Synthesis and Biological Profile of the Enantiomers of [4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-*cis*-octahydroquinoxalin-1-yl]furan-2-ylmethanone (Cyclazosin), a Potent Competitive α_{1B}-Adrenoceptor Antagonist

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Received July 15, 1996⊗

The enantiomers of [4-(4-amino-6,7-dimethoxyquinazolin-2-yl)-*cis*-octahydroquinoxalin-1-yl]-furan-2-ylmethanone (cyclazosin, **1**) were synthesized from the chiral furan-2-yl(*cis*-octahydroquinoxalin-1-yl)methanone [(+)-**2** and (−)-**2**], which were obtained by resolution of the racemic amine with (*S*)-(+)- and (*R*)-(−)-mandelic acid. The binding profile of the enantiomers of **1** was assessed at α_1-, α_2-, D_2, and 5-HT_{1A} receptors as well as at native α_{1A}- and α_{1B}- and cloned α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptor subtypes in comparison with prazosin, spiperone, and AH11110A. (+)-**1** displayed a 40−90-fold selectivity for the α_{1B}(α_{1b})-adrenoceptor relative to α_{1A}(α_{1a}) and α_{1d} subtypes. A significant enantioselectivity was observed at the α_{1A}(α_{1a})-adrenoceptor and particularly at α_{1d}-adrenoceptors since (−)-**1** was 11−14- and 47-fold, respectively, more potent than (+)-**1**. Furthermore the enantiomer (+)-**1** displayed selectivities of 1100-, 19000-, and 12000-fold in binding to α_{1b}-adrenoceptors relative to α_2-adrenoceptors and 5-HT_{1A} and D_2 receptors. These results indicate that (+)-**1**, [(+)-cyclazosin] is the most potent and selective ligand for the α_{1B}-adrenoceptor subtype so far described and may be a valuable tool in the characterization of α_1-adrenoceptor subtypes.

Introduction

Pharmacological and binding studies have shown that α_1-adrenoceptors can be classified into at least three subtypes, namely, α_{1A}, α_{1B}, and α_{1D}.[1] 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).[2] The α_{1B} subtype displays lower affinity for the above antagonists but is preferentially inactivated by the alkylating agent CEC,[2] whereas the α_{1D} subtype has high affinity for the antagonist BMY7378.[3] Current evidence indicates that rat submaxillary gland,[4] human liver,[5] and various tissues such as prostatic rat vas deferens,[6] rabbit prostate, and prostatic urethra[7] contain predominantly the α_{1A}-adrenoceptor, whereas rat liver and spleen[8] are considered α_{1B}-adrenoceptor preparations, and the α_{1D}-adrenoceptor mediates the contraction in rat aorta.[9,10] Cloning studies have confirmed the existence of three distinct α_1-adrenoceptors, which are now designated as α_{1a}, α_{1b}, and α_{1d} subtypes.[11−14] The recombinant α_{1a}-adrenoceptor (formerly designated as α_{1c})[12,15] corresponds to the native α_{1A}-adrenoceptor, 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}-adrenoceptor recently characterized in rat aorta. Thus, α_1-adrenoceptors 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 receptors, respectively.[1,16,17]

Whereas several selective α_{1A}-adrenoceptor antagonists have been discovered,[2] for example, WB 4101, 5-methylurapidil, (+)-niguldipine, and SNAP 5089, only a few selective α_{1B}-adrenoceptor antagonists are available. Besides spiperone, which is 10-fold selective for α_{1B}- versus α_{1A}-adrenoceptors,[4] a new compound, AH11110A, has been recently described as a selective α_{1b}-adrenoceptor ligand.[18] Our research group has previously been involved in designing new α_1-adrenoceptor antagonists structurally related to prazosin 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-adrenoceptor.[19,20] Among a variety of structural modifications performed on prazosin, we have demonstrated that the piperazine ring may not be essential for activity at α_1-adrenoceptors.[19] The replacement of the piperazine ring of prazosin with a *cis*-decahydroquinoxaline moiety afforded [4-(4-amino-6,7-dimethoxyquinazolin-2-yl)-*cis*-octahydroquinoxalin-1-yl]furan-2-ylmethanone (cyclazosin, **1**) which displayed a significant selectivity for α_{1B}- relative to α_{1A}-adrenoceptors while failing to discriminate between α_{1B}- and α_{1D}-adrenoceptors.[20,21] It is well known that stereochemistry has a relevant role in the drug−receptor interaction process. Among α_1-adrenoceptor ligands, the enantiomers of niguldipine, a potent calcium channel antagonist also displaying high affinity for α_1-adrenoceptors,[2] were selective for the α_{1A}-adrenoceptor. (*R*)-(+)-Niguldipine was 600-fold selective for α_{1A}- versus α_{1B}-adrenoceptors, whereas the *S*-(−)-enantiomer was about 30-fold less selective for the same subtype.[22] Very recently, another antagonist, (−)-mephendioxan,[23] has been shown to possess significant enantioselectivity. It was 36-fold selective for the α_{1A}- versus α_{1B}-adrenoceptor and 60- and 20-fold selective

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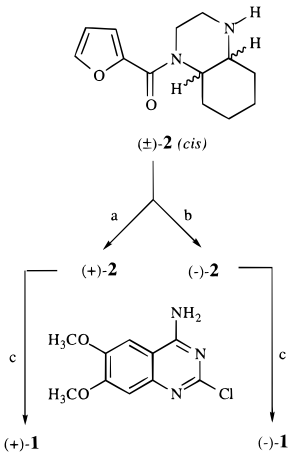
⊗ Abstract published in *Advance ACS Abstracts,* October 15, 1996.

S0022-2623(96)00510-9 CCC: $12.00 © 1996 American Chemical Society

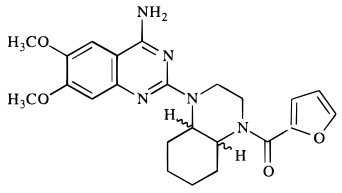
Scheme 1[a]



[a] (a) (*S*)-(+)-Mandelic acid, MeOH, room temperature; (b) (*R*)-(−)-mandelic acid, MeOH, room temperature; (c) *N*,*N*-diisopropylethylamine, *i*-AmOH, reflux, 72 h.

in binding to the α_{1a}-adrenoceptor relative to α_{1b} and α_{1d} subtypes.[23] Concerning prazosin-related antagonists, only the enantiomers of terazosin have been recently studied at α_1-adrenoceptor subtypes without their displaying, however, a marked enantioselectivity.[24]

Since cyclazosin (**1**) incorporates a decahydroquinoxaline nucleus in a cis relationship, which is responsible for the high affinity for α_1-adrenoceptors and bears two chiral centers in a different position relative to terazosin, we thought it of interest to investigate whether the enantiomers of **1** might be able to discriminate among α_1-adrenoceptor subtypes. We report here the synthesis of the two enantiomers of **1** and their biological profile assessed by binding assays.



Cyclazosin (*cis* isomer) **1**

Chemistry

Enantiomers (+)- and (−)-**1** were prepared with a procedure slightly modified with regard to that used for the synthesis of racemic **1** and were characterized as hydrochloride salts. 4-Amino-2-chloro-6,7-dimethoxyquinazoline was coupled separately with the homochiral amines furan-2-yl(*cis*-octahydroquinoxalin-1-yl)-methanone [(+)- and (−)-**2**] in the presence of *N*,*N*-diisopropylethylamine (Scheme 1). These were obtained by resolving racemic **2**[20] with (*S*)-(+)- and (*R*)-(−)-mandelic acid. The enantiomeric purity was assessed, in comparison with racemic **2**, by HPLC upon their transformation with (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate into the corresponding diastereomeric ureas 4-(furan-2-ylcarbonyl)-*cis*-octahydroquinoxaline-1-carboxylic acid (1-naphthalen-1-ylethyl)amide.

MS spectra revealed for all three ureas the absence of the molecular ion and the cleavage of the molecules into the starting materials. The HPLC analysis (reversed phase, C_{18} column, programmed elution with water/acetonitrile from 95−5%, v/v, to 63−37%, v/v) showed a single peak with a retention time of 30.24 and

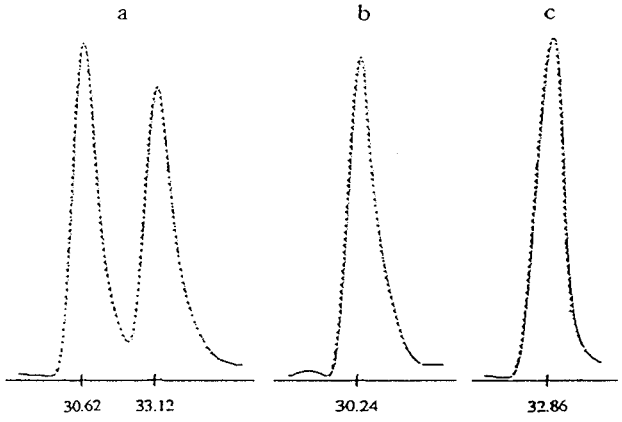


Figure 1. Analytical HPLC chromatograms of the diastereomeric naphthylethyl ureas of racemic **2** (a) and the enantiomers (−)-**2** (b) and (+)-**2** (c). Chromatographic conditions: reversed phase, C_{18} column, Beckmann ultrasphere ODS, 5 μm (150 × 4.6 i.d.); programmed elution, water/acetonitrile from 95:5 to 63:37 (v/v) at a flow rate of 1 mL/min; detection, UV at 254 nm; chart speed, 7.8 mm/min. Retention times (min) are indicated at each peak.

32.86 min for the ureas obtained from (−)- and (+)-**2**, respectively. In the same conditions, the urea from racemic **2** showed two peaks with a retention time of 30.62 and 33.12 min, respectively (Figure 1). Since a purposely prepared mixture of 98% of urea from (+)-**2** and 2% of urea from (−)-**2**, or vice versa, showed a detectable appearance of the less abundant diastereomer, we attributed to the amines (+)- and (−)-**2** a stereochemical purity higher than 98%. In addition, the experimental reaction conditions to final products, shown in Scheme 1, should not give racemization; thus, it can be assumed that the configuration of compounds (+)- and (−)-**1** is retained.

Biology

Receptor subtype selectivity of the enantiomers of **1** was determined by employing receptor binding assays. [^{3}H]Prazosin was used to label α_1-adrenoceptor binding sites of rat cerebral cortex homogenates as well as α_{1A}- and α_{1B}-adrenoceptor subtypes of CEC-pretreated rat hippocampus and liver membranes. In addition, competition assays were performed using [^{3}H]prazosin on membranes prepared from COS-7 cells expressing bovine α_{1a}-, hamster α_{1b}-, or rat α_{1d}-adrenoceptor subtypes and membranes prepared from CHO cells expressing human α_1-adrenoceptor subtypes. Finally, [^{3}H]rauwolscine, [^{3}H]-8-OH-DPAT, and [^{3}H]spiperone were used to label α_2-adrenoceptors in rat cerebral cortex, 5-HT_{1A} receptors in rat hippocampus, and D_2 receptors in rat striatum, respectively. Binding affinities are expressed as pK_i values derived using the Cheng−Prusoff equation.[25] Prazosin, spiperone, and AH11110A were used as the reference compounds.

Results and Discussion

The pharmacological results of radioligand binding assays performed on (+)- and (−)-**1** are reported in Tables 1 and 2 and Figure 2 in comparison to racemic **1**, prazosin, spiperone, and AH11110A as reference compounds.

A previous study had shown that cyclazosin (**1**) is a potent competitive antagonist of rat vas deferens α-adrenoceptors endowed with a marked selectivity for α_1-

Table 1. Affinity Constants, Expressed as pK_i, of the Enantiomers of Cyclazosin (**1**) for Native and Cloned α_1-Adrenoceptor Subtypes, Native α_1- and α_2-Adrenoceptors, and 5-HT_{1A} and D_2 Receptors in Comparison to Reference Compounds[a]

	pK_i, native receptors (rat)						pK_i, 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**	8.28 ± 0.05	7.73 ± 0.12	9.68 ± 0.04	6.13 ± 0.04	4.89 ± 0.02	5.08 ± 0.07	7.48 ± 0.05	9.16 ± 0.02	7.57 ± 0.003
(−)-**1**	9.10 ± 0.13	8.77 ± 0.07	9.85 ± 0.11	5.86 ± 0.03	5.21 ± 0.10	≪5	8.62 ± 0.12	9.51 ± 0.15	9.24 ± 0.06
(±)-**1**	9.25 ± 0.09	8.41 ± 0.03	9.57 ± 0.01	6.17 ± 0.05	5.16 ± 0.08	<5	8.18 ± 0.14	9.23 ± 0.04	9.28 ± 0.05
PRA[b]	9.13 ± 0.02	9.03 ± 0.09	9.44 ± 0.12	6.83 ± 0.07	5.63 ± 0.07	<5	9.14 ± 0.04	9.34 ± 0.12	8.86 ± 0.05
SPIP[c]	8.16 ± 0.01	7.42 ± 0.07	8.81 ± 0.08	6.87 ± 0.15	7.60 ± 0.11	9.24 ± 0.03	7.87 ± 0.11	8.15 ± 0.01	7.66 ± 0.13
AH[d]	6.65 ± 0.14	6.19 ± 0.12	7.38 ± 0.02	5.54 ± 0.15	<5	<5	6.79 ± 0.13	7.40 ± 0.04	6.42 ± 0.12

[a] Values are the mean ± SE of two to three separate experiments performed in triplicate. The pseudo-Hill coefficients (*n*H) were not significantly different from unity ($p > 0.05$) with the exception of spiperone and prazosin respectively at α_1- and α_2-adrenoceptors of rat cerebral cortex. Equilibrium dissociation constants (K_i) were derived using the Cheng−Prusoff equation.[25] Scatchard plots were linear or almost linear in all preparations tested. The affinity estimates were derived from displacement of [^{3}H]prazosin from α_1-adrenoceptors, [^{3}H]rauwolscine from α_2-adrenoceptors, [^{3}H]spiperone from D_2 receptors, and [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetraline from 5-HT_{1A} receptors. [b] PRA, prazosin. [c] SPIP, spiperone. [d] AH, AH11110A.

Table 2. Affinity Constants, Expressed as pK_i, of (+)-**1** and AH11110A for Human Cloned α_1-Adrenoceptor Subtypes[a]

	pK_i, cloned receptors		
compd	human hippocampus, α_{1a}	human hippocampus, α_{1b}	human hippocampus, α_{1d}
(+)-**1**	7.91 ± 0.16	9.87 ± 0.01	8.49 ± 0.05
AH11110A	7.02 ± 0.10	7.73 ± 0.03	6.36 ± 0.05

[a] Values are the mean ± SE of two to three separate experiments performed in triplicate. The pseudo-Hill coefficients (*n*H) were not significantly different from unity ($p > 0.05$). Equilibrium dissociation constants (K_i) were derived using the Cheng−Prusoff equation.[25] Scatchard plots were linear or almost linear in all preparations tested. The affinity estimates were derived from displacement of [^{3}H]prazosin from α_1-adrenoceptors.

versus α_2-adrenoceptors (pA_2 α_1 = 8.97 ± 0.02; pA_2 α_2 = 5.08 ± 0.10), higher than that displayed by prazosin (α_1/α_2 selectivity ratio of 7800 and 1200, respectively).[20] Binding assays[21] confirmed its specificity and affinity for α_1-adrenoceptors (pK_i = 9.25 ± 0.09) relative to α_2-adrenoceptors (pK_i = 6.17 ± 0.05) and 5-HT_{1A} (pK_i = 5.16 ± 0.08) and D_2 (pK_i < 5) receptors. In addition, cyclazosin showed a 10−15-fold selectivity for $\alpha_{1B}(\alpha_{1b})$-adrenoceptors with respect to the $\alpha_{1A}(\alpha_{1a})$ subtype [pK_i = (9.23 ± 0.04) − (9.57 ± 0.01) and (8.18 ± 0.14) − (8.41 ± 0.03), respectively]. However, it failed to discriminate between cloned α_{1b}- and α_{1d}-adrenoceptors (pK_i = 9.23 ± 0.04 and 9.28 ± 0.05, respectively).

In the present work, (+)- and (−)-**1** inhibited with a 7-fold difference the [^{3}H]prazosin binding at α_1-adrenoceptors of rat cerebral cortex membrane as revealed by their respective pK_i values of 8.28 ± 0.05 and 9.10 ± 0.13, whereas their affinity was markedly lower, albeit similar, at cortical α_2-adrenoceptors as well as at 5-HT_{1A} and D_2 receptors (Table 1). Cyclazosin enantiomers also proved weakly active at cortical 5-HT_{2A} receptors (results not shown). Racemic cyclazosin and prazosin, but not spiperone, displayed an α_1-adrenoceptor specificity similar to that of (+)- and (−)-**1**.

The analysis of the affinity profile displayed by (+)- and (−)-**1** at native α_{1A}- and α_{1B}- as well as at cloned α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptor subtypes is rather interesting (Tables 1 and 2 and Figure 2). The enantiomer (−)-**1**, although more potent than (+)-**1** at all subtypes, was nearly devoid, like prazosin, of subtype selectivity with the exception of a 12-fold higher affinity at native α_{1B}- relative to α_{1A}-adrenoceptors. On the contrary, (+)-**1** inhibited [^{3}H]prazosin binding with high affinity (pK_i = 9.16 ± 0.02) at cloned α_{1b}-adrenoceptors of COS-7 cells and a significantly lower potency at both α_{1a} and α_{1d} subtypes (pK_i = 7.48 ± 0.05 and 7.57 ± 0.003, respectively). Thus, (+)-**1** displayed a 40−50-fold selectivity for α_{1b}- versus α_{1a}- and α_{1d}-adrenoceptors of the above cells and an even higher selectivity (90-fold) for the native α_{1B}-adrenoceptor (pK_i = 9.68 ± 0.04) and cloned human α_{1b}-adrenoceptor (pK_i = 9.87 ± 0.01) relatively to the native α_{1A} (pK_i = 7.73 ± 0.12) and human cloned α_{1a} (pK_i = 7.91 ± 0.16), although the selectivity with respect to the α_{1d} subtype of latter species (pK_i = 8.49 ± 0.05) was lower (24-fold). Clearly, (+)-cyclazosin [(+)-**1**] emerges as the most interesting ligand of prazosin-related antagonists as it displayed high affinity, in the nanomolar range like prazosin, and an unprecedented selectivity for $\alpha_{1b}(\alpha_{1B})$-adrenoceptors, which is lacking in the antagonists presently available.[21]

Among 2,4-diamino-6,7-dimethoxyquinazoline derivatives, only the enantiomers of terazosin have been investigated at α_1-adrenoceptor subtypes. However, it has been shown that they are not selective as they showed the same potency at each α_1-adrenoceptor subtype.[24]

In comparison with the antipsychotic drug spiperone, which has been considered for some time the most selective competitive antagonist for α_{1B}-adrenoceptors,[2] (+)-cyclazosin proved about 10-fold more potent at the $\alpha_{1B}(\alpha_{1b})$ subtype and significantly more selective than spiperone for $\alpha_{1B}(\alpha_{1b})$- versus both $\alpha_{1A}(\alpha_{1a})$- and α_{1d}-adrenoceptors (Table 1). However, the low selectivity of spiperone for the α_{1b}-adrenoceptor found in this study confirms the data from other laboratories.[26,27] On the other hand, the amidine AH11110A, which is claimed to display a 33−37-fold selectivity for α_{1b} versus α_{1a} and α_{1d} subtypes,[18] in our hands showed a lower selectivity on the same clones (Table 1), which was also confirmed in human clonal α_1-adrenoceptors (Table 2). (+)-Cyclazosin proved 60−140 times more potent than AH11110A at α_{1b}-adrenoceptors (pK_i = 9.16 ± 0.02 vs 7.40 or pK_i = 9.87 ± 0.01 vs 7.73 ± 0.03) while displaying a higher selectivity than AH11110A versus α_{1a}- and α_{1d}-adrenoceptors. Whether or not the different selectivity found by us for AH11110A could depend on the use of a different radioligand ([^{125}I]HEAT was used in ref 18) is an open issue.

An analysis of the affinity profile of the two enantiomers of cyclazosin reveals that stereochemistry plays a significantly different role at the three α_1-adrenoceptor subtypes. Clearly, $\alpha_{1A}(\alpha_{1a})$ and α_{1d} subtypes, but not the $\alpha_{1B}(\alpha_{1b})$-adrenoceptor, display a significant enanti-

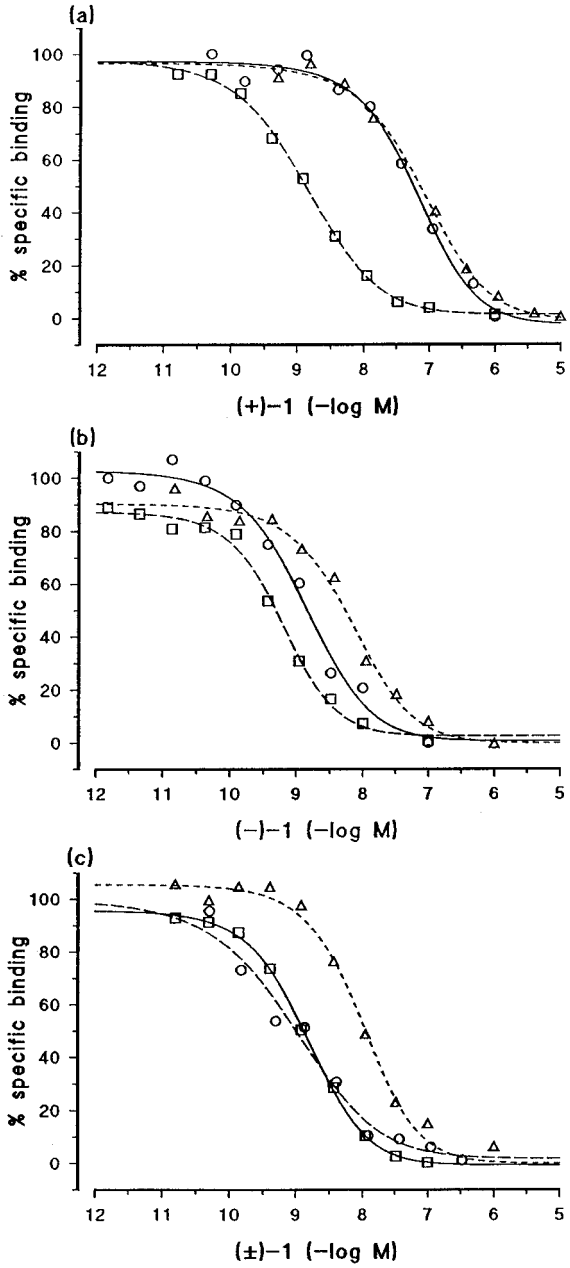


Figure 2. Representative experiments showing (+)-**1** (a), (−)-**1** (b), and (±)-**1** (c) competition binding curves for cloned α_{1a}- (△), α_{1b}- (□), and α_{1d}- (○) adrenoceptor subtypes labeled with [^{3}H]prazosin. Data were analyzed using Allfit.[29] Differences in slopes of the curves were determined by the test of parallelism and were not different ($p > 0.05$).

oselectivity for the two enantiomers. It appears that the stereochemical requirements for the $\alpha_{1B}(\alpha_{1b})$ subtype are satisfied by both enantiomers, whereas the $\alpha_{1A}(\alpha_{1a})$ and mostly the α_{1d} subtype are markedly sensitive to the configuration of the *cis*-decahydroquinoxaline nucleus. It follows that the knowledge of the absolute configuration of cyclazosin enantiomers may acquire relevance for designing new derivatives with improved selectivity for α_{1B}-adrenoceptors. Work is in progress to address this crucial issue.

In conclusion, the present investigation has demonstrated that the replacement of the piperazine ring of prazosin with a *cis*-decahydroquinoxaline moiety affording (+)-**1** does not affect the affinity for α_{1B}-adrenoceptors, while it significantly decreases the affinity for $\alpha_{1A}(\alpha_{1a})$ and α_{1d} subtypes in comparison to prazosin. The overall result of this structural modification is a significant improvement in selectivity toward α_{1B}-adrenoceptors. Thus, (+)-cyclazosin [(+)-**1**] emerges as a valuable tool for the characterization of α_1-adrenoceptor subtypes owing to its unprecedented selectivity for α_{1B}-adrenoceptors, associated with high potency.

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin-Elmer 297 and Varian Gemini 200 instruments, respectively. 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. The elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Mass spectra were performed with a Hewlett Packard instrument consisting of model 5890A for the separation section and model 5971A for the mass section. HPLC analysis was carried out on a Hewlett-Packard 1090 apparatus series II, with a UV 254 detector, using a Beckmann reversed phase C_{18} column, ultrasphere ODS, 5 μm (150 × 4.6 i.d.). The optical rotation was measured on a Perkin-Elmer 241 MC polarimeter. Chromatographic separations were performed on silica gel columns by flash chromatography (Kieselgel 40, 0.040−0.063 mm; Merck). R_f values were determined with silica gel TLC plates (Kieselgel 60 F_{254}, layer thickness 0.25 mm; Merck). The composition and volumetric ratio of eluting mixtures were as follows: A, petroleum ether−ethyl acetate−methanol−28% ammonia (8:6:2:0.2); B, chloroform−ethyl acetate (9:1). 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. Compounds were named following IUPAC rules as applied by AUTONOM, a PC software for systematic names in organic chemistry (Beilstein-Institut and Springer-Verlag).

Resolution of (±)-Furan-2-yl(*cis*-octahydroquinoxalin-1-yl)methanone (2). A solution of racemic **2**[20] (5.31 g, 22.7 mmol) in MeOH (50 mL) was treated with a solution of (*S*)-(+)-mandelic acid (3.49 g, 22.7 mmol) in MeOH (50 mL). The mixture was evaporated to dryness to give a residue that was crystallized by dissolving the solid in 600 mL of hot ethyl acetate and then reducing by evaporation the volume to 300 mL. The precipitate was recrystallized six other times with the same solvent and analogous technique to give 0.91 g of (+)-mandelate salt: mp 188−190 °C; $[\alpha]^{20}_D = +79.4°$ ($c = 1$, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.18−2.20 (m, 8H, H_{5-8} octahydroquinoxaline), 2.36−2.58 (m, 1H, H_3 octahydroquinoxaline), 2.86−3.62 (m, 3H, H_2, H_3, H_{4a} octahydroquinoxaline), 4.12−4.70 (m, 2H, H_2, H_{8a} octahydroquinoxaline), 4.90 (s, 1H, C*H*OH), 5.60 (br s, 3H, OH, NH, exchangeable with D_2O), 6.55 (m, 1H, H_4 furan), 7.07 (m, 1H, H_3 furan), 7.12−7.30 (m, 3H, arom), 7.36−7.46 (m, 2H, arom), 7.52 (m, 1H, H_5 furan). Anal. ($C_{21}H_{26}N_2O_5$) C, H, N.

The salt was dissolved in water, the ice-cooled solution made basic with 2 N NaOH, and the resulting mixture extracted with chloroform (3 × 50 mL). Removal of dried solvent gave (+)-furan-2-yl(*cis*-octahydroquinoxalin-1-yl)methanone [(+)-**2**] as a waxy solid: 0.48 g; mp 47−50 °C; $[\alpha]^{20}_D = +70.1°$ ($c = 1$, MeOH); R_f 0.36 (mixture A); MS (EI) *m*/*z* (rel int) 54 (12), 67 (13), 81 (13), 95 (100), 110 (31), 123 (17), 139 (7), 163 (5), 177 (1), 191 (1), 205 (1), 234 (11) $[M]^+$; ^{1}H NMR ($CDCl_3$) δ 1.22−1.90 (m, 9H, H_{5-8} octahydroquinoxaline, NH, exchangeable with D_2O), 1.93−2.43 (m, 1H, H_3 octahydroquinoxaline), 2.76−3.18 (m, 3H, H_2, H_3, H_{4a} octahydroquinoxaline), 4.02−4.65 (m, 2H, H_2, H_{8a} octahydroquinoxaline), 6.46 (m, 1H, H_4 furan), 6.92 (m, 1H, H_3 furan), 7.47 (m, 1H, H_5 furan).

The amine recovered by a similar alkaline treatment from the combined mother liquors of the above mandelate (3.92 g, 16.7 mmol) was dissolved in MeOH (50 mL) and treated with a solution of (*R*)-(−)-mandelic acid (2.54 g, 16.7 mmol) in MeOH (50 mL). The resulting mixture was evaporated to dryness to give a residue that was crystallized six times with ethyl acetate as described for the other enantiomer. We

obtained 1.99 g of (−)-mandelate salt: mp 188−190 °C; $[\alpha]^{20}_D$ = −79.4° (*c* = 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.18−2.20 (m, 8H, H_{5-8} octahydroquinoxaline), 2.36−2.58 (m, 1H, H_3 octahydroquinoxaline), 2.86−3.62 (m, 3H, H_2, H_3, H_{4a} octahydroquinoxaline), 4.12−4.70 (m, 2H, H_2, H_{8a} octahydroquinoxaline), 4.90 (s, 1H, C*H*OH), 5.60 (br s, 3H, OH, NH, exchangeable with D_2O), 6.55 (m, 1H, H_4 furan), 7.07 (m, 1H, H_3 furan), 7.12−7.30 (m, 3H, arom), 7.36−7.46 (m, 2H, arom), 7.52 (m, 1H, H_5 furan). Anal. ($C_{21}H_{26}N_2O_5$) C, H, N.

This salt was treated as described for the other enantiomer to give (+)-furan-2-yl(*cis*-octahydroquinoxalin-1-yl)methanone [(−)-**2**] as a waxy solid: 1.2 g; mp 47−50 °C; $[\alpha]^{20}_D$ = −70.7° (*c* = 1, MeOH); R_f 0.36 (mixture A); MS (EI) *m*/*z* (rel int) 54 (10), 67 (13), 81 (15), 95 (100), 110 (29), 123 (18), 139 (7), 163 (3), 177 (1), 191 (1), 205 (1), 234 (10) $[M]^+$; ^{1}H NMR ($CDCl_3$) δ 1.22−1.90 (m, 9H, H_{5-8} octahydroquinoxaline, NH, exchangeable with D_2O), 1.93−2.43 (m, 1H, H_3 octahydroquinoxaline), 2.76−3.18 (m, 3H, H_2, H_3, H_{4a} octahydroquinoxaline), 4.02−4.65 (m, 2H, H_2, H_{8a} octahydroquinoxaline), 6.46 (m, 1H, H_4 furan), 6.92 (m, 1H, H_3 furan), 7.47 (m, 1H, H_5 furan).

Determination of Optical Purity of (+)- and (−)-2. A mixture of racemic **2**[20] (0.05 g, 0.21 mmol) and (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate (0.042 g, 0.21 mmol) in dry dichloromethane (10 mL) was stirred for 4 h at room temperature. Removal of solvent gave a residue which was purified by column chromatography. Eluting with mixture B afforded the urea 4-(furan-2-ylcarbonyl)-*cis*-octahydroquinoxaline-1-carboxylic acid (1-naphthalen-1-ylethyl)amide: 0.075 g (oil); R_f 0.28; MS (EI) *m*/*z* (rel int) 127 (36), 155 (40), 182 (100), 197 (70) [$M^+$of naphthylethyl isocyanate], 110 (100), 123 (55), 139 (18), 163 (7), 191 (4), 234 (39) [$M^+$of **2**]; ^{1}H NMR ($CDCl_3$) δ 1.28−2.30 (m, 11H, H_{5-8} octahydroquinoxaline, C*H*$_3$CH), 3.38−3.60 (m, 1H, H_3 octahydroquinoxaline), 3.62−3.98 (m, 2H, H_2, H_3 octahydroquinoxaline), 4.00−4.20 (m, 2H, H_2, H_{4a} (or H_{8a}) octahydroquinoxaline), 4.38−4.68 (m, 2H, H_{8a} (or H_{4a}) octahydroquinoxaline, NH), 5.70−5.92 (m, 1H, CH$_3$C*H*), 6.48 (m, 1H, H_4 furan), 7.02 (m, 1H, H_3 furan), 7.40−7.60 (m, 5H, H_5 furan, 4 arom), 7.74−7.92 (m, 2H, arom), 8.10−8.24 (m, 1H, arom). HPLC analysis was performed with water/acetonitrile as the eluting mixture, at a flow rate of 1 mL/min and adopting a programmed procedure that, starting from a 95−5% (v/v) ratio, reached, in 10 min, the composition 63−37% (v/v) that was maintained throughout the experiment. Under these conditions, injected samples (1.5 μL, *c* = 5 mg/mL), which were detected by absorbance at 254 nm, gave two peaks with retention times of 30.62 and 33.12 min, as shown in the chromatogram of Figure 1.

The urea derivative of (+)-**2** was prepared as described for racemic **2**: oil; R_f 0.30 (mixture B); MS (EI) *m*/*z* (rel int) 127 (95), 155 (68), 182 (100), 197 (46) [$M^+$of naphthylethyl isocyanate], 110 (100), 123 (50), 139 (20), 163 (8), 191 (3), 234 (20) [$M^+$of **2**]; ^{1}H NMR ($CDCl_3$) δ 1.30−2.30 (m, 11H, H_{5-8} octahydroquinoxaline, C*H*$_3$CH), 3.38−3.50 (m, 1H, H_3 octahydroquinoxaline), 3.62−3.97 (m, 2H, H_2, H_3 octahydroquinoxaline), 4.00−4.22 (m, 2H, H_2, H_{4a} (or H_{8a}) octahydroquinoxaline), 4.30−4.70 (m, 2H, H_{8a} (or H_{4a}) octahydroquinoxaline, NH), 5.72−5.96 (m, 1H, CH$_3$C*H*), 6.48 (m, 1H, H_4 furan), 7.03 (m, 1H, H_3 furan), 7.40−7.65 (m, 5H, H_5 furan, arom), 7.75−7.96 (m, 2H, arom), 8.10−8.25 (m, 1H, arom). HPLC analysis revealed a single peak with retention time of 32.86 min (Figure 1).

The urea derivative of (−)-**2** was prepared as described for racemic **2**: oil; R_f 0.23 (mixture B); MS (EI) *m*/*z* (rel int) 127 (68), 155 (65), 182 (100), 198 (47) [$M^+$of naphthylethyl isocyanate], 110 (100), 123 (35), 139 (21), 163 (13), 191 (7), 234 (40) [$M^+$of **2**]; ^{1}H NMR ($CDCl_3$) δ 1.25−2.28 (m, 11H, H_{5-8} octahydroquinoxaline, C*H*$_3$CH), 3.42−3.60 (m, 1H, H_3 octahydroquinoxaline), 3.62−3.95 (m, 2H, H_2, H_3 octahydroquinoxaline), 3.98−4.15 (m, 2H, H_3, H_{4a} (or H_{8a}) octahydroquinoxaline), 4.40−4.52 (m, 1H, H_{8a} (or H_{4a}) octahydroquinoxaline), 4.55−4.70 (m, 1H, NH), 5.72−5.93 (m, 1H, CH$_3$C*H*), 6.48 (m, 1H, H_4 furan), 7.02 (m, 1H, H_3 furan), 7.38−7.62 (m, 5H, H_5 furan, arom), 7.74−7.91 (m, 2H, arom), 8.08−8.17 (m, 1H, arom). HPLC analysis revealed a single peak with retention time of 30.24 min (Figure 1).

(+)-[4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-*cis*-octahydroquinoxalin-1-yl]furan-2-ylmethanone Hydrochloride [(+)-1]. A mixture of (+)-**2** (0.33 g, 1.41 mmol), 4-amino-2-chloro-6,7-dimethoxyquinazoline (0.28 g, 1.17 mmol), and *N*,*N*-diisopropylethylamine (0.31 g, 2.35 mmol) in *i*-AmOH (20 mL) was refluxed for 72 h. After cooling, the mixture was left at 0 °C overnight; then the solid was collected, triturated with cold 2 N NaOH, filtered, washed with water, and transformed into the hydrochloride salt. Crystallization from 15% MeOH/EtOH gave (+)-**1**: 0.09 g; mp 262−264 °C; $[\alpha]^{20}_D$ = +74.4° (*c* = 1, MeOH); R_f 0.43 (mixture A); ^{1}H NMR (DMSO-d_6) δ 1.28−2.45 (m, 8H, H_{5-8} octahydroquinoxaline), 3.68−4.00 (m, 8H, OCH_3, H_3 octahydroquinoxaline), 4.05−4.30 (m, 2H, H_2 octahydroquinoxaline), 4.38−4.50 (m, 1H, H_{4a} octahydroquinoxaline), 4.58−4.75 (m, 1H, H_{8a} octahydroquinoxaline), 6.70 (m, 1H, H_4 of furan), 7.14 (m, H_3 of furan), 7.55 (s, 1H, arom), 7.76 (s, 1H, arom), 7.90 (m, 1H, H_5 of furan), 8.67 (br s, 1H, NH, exchangeable with D_2O), 8.90 (br s, 1H, NH, exchangeable with D_2O), 11.92 (br s, 1H, NH, exchangeable with D_2O). Anal. ($C_{23}H_{27}N_5O_4$·HCl·1.5H_2O) C, H, N.

(−)-[4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-*cis*-octahydroquinoxalin-1-yl]furan-2-ylmethanone Hydrochloride [(−)-1]. This was obtained from (−)-**2** (0.5 g, 2.13 mmol) as described for the enantiomer (+)-**1**: 0.1 g; mp 264−266 °C; $[\alpha]^{20}_D$ = −74.8° (*c* = 1, MeOH); R_f 0.43 (mixture A); ^{1}H NMR (DMSO-d_6) δ 1.28−2.45 (m, 8H, H_{5-8} octahydroquinoxaline), 3.68−4.00 (m, 8H, OCH_3, H_3 octahydroquinoxaline), 4.05−4.30 (m, 2H, H_2 octahydroquinoxaline), 4.38−4.50 (m, 1H, H_{4a} octahydroquinoxaline), 4.58−4.75 (m, 1H, H_{8a} octahydroquinoxaline), 6.70 (m, 1H, H_4 furan), 7.16 (m, H_3 furan), 7.56 (s, 1H, arom), 7.76 (s, 1H, arom), 7.92 (m, 1H, H_5 furan), 8.65 (br s, 1H, NH, exchangeable with D_2O), 8.90 (br s, 1H, NH, exchangeable with D_2O), 11.95 (br s, 1H, NH, exchangeable with D_2O). Anal. ($C_{23}H_{27}N_5O_4$·HCl·1.5H_2O) C, H, N.

Radioligand Binding Assays at Native Receptors. Binding studies on native α_1-, α_{1A}-, α_{1B}-, and α_2-adrenergic, 5-HT_{1A} serotoninergic, and D_2 dopaminergic receptors were carried out in membranes of rat cerebral cortex (α_1 and α_2), hippocampus (5-HT_{1A}), striatum (D_2), hippocampus pretreated with chloroethylclonidine (α_{1A}),[7] and liver (α_{1B}).[7]

Male Sprague−Dawley rats (200−300 g; Charles River, Italy) were killed by cervical dislocation, and different tissues were excised, immediately frozen, and stored at −70 °C until use. Tissues were homogenized (2 × 20 s) in 50 vol of cold Tris-HCl buffer, pH 7.4 (except for D_2 receptor membrane preparations), using a Polytron homogenizer (speed 7). Homogenates were centrifuged at 49000*g* for 10 min, resuspended in 50 vol of the same buffer, incubated at 37 °C for 15 min (30 min with 10 μM CEC for α_{1A} preparations), and centrifuged and resuspended twice more. The final pellets were suspended in 100 vol of Tris-HCl buffer, pH 7.4, containing 10 μM pargyline and 0.1% ascorbic acid. Membranes were incubated in a final volume of 1 mL for 30 min at 25 °C with 0.1−0.5 nM [^{3}H]prazosin (α_1-adrenoceptor), 0.5−1.5 nM [^{3}H]rauwolscine (α_2-adrenoceptor), or 0.5−1.5 nM [^{3}H]-8-OH-DPAT (5-HT_{1A}) in the absence or presence of competing drugs.

For D_2 membrane preparations, rat striata were homogenized (2 × 20 s) in 30 vol of cold Tris-HCl buffer, pH 7.4, using a Polytron homogenizer (speed 7) and centrifuged at 49000*g* for 10 min. The final pellets were suspended in 200 vol of Tris-HCl incubation buffer containing 10 μM pargyline, 0.1% ascorbic acid, and the following saline concentrations: NaCl, 120 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1 mM; then membranes were incubated for 15 min at 37 °C with 0.2−0.6 nM [^{3}H]spiperone.

Nonspecific binding was determined in the presence of 10 μM phentolamine (α-adrenoceptors), 10 μM 5-HT (5-HT_{1A} receptor), and 1 μM (+)-butaclamol (D_2 receptor). The 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. The filters were then washed with ice-cold buffer, and the radioactivity retained on the filters was counted by liquid scintillation spectrometry.

Radioligand Binding Assays at Cloned Receptors. [^{3}H]Prazosin binding at cloned bovine α_{1a}-, hamster α_{1b}-, and

rat α_{1d}-adrenoceptors was performed in membranes of COS-7 cells (CV-1 monkey kidney epithelial cells) expressing transiently the above α_1-adrenoceptor subtypes. Construction and transfection of individual α_1-adrenoceptor subtypes were carried out by Dr. S. Cotecchia (Université de Lausanne, Switzerland) as previously described.[11−13] COS-7 cell membranes (35, 35, and 70 μg of protein/sample for α_{1b}, α_{1a}, and α_{1d}, respectively) were incubated in 50 mM Tris-HCl, pH 7.4, containing 10 μM pargyline and 0.1% ascorbic acid, with 0.1−0.4 nM [^{3}H]prazosin, in a final volume of 0.22 mL for 30 min at 25 °C, in the absence or presence of the competing drugs (1 pM−10 μM). Nonspecific binding was determined in the presence of 100 μM phentolamine. The 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.

Binding to cloned human α_1-adrenoceptor subtypes was performed in membranes from CHO cells (Chinese hamster ovary cells) transfected by electroporation with DNA expressing the gene encoding each α_1-adrenoceptor subtype. Cloning and stable expression of the human α_1-adrenoceptor gene was performed as previously described.[28] CHO cell membranes (30 μg of proteins) 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 pM−10 μM). Nonspecific binding was determined in the presence of 10 μM phentolamine. The 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. The inhibition of specific binding of the radioligands by tested drugs was analyzed to estimate the IC_{50} value by using the nonlinear curve-fitting program Allfit.[29] The IC_{50} value is converted to an affinity constant (K_i) by the Cheng−Prusoff equation.[25] Data are expressed as mean of pK_i ± SE.

Acknowledgment. This work was supported by a grant from MURST. The authors wish to thank Dr. Luciano Barboni and Dr. Massimo Ricciutelli for the analytical HPLC measurements.

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JM960510X