Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 2334–2345
Bioorganic & Medicinal Chemistry

Synthesis and α_1-adrenoceptor antagonist activity of derivatives and isosters of the furan portion of (+)-cyclazosin

Gianni Sagratini,[a] Piero Angeli,[a] Michela Buccioni,[a] Ugo Gulini,[a] Gabriella Marucci,[a] Carlo Melchiorre,[b] Amedeo Leonardi,[c] Elena Poggesi[c] and Dario Giardinà[a,*]

[a]*Department of Chemical Sciences, University of Camerino, 62032 Camerino, Italy*
[b]*Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy*
[c]*Pharmaceutical R&D Division, Recordati SpA, Milano, Italy*

Received 13 September 2006; revised 3 January 2007; accepted 17 January 2007
Available online 19 January 2007

Abstract—α_1-Adrenoceptor selective antagonists are crucial in investigating the role and biological functions of α_1-adrenoceptor subtypes. We synthesized and studied the α_1-adrenoceptor blocking properties of new molecules structurally related to the α_{1B}-adrenoceptor selective antagonist (+)-cyclazosin, in an attempt to improve its receptor selectivity. In particular, we investigated the importance of substituents introduced at position 5 of the 2-furan moiety of (+)-cyclazosin and its replacement with classical isosteric rings. The 5-methylfuryl derivative (+)-**3**, [(+)-metcyclazosin], improved the pharmacological properties of the progenitor, displaying a competitive antagonism and an 11 fold increased selectivity for α_{1B} over α_{1A}, while maintaining a similar selectivity for the α_{1B}-adrenoceptor relative to the α_{1D}-adrenoceptor. Compound (+)-**3** may represent a useful tool for α_{1B}-adrenoceptor characterization in functional studies.

1. Introduction

α_1-Adrenoceptors are members of the G-protein coupled superfamily of receptors which play critical roles in the regulation of a variety of physiological processes. At present, three native subtypes, α_{1A}, α_{1B} and α_{1D}, have been pharmacologically detected,[1] and a fourth α_1-adrenoceptor, α_{1L}, displaying low affinity for prazosin[2] and conformationally related to the α_{1A}-subtype,[3] has been reported.

α_1-Adrenoceptors are of therapeutic interest because of their important role in the control of blood pressure and in the contraction and growth of smooth and cardiac muscle.[4] However, the functional role of specific α_1-adrenoceptor subtypes is not completely defined because of the lack of ligands that are functionally selective for the α_1-adrenoceptor subtypes. There is a particular need for selective α_{1B}-adrenoceptor antagonists which, to date, have been discovered in very restricted number, in comparison to the selective α_{1A}- and α_{1D}-adrenoceptor ligands.

In fact, besides the alkylating agent chloroethylclonidine that was originally identified as selective α_{1B}-adrenoceptor antagonist,[5] only a few reversible antagonists have been reported to be selective for the α_{1B}-adrenoceptor, including spiperone,[6] risperidone,[7] (±)-cyclazosin,[8] L-765,314[9,10] and AH11110A,[11] but their moderate selectivity, observed in radioligand binding assays, was not confirmed, with the exception of L-765,314, in functional experiments.[12]

Recently, we reported the quinazoline compound (+)-cyclazosin, (+)-2-[(4a*S*,8a*R*)-4-(2-furoyl)octahydroquinoxalin-1(2*H*)-yl]-6,7-dimethoxyquinazolin-4-amine hydrochloride [(+)-**1**], which was characterized both for α_1-adrenoceptor binding activity[13] and chemical structure[14,15] (Fig. 1). In binding assays, (+)-**1** displayed high affinity and selectivity for α_{1b}-adrenoceptor both in animal and human cloned α_1-subtypes (pK_i α_{1b} = 9.16–9.87; selectivity ratios, α_{1b}/α_{1a} = 48–91 and

Keywords: α_1-Adrenoceptor subtypes; α_1-Adrenoceptor antagonists; α_{1B}-Adrenoceptor selective antagonists; (+)-Cyclazosin analogues.
* Corresponding author. Tel.: +39 0737 402265; fax: +39 0737 637345; e-mail: dario.giardina@unicam.it

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.01.028

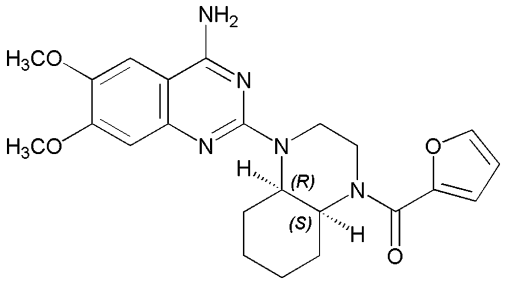


Figure 1. Structure of (+)-cyclazosin, (+)-2-[(4a*S*,8a*R*)-4-(2-furoyl)-octahydroquinoxalin-1 (*2H*)-yl]-6,7-dimethoxyquinazolin-4-amine.

α_{1b}/α_{1d} = 24–39).[13] Its α_{1B}-adrenoceptor selectivity was also confirmed in functional experiments performed in rat and rabbit tissues.[16] It displayed competitive antagonism and higher potency at the α_{1B}-adrenoceptor (pA_2 = 8.85) than at α_{1A} (pA_2 = 7.75) and α_{1D} (pA_2 = 7.27) subtypes, with α_{1B}/α_{1A}- and α_{1B}/α_{1D}-selectivity ratio values of 13 and 38, respectively.

To improve the α_{1B}-adrenoceptor selectivity of (+)-**1**, we performed two kinds of structural modifications on its furan ring, while keeping the same structural configuration of carbon atoms 4a and 8a of the octahydroquinoxaline moiety: (i) introduction of selected substituents at position 5 of the furan ring, and (ii) replacement of the furan moiety with classical isosteric rings (Scheme 1). Thus, we report here the synthesis and the α_1-adrenoceptor antagonist activity of the (+)-**1** derivatives (+)-**2** - (+)-**5**, bearing the bromo, methyl, methoxy or acetyl substituents at furan position 5,[17] and the analogues (+)-**6** - (+)-**9**, obtained by exchanging the furan moiety with a thiophene, pyrrole, pyridine or phenyl ring.

2. Chemistry

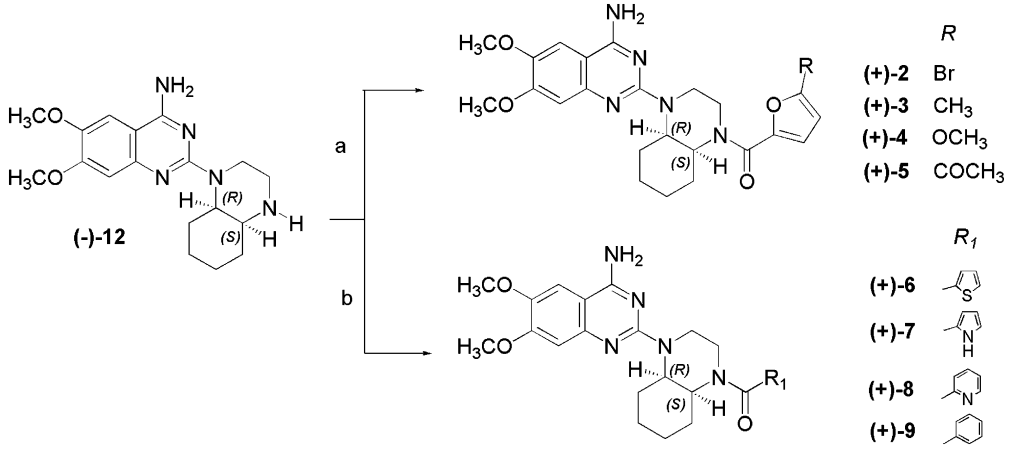
The two series of compounds (+)-**2** - (+)-**5** and (+)-**6** - (+)-**9**, bearing the same stereochemical configuration of prototype (+)-**1**, were prepared by acylation of the key intermediate (−)-**12** with the appropriate 5-substituted-2-furoyl chloride or aroyl chloride (Scheme 1).

Mono Cbz-protected (±)-**10**, synthesized by reaction between *cis*-decahydroquinoxaline[18] and benzylchloroformate, was separated into the enantiomers (+)-**10** and (−)-**10** through fractional crystallization of the diastereomer salt mixture obtained by its treatment with (*S*)-(+)- or (*R*)-(−)-mandelic acid (Scheme 2).

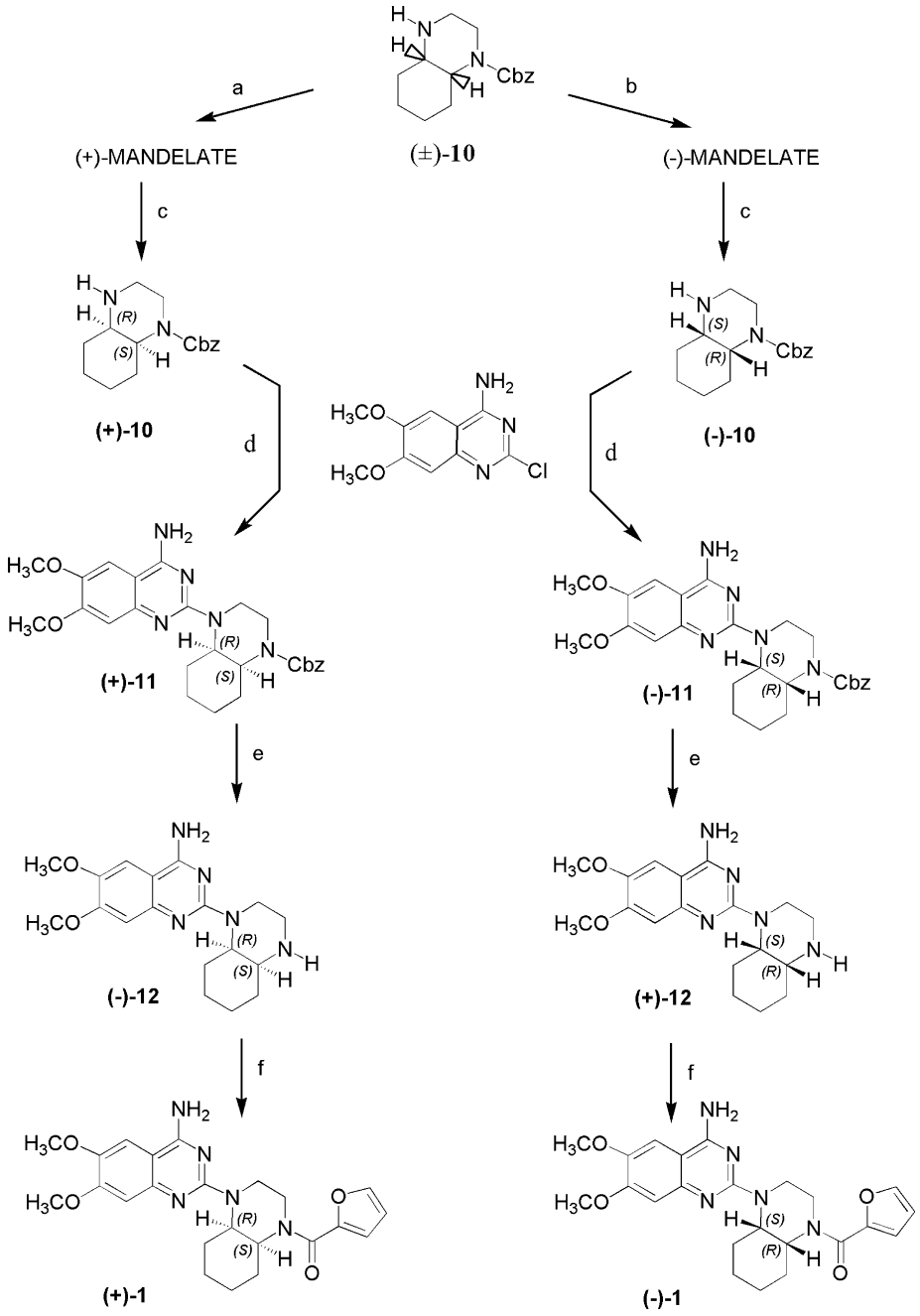
The configuration of (+)-**10** and (−)-**10** was deduced by synthesizing (+)-**1** and (−)-**1**, whose absolute configuration is already known[14] (Scheme 2). Thus, (+)-**10** and (−)-**10** were reacted with 2-chloro-6,7-dimethoxyquinazolin-4-amine to give (+)-**11** and (−)-**11**, respectively. The subsequent removal of the Cbz protecting group by catalytic hydrogenation with cyclohexene and Pd/C in ethanol afforded the chiral amines (−)-**12** and (+)-**12**, with inversion of optical rotation. Their acylation with 2-furoyl chloride gave two compounds that were identified by elemental analysis, ^{1}H NMR and optical rotatory parameters to be (+)-**1** and (−)-**1**, respectively.

The enantiomeric purity of (+)-**10** and (−)-**10** was assessed by performing an HPLC analysis (HP Hypersil column, eluent mixture ethyl acetate/*n*-hexane) on the corresponding diastereomeric amides prepared by reaction with (*S*)-(−)-1-[(4-methylphenyl)sulfonyl]prolyl chloride,[19,20] in comparison with the amide mixture obtained from (±)-**10**. Thus, the mixture obtained from (±)-**10** showed a diagram with two peaks (retention time, 7.60 and 8.75 min), whereas the diastereomeric amides deriving from (+)-**10** and (−)-**10** displayed only one peak at retention time of 7.49 and 8.61 min, respectively (Fig. 2). The optical purity of (+)-**10** and (−)-**10** was found to be higher than 98%, since the analysis of an actual 98:2 mixture of these diastereomeric amides displayed, besides the principal peak, a significant and detectable small peak relative to the less abundant diastereomer.

The new chiral compounds (+)-**2**, (+)-**3** and (+)-**5** - (+)-**9** were obtained as hydrochloride salts, purified by crystallization and characterized by elemental analysis, ^{1}H



Scheme 1. Reagents: (a) 5-substituted-2-furoyl chlorides, CH_2Cl_2, Et_3N; (b) R_1COCl, CH_2Cl_2, Et_3N.



Scheme 2. Reagents and conditions: (a) (*S*)-(+)-mandelic acid, MeOH; (b) (*R*)-(−)-mandelic acid, MeOH; (c) 2 N NaOH, $H_2O/CHCl_3$; (d) *i*-AmOH, reflux; (e) cyclohexene, Pd/C, MeOH/EtOH, reflux; (f) 2-furoyl chloride, Et_3N, CH_2Cl_2.

NMR, specific rotation and chromatographic parameters. Compound (+)-**4** was isolated and characterized as the free base because of stability problem of the salt.

3. Pharmacology

Compounds(+)-**2** - (+)-**9** were studied in rat isolated tissues to assess their antagonist activity and selectivity at α_1-adrenoceptor subtypes in comparison with the prototype (+)-**1**. α_{1A}- and α_{1D}-Adrenoceptor blocking activities were evaluated by antagonism of (−)-noradrenaline-induced contractions of prostatic vas deferens[21] and aorta,[22] respectively.

Stam et al.[23] reported a significantly lower affinity ($pK_B = 7.96$) for (+)-**1** in α_{1B}-adrenoceptor rat spleen with respect to our results found in α_{1B}-adrenoceptor rabbit thoracic aorta ($pK_B = 8.85$),[16] or in binding experiments on hamster cloned α_{1b}-adrenoceptors ($pK_i = 9.16$).[13] For this reason, we decided to investigate the α_{1B}-adrenoceptor antagonism of (+)-**1** and of its related compounds (+)-**2** - (+)-**9** in the rat spleen by using the Buckner procedure[24] with phenylephrine as agonist and an incubation period of 30 min (instead of 60 min as adopted by Stam et al.[23]) because, in our experiments, the reproducibility of phenylephrine concentration–response curves was not observed when the curve was constructed after 45 and 60 min from the

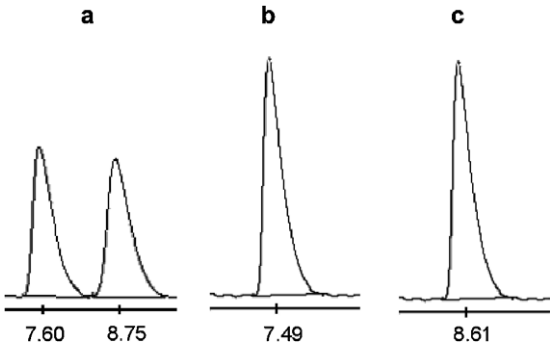


Figure 2. Analytical HPLC chromatograms of diastereomeric l-[(4-methylphenyl)sulfonyl]prolyl amides obtained from racemic **10** (a) and from enantiomers (+)-**10** (b) and (−)-**10** (c). Chromatographic conditions: HP Hipersil column, 5 μm (200 × 2.1 mm, i.d.); hexane/ethyl acetate (70:30, v/v) as eluting mixture at a flow rate of 1 mL/min; detection, UV at 254 nm. Retention times (min) are indicated at each peak.

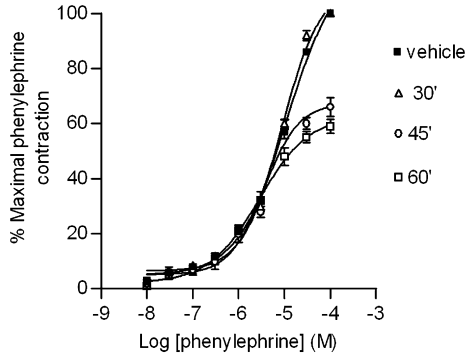


Figure 3. Time-dependent change of phenylephrine concentration–response curves in rat spleen, taken 30, 45, and 60 min after the curve used as control. Data are means ± SEM of six separate experiments.

one taken as control, owing to a 34–41% decrease of the maximal response to the agonist (Fig. 3).

The antagonist potency at α_1-adrenoceptor subtypes was expressed as pA_2 values calculated by Schild plots at three different concentrations according to Arunlakshana and Schild.[25] However, when the slope of the Schild plot was significantly different from unity, the potency was expressed as pK_B values, according to van Rossum.[26] In this case, the pK_B value was calculated at the lowest antagonist concentration giving a significant rightward shift of the agonist concentration–response curve [log (concentration ratio -1) $\geqslant 0.5$].

The affinity profile of the new compounds was also evaluated in radioreceptor binding assays on human cloned α_1-adrenoceptors in comparison with (+)-**1**. Competition experiments were performed using [^{3}H]prazosin to label α_1-adrenoceptor binding sites on membranes of Chinese hamster ovary (CHO) cells expressing human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes.[27] Binding affinities were expressed as pK_i values derived using the Cheng–Prusoff equation.[28]

Experimental data were subjected to statistical analysis by means of Student's *t*-test. A p value <0.05 was taken to indicate a statistically significant difference.

4. Results and discussion

The pharmacological results of functional antagonism and radioligand binding assays are reported in Tables 1 and 2, respectively.

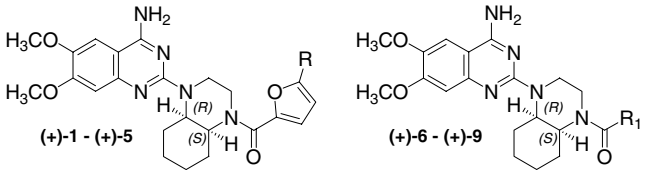
In functional assays, all the new compounds (+)-**2** - (+)-**9** were either competitive (mainly at α_{1D} and α_{1A}-adrenoceptors) or non-competitive antagonists (mainly at α_{1B}-adrenoceptor). In contrast with the competitive behaviour at rabbit aorta α_{1B}-adrenoceptor ($pA_2 = 8.85$[16]), in the rat spleen α_{1B}-adrenoceptor (+)-**1** exhibited a non-competitive antagonism while displaying a similar potency ($pK_B = 8.86$). In this latter tissue, apart from (+)-**3** which competitively antagonized the phenylephrine-induced contractions in the 10–100 nM range, all new compounds were non-competitive antagonists, because the slope of the Schild plot was different from unity and the maximum response to the agonist was always reduced. Furthermore, it should be noted that compounds (+)-**2** - (+)-**5** displayed pK_B values different from those previously reported likely because an increased number of experiments were performed using higher concentrations of each compound.[17]

Both series of compounds revealed the same preferential antagonist activity for the α_{1B}-adrenoceptor over the α_{1A}- and α_{1D}-subtypes. The pK_B or pA_2 values of (+)-**2** - (+)-**5** at α_{1B} and α_{1D}-adrenoceptors ranged within 8.13–8.65 and 6.67–7.23, respectively, which are comparable with those displayed by (+)-**1** at the same adrenoceptor subtypes (8.86 and 7.27, respectively). These compounds displayed an α_{1B}-adrenoceptor selectivity relative to the α_{1D}-adrenoceptor similar to that of (+)-**1** (19–41 vs 39). In contrast, (+)-**3** and (+)-**4**, owing to a lower antagonism at the α_{1A}-adrenoceptor (6.48 and 6.85 vs 7.77), displayed an α_{1B}/α_{1A} selectivity ratio higher than that of (+)-**1** (148 and 45, respectively, vs 13).

The replacement of the furan ring of (+)-**1** with other isosteric aromatic moieties, affording the series (+)-**6** - (+)-**9**, did not modify the potency at the α_{1D}-adrenoceptor (7.12–7.76 vs 7.27). In addition, the activity was maintained also at the α_{1B} and α_{1A}-adrenoceptors with the exception of the slight decrease (5- to 6-fold) observed for the pyrrole analogue (+)-**7** at both subtypes and for the pyridine analogue (+)-**8** at the α_{1A}-adrenoceptor. Consequently, (+)-**8** was the most selective of this series with α_{1B}/α_{1D} and α_{1B}/α_{1A} selectivity ratios of 33 and 40, respectively.

In binding assays, the two series of compounds displayed higher affinity, although lower than that of prototype (+)-**1**, for the α_{1b}-adrenoceptor with respect to the other subtypes. Only the pyrrole analogue (+)-**7** showed similar affinity at α_{1b}- and α_{1d}-adrenoceptors. Moreover, these compounds displayed an affinity comparable to that of (+)-**1** at α_{1a}- and α_{1d}-adrenoceptor subtypes with the exception of (+)-**8** that was 8 fold less active than (+)-**1**

Table 1. Functional antagonist affinities, expressed as pA_2 or pK_B, of compounds (+)-**2** - (+)-**9**, and (+)-**1** as reference, at α_1-adrenoceptor subtypes of isolated rat prostatic vas deferens (α_{1A}), spleen (α_{1B}) and thoracic aorta (α_{1D})



Compound	R/R_1	pA_2[a] or pK_B[b]			Selectivity ratio[c]		
		α_{1A}	α_{1B}	α_{1D}	α_{1B}/α_{1A}	α_{1B}/α_{1D}	α_{1D}/α_{1A}
(+)-**1**	Hydrogen	7.77 ± 0.03[a]	8.86 ± 0.03[b]	7.27 ± 0.07[a]	13	39	0.3
(+)-**2**	Bromo	7.53 ± 0.04[b]	8.50 ± 0.02[b]	7.23 ± 0.02[a]	9	19	0.5
(+)-**3**	Methyl	6.48 ± 0.12[a]	8.65 ± 0.01[a]	7.04 ± 0.03[a]	148	41	4
(+)-**4**	Methoxy	6.85 ± 0.02[a]	8.50 ± 0.16[b]	7.05 ± 0.02[a]	45	28	2
(+)-**5**	Acetyl	7.05 ± 0.04[b]	8.13 ± 0.12[b]	6.67 ± 0.09[a]	12	29	0.4
(+)-**6**	2-Thienyl	7.44 ± 0.07[b]	8.78 ± 0.09[b]	7.45 ± 0.06[a]	22	21	1
(+)-**7**	2-Pyrrolyl	7.06 ± 0.06[b]	8.05 ± 0.02[b]	7.33 ± 0.02[b]	10	5	2
(+)-**8**	2-Pyridyl	7.04 ± 0.13[b]	8.64 ± 0.14[b]	7.12 ± 0.02[b]	40	33	1
(+)-**9**	Phenyl	7.51 ± 0.01[a]	8.83 ± 0.07[b]	7.76 ± 0.04[b]	21	12	2

[a] pA_2 values, expressed as means ± SEM of three different concentrations, each tested at least four times.
[b] pK_B values (±SEM) calculated according to van Rossum.
[c] Calculated by the antilog of the difference between pA_2 or pK_B values at different α_1-adrenoceptor subtypes.

Table 2. Binding affinity constants, expressed as pK_i, of compounds (+)-**2** - (+)-**9**, and (+)-**1** as reference, at cloned human α_1-adrenoceptor subtypes expressed in CHO cells

Compound	pK_i[a]			Selectivity ratio[b]		
	α_{1a}	α_{1b}	α_{1d}	α_{1b}/α_{1a}	α_{1b}/α_{1d}	α_{1d}/α_{1a}
(+)-**1**	7.91	9.87	8.49	91	24	4
(+)-**2**	8.46	9.51	8.81	11	5	2
(+)-**3**	7.63	9.06	8.32	27	6	5
(+)-**4**	7.56	9.06	8.44	32	4	8
(+)-**5**	7.59	8.86	8.13	19	5	4
(+)-**6**	8.37	9.62	8.63	18	10	2
(+)-**7**	7.98	8.50	8.82	3	0.5	7
(+)-**8**	7.31	8.23	7.57	8	5	2
(+)-**9**	8.38	9.31	8.49	8.5	7	1

[a] Equilibrium dissociation constants (K_i) were calculated from IC_{50} values using the Cheng–Prusoff equation. The affinity estimates, derived from displacement of [^{3}H]prazosin binding from α_1-adrenoceptors, were from two to three experiments performed in triplicate, which agreed within ±20%.
[b] Calculated by the antilog of the difference between pK_i values at different α_1-adrenoceptor subtypes.

at α_{1d}-adrenoceptors. Consequently, the new compounds maintained, although to a lesser extent, the α_{1b}-adrenoceptor selectivity of (+)-**1** with the exception of (+)-**7** that was 7-fold selective for the α_{1d}-adrenoceptor over the α_{1a} subtype. The most selective compounds of the series were (+)-**3** and (+)-**4** with an α_{1b}/α_{1a} selectivity ratio of 27 and 32, respectively, and the thienyl analogue (+)-**6** with α_{1b}/α_{1a} and α_{1b}/α_{1d} selectivity ratios of 18 and 10.

A comparison between binding and functional data clearly shows that the results are not comparable because pK_B or pA_2 values are lower than the pK_i values at all α_1-adrenoceptor subtypes. This discrepancy was already observed for other α_1-adrenoceptor antagonists, including prazosin-related compounds, and different hypotheses were taken into account for a possible explanation: diffusion-related temporal inequilibrium,[29] inverse agonism and heterodimer formation.[30–32] Thus, the discrepancy observed in the present work may be the consequence of different bioavailabilities of compounds at the receptor level or of species differences in affinity.[32]

5. Conclusion

The results of the present investigation indicate that, besides the unsubstituted and 5-substituted furans, other aromatic rings, like thiophene, pyridine and phenyl, confer high potency towards the α_{1B}-subtype. Among the studied compounds, the 5-methyl furyl derivative (+)-**3**, [(+)-metcyclazosin], displayed the most interesting

pharmacological profile. In contrast to the progenitor and other analogues, it displayed a competitive antagonism at all three α_1-adrenoceptor subtypes and improved selectivity for the α_{1B}-adrenoceptor. In particular, (+)-**3** showed 11-fold higher α_{1B}/α_{1A} selectivity ratio (148) relative to (+)-**1** (13), while keeping similar selectivity for the α_{1B}-adrenoceptor over the α_{1D}-adrenoceptor subtype (ratio 41 vs 39). Since very few selective α_{1B}-adrenoceptor ligands are available, (+)-**3** may represent a useful tool for α_{1B}-adrenoceptor characterization in functional studies.

6. Experimental

6.1. 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 VXR 300 instruments, respectively. The IR spectra, not included, were consistent with all the assigned structures. The elemental analyses of compounds, performed on a Fisons instrument mod. EA1108CHNS-O, agreed with the calculated values within the range ±0.4%. Electron impact ionization (EI) mass spectra were obtained with a Hewlett-Packard instrument, consisting of model 5890 A for the separation section and model 5971 A for the mass section. Electronspray (ESI) mass spectra were recorded on a Hewlett-Packard series 1100 MSD spectrometer. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. Analytical HPLC was performed on a Hewlett-Packard 1090 apparatus, series II, with UV detector, equipped with a HP Hypersil column, 5 μm (200 × 2.1 mm, i.d.). Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. 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: A, petroleum ether–ethyl acetate–methanol–28% ammonia (8:6:2:0.2); B, petroleum ether–ethyl acetate–methanol–28% ammonia (8:6:2:0.1); C, petroleum ether–ethyl acetate–methanol–28% ammonia (9:6:1.5:0.1); D, petroleum ether–ethyl acetate–methanol–28% ammonia (8:6:2:0.05); E, petroleum ether–ethyl acetate–methanol–7% ammonia (6:4:2:0.2); F, cyclohexane–ethyl acetate–methanol–14% ammonia (6:4:1.5:0.1); G, ethyl acetate–cyclohexane–chloroform–methanol (7:3:2:1); H, ethyl acetate–cyclohexane (5:5). 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 ACD/Name software, version 7.0 (Advanced Chemistry Development, Inc., Toronto, Canada). Solid compounds were purified by crystallization using the following solvents or mixture of solvents: MeOH, (+)-**2**, (+)-**6**, (+)-**7**; EtOH, (+)-**5**, (+)-**9**, (−)-**11**; *i*-PrOH, (+)-**3**, (+)-**11**; MeOH/EtOH, (+)-**1**, (−)-**1**; MeOH/*i*-PrOH, (+)-**12**, (−)-**12**. Yields are of purified products and were not optimized. Chemicals and reagents were purchased from Sigma–Aldrich Srl (Milano, Italy) or Lancaster research chemicals (Chiminord, Srl, Cusano Milanino, Milano, Italy).

6.1.1. (±)-Benzyl *cis*-octahydroquinoxaline-1(2*H*)-carboxylate [(±)-10]. A solution of methanesulfonic acid (10 g, 104 mmol) in water (10 mL) was added to a mixture of *cis*-decahydroquinoxaline (8.21 g, 5.6 mmol), H_2O (12 mL) and EtOH (35 mL). Next, a solution of benzyl chloroformate (8.50 g, 49.8 mmol) in 1,2-dimethoxyethane (18 mL) and a 50% water solution of potassium acetate (6 mL) were alternatively added dropwise to the solution. The mixture was heated at 60 °C for 2 h and then evaporated under vacuum. The residue was treated with water and the resulting precipitate was removed by filtration. The solution was basified with 2 N NaOH and extracted with $CHCl_3$. The removal of dried solvent gave a crude residue that was purified by column chromatography eluting with mixture E to give (±)-**10** as an oily product: 10.5 g (68%); R_f = 0.28 (mixture F), MS (EI) *m*/*z* = 274 $[M]^+$, ^{1}H NMR ($CDCl_3$): δ 1.22–2.21 (m, 9H, H_{5-8} octahydroquinoxaline and NH, exchangeable with D_2O), 2.68–3.20 (m, 4H, H_{2-3} octahydroquinoxaline), 3.78–4.15 (m, 2H, H_{4a} and H_{8a} octahydroquinoxaline), 5.18 (br s, 2H, OCH_2), 7.24–7.41 (m, 5H, aromatics).

6.1.1.1. Optical resolution of (±)-benzyl *cis*-octahydroquinoxaline-1(2*H*)-carboxylate [(±)-10]. A solution of racemic **10** (22.8 g, 83 mmol) in MeOH (100 mL) was treated with a solution of (*S*)-(+)-mandelic acid (12.6 g, 83 mmol) in MeOH (100 mL). Removal of the solvent gave a residue that was crystallized seven times from ethyl acetate, affording the less soluble (+)-mandelate salt: 5.5 g; mp 140–143 °C; $[\alpha]_D^{20}$ +61.6 (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.15–2.08 (m, 8H, H_{5-8} octahydroquinoxaline), 2.61–3.15 (m, 5H, H_{2-3} octahydroquinoxaline and OH, exchangeable with D_2O), 3.70–4.00 (m, 2H, H_{4a} and H_{8a} octahydroquinoxaline), 4.88 (s, 1H, CH mandelic acid), 5.10 (br s, 2H, OCH_2), 6.60 (br signal, 2H, NH_2^+, exchangeable with D_2O), 7.18–7.41 (m, 10H, aromatics). Anal. Calcd for $C_{24}H_{30}N_2O_5$: C, 67.59; H, 7.09; N, 6.57. Found: C, 67.44; H, 7.45; N, 6.63.

Following a similar procedure, the other diastereomer mandelate was obtained from racemic **10** (8 g, 29 mmol) and an equimolar amount of (*R*)-(−)-mandelic acid: 2.7 g; mp 140–142 °C; $[\alpha]_D^{20}$ −61.1 (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.12–2.10 (m, 8H, H_{5-8} octahydroquinoxaline), 2.59–3.15 (m, 4H, H_{2-3} octahydroquinoxaline), 3.43 (br signal, 1H, OH, exchangeable with D_2O), 3.65–4.00 (m, 2H, H_{4a} and H_{8a} octahydroquinoxaline), 4.90 (s, 1H, CH mandelic acid), 5.12 (br s, 2H, OCH_2), 6.66 (br signal, 2H, NH_2^+, exchangeable with D_2O), 7.18–7.46 (m, 10H, aromatics). Anal. Calcd for $C_{24}H_{30}N_2O_5$: C, 67.59; H, 7.09; N, 6.57. Found: C, 67.48; H, 7.39; N, 6.61.

6.1.1.2. (+)-Benzyl (4a*R*,8a*S*)-octahydroquinoxaline-1(2*H*)-carboxylate [(+)-10]. The (+)-mandelate salt (5.30 g) was dissolved in H_2O and then displaced in basic medium by addition of 2 N NaOH at low temperature (0 °C). The free base was extracted with $CHCl_3$, the solvent dried and then evaporated to give 3.5 g of (+)-**10** as an oil: $[\alpha]_D^{20}$ +35.8 (*c* 0.5, MeOH); R_f = 0.28 (mixture

F); MS (EI) $m/z = 274$ $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.22-2.21 (m, 9H, H_{5-8} octahydroquinoxaline, NH exchangeable with D_2O), 2.68–3.20 (m, 4H, H_{2-3} octahydroquinoxaline), 3.78–4.15 (m, 2H, H_{4a} and H_{8a} octahydro-quinoxaline), 5.18 (br s, 2H, OCH_2), 7.24–7.41 (m, 5H, aromatics).

6.1.1.3. (−)-Benzyl (4a*S*,8a*R*)-octahydroquinoxaline-1(2*H*)-carboxylate [(−)-10]. The (−)-mandelate salt (2.28 g) was dissolved in H_2O and then displaced in basic medium by addition of 2 N NaOH at low temperature (0 °C). The free base was extracted with $CHCl_3$ that was dried and then evaporated to give 1.27 g of (−)-**10** as an oil: $[\alpha]_D^{20}$ −36.0 (*c* 0.5, MeOH); $R_f = 0.28$ (mixture F); MS (EI) $m/z = 274$ $[M]^+$; ^1H NMR ($CDCl_3$): δ 1.20–2.20 (m, 9H, H_{5-8} octahydroquinoxaline, NH exchangeable with D_2O), 2.66–3.18 (m, 4H, H_{2-3} octahydroquinoxaline), 3.75–4.15 (m, 2H, H_{4a} and H_{8a} octahydroquinoxaline), 5.15 (br s, 2H, OCH_2), 7.21–7.40 (m, 5H, aromatics).

6.1.1.4. Determination of the optical purity of (+)-10 and (−)-10. A solution of (*S*)-(−)-1-[(4-methylphenyl)sulfonyl]prolyl chloride[33] (0.31 g, 1.09 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise and under nitrogen to a cooled (0 °C) and stirred solution of racemic **10** (0.2 g, 0.73 mmol) and Et_3N (0.15 mL) dissolved in CH_2Cl_2 (12 mL). Then the mixture was stirred at room temperature for 3 h, the solvent evaporated and the crude residue purified by column chromatography (mixture H) to give an oily mixture of the two diastereomers of benzyl 4-{1-[(4-methylphenyl)sulfonyl]prolyl}-*cis*-octahydroquinoxaline-1(2*H*)-carboxylate: 0.30 g; MS (ESI) m/z = 526 $([M+H]^+)$; ^1H NMR ($CDCl_3$): δ 1.25–2.60 (m, 15H, H_{5-8} octahydroquinoxaline, H_{3-4} proline and CH_3), 3.25–4.46 (m, 8H, H_{2-3}, H_{4a} and H_{8a} octahydroquinoxaline, H_5 proline), 4.62–4.80 (m, 1H, H_2 proline), 5.18 (s, 2H, OCH_2), 7.22–7.51 (m, 7H, aromatics), 7.70–7.83 (m, 2H, aromatics).

The two diastereomeric amides were synthesized as vitreous compounds by reaction of (*S*)-(−)-1-[(4-methylphenyl)sulfonyl]prolyl chloride alternatively with (+)-**10** and (−)-**10**.

Amide from (+)-**10**: MS (ESI) m/z = 526 $([M+H]^+)$; ^1H NMR ($CDCl_3$): δ 1.25-2.60 (m, 15H, H_{5-8} octahydroquinoxaline, H_{3-4} proline and CH_3), 3.23–4.15 (m, 7H, H_{2-3}, H_{4a} or H_{8a} octahydroquinoxaline and H_5 proline), 4.25–4.42 (m, 1H, H_{8a} or H_{4a}), 4.62–4.72 (m, 1H, H_2 proline), 5.18 (s, 2H, OCH_2), 7.20–7.43 (m, 7H, aromatics), 7.72-7.78 (m, 2H, aromatics).

Amide from (−)-**10**: MS (ESI) m/z = 526 $([M+H]^+)$; ^1H NMR ($CDCl_3$): δ 1.25–2.60 (m, 15H, H_{5-8} octahydroquinoxaline, H_{3-4} proline and CH_3), 3.27–4.46 (m, 8H, H_{2-3}, H_{4a} and H_{8a} octahydroquinoxaline and H_5 proline), 4.62–4.78 (m, 1H, H_2 proline), 5.18 (s, 2H, OCH_2), 7.22–7.43 (m, 7H, aromatics), 7.71–7.82 (m, 2H, aromatics).

The HPLC analysis of the two single diastereomers, in comparison with a diastereomeric mixture of them, was performed on a HP Hipersil column, 5 μm (200 × 2.1 mm, i.d.), with hexane/ethyl acetate (70:30, v/v) as eluting mixture at a flow rate of 1 mL/min. Under these conditions the injected samples of mixtures (3μL, *c* 2.5 mg/mL), detectable by absorbance at 254 nm, produced two peaks with retention time of 7.60 and 8.75 min, whereas the two diastereomers obtained from (+)-**10** and (−)-**10** displayed single peaks at retention time of 7.49 and 8.61 min, respectively.

6.1.2. (+)-Benzyl (4a*R*,8a*S*)-4-(4-amino-6,7-dimethoxyquinazolin-2-yl)octahydroquinoxaline-1 (2*H*)-carboxylate hydrochloride [(+)-11]. A mixture of 2-chloro-6,7-dimethoxyquinazolin-4-amine (1.27 g, 5.31 mmol), (+)-**10** (1.60 g, 5.84 mmol) and Et_3N (2.25 mL, 15.93 mmol) in *i*-AmOH (40 mL) was stirred under reflux for seven days. The solvent was removed in vacuum, then the crude residue was triturated with cold 2 N NaOH and the precipitate collected. Purification by column chromatography, eluting with mixture G, afforded a solid that was transformed into (+)-**11** as hydrochloride salt: 1.3 g (48%); mp 243–245 °C; $R_f = 0.37$ (mixture F); $[\alpha]_D^{20}$ +14.9 (*c* 1, MeOH); ^1H NMR (DMSO-d_6): δ 1.25–1.98 (m, 7H, H_{5-8} octahydroquinoxaline), 2.28–2.47 (m, 1H, H_{5-8} octahydroquinoxaline), 3.63–4.28 (m, 11H, OCH_3, H_{2-3} and H_{4a} octahydroquinoxaline), 4.60–4.87 (m, 1H, H_{8a} octahydroquinoxaline), 5.05–5.24 (m, 2H, OCH_2), 7.21–7.46 (m, 5H, aromatics), 7.65 (s, 1H, aromatics), 7.78 (s, 1H, aromatics), 8.68 (br s, 1H, NH, exchangeable with D_2O), 8.92 (br s, 1H, NH, exchangeable with D_2O), 12.10 (br s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{26}H_{31}N_5O_4 \cdot HCl \cdot 0.75H_2O \cdot C_3H_8O$: C, 59.28; H, 7.12; N, 11.92. Found: C, 59.42; H, 7.39; N, 12.30.

6.1.3. (−)-Benzyl (4a*S*,8a*R*)-4-(4-amino-6,7-dimethoxyquinazolin-2-yl)octahydroquinoxaline-1(2*H*)-carboxylate hydrochloride [(−)-11]. The title compound was synthesized from 2-chloro-6,7-dimethoxyquinazolin-4-amine (0.99 g, 4.2 mmol) and (−)-**10** (1.27 g, 4.6 mmol) following the procedure described for (+)-**11**: 1.5 g (71%); mp 242–244 °C; $R_f = 0.37$ (mixture F); $[\alpha]_D^{20}$ −15.3 (*c* 1, MeOH); ^1H NMR (DMSO-d_6): δ 1.23–2.00 (m, 7H, H_{5-8} octahydroquinoxaline), 2.28–2.47 (m, 1H, H_{5-8} octahydroquinoxaline), 3.70–4.20 (m, 11H, OCH_3, H_{2-3} and H_{4a} octahydroquinoxaline), 4.62–4.80 (m, 1H, H_{8a} octahydroquinoxaline), 5.03–5.20 (m, 2H, OCH_2), 7.25–7.40 (m, 5H, aromatics), 7.63 (s, 1H, aromatics), 7.75 (s, 1H, aromatics), 8.62 (br s, 1H, NH, exchangeable with D_2O), 8.91 (br s, 1H, NH, exchangeable with D_2O), 12.02 (br s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{26}H_{31}N_5O_4 \cdot HCl \cdot H_2O \cdot 0.25C_2H_6O$: C, 58.56; H, 6.58; N, 12.88. Found: C, 58.58; H, 6.40; N, 12.90.

6.1.4. (−)-6,7-Dimethoxy-2-[(4a*S*,8a*R*)-octahydroquinoxalin-1(2*H*)-yl]quinazolin-4-amine dihydrochloride [(−)-12]. Cyclohexene (42 mL) and 10% Pd/C (0.56 g) were consecutively added to a solution of (+)-**11** (1.2 g, 2.34 mmol) in MeOH (10 mL) and EtOH (30 mL). The mixture was stirred while heating at 80 °C for 6 h. After cooling, the catalyst was filtered off and the solvent evaporated to dryness to give a residue that was transformed into (−)-**12** as the hydrochloride salt: 0.79 g

(81%); mp 287-290 °C; $R_f = 0.24$ (mixture A); $[\alpha]_D^{20}$ −60.1 (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.35–2.40 (m, 8H, H_{5-8} octahydroquinoxaline), 2.97–3.65 (m, 4H, H_{2-3} octahydroquinoxaline), 3.85 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 4.62–4.83 (m, 1H, H_{4a} octahydroquinoxaline), 4.88–5.20 (m, 1H, H_{8a} octahydroquinoxaline), 7.80 (s, 2H, aromatics), 8.74 (br s, 1H, NH, exchangeable with D_2O), 9.07 (br s, 1H, NH, exchangeable with D_2O), 9.60 (br s, 1H, NH exchangeable with D_2O), 10.05 (br s, 1H, NH exchangeable with D_2O), 12.82 (br s, 1H, NH exchangeable with D_2O). Anal. Calcd for $C_{18}H_{25}N_5O_2{\cdot}2HCl{\cdot}1.5H_2O$: C, 48.76; H, 6.82; N, 15.80. Found: C, 48.99; H, 6.76; N, 15.85.

6.1.5. (+)-6,7-Dimethoxy-2-[(4a*R*,8a*S*)-octahydroquinoxalin-1(2*H*)-yl]quinazolin-4-amine dihydrochloride [(+)-12]. Obtained from (−)-**11** (1.73 g, 3.82 mmol) following the procedure described for (−)-**12**: 0.72 g (55%); mp 290–292 °C; $R_f = 0.24$ (mixture A); $[\alpha]_D^{20}$ +59.6 (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.35–2.40 (m, 8H, H_{5-8} octahydroquinoxaline), 3.00–3.68 (m, 4H, H_{2-3} octahydroquinoxaline), 3.85 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 4.62–4.83 (m, 1H, H_{4a} octahydroquinoxaline), 4.90–5.20 (m, 1H, H_{8a} octahydroquinoxaline), 7.80 (s, 2H, aromatics), 8.75 (br s, 1H, NH, exchangeable with D_2O), 9.00 (br s, 1H, NH, exchangeable with D_2O), 9.62 (br s, 1H, NH, exchangeable with D_2O), 10.00 (br s, 1H, NH, exchangeable with D_2O), 12.82 (br s, 1H, NH exchangeable with D_2O). Anal. Calcd for $C_{18}H_{25}N_5O_2{\cdot}2HCl{\cdot}1H_2O$: C, 49.77; H, 6.73; N, 16.12. Found: C, 49.73; H, 6.88; N, 16.06.

6.1.6. (+)-2-[(4a*S*,8a*R*)-4-(2-Furoyl)octahydroquinoxalin-1(2*H*)-yl]-6,7-dimethoxyquinazolin-4-amine hydrochloride [(+)-1]. A solution of 2-furoyl chloride (0.034 g, 0.26 mmol) in dry CH_2Cl_2 (2.5 mL) was added dropwise to a cooled (0 °C) and stirred solution of (−)-**12** free base (0.060 g, 0.17 mmol) and Et_3N (0.02 mL) in dry CH_2Cl_2 (5 mL). Then the mixture was stirred for 3 h at room temperature, the solvent removed and the residue purified by column chromatography eluting with mixture C. The product was transformed into (+)-**1** as hydrochloride salt: 0.022 g; mp 263–264 °C; $R_f = 0.43$ (mixture A); $[\alpha]_D^{20}$ +74.1 (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.25–2.45 (m, 8H, H_{5-8} octahydroquinoxaline), 3.70–4.30 (m, 10H, H_{2-3} octahydroquinoxaline, OCH_3), 4.37–4.48 (m, 1H, H_{8a} octahydroquinoxaline), 4.58–4.76 (m, 1H, H_{4a} octahydroquinoxaline), 6.72 (m, 1H, H_4 furan), 7.14 (m, 1H, H_3 furan), 7.54 (s, 1H, aromatics), 7.76 (s, 1H, aromatics), 7.90 (m, 1H, H_5 furan), 8.62 (br s, 1H, NH, exchangeable with D_2O), 8.88 (br s, 1H, NH, exchangeable with D_2O), 11.90 (br s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{23}H_{27}N_5O_4{\cdot}HCl{\cdot}1.5H_2O$: C, 55.14; H, 6.24; N, 13.98. Found: C, 55.29; H, 6.56; N, 14.16.

6.1.7. (−)-2-[(4a*R*,8a*S*)-4-(2-Furoyl)octahydroquinoxalin-1(2*H*)-yl]-6,7-dimethoxyquinazolin-4-amine hydrochloride [(−)-1]. Obtained from (+)-**12** free base (0.072 g, 0.21 mmol) following the procedure described for (+)-**1**: 0.018 g; mp 263–265 °C; $R_f = 0.43$ (mixture A); $[\alpha]_D^{20}$ −73.9 (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.25–2.45 (m, 8H, H_{5-8} octahydroquinoxaline), 3.70–4.30 (m, 10H, H_{2-3} octahydroquinoxaline, OCH_3), 4.37–4.50 (m, 1H, H_{8a} octahydroquinoxaline), 4.52–4.76 (m, 1H, H_{4a} octahydroquinoxaline), 6.72 (m, 1H, H_4 furan), 7.14 (m, 1H, H_3 furan), 7.56 (s, 1H, aromatics), 7.76 (s, 1H, aromatics), 7.90 (m, 1H, H_5 furan), 8.64 (br s, 1H, NH, exchangeable with D_2O), 8.90 (br s, 1H, NH, exchangeable with D_2O), 11.90 (br s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{23}H_{27}N_5O_4{\cdot}HCl{\cdot}1.5H_2O$: C, 55.14; H, 6.24; N, 13.98. Found: C, 55.40; H, 6.12; N, 14.27.

6.1.8. General procedure for the synthesis of [(+)-2 - (+)-9]. A solution of proper acyl chloride (1.05 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise to a stirred and cooled (0 °C) solution of (−)-**12** free base (1 mmol) and Et_3N (0.15 mL, 1.5 mmol) dissolved in dry CH_2Cl_2 (10 mL). Then the mixture was stirred at room temperature for 3 h, the solvent evaporated and the residue purified by column chromatography. The products, eluted as free bases, were transformed into the corresponding hydrochloride salts and crystallized. However, (+)-**4** was isolated and characterized as free base.

6.1.8.1. (+)-2-[(4a*S*,8a*R*)-4-(5-Bromo-2-furoyl)octahydroquinoxalin-1(2*H*)-yl]-6,7-dimethoxyquinazolin-4-amine hydrochloride [(+)-2]. Obtained from 5-bromo-2-furoyl chloride[34] (0.16 g, 0.77 mmol) and (−)-**12** free base (0.25 g, 0.73 mmol): 0.08 g (21%); mp 270–272 °C; R_f = 0.56 (eluting mixture B); $[\alpha]_D^{20}$ +90.8 (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.20–2.10 (m, 7H, H_{5-8} octahydroquinoxaline), 2.35–2.42 (m, 1H, H_{5-8} octahydroquinoxaline), 3.78–4.32 (m, 10H, H_{2-3} octahydroquinoxaline, OCH_3), 4.38–4.50 (m, 1H, H_{8a} octahydroquinoxaline), 4.57–4.80 (m, 1H, H_{4a} octahydroquinoxaline), 6.82 (m, 1H, H_4 furan), 7.21 (m, 1H, H_3 furan), 7.55 (s, 1H, aromatics), 7.76 (s, 1H, aromatics), 8.65 (br s, 1H, NH, exchangeable with D_2O), 8.90 (br s, 1H, NH, exchangeable with D_2O), 12.00 (br s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{23}H_{26}BrN_5O_4{\cdot}HCl{\cdot}0.25H_2O$: C, 49.56; H, 4.97; N, 12.57. Found: C, 49.41; H, 5.15; N, 12.41.

6.1.8.2. (+)-6,7-Dimethoxy-2-[(4a*S*,8a*R*)-4-(5-methyl-2-furoyl)octahydroquinoxalin-1(2*H*)-yl]quinazolin-4-amine hydrochloride [(+)-3]. Obtained from 5-methyl-2-furoyl chloride[34] (0.11 g, 0.76 mmol) and (−)-**12** free base (0.25 g, 0.73 mmol): 0.075 g (20%); mp 273–275 °C; R_f = 0.45 (eluting mixture B); $[\alpha]_D^{20}$ +70.2 (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.20–2.10 (m, 7H, H_{5-8} octahydroquinoxaline), 2.20–2.45 (m, 4H, H_{5-8} octahydroquinoxaline and CH_3), 3.73–4.32 (m, 10H, H_{2-3} octahydroquinoxaline, OCH_3), 4.38–4.50 (m, 1H, H_{8a} octahydroquinoxaline), 4.57–4.73 (m, 1H, H_{4a} octahydroquinoxaline), 6.30 (m, 1H, H_4 furan), 7.00 (m, 1H, H_3 furan), 7.58 (s, 1H, aromatics), 7.78 (s, 1H, aromatics), 8.68 (br s, 1H, NH, exchangeable with D_2O), 8.90 (br s, 1H, NH, exchangeable with D_2O), 11.98 (br s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{24}H_{29}N_5O_4{\cdot}HCl{\cdot}3.5H_2O{\cdot}0.25C_3H_8O$: C, 52.51; H, 6.94; N, 12.37. Found: C, 52.36; H, 6.60; N, 12.03.

6.1.8.3. (+)-6,7-Dimethoxy-2-[(4a*S*,8a*R*)-4-(5-methoxy-2-furoyl)octahydroquinoxalin-1(2*H*)-yl]quinazolin-4-amine [(+)-4]. Obtained from 5-methoxy-2-furoyl chloride[35]

(0.12 g, 0.76 mmol) and (−)-**12** free base (0.25 g, 0.73 mmol). Because of stability problem the product was isolated and characterized as free base: 0.05 g (12%); mp 139–141 °C; R_f = 0.27 (eluting mixture B); $[\alpha]_D^{20}$ +13.3 (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.21–2.18 (m, 7H, H_{5-8} octahydroquinoxaline), 2.20–2.42 (m, 1H, H_{5-8} octahydroquinoxaline), 3.70–4.18 (m, 13H, H_{2-3} octahydroquinoxaline, OCH_3), 4.22–4.38 (m, 1H, H_{8a} octahydroquinoxaline), 4.42–4.60 (m, 1H, H_{4a} octahydroquinoxaline), 5.60 (m, 1H, H_4 furan), 6.78 (br s, 1H, aromatics), 7.05 (m, 1H, H_3 furan), 7.20 (br s, 2H, NH, exchangeable with D_2O), 7.42 (br s, 1H, aromatics). Anal. Calcd for $C_{24}H_{29}N_5O_5{\cdot}1.5H_2O{\cdot}0.25C_6H_{14}$: C, 59.35; H, 6.93; N, 13.57. Found: C, 59.43; H, 6.75; N, 13.43.

6.1.8.4. (+)-1-(5-{[(4a*R*,8a*S*)-4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)octahydroquinoxalin-1(2*H*)-yl]carbonyl}-2-furyl)ethanone hydrochloride [(+)-5]. Obtained from 5-acetyl-2-furoyl chloride[36] (0.10 g, 0.61 mmol) and (−)-**12** free base (0.20 g, 0.58 mmol): 0.12 g (42%); mp 246–249 °C; R_f = 0.51 (eluting mixture B); $[\alpha]_D^{20}$ +110.2 (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.30–2.08 (m, 7H, H_{5-8} octahydroquinoxaline), 2.38–2.55 (m, 4H, H_{5-8} octahydroquinoxaline and CH_3), 3.78–4.30 (m, 10H, H_{2-3} octahydroquinoxaline, OCH_3), 4.38–4.50 (m, 1H, H_{4a} octahydroquinoxaline), 4.62–4.80 (m, 1H, H_{8a} octahydroquinoxaline), 7.25 (m, 1H, H_3 furan), 7.50–7.62 (m, 2H, H_4 furan and aromatics), 7.80 (s, 1H, aromatics), 8.65 (br s, 1H, NH, exchangeable with D_2O), 8.90 (br s, 1H, NH, exchangeable with D_2O), 12.05 (br s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{25}H_{29}N_5O_5{\cdot}HCl{\cdot}3.25H_2O$: C, 52.26; H, 6.40; N, 12.19. Found: C, 52.02; H, 6.61; N, 12.12.

6.1.8.5. (+)-6,7-Dimethoxy-2-[(4a*S*,8a*R*)-4-(2-thienylcarbonyl)octahydroquinoxalin-1(2*H*)-yl]quinazolin-4-amine hydrochloride [(+)-6]. Obtained from thiophene-2-carbonyl chloride (0.089 g, 0.61 mmol) and (−)-**12** free base (0.2 g, 0.58 mmol): 0.06 g (21%); mp 280–282 °C; R_f = 0.42 (eluting mixture B); $[\alpha]_D^{20}$ +46.4 (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.25–2.10 (m, 7H, H_{5-8} octahydroquinoxaline), 2.25–2.48 (m, 1H, H_{5-8} octahydroquinoxaline), 3.70–4.22 (m, 10H, H_{2-3} octahydroquinoxaline, OCH_3), 4.25–4.42 (m, 1H, H_{8a} octahydroquinoxaline), 4.57–4.78 (m, 1H, H_{4a} octahydroquinoxaline), 7.18 (m, 1H, H_4 thiophene), 7.42–7.65 (m, 2H, $H_{3,5}$ thiophene), 7.78 (s, 1H, aromatics), 7.82 (m, 1H, aromatics), 8.62 (br s, 1H, NH, exchangeable with D_2O), 8.90 (br s, 1H, NH, exchangeable with D_2O), 11.90 (br s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{23}H_{27}N_5O_3S{\cdot}HCl{\cdot}2.5H_2O$: C, 51.63; H, 6.22; N, 13.09. Found: C, 51.45; H, 6.16; N, 13.04.

6.1.8.6. (+)-6,7-Dimethoxy-2-[(4a*S*,8a*R*)-4-(1*H*-pyrrol-2-ylcarbonyl)octahydroquinoxalin-1(2*H*)-yl]quinazolin-4-amine hydrochloride [(+)-7]. Obtained from 1*H*-pyrrole-2-carbonyl chloride[37] (0.079 g, 0.61 mmol) and (−)-**12** free base (0.2 g, 0.58 mmol): 0.014 g (5%); mp 284–286 °C; R_f = 0.46 (eluting mixture D), $[\alpha]_D^{20}$ +45.0 (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.22–2.10 (m, 7H, H_{5-8} octahydroquinoxaline), 2.30–2.45 (m, 1H, H_{5-8} octahydroquinoxaline), 3.70–4.42 (m, 11H, H_{2-3} and H_{8a} octahydroquinoxaline, OCH_3), 4.50–4.72 (m, 1H, H_{4a} octahydroquinoxaline), 6.18 (m, 1H, H_4 pyrrole), 6.62 (m, 1H, H_3 pyrrole), 6.90 (m, 1H, H_5 pyrrole), 7.50 (s, 1H, aromatics), 7.75 (s, 1H, aromatics), 8.65 (br s, 1H, NH, exchangeable with D_2O), 8.94 (br s, 1H, NH, exchangeable with D_2O), 11.52 (br s, 1H, NH, exchangeable with D_2O), 11.85 (br s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{23}H_{28}N_6O_3{\cdot}HCl{\cdot}2.75H_2O$: C, 52.87; H, 6.66; N, 16.08. Found: C, 53.18; H, 6.53; N, 15.68.

6.1.8.7. (+)-6,7-Dimethoxy-2-[(4a*S*,8a*R*)-4-(pyridin-2-ylcarbonyl)octahydroquinoxalin-1(2*H*)-yl]quinazolin-4-amine dihydrochloride [(+)-8]. Obtained from pyridine-2-carbonyl chloride[38] (0.11 g, 0.76 mmol) and (−)-**12** free base (0.25 g, 0.73 mmol): 0.074 g (19%); mp 287–289 °C; R_f = 0.29 (eluting mixture B); $[\alpha]_D^{20}$ +73.2 (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.30–2.15 (m, 7 H, H_{5-8} octahydroquinoxaline), 2.25–2.48 (m, 1H, H_{5-8} octahydroquinoxaline), 3.65–4.15 (m, 10H, H_{2-3} octahydroquinoxaline, OCH_3), 4.35–4.42 (m, 1H, H_{8a} octahydroquinoxaline), 4.70–4.90 (m, 1H, H_{4a} octahydroquinoxaline), 7.50–7.80 (m, 4H, H_3, H_5 pyridine and aromatics), 7.92–8.10 (m, 1H, H_4 pyridine), 8.60-8.76 (m, 2H, H_6 pyridine and NH exchangeable with D_2O), 8.95 (br s, 1H, NH, exchangeable with D_2O), 12.05 (br s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{24}H_{28}N_6O_3{\cdot}2HCl{\cdot}3H_2O$: C, 50.09; H, 6.31; N, 14.60. Found: C, 50.18; H, 6.47; N, 14.31.

6.1.8.8. (+)-2-[(4a*S*,8a*R*)-4-Benzoyloctahydroquinoxalin-1(2*H*)-yl]-6,7-dimethoxyquinazolin-4-amine hydrochloride [(+)-9]. Obtained from benzoyl chloride (0.094 g, 0.67 mmol) and (−)-**12** free base (0.22 g, 0.64 mmol): 0.08 g (26%); mp 281–283 °C; R_f = 0.45 (eluting mixture A); $[\alpha]_D^{20}$ +36.8 (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6): δ 1.28–2.15 (m, 7H, H_{5-8} octahydroquinoxaline), 2.40–2.50 (m, 1H, H_{5-8} octahydroquinoxaline), 3.62–4.12 (m, 10H, H_{2-3} octahydroquinoxaline, OCH_3), 4.22–4.43 (m, 1H, H_{8a} octahydroquinoxaline), 4.62–4.85 (m, 1H, H_{4a} octahydroquinoxaline), 7.38–7.67 (m, 6H, aromatics), 7.75 (s, 1H, aromatics), 8.62 (br s, 1H, NH, exchangeable with D_2O), 8.90 (br s, 1H, NH, exchangeable with D_2O), 12.00 (br s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{25}H_{29}N_5O_3{\cdot}HCl{\cdot}1.75H_2O$: C, 58.25; H, 6.55; N, 13.59. Found: C, 58.16; H, 6.87; N, 13.48.

6.2. Pharmacology

6.2.1. Functional experiments. Tissues for experiments were taken from male Wistar rats (275–300 g; Charles River, Como, Italy). After killing by cervical dislocation, the required organs were isolated. Vas deferens prostatic portion, spleen and aorta were freed from adhering connective tissue and set up rapidly, under a suitable tension, in 20-mL organ baths. The bath medium, containing physiological salt solution (pH 7.4), was kept at 37 °C and aerated with 5% CO_2/95% O_2. Concentration–response curves were constructed by cumulative addition of agonist. The agonist concentration in the bath was increased approximately 3-fold at each step, with each addition being made only after the response of the previous addition had attained a maximal level and remained steady. Contractions were

recorded by means of a force displacement transducer connected to the MacLab System PowerLab/800.

In all experiments a control agonist concentration–response curve (vehicle) was constructed in the presence of the maximum DMSO concentration (0.5%) contained in the bathing solutions being the solvent used for dissolution of tested antagonists on preparing the initial stock solution. These curves were not different from the previous one indicating no interference of solvent in the agonist effect. The agonist-elicited concentration–response curves obtained in the presence of the tested concentrations of antagonist were related to the vehicle control curve, of which the maximal response was taken as 100%. Parallel experiments in which tissues did not receive any antagonist were run in order to check any variation in sensitivity. The experimental conditions used for the investigation at α_1-adrenoceptor subtypes are procedures taken from the quoted literature.

All pharmacological graphics were drawn by a Prism 3.0 computer program (GraphPad Software, Inc., San Diego, CA, USA). Chemicals, (−)-noradrenaline bitartrate, (−)-phenylephrine hydrochloride, cocaine hydrochloride, normetanephrine hydrochloride and (±)-propranolol hydrochloride were purchased from Sigma–Aldrich Srl (Milano, Italy).

6.2.1.1. Prostatic rat vas deferens. Affinity at α_{1A} adrenoceptor was evaluated on prostatic rat vas deferens according to a reported procedure.[21] Prostatic portions of 2 cm length were mounted under 0.35 g tension at 37 °C in Tyrode solution of the following composition (mM): NaCl, 130; KCl, 2; $CaCl_2$, 1.8; $MgCl_2$, 0.89; NaH_2PO_4, 0.42; $NaHCO_3$, 25; glucose, 5.6. To prevent the neuronal uptake of noradrenaline, used as agonist, cocaine hydrochloride (10 μM) was added to the Tyrode solution 20 min before the agonist cumulative concentration–response curve was recorded. Vas deferens was equilibrated for 45 min, with washing every 15 min. After the equilibration period, tissues were primed twice by addition of 10 μM noradrenaline in order to obtain a constant response. After another washing and equilibration period of 45 min, a cumulative isotonic noradrenaline concentration–response curve was constructed to determine the relationship between agonist concentrations and contractile response. When measuring the effect of the antagonist, it was allowed to equilibrate with the tissue for 30 min before constructing a new concentration–response curve to the agonist. The noradrenaline solution contained 0.05% $Na_2S_2O_5$ to prevent oxidation.

6.2.1.2. Aorta. Affinity at rat aorta α_{1D} adrenoceptor was evaluated using a procedure adapted from that already reported.[22] Two strips (15 mm × 3 mm) were cut helically from rat thoracic aorta beginning from the end most proximal to the heart. The endothelium was removed by rubbing with filter paper: the absence of 100 μM acetylcholine-induced relaxation to preparations contracted with 1 μM noradrenaline was taken as an indicator that the vessel was denuded successfully. The strips were then tied with surgical thread and suspended in an organ bath containing 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. Cocaine hydrochloride (10 μM), normetanephrine hydrochloride (1 μM) and propranolol hydrochloride (1 μM) were added to prevent the neuronal and extraneuronal uptake of the agonist noradrenaline and to block the β-adrenoceptors, respectively. In the absence of these inhibitors the noradrenaline concentration–response curve was significantly displaced to the right (data not shown).

After an equilibration period of at least two hours under an optimal tension of 1 g, cumulative noradrenaline concentration–response curves were recorded isometrically at 1 h intervals, the first being discarded and the second one taken as control. After inspection of vehicle activity, the antagonist was allowed to equilibrate with the tissue for 30 min before generation of the third cumulative concentration–response curve to the agonist. Noradrenaline solutions contained 0.05% K_2EDTA in 0.9% NaCl to prevent oxidation.

6.2.1.3. Spleen. The spleen was removed and bisected longitudinally in 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; EDTA, 0.01. Propranolol hydrochloride (4 μM) was added to block β-adrenoceptors. Following a reported procedure the spleen strips were placed under 1 g resting tension and equilibrated for 2 h.[24] A first cumulative concentration–response curve to the agonist phenylephrine was quickly taken isometrically, followed by 30 min washing. Subsequently, a second cumulative curve was constructed followed by 30 min washing. Each tissue was then incubated either with vehicle or different antagonist concentrations and a new agonist concentration–response curve constructed.

To test the reproducibility in the tissue, a new concentration–response curve to phenylephrine, after the second, was repeated at different successive times, that is, 30, 45 and 60 min, corresponding to different possible periods of incubation with the antagonist. The third concentration–response curve, recorded after 30 min, did not modify significantly either the pD_2 value or the maximum response of the agonist, whereas a significant decrease of the maximum response from the control curve was observed after 45 and 60 min (34% and 41%, respectively).

On the basis of these results, a time of 30 min was selected as the period for antagonist incubation. As a consequence, measuring the antagonism of (+)-cyclazosin and related new compounds, the various compound concentrations were equilibrated for 30 min with tissue before constructing the new phenylephrine concentration–response curve (third curve).

6.2.2. Binding assays. Competition binding assays to cloned human α_{1a}, α_{1b} and α_{1d}-adrenoceptor subtypes

were performed in membrane preparations from CHO (Chinese hamster ovary) cell lines transfected by electroporation with DNA expressing the gene encoding each α_1-adrenoceptor. Cloning and stable expression of the human α_1-adrenoceptor gene was performed as previously described.[27] Briefly, CHO cell membranes (30 μg proteins) were incubated in 50 mM Tris–HCl buffer, 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). Non-specific 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.

6.2.3. Data analysis. In functional studies, responses were expressed as percentage of the maximal contraction observed in the agonist concentration–response curve taken as control. Each response was plotted graphically as a mean from at least four separate experiments. Curves were fitted to all the data by a non-linear regression using the Prism 3.0 program to calculate pEC_{50} values. In all cases, 50% of the maximum for each concentration–response curve was used to evaluate the EC_{50}. This value, calculated in presence and in absence of antagonist in a single tissue, was used to determine the concentration ratio.

Schild plots were constructed to estimate the pA_2 values and the slope of the regression line using experimental series obtained from at least three different concentrations.[25] The Schild diagrams were constructed by plotting the log (concentration ratio − 1) against the log [antagonist] and deriving it from a linear regression using the Prism 3.0 program. When the Schild plot slope was not significantly different from unity ($p > 0.05$), the regression was recalculated with a constrained slope of 1 and the result given as a pA_2 value. In a number of cases, Schild analysis could not be performed due to the non-parallel slopes of concentration–response curves and variable depression. As a consequence, pK_B values were calculated at only one concentration, according to van Rossum.[26]

Thus, a pA_2 value was determined for (+)-**1**, (+)-**3**, (+)-**4** and (+)-**9** at the α_{1A}-adrenoceptor, for(+)-**3** at the α_{1B}-adrenoceptor and for (+)-**1** - (+)-**6** at the α_{1D}-adrenoceptor. In all other cases the antagonist potency was expressed by pK_B values calculated with the equation $pK_B = \log$ (concentration ratio − 1)/[antagonist] at the lowest antagonist concentration giving a significant rightward shift ($\geqslant 0.5$) of the agonist concentration–response curve.

Data were compared by Student's *t*-test and presented as means ± SEM of 4–6 experiments. A p value <0.05 was taken to indicate a statistically significant difference.

Data from binding assays were analysed using a non-linear curve-fitting program Allfit.[39] Scatchard plots were linear in all preparations and the pseudo-Hill coefficients non-significantly different from the unity ($p > 0.05$). The inhibition of the radioligand specific binding by tested compounds allowed the estimation of IC_{50} values that were converted to affinity constants (K_i) by the Cheng–Prusoff equation[28]: $K_i = IC_{50}/(1 + L/K_d)$, where L and K_d are the concentration and the equilibrium dissociation constant of the radioligand. Data are expressed as means of pK_i values of 2–3 separate experiments performed in triplicate.

Acknowledgments

The present work was supported by a grant from the University of Camerino and Cofinanziamento MIUR 2005 (Italy).

References and notes

1. Hieble, J. P.; Bylund, D. B.; Clarke, D. E.; Eikenburg, D. C.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Ruffolo, R. R., Jr. *Pharmacol. Rev.* **1995**, *47*, 267–270.
2. Muramatsu, I.; Ohmura, T.; Hashimoto, S.; Oshita, M. *Pharmacol. Commun.* **1995**, *6*, 23–28.
3. Ford, A. P. D. W.; Daniels, D. V.; Chang, D. J.; Gever, J. R.; Jasper, J. R.; Lesnick, J. D.; Clarke, D. E. *Br. J. Pharmacol.* **1997**, *121*, 1127–1135.
4. Zhong, H.; Minneman, K. P. *Eur. J. Pharmacol.* **1999**, *375*, 261–276.
5. Han, C.; Abel, P. W.; Minneman, K. P. *Mol. Pharmacol.* **1987**, *32*, 505–510.
6. Hancock, A. A. *Drug Develop. Res.* **1996**, *39*, 54–107.
7. Sleight, A. J.; Koek, W.; Bigg, D. C. H. *Eur. J. Pharmacol.* **1993**, *238*, 407–410.
8. Giardinà, D.; Crucianelli, M.; Melchiorre, C.; Taddei, C.; Testa, R. *Eur. J. Pharmacol.* **1995**, *287*, 13–16.
9. Chang, R. S. L.; Chen, T. B.; O'Malley, S. S.; Lagu, B.; Nagarathnam, D.; Forray, C.; Marzabadi, M.; Wong, W.; Murali Dhar, T.; Hong, X.; Gluchowski, C.; Disalvo, J.; Patane, M.; Bock, M. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1998**, *358* (Suppl. 2), R593.
10. Patane, M. A.; Scott, A. L.; Broten, T. P.; Chang, R. S. L.; Ransom, R. W.; Disalvo, J.; Forray, C.; Bock, M. G. *J. Med. Chem.* **1998**, *41*, 1205–1208.
11. King, H. K.; Goetz, A. S.; Ward, S. D. C.; Saussy, D. L., Jr. *Soc. Neurosci. Abstr.* **1994**, *20*, 526.
12. Eltze, M.; König, H.; Ullrich, B.; Grebe, T. *Eur. J Pharmacol.* **2001**, *415*, 265–276.
13. Giardinà, D.; Crucianelli, M.; Romanelli, R.; Leonardi, A.; Poggesi, E.; Melchiorre, C. *J. Med. Chem.* **1996**, *39*, 4602–4607.
14. Giardinà, D.; Sagratini, G.; Grelli, M.; Gulini, U.; Melchiorre, C.; Antolini, L. *Il Farmaco* **2004**, *59*, 965–969.
15. According to the IUPAC rules, as applied by recent ACD/Name software, version 7.0, in the present work, the chemical nomenclature of (+)-cyclazosin is different from the one previously reported: [4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-(4a*R*,8a*S*)-perhydro-1-quinoxalinyl](2-furyl) methanone.
16. Marucci, G.; Angeli, P.; Buccioni, M.; Gulini, U.; Melchiorre, C.; Sagratini, G.; Testa, R.; Giardinà, D. *Eur. J. Pharmacol.* **2005**, *522*, 100–107.
17. Preliminary biological results of compounds (+)-**2** - (+)-**5** were reported at the 14th Camerino-Noordwijkerhout Symposium (Camerino, Italy, 7–11 September 2003) and published in Ref. 31 without experimental chemistry and pharmacology description.
18. Brill, E.; Schultz, H. P. *J. Org. Chem.* **1964**, *29*, 579–581.

19. Beecham, A. F. *J. Am. Chem. Soc.* **1957**, *79*, 3257–3261.
20. Gerster, J. F.; Rohlfing, S. R.; Pecore, S. E.; Winandy, R. M.; Stern, R. M.; Landmesser, J. E.; Olsen, R. A.; Gleason, W. B. *J. Med. Chem.* **1987**, *30*, 839–843.
21. Eltze, M.; Boer, R.; Sanders, K. H.; Kolassa, N. *Eur. J. Pharmacol.* **1991**, *202*, 33–44.
22. Ko, F. N.; Guh, J. H.; Yu, S. M.; Hou, Y. C.; Wu, Y. C.; Teng, C. M. *Br. J. Pharmacol.* **1994**, *112*, 1174–1180.
23. Stam, W. B.; Van Der Graaf, P. H.; Saxena, P. R. *Eur. J. Pharmacol.* **1998**, *361*, 79–83.
24. Buckner, S. A.; Oheim, K. W.; Morse, P. A.; Knepper, S. M.; Hancock, A. A. *Eur. J. Pharmacol.* **1996**, *297*, 241–248.
25. Arunlakshana, D.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48–58.
26. Van Rossum, J. M. *Archives internationales de pharmacodynamie et de therapie* **1963**, *143*, 299–330.
27. Testa, R.; Taddei, C.; Poggesi, E.; Destefani, C.; Cotecchia, S.; Hieble, J. P.; Sulpizio, A. C.; Naselsky, D.; Bergsma, D.; Ellis, C.; Swift, A.; Ganguly, S.; Ruffolo, R. R., Jr.; Leonardi, A. *Pharmacol. Commun.* **1995**, *6*, 79–86.
28. Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
29. Kenakin, T. In *Pharmacological Analysis of Drug–Receptor Interaction*; Raven Press: New York, 1993; pp 278–321.
30. Melchiorre, C.; Bolognesi, M. L.; Budriesi, R.; Chiarini, A.; Giardinà, D.; Minarini, A.; Quaglia, W.; Leonardi, A. *Il Farmaco* **1998**, *53*, 278–286.
31. Sagratini, G.; Buccioni, M.; Gulini, U.; Marucci, G.; Melchiorre, C.; Leonardi, A.; Testa, R.; Giardinà, D. *Med. Chem. Res.* **2004**, *13*, 190–199.
32. Antonello, A.; Hrelia, P.; Leonardi, A.; Marucci, G.; Rosini, M.; Tarozzi, A.; Tumiatti, V.; Mechiorre, C. *J. Med. Chem.* **2005**, *48*, 28–31.
33. Beecham, A. *J. Am. Chem. Soc.* **1967**, *139*, 553–570.
34. Fisichella, S.; Alberghina, G. *J. Chem. Soc., Perkin Trans. 2* **1978**, *6*, 567–571.
35. Manly, D. G.; Amstutz, E. D. *J. Org. Chem.* **1956**, *21*, 516–519.
36. Reiter, L. A. U.S. Patent 4,814,341, 1989. *Chem. Abstr.* **1989**, *111*, 77998z.
37. Boatman, R. J.; Whitlock, H. W. *J. Org. Chem.* **1976**, *41*, 3050–3051.
38. Wingfield, H. N., Jr.; Harlan, W. R.; Hanmer, H. R. *J. Am. Chem. Soc.* **1953**, *75*, 4364.
39. De Lean, A.; Munson, P. J.; Rodbard, D. *Am. J. Physiol.* **1978**, *235*, E97–E102.