmol). CEC pretreated rat hippocampus and liver membranes were used to determine [^{3}H]prazosin binding to native α_{1A}- and α_{1B}-adrenoceptor subtypes, respectively, as previously described.[26]

Cloned Receptors. [^{3}H]Prazosin binding to cloned α_1-adrenoceptor subtypes was performed in COS-7 cells expressing transiently bovine α_{1a}- (previously named α_{1c}), hamster α_{1b}-, and rat α_{1d}-adrenoceptors. Construction and transfection of individual α_1-adrenoceptor subtypes were carried out by Dr. S. Cotecchia (Université de Lausanne, Switzerland) as previously described.[3−5] COS-7 cell membranes (35−70 μg of proteins) were incubated in 50 mM Tris-HCl, pH 7.4, containing 10 μM pargyline and 0.1% ascorbic acid, with 0.3−0.6 nM [^{3}H]prazosin, in the absence or presence of the displacing drug, in a final volume of 0.22 mL. Nonspecific binding was determined in presence of 100 μM phentolamine. The reaction mixture was incubated for 30 min at 25 °C and then stopped by addition of ice cold Tris-HCl buffer and rapid filtration through Whatman GF/B filters.

Data Analysis. The dissociation constants (pA_2 values, Table 1) were determined by Schild plots[18] obtained from the dose ratios at the EC_{50} values of the agonists calculated at three antagonist concentrations. Each concentration was tested five times, and Schild plots were constrained to slope −1.[19] The compounds were tested at only one concentration when determining α_2-adrenoreceptor blocking activity because of their low affinity for this receptor. In these cases, pA_2 (−log K_B) values were calculated according to van Rossum.[20] Data are presented as the mean ± SE of *n* experiments. Differences between mean values were tested for significance by student's *t*-test.

Binding data were analyzed using a nonlinear curve-fitting program Allfit.[27] Scatchard plots were linear or almost linear in all preparations. All pseudo-Hill coefficients (nH) were not significantly different from unity ($p > 0.05$). Equilibrium dissociation constants (K_i) were derived from the Cheng−Prusoff equation,[21] $K_i = IC_{50}/(1 + L/K_d)$, where L and K_d are the concentration and the equilibrium dissociation constant of the radioligand, respectively. pK_i values (Table 2) are the mean ± SE of three separate experiments performed in triplicate.

Acknowledgment. This work was supported by a grant from MURST. We express our thanks to Prof. G. P. Spada (University of Bologna) for performing CD measurements and for valuable discussions.

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JM960069A