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Searching for cyclazosin analogues as α_{1B}-adrenoceptor antagonists

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Abstract

A series of quinazoline derivatives, **2**–**20**, structurally related to the racemic α_1-adrenoceptor antagonist cyclazosin (**1**), were synthesized and evaluated for their functional antagonism at α_1- and α_2-adrenoceptors and for their binding affinity at human cloned α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes. They displayed, like **1**, preferential antagonism and selectivity for α_1 versus α_2-adrenoceptors. Compounds **10**, **13**, and **18** showed high potency at α_1-adrenoceptors similar to that of **1** (pK_B values 8.47–8.89 versus 8.67), whereas **13** and **15** were endowed with the highest α_1-adrenoceptor selectivity, only 3- to 4-fold lower than that of **1**. In binding experiments, all of the compounds displayed an affinity practically similar to that found for **1**, with the exception of **19** and **20** that were definitely less potent. The *s*-triazine analogue **18** was the most potent of the series with pK_i values of 10.15 (α_{1a}), 10.22 (α_{1b}) and 10.40 (α_{1d}), resulting 77-fold more potent than **1** at α_{1a}-adrenoceptors. In addition, the majority of compounds, like prototype **1**, showed the same trend of preferential affinity for α_{1d}- and α_{1b}-adrenoceptors that α_{1a}-subtype. In conclusion, we identified compounds **2**–**5**, **10**, **12** and **13**, bearing either an aliphatic- or an arylalkyl- or aryloxyalkyl-acyl function, with an interesting subtype-selectivity profile, which makes them suitable candidates for their resolution as enantiomers structurally related to (+)-cyclazosin.
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1. Introduction

α_1-Adrenoceptors constitute a heterogeneous family of receptors belonging to the superfamily of G-protein coupled receptors. At present, three native and functional subtypes, α_{1A}, α_{1B} and α_{1D}, can be pharmacologically distinguished which exhibit equivalency to the cloned α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors expressed in various tissues and cell lines [1]. Also 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 own therapeutic interest because of their important role in control of blood pressure and contraction and growth of smooth and cardiac muscle [4]. However, the functional role of specific α_1-adrenoceptor subtypes is not completely defined. Many pharmacological evidences indicate that the α_{1A}-adrenoceptor is the most important subtype in mediating the human prostate smooth muscle contraction making it a potential important target for treatment of benign prostatic hypertrophy (BPH) [5]. In addition, functional studies seem to indicate that, in animal species, the α_{1A}- and α_{1D}-adrenoceptors are those mainly involved in the contraction of main arteries, whereas the α_{1B}-subtype controls the small resistance vessels [6]. On the contrary, available information regarding α_1-adrenoceptor subtypes mediating vasoconstriction in humans is still very scarce [6].

Thus, the quantitative evaluation of subtype distribution and the definition of the proper functional role in different tissues need further studies with new potent and subtype-selective α_1-adrenoceptor ligands. Particu-

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larly, selective α_{1B}- and α_{1D}-antagonists are needed because, in comparison with α_{1A} antagonists, only a few of them are available.

With respect to α_{1B}-selective ligands, besides the alkylating agent chloroethylclonidine (CEC) [7], the compounds spiperone, risperidone and AH11110A were first indicated as reversible selective ligands, but their selectivity was lowered or not confirmed in subsequent experiments [8]. In contrast, the chiral 2,4-diaminoquinazoline compound L-765,314 was shown to be α_{1B}-selective both in binding and functional experiments [9]. In addition, we recently discovered [10] an other chiral quinazoline derivative, (+)-cyclazosin, endowed with high binding affinity (pK_i = 9.16–9.87) and selectivity for native and cloned $\alpha_{1B/b}$-adrenoceptor with respect to the $\alpha_{1A/a}$- and α_{1d}-subtypes (up to 90- and 40-fold, respectively). However, a functional study in isolated rat tissues reported that (+)-cyclazosin was endowed with low antagonist activity at all three α_1-adrenoceptors, while being unexpectedly devoid of selectivity for the α_{1B}-subtype [11]. At present, a further pharmacological investigation is in progress to clarify the discrepancy observed between binding and functional results obtained for (+)-cyclazosin. In comparison with the (+)-enantiomer, racemic cyclazosin, ([4-(4-amino-6,7-dimethoxyquinazolin-2-yl)-*cis*-perhydroquinoxalin-1-yl]furan-2-ylmethanone), (**1**) (Fig. 1) displayed a moderate selectivity towards two specific subtypes. In particular it showed a preferential binding affinity for cloned α_{1d}- and α_{1b}- adrenoceptors relative to the α_{1a}-subtype both in animal and human species (10- to 15-fold [12] and 17- to 32-fold [13], respectively).

The aim of present work was to search for new (+)-cyclazosin analogues possibly endowed with increased affinity and selectivity for α_{1b}-adrenoceptors. The approach to this project consisted in the synthesis and the pharmacological evaluation at α_1-adrenoceptor subtypes of a number of racemic compounds, bearing different substituents as an alternative to the 2-furoyl moiety of cyclazosin. Among these racemic derivatives, molecules displaying a pharmacological profile comparable or, hopefully, better than that of racemic cyclazosin will be selected for a subsequent development as optically pure enantiomers.

Here we report the synthesis and the pharmacological data of racemic cyclazosin analogues (**2**–**20**), in which the 2-furoyl moiety of cyclazosin has been exchanged for aliphatic and alicyclic acyl groups (**2**–**6**), a benzoyl function (**7**), a series of substituted and non-substituted arylalkyl-, benzyloxy- or phenoxyalkyl-carbonyl groups (**8**–**13**), 5-methyl-2-furyl-, tetrahydro-2-furyl-, chroman-2-yl-carbonyl groups (**14**–**16**), disubstituted *s*-triazines (**17** and **18**), an hydrogen atom (**19**), or a methyl group (**20**) (Fig. 1).

2. Chemistry

As shown in Scheme 1, the 6,7-dimemoxy-2-*cis*-perhydro-1-quinoxalinyl-4-quinazolinamine **19** was first synthesized and used as common precursor for all other compounds. It was obtained as already reported [14] by reaction of 2-chloro-6,7-dimethoxy-4-quinazolinylamine with the *cis*-perhydroquinoxaline [15]. Compounds **2**–**11**, **12**–**15** [14], and **16** were synthesized by acylation of **19** with appropriate acyl chloride in dichloromethane and in presence of triethylamine. Most of acyl chlorides were commercial, other were synthesized as reported in literature or by standard procedure. The alkylation of **19** with cyanuric acid and with 2-chloro-4,6-dimethoxy-*s*-triazine [16], in basic medium, gave the respective triazine analogues **17** and **18**. Finally, the methyl derivative **20** was synthesized, by reductive alkylation of **19** with formaldehyde and sodium cyanoborohydride, in acetonitrile. With the exception of **17** and **18** all compounds were obtained as hydrochloride salt and purified by crystallization with proper solvent.

3. Biology

3.1. Functional experiments

Compounds **2**–**20** were studied in animal isolated tissues in order to test their α-adrenoceptor antagonist activity and selectivity, in comparison with cyclazosin used as reference. The α_1-adrenoceptor antagonism was assessed evaluating the inhibition of the noradrenaline (NA) induced contraction of guinea pig spleen, a tissue expressing the α_{1B}-subtype [17]. The α_2-adrenoceptor blocking activity was determined by antagonism of the clonidine-induced inhibition of the twitch response on field-stimulated prostatic portion of rat vas deferens [18,19].

The α_1-adrenoceptor antagonist potency was expressed as pA_2 values according to Arunlakshana and Schild [20,21]. 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 [22]. The pK_B values, representing the negative logarithm of the apparent dissociation constant K_B, were calculated at the lowest antagonist concentration giving a significant rightward shift of the NA concentration–response curve.

At α_2-adrenoceptor the antagonism (pK_B) was evaluated at a single concentration for compound **2**, whereas only an estimated affinity was given for the majority of compounds because of their low activity.

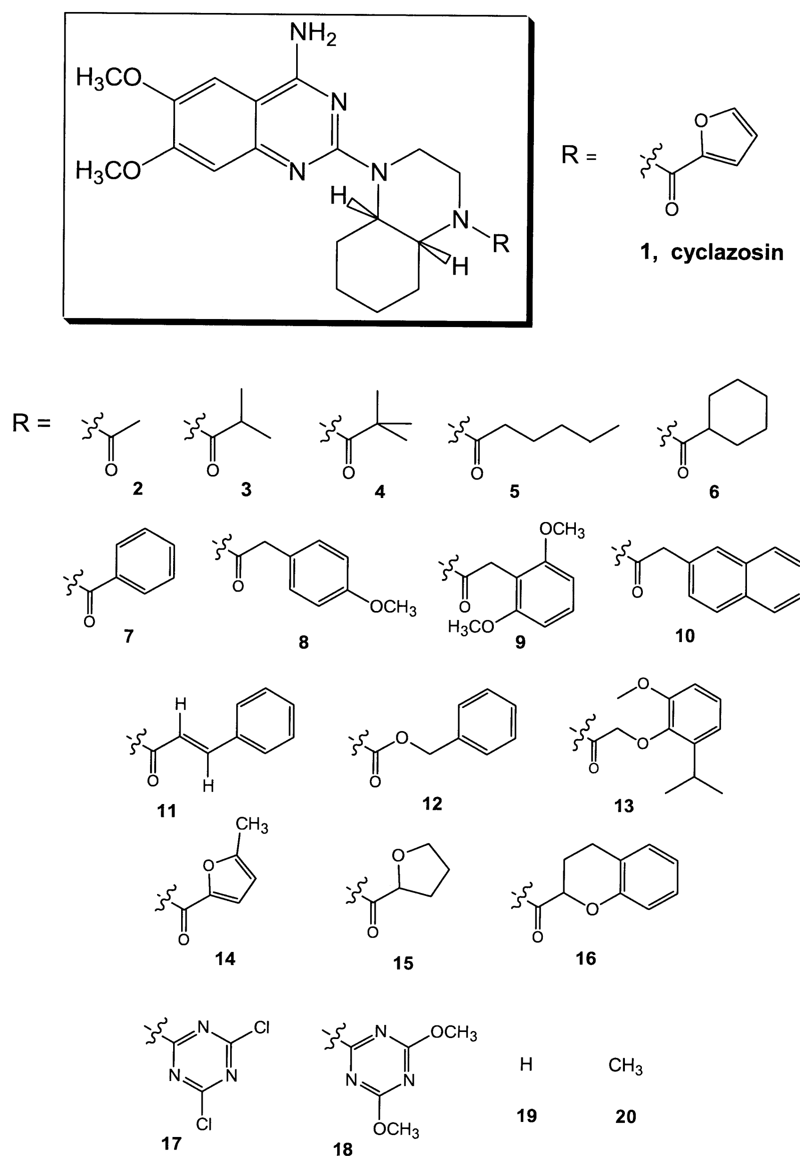


Fig. 1.

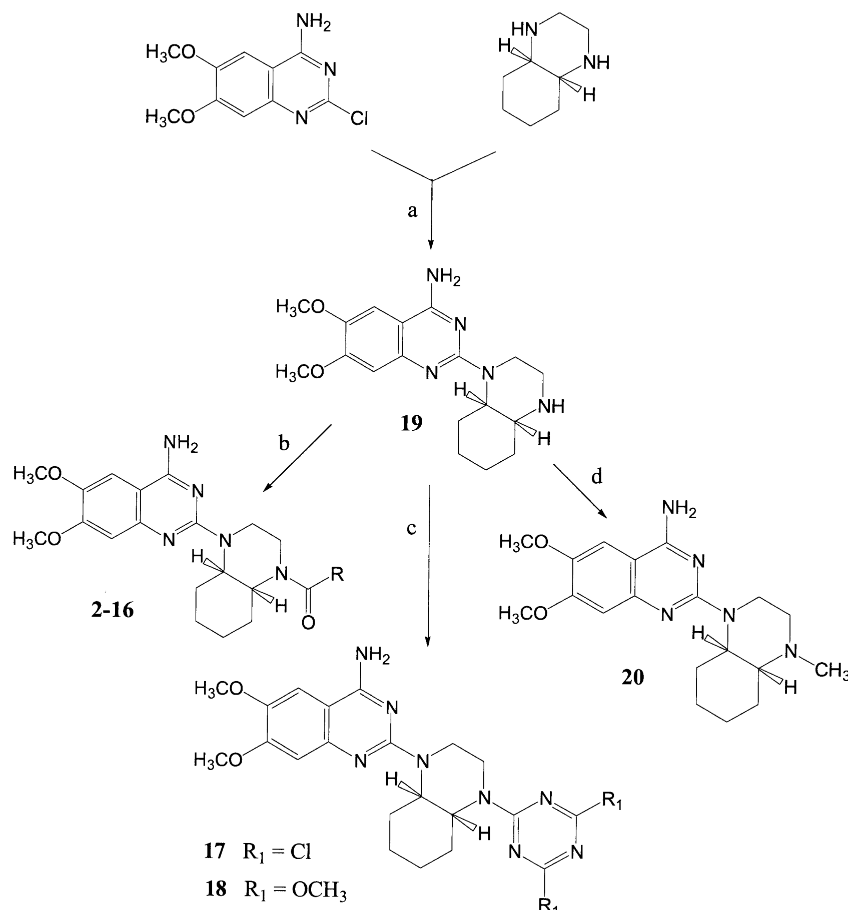
3.2. Binding studies

The pharmacological profile of studied compounds was also evaluated in binding assays on cloned α_1-adrenoceptors, in comparison with the standard cyclazosin. Competition assays were performed using [^{3}H]prazosin to label cloned human α_1-adrenoceptors on membranes prepared from chinese hamster ovary (CHO) cells expressing human α_{1a}-, α_{1b}- and α_{1d}-subtypes [23]. Binding affinities were expressed as pK_i values derived using the Cheng–Prusoff equation [24].

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. The pharmacological results are reported in Tables 1 and 2.

4. Results and discussion

Compounds **2**–**20** inhibited α_1-adrenoceptors in a non-competitive manner with the exception of **7**, **14** and **15**, which in the guinea pig spleen behaved as competitive antagonists, albeit in a narrow range of concentrations (10-fold) (Table 1). They displayed, like cyclazosin (**1**), a preferential antagonism and selectivity for α_1- versus α_2-adrenoceptors. The majority of compounds were less potent than **1** with pK_B values ranging



Scheme 1. Reagents: (a) *i*-AmOH, reflux; (b) RCOCl, Et_3N, CH_2Cl_2; (c) cyanuric chloride, Na_2CO_3, CH_2Cl_2 for **17**; 2-chloro-4,6-dimethoxy-*s*-triazine, Et_3N, CH_2Cl_2 for **18**; (d) 37% formaldehyde, $NaBH_3CN$, CH_3CN. Specifications for R in **2**–**16** are as reported in Fig. 1.

from 6.52 to 7.99. However, compounds **10**, **13**, and **18** showed high α_1-adrenoceptor antagonism similar to that displayed by **1** (pK_B = 8.89, 8.71, 8.47 and 8.67, respectively). Compounds **13** and **15** were the most selective antagonists for α_1-adrenoceptors.

Binding experiments on cloned human α_{1a}, α_{1b} and α_{1d}-adrenoceptor subtypes showed that all the acylated and *s*-triazine non-acylated cyclazosin analogues displayed an affinity similar to that found for **1** (Table 2).

Among the acylated analogues, the affinity at the α_{1a}-subtype was slightly higher (2- to 9-fold) than that of **1**, with the exception of the short-chain aliphatic acyl analogues **2**–**4** and the 2-naphtylmethyl-carbonyl compound **10**, which were almost equiactive to **1**. At the α_{1b}-subtype, the majority of compounds were practically as potent as **1**, whereas compounds **2**, **4**, **8** and **10** were slightly less active (4 to 8-fold). Similarly, most of the compounds displayed at α_{1d}-adrenoceptors an affinity similar to that of cyclazosin with the exception of **4** and **16**, which were about 5-fold less potent than **1**, and compound **13**, which resulted 3-fold more potent.

Concerning the non-acylated analogues **17**–**20**, *s*-triazines **17** and **18** showed high affinity, but not selectivity, at all three subtypes. Their affinity at α_{1b}- and α_{1d}-adrenoceptor was comparable with that of **1** and other acylated compounds but was 16- to 77-fold higher at α_{1a}-subtype. The 4,6-dimethoxy-substituted triazine **18** was the most potent compound of the series at α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors with pK_i values of 10.15, 10.22 and 10.40, respectively. On the contrary, the *N*-unsubstituted compound **19** and its *N*-methyl derivative **20** showed very low affinity at all three subtypes.

This latter result clearly suggests that a carboxamide function (**2**–**16**) or an electron rich moiety like an *s*-triazine ring (**17** and **18**) may play an important role in the interaction of these cyclazosin-related compounds with the receptor.

Altogether, the binding results indicate that the affinity for α_{1b}- and α_{1d}-adrenoceptors can be retained by exchanging the hydrogen atom of the piperazine NH function of compound **19** for a variety of substituents, supporting the view of a receptor binding site with no particular constraint for the substituent linked to the piperazine ring. On the contrary, the α_{1a}-adrenoceptor would express a more specific structural demand in the *N*-substituent for optimum interaction, as revealed by

Table 1
Functional α_1- and α_2-adrenoceptor antagonist activity of compounds **2-20**, and cyclazosin (**1**) as reference, at guinea pig spleen and prostatic rat vas deferens, respectively

Comp.	α Adrenoceptors		Selectivity ratio [c]
	pK_B α_1 versus NA [a]	pK_B α_2 versus clonidine [b]	α_1/α_2
1	8.67±0.06 [d]	4.90±0.03 [e]	5890
2	7.56±0.17	4.99±0.18 [e]	371
3	7.05±0.08	< 5	> 112
4	7.93±0.04	< 5	> 850
5	7.99±0.05	< 5.52	> 295
6	7.49±0.04	< 5.52	> 93
7	7.74±0.06 [f]	< 5.52	> 166
8	7.33±0.10	< 5.52	> 64
9	7.86±0.08	< 6	> 72
10	8.89±0.07	< 6	> 776
11	6.52±0.05	< 5.52	> 10
12	7.36±0.05	< 5.52	> 69
13	8.71±0.07	< 5.52	> 1549
14	7.95±0.03 [g]	< 5.52	> 269
15	7.77±0.02 [h]	< 4.52	> 1780
16	7.37±0.06	< 5.52	> 71
17	7.88±0.12 [i]	< 5.52	> 229
18	8.47±0.13	< 5.52	> 891
19	6.88±0.05	< 5	> 76
20	7.17±0.04	< 5	> 148

[a] pK_B values ±SE were calculated by the equation pK_B = log(DR − 1) − log[B] [22], at the lowest significant antagonist concentration: 1 μM (**3**–**5**, **11**, **19**, **20**), 0.1 μM (**2**, **6**, **8**, **12**, **16**, **17**), 0.03 μM (**9**), 0.01 μM (**1**, **3**, **13**, **18**), 0.003 μM (**10**), tested three to six times.
[b] Estimated pK_B values because compounds were inactive at low concentrations whereas at higher concentrations (from 3 to 100 μM) they gave inhibition of the twitch responses of the electrically stimulated tissue.
[c] Calculated by the antilog of the difference between pK_B values at α_1 and α_2-adrenoceptors.
[d] Calculated at the concentration 0.01 μM.
[e] Calculated at the concentration 30 μM.
[f] Normalized pA_2 value; $n = -1.05 \pm 0.21$.
[g] Normalized pA_2 value; $n = -0.95 \pm 0.12$.
[h] Normalized pA_2 value; $n = -0.94 \pm 0.10$.
[i] A concomitant 30% depression of NA curve was observed.

the high affinity observed for **17** and **18**, which bear a substituted *s*-triazine moiety.

Concerning the selectivity towards α_1-adrenoceptor subtypes, the majority of compounds showed a trend of preferential affinity for α_{1d}- and α_{1b}-adrenoceptors, in analogy with the precursor cyclazosin. In particular the acetyl and the benzyloxycarbonyl analogues, **2** and **12**, displayed a slight to moderate selectivity for α_{1d}-adrenoceptors over α_{1a}- and α_{1b}-subtypes (96- to 13-fold and 13- to 6-fold, respectively).

In conclusion, we identified compounds **2**–**5**, **10**, **12** and **13**, bearing either an aliphatic acyl function or an arylalkyl- or aryloxyalkyl-carbonyl group, that, in binding assays, showed an interesting subtype-selectivity profile, which makes them suitable candidates for resolution in the chiral form structurally related to (+)-cyclazosin.

5. Experimental

5.1. Chemistry

Melting points were taken in glass capillary tubes on a Buchi SMP-20 apparatus and are unconnected. 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 agreed with the calculated values within the range ±0.4%. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography, whereas the analytical control was made 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) petrol ether–ethyl acetate–methanol–28% ammonia (8:6:2:0.2); (B) ethyl acetate–methanol–chloroform (6:3:1); (C) ethyl acetate–petrol ether–methanol–7% ammonia (7:7:1:0.05); (D) cyclohexane–ethyl acetate–methanol–7% ammonia (6:3:1:0.05); (E) petrol ether–ethyl acetate–methanol–7% ammonia (9:7:1:0.1); (F) ethyl acetate–cyclohexane–methanol (6:3:1); (G) petrol ether–ethyl acetate–methanol–14% ammonia (8:8:2:0.2); (H) petrol ether–ethyl acetate–methanol–14% ammonia (8:5:0.6:0.025); (I) petrol ether–ethyl acetate–methanol–7% ammonia (8:4:1:0.05); (L) ethyl acetate–petrol ether–methanol–28% ammonia (8:8:2:0.1); (M) petrol ether–ethyl acetate–methanol–14% ammonia (8:6:2:0.1); (N) petrol ether–ethyl acetate–methanol–7% ammonia (8:6:2:0.2); (O) ethyl acetate–dichloromethane–acetone (5:3:2); (P) ethyl acetate–cyclohexane–methanol (6.5:3:0.5); (Q) chloroform–methanol (9:1); (R) petrol ether–chloroform–methanol–28% ammonia (8:6:2:0.15). 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, a PC IUPAC name generator, version 1997, Advanced Chemistry Development, Toronto, Canada.

Most of acyl chlorides were commercial, other were prepared as indicated in the literature. The 2-(2,6-dimethoxyphenyl)ethanoyl chloride and 2-(2-isopropyl-6-methoxyphenoxy)ethanoyl chloride were synthesized as usual with $SOCl_2$ in dry benzene starting from the corresponding carboxylic acid.

Table 2
Binding affinity constants, expressed as pK_i of compounds **2**–**20**, and cyclazosin (**1**) as reference, for human cloned α_1-adrenoceptor subtypes [a]

Comp	α_1-Adrenoceptors					
	pK_i			Selectivity ratio [b]		
	α_{1a}	α_{1b}	α_{1d}	α_{1d}/α_{1a}	α_{1b}/α_{1a}	α_{1d}/α_{1b}
1	8.26±0.05	9.49±0.01	9.77±0.04	31	17	2
2	7.74±0.08	8.61±0.06	9.72±0.01	96	7	13
3	8.29±0.03	9.15±0.04	9.64±0.03	22	7	3
4	8.07±0.10	8.92±0.06	9.06±0.02	10	7	1.5
5	8.77±0.04	9.74±0.07	9.92±0.01	14	9	1.5
6	9.17±0.01	9.68±0.03	9.77±0.03	4	3	1
7	9.13±0.04	9.57±.05	10.05±0.02	8	3	3
8	8.93±0.01	8.95±0.09	9.68±0.01	5	1	5
9	9.21±0.06	9.49±0.02	9.51±0.04	2	2	1
10	8.06±0.12	8.97±.02	9.36±0.06	20	8	2
11	8.89±0.03	9.38±0.05	9.74±0.02	7	3	2
12	8.83±0.01	9.19±0.04	9.96±0.03	13	2	6
13	9.11±0.06	9.79±0.04	10.30±0.10	16	5	3
14	8.90±0.02	9.62±0.07	9.96±0.01	11	5	2
15	8.54±0.02	9.38±0.01	9.42±0.02	8	7	1
16	8.85±0.05	9.42±0.01	9.14±0.07	2	4	0.5
17	9.46±0.03	8.86±0.11	9.62±0.04	1.5	0.2	6
18	10.15±0.01	10.22±0.02	10.40±0.02	2	1	1.5
19	< 6	7.00±0.01	6.96±0.05	> 9	> 10	1
20	7.05±0.08	7.13±0.01	7.45±0.05	2	1	2

[a] Equilibrium dissociation constants (K_i) were derived from IC_{50} values using the Cheng–Prusoff equation [24]. The affinity estimates were derived from displacement of $[^3H]$prazosin binding from α_1-adrenoceptors. pK_i values are the mean ±SE of two to three separate experiments each performed in triplicate.

[b] The selectivity ratio is the antilog of the difference between pK_i values at different α_1-adrenoceptor subtypes.

*5.1.1. 6,7-Dimethoxy-2-cis-perhydro-1-quinoxalinyl-4-quinazolinamine dihydrochloride (**19**)*

A mixture of 2-chloro-6,7-dimethoxy-4-quinazolinamine (7.85 g, 32.80 mmol), *cis*-perhydroquinoxaline (11.5 g, 82 mmol), triethylamine (13.3 g, 131 mmol) and dimethylamino pyridine (0.4 g, 3.2 mmol) was refluxed for 72 h in *i*-AmOH (80 ml) then the solvent removed in vacuo and the residue purified by column chromatography eluting with mixture of solvent A. The coarse solid was transformed into the hydrochloride salt and crystallized from MeOH–*i*-PrOH (1:1). Obtained 14.7 g (73%) of product as dihydrochloride, m.p.: 290–294 °C.^1H NMR (DMSO-d_6): δ 1.35–2.40 (m, 8H, H_{5-8} perhydroquinoxaline), 3.00–3.70 (m, 4H, H_{2-3} perhydroquinoxaline), 3.86 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.63–4.86 (m, 1H, H_{4a} perhydroquinoxaline), 4.92–5.12 (m, 1H, H_{8a} perhydroquinoxaline), 7.68 (s, 1H, arom), 7.65 (s, 1H, arom), 8.77 (s, br, 1H, NH exchangeable with D_2O), 9.00 (s, br, 1H, NH exchangeable with D_2O), 9.56 (s, br, 1H, NH exchangeable with D_2O), 9.95 (s, br, 1H, NH exchangeable with D_2O), 12.70 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{18}H_{25}N_5O_2 \cdot 2HCl \cdot 2.5H_2O$) (C, H, N).

*5.1.2. General procedure for the synthesis of **2**–**16***

The proper acyl chloride (1.47 mmol) was dissolved in dry CH_2Cl_2 (5 ml) and added dropwise to a stirred and cooled (0 °C) solution of **19** free base (0.5 g, 1.4 mmol) and Et_3N (0.2 g, 2.1 mmol) in dry CH_2C1_2 (10 ml). The mixture was stirred 3 h at room temperature (r.t.) then cooled at 0 °C for a night. The precipitate was collected and purified by column chromatography eluting with the appropriate solvent mixture. The eluted free base was transformed into the hydrochloride salt and crystallized from specific solvent. In the case of compounds **7** and **8** the initial precipitate obtained after cooling was transformed into the hydrochloride salt and crystallized. Concerning compound **13**, after 3 h stirring the reaction mixture was distilled and the residue purified by column chromatography.

*5.1.3. 1-[4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl]-1-ethanone hydrochloride (**2**)*

Acetyl chloride was used; eluting mixture B; 69% yield; m.p.: 212–215 °C (*i*-PrOH);^1H NMR (DMSO-d_6): δ 1.30–2.30 (m, 11H, H_{5-8} perhydroquinoxaline and CH_3CO), 3.60–4.40 (m, 11H, H_{2-3} and H_{4a} perhydroquinoxaline, OCH_3), 4.60–4.80 (m, 1H, H_{8a} perhydroquinoxaline), 7.65 (s, 1H, arom), 7.78 (s, 1H, arom), 8.64 (s, br, 1H, NH exchangeable with D_2O), 8.96 (s, br, 1H, NH exchangeable with D_2O), 12.21 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{20}H_{27}N_5O_3 \cdot HCl \cdot 0.25i\text{-}PrOH \cdot 2.5H_2O$) (C, H, N).

5.1.4. 1-[4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl]-2-methyl-1-propanone hydrochloride (**3**)

The 2-methylpropanoyl chloride was used; eluting mixture C; 22% yield; m.p.: 275–278 °C (EtOH);^{1}H NMR (DMSO-d_6): δ 0.98–1.12 (m, 6H, CH(CH_3)$_2$), 1.30–2.25 (m, 8H, H_{5-8} perhydroquinoxaline), 2.78–3.03 (m, 1H, CH(CH$_3$)$_2$), 3.65–4.15 (m, 10H, OCH_3 and H_{2-3} perhydroquinoxaline), 4.20–4.38 (m, 1H, H_{4a} perhydroquinoxaline), 4.55–4.72 (m, 1H, H_{8a} perhydroquinoxaline), 7.50 (s, 1H, arom), 7.73 (s, 1H, arom), 8.68 (s, br, 1H, NH exchangeable with D_2O), 8.88 (s, br, 1H, NH exchangeable with D_2O), 11.91 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{22}H_{31}N_5O_3 \cdot HCl \cdot H_2O$) (C, H, N).

5.1.5. 1-[4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl]-2,2-dimethyl-1-propanone hydrochloride (**4**)

The 2,2-dimethylpropanoyl chloride was used; eluting mixture C; 23% yield; m.p.: 308–310 °C (MeOH–i-PrOH);^{1}H NMR (DMSO-d_6): δ 1.20–2.00 (m, 16H, H_{5-8} perhydroquinoxaline and $C(CH_3)_3$), 2.10–2.30 (m, 1H, H_{5-8} perhydroquinoxaline), 3.65–4.00 (m, 9H, OCH_3 and H_{2-3} perhydroquinoxaline), 4.08–4.34 (m, 2H, H_{2-3} and H_{4a} perhydroquinoxaline), 4.53–4.75 (m, 1H, H_{8a} perhydroquinoxaline), 7.40 (s, 1H, arom), 7.70 (s, 1H, arom), 8.62 (s, br, 1H, NH exchangeable with D_2O), 8.81 (s, br, 1H, NH exchangeable with D_2O), 11.72 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{23}H_{33}N_5O_3 \cdot HCl \cdot 0.5H_2O \cdot 0.25i$-PrOH) (C, H, N).

5.1.6. 1-[4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl]-1-hexanone hydrochloride (**5**)

Hexanoyl chloride was used; eluting mixture D; 20% yield; m.p.: 215–217 °C (i-PrOH);^{1}H NMR (DMSO-d_6): δ 0.90–1.00 (m, 3H, CH_3), 1.12–2.45 (m, 16H, H_{5-8} perhydroquinoxaline and $CO(CH_2)_4$), 3.60–4.18 (m, 10H, H_{2-3} perhydroquinoxaline and OCH_3), 4.20–4.41 (m, 1H, H_{4a} perhydroquinoxaline), 4.55–4.78 (m, 1H, H_{8a} perhydroquinoxaline), 7.52 (s br, 1H, arom), 7.76 (s br, 1H, arom), 8.64 (s, br, 1H, NH exchangeable with D_2O), 8.89 (s, br, 1H, NH exchangeable with D_2O), 11.98 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{24}H_{35}N_5O_3 \cdot HCl \cdot 2H_2O$) (C, H, N).

5.1.7. [4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl](cyclohexyl)methanone hydrochloride (**6**)

Cyclohexyl chloride was used; eluting mixture E; 67% yield; m.p.: 283–286 °C (MeOH); ^{1}H NMR (DMSO-d_6): δ 1.00–2.22 (m, 18H, H_{5-8} perhydroquinoxaline and cyclohexyl), 2.52–2.71 (m, 1H, cyclohexyl), 3.62–4.15 (m, 10H, H_{2-3} perhydroquinoxaline and OCH_3), 4.24–4.38 (m, 1H, H_{4a} perhydroquinoxaline), 4.60–4.75 (m, 1H, H_{8a} perhydroquinoxaline), 7.53 (s, 1H, arom), 7.77 (s, 1H, arom), 8.68 (s, br, 1H, NH exchangeable with D_2O), 8.90 (s, br, 1H, NH exchangeable with D_2O), 11,96 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{25}H_{35}N_5O_3 \cdot HCl \cdot 0.25H_2O$) (C, H, N).

5.1.8. [4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl](phenyl)methanone hydrochloride (**7**)

Benzoyl chloride was used; 43% yield; m.p.: 280–282 °C (EtOH); ^{1}H NMR (DMSO-d_6): δ 1.40–1.61 (m, 4H, H_{5-8} perhydroquinoxaline), 1.70–2.12 (m, 4H, H_{5-8} perhydroquinoxaline), 3.70–4.19 (m, 10H, H_{2-3} perhydroquinoxaline, OCH_3), 4.32–4.44 (m, 1H, H_{4a} perhydroquinoxaline), 4.61–4.80 (m, 1H, H_{8a} perhydroquinoxaline), 7.43–7.62 (m, 6H, arom), 7.75 (s, 1H, arom), 8.67 (s, br, 1H, NH exchangeable with D_2O), 8.89 (s, br, 1H, NH exchangeable with D_2O), 11.74 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{25}H_{29}N_5O_3 \cdot HCl \cdot 0.5H_2O$) (C, H, N).

5.1.9. 1-[4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl]-2-(4-methoxyphenyl)-1-ethanone hydrochloride (**8**)

The 2-(4-methoxyphenyl)ethanoyl chloride was used; 52% yield; m.p.: 204–207 °C (EtOH); ^{1}H NMR (DMSO-d_6): δ 1.30–2.30 (m, 8H, H_{5-8} perhydroquinoxaline), 3.57–4.13 (m, 15H, H_{2-3} perhydroquinoxaline, OCH_3 and CH_2CO), 4.25–4.45 (m, 1H, H_{4a} perhydroquinoxaline), 4.53–4.72 (m, 1H, H_{8a} perhydroquinoxaline), 6.88 (d, $J = 9.0$ Hz, 2H, arom), 7.20 (d, $J = 9.0$ Hz, 2H, arom), 7.49 (s, br, 1H, arom), 7.77 (s, br, 1H, arom), 8.68 (s, br, 1H, NH exchangeable with D_2O), 8.90 (s, br, 1H, NH exchangeable with D_2O), 11.93 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{27}H_{33}N_5O_4 \cdot HCl \cdot 2H_2O$) (C, H, N).

5.1.10. 1-[4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl]-2-(2,6-dimethoxyphenyl)-1-ethanone hydrochloride (**9**)

The 2-(2,6-dimethoxyphenyl)ethanoyl chloride was synthesized from the 2-(2,6-dimethoxyphenyl)acetic acid [25] and $SOCl_2$ by standard method and used for reaction. Eluting mixture F; 70% yield; m.p.: 208–210 °C (i-PrOH). ^{1}H NMR (DMSO-d_6): δ 1.30–2.37 (m, 8H, H_{5-8} perhydroquinoxaline), 3.65 (s, 2H, CH_2CO), 3.68–4.18 (m, 16H, H_{2-3} perhydroquinoxaline and OCH_3), 4.22–4.40 (m, 1H, H_{4a} perhydroquinoxaline), 4.57–4.73 (m, 1H, H_{8a} perhydroquinoxaline), 6.62 (d, $J = 9.5$ Hz, 2H, arom), 7.21 (t, $J = 9.5$ Hz, 1H, arom), 7.50 (s, 1H, arom), 7.78 (s, 1H, arom), 8.69 (s, br, 1H, NH exchangeable with D_2O), 8.87 (s, br, 1H, NH exchangeable with D_2O), 11.90 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{28}H_{35}N_5O_5 \cdot HCl \cdot 2H_2O$) (C, H, N).

*5.1.11. [4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl](2-naphthyl)-1-ethanone hydrochloride (**10**)*

The 2-(2-naphthyl)ethanoyl chloride [26] was used; eluting mixture G, 10% yield, m.p.: 256–258 °C (MeOH); ^{1}H NMR (DMSO-d_6): δ 1.28–2.32 (m, 8H, H_{5-8} perhydroquinoxaline), 3.68–4.18 (m, 12H, H_{2-3} perhydroquinoxaline, OCH_3 and CH_2CO), 4.32–4.48 (m, 1H, H_{4a} perhydroquinoxaline), 4.60–4.76 (m, 1H, H_{8a} perhydroquinoxaline), 7.36–7.57 (m, 4H, arom), 7.70–7.97 (m, 5H, arom), 8.62 (s, br, 1H, NH exchangeable with D_2O), 8.90 (s, br, 1H, NH exchangeable with D_2O), 11.97 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{30}H_{33}N_5O_3 \cdot HCl \cdot 2H_2O$) (C, H, N).

*5.1.12. (E)-1-[4-(4-amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl]-3-phenyl-2-propen-1-one hydrochloride (**11**)*

The (*E*)-3-phenyl-2-propenoyl chloride was used; eluting mixture E; 41% yield; m.p.: 246–250 °C (EtOH–AcOEt, 1:1); ^{1}H NMR (DMSO-d_6): δ 1.35–2.37 (m, 8H, H_{5-8} perhydroquinoxaline), 3.78–4.12 (m, 9H, OCH_3 and H_{2-3} perhydroquinoxaline), 4.20–4.40 (m, 1H, H2.3 perhydroquinoxaline), 4.46–4.60 (m, 1H, H_{4a} perhydroquinoxaline), 4.63–4.80 (m, 1H, H_{8a} perhydroquinoxaline), 7.20 (d, $J = 16.7$ Hz, 1H, $C_6H_5CH{=}CH$), 7.32–7.48 (m, 4H, arom), 7.56 (d, $J =$ 16.7 Hz, 1H, $C_6H_5CH{=}CH$), 7.67–7.80 (m, 3H, arom), 8.70 (s, br, 1H, NH exchangeable with D_2O), 8.88 (s, br, 1H, NH exchangeable with D_2Q), 11.82 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{27}H_{31}N_5O_3 \cdot HCl \cdot 0.75H_2O$) (C, H, N).

*5.1.13. Benzyl 4-(4-amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxaline carboxylate hydrochloride (**12**)*

Benzyl chloromethanoate was used; eluting mixture H; 14% yield; m.p.: 243–245 °C (EtOH); ^{1}H NMR (DMSO-d_6): δ 1.20–2.50 (m, 8H, H_{5-8} perhydroquinoxaline), 3.60–4.30 (m, 11H, H_{2-3} and H_{4a} perhydroquinoxaline, OCH_3), 4.62–4.82 (m, 1H, H_{8a} perhydroquinoxaline), 5.18 (s, 2H, OCH_2), 7.25–7.42 (m, 5H, arom), 7.70 (s, 1H, arom), 7.80 (s, 1H, arom), 8.60 (s, br, 1H, NH exchangeable with D_2O), 8.95 (s, br, 1H, NH exchangeable with D_2O), 12.18 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{26}H_{31}N_5O_4 \cdot HCl \cdot 0.75H_2O$) (C, H, N).

*5.1.14. 1-[4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl]-2-(2-isopropyl-6-methoxyphenoxy)-1-ethanone hydrochloride (**13**)*

The 2-(2-isopropyl-6-methoxyphenoxy)ethanoyl chloride was synthesized from 2-(2-isopropyl-6-methoxyphenoxy)acetic acid [27] and $SOCl_2$ by standard method and used for reaction. Eluting mixture I; 21% yield; m.p.: 208–209 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6): δ 1.13 (d, $J = 6.90$ Hz, 6H, $(CH_3)_2CH$), 1.30–2.40 (m, 8H, H_{5-8} perhydroquinoxaline), 3.20–3.45 (m, 1H, $(CH_3)_2CH$), 3.70–3.95 (m, 11H, H_{2-3} perhydroquinoxaline and OCH_3), 4.00–4.30 (m, 2H, H_{2-3} perhydroquinoxaline), 4.31–4.43 (m, 1H, H_{4a} perhydroquinoxaline), 4.60 (m, 2H, $COCH_2$), 4.64–4.80 (m, 1H, H_{8a} perhydroquinoxaline), 6.83–6.95 (m, 2H, arom), 7.08 (t, $J = 7.90$ Hz, 1H, arom), 7.40 (s, 1H, arom), 7.75 (s, 1H, arom), 8.70 (s, br, 1H, NH exchangeable with D_2O), 8.88 (s br, 1H, NH exchangeable with D_2O), 11.77 (s br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{30}H_{39}N_5O_5 \cdot HCl \cdot 2.5H_2O$) (C, H, N).

*5.1.15. [4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl](5-methyl-2-furyl) methanone hydrochloride (**14**)*

The 5-methyl-2-furancarbonyl chloride [28] was used; eluting mixture L; 27% yield; m.p.: 220–223 °C (*i*-PrOH); ^{1}H NMR (DMSO-d_6): δ 1.20–2.10 (m, 7H, H_{5-8} perhydroquinoxaline), 2.30–2.47 (m, 4H, H_{5-8} perhydroquinoxaline and CH_3), 3.73–4.32 (m, 10H, H_{2-3} perhydroquinoxaline, OCH_3), 4.38–4.50 (m, 1H, H_{4a} perhydroquinoxaline), 4.57–4.75 (m, 1H, H_{8a} perhydroquinoxaline), 6.30 (m, 1H, H_4 furan), 7.05 (m, 1H, H_3 furan), 7.58 (s, 1H, arom), 7.76 (s, 1H, arom), 8.68 (s, br, 1H, NH exchangeable with D_2O), 8.90 (s, br, 1H, NH exchangeable with D_2O), 11.95 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{24}H_{29}N_5O_4 \cdot HCl \cdot 2.5H_2O$) (C, H, N).

*5.1.16. [4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl](tetrahydro-2-furanyl) methanone hydrochloride (**15**)*

Tetrahydro-2-furancarbonyl chloride [29] was used; eluting mixture M; 21% yield; m.p.: 220–223 °C (EtOH); ^{1}H NMR (DMSO-d_6): δ 1.24–2.35 (m, 12H, H_{5-8} perhydroquinoxaline and H_{3-4} tetrahydrofuran), 3.62–4.18 (m, 12H, H_{2-3} perhydroquinoxaline, H_5 tetrahydrofuran and OCH_3), 4.22–4.3 8 (m, 2H, H_{4a} perhydroquinoxaline and H_2 tetrahydrofuran), 4.55–4,80 (m, 1H, H_{8a} perhydroquinoxaline), 7.40 (s, 1H, arom), 7.70 (s, 1H, arom), 8.66 (s, br, 1H, NH exchangeable with D_2O), 8.85 (s, br, 1H, NH exchangeable with D_2O), 11,78 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{23}H_{31}N_5O_4 \cdot HCl \cdot 2.5H_2O$) (C, H, N).

*5.1.17. [4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-cis-perhydro-1-quinoxalinyl] (3,4-dihydro-2H-2-chromenyl) methanone hydrochloride (**16**)*

The 2-chromanecarbonyl chloride [30] was used; eluting mixture N; 44% yield; m.p.: 238–240 °C (MeOH); ^{1}H NMR (DMSO-d_6): δ 1.02–2.42 (m, 10H, H_{5-8} perhydroquinoxaline and $CHCH_2$ chromenyl), 2.68–2.95 (m, 2H, CH_2Ar chromenyl), 3.71–4.40 (m, 11H, H_{2-3} and H_{4a} perhydroquinoxaline, OCH_3), 4.60–4.92 (m, 1H, H_{8a} perhydroquinoxaline), 5.08–5.27 (m,

1H, CH chromenyl), 6.80–6.94 (m, 2H, arom), 7.02–7.20 (m, 2H, arom), 7.61 (s, 1H, arom), 7.75 (s, 1H, arom), 8.66 (s, br, 1H, NH exchangeable with D_2O), 8.91 (s, br, 1H, NH exchangeable with D_2O), 12.15 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{28}H_{33}N_5O_4 \cdot HCl \cdot 0.5H_2O$) (C, H, N).

5.1.18. 2-[4-(4,6-Dichloro-1,3,5-triazin-2-yl)-cis-perhydro-1-quinoxalinyl]-6,7 dimethoxy-4-quinazolinamine (17)

Compound **19** (0.5 g, 1.4 mmol) and Na_2CO_3 (0.16 g, 1.5 mmol) were added at 0–5 °C to a solution of 2,4,6-trichloro-1,3,5-triazine (0.36 g, 1.95 mmol) in water–acetone (14/10 v/v). The mixture was stirred at r.t. for 1 h then the organic solvent evaporated and the residue extracted with chloroform. The removal of dried solvent gave a crude product that was purified by column chromatography eluting with mixture O. Obtained 0.9 g (63%) of product; m.p.: 137–140 °C (dec); ^1H NMR ($CDCl_3$-d_6): δ 1.36–2.17 (m, 7H, H_{5-8} perhydroquinoxaline), 2.32–2.55 (m, 1H, H_{5-8} perhydroquinoxaline), 3.70–3.88 (m, 2H, H_{2-3} perhydroquinoxaline), 3.94 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.0–4.20 (m, 1H, H_{2-3} perhydroquinoxaline), 4.56–4.80 (m, 3H, H_{2-3}, H_{4a} and H_{8a} perhydroquinoxaline), 5.14 (s, br, 2H, NH exchangeable with D_2O), 6.81 (s, 1H, arom), 6.93 (s, 1H, arom). *Anal.* ($C_{21}H_{24}Cl_2N_8O_2 \cdot 0.5C_4H_8O_2$) (C, H, N).

5.1.19. 2-[4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-cis-perhydro-1-quinoxalinyl]-6,7-dimethoxy-4-quinazolinamine (18)

A solution of 2-chloro-4,6-dimethoxy-*s*-triazine [16] (0.31 g, 1.74 mmol) in CH_2Cl_2 (5 ml) was added dropwise to a stirred solution of **19** (0.5 g, 1.46 mmol) and Et_3N (0.26 g, 2.6 mmol) dissolved in CH_2Cl_2 (10 ml). The mixture was stirred at reflux for 3 h then cooled and left at 0 °C for a night. The crude precipitate was collected and purified by two consecutive column chromatography eluting with mixture P in the first and Q in the second. Obtained 0.14 g (18% yield) of product; m.p.: 147–150 °C (dec); ^1H NMR ($CDCl_3$-d_6): δ 1.34–2.12 (m, 7H, H_{5-8} perhydroquinoxaline), 2.30–2.51 (m, 1H, H_{5-8} perhydroquinoxaline), 3.70–3.88 (m, 2H, H_{2-3} perhydroquinoxaline), 3.91–4.01 (m, 12H, OCH_3), 4.10–4.34 (m, 1H, H_{2-3} perhydroquinoxaline), 4.48–4.73 (m, 3H, H_{2-3}, H_{4a} and H_{8a} perhydroquinoxaline), 5.08 (s, br, 2H, NH exchangeable with D_2O), 6.80 (s, 1H, arom), 6.92 (s, 1H, arom). *Anal.* ($C_{23}H_{30}N_8O_4 \cdot 0.5C_4H_8O_2$) (C, H, N).

5.1.20. 2-[4-Methyl-cis-perhydro-1-quinoxalinyl]-6,7-dimethoxy-4-quinazolinamine dihydrochloride (20)

A solution of 37% formaldehyde (0.11 g, 3.64 mmol) was added dropwise into a solution of **19** (0.25 g, 0.73 mmol) in acetonitrile (15 ml), followed of addition of $NaBH_3CN$ (0.073 g, 1.16 mmol) and acidification (pH 6.5) with acetic acid. The mixture was stirred at r.t. for 1 h then the solvent removed and the residue purified by column chromatography eluting with the mixture R. The free base was transformed into the hydrochloride salt and crystallized from MeOH–*i*-PrOH (1:1). Obtained 0.08 g (31%); m.p.: 290–293 °C; ^1H NMR (DMSO-d_6): δ 1.40–1.95 (m, 6H, H_{5-8} perhydroquinoxaline), 2.20–2.45 (m, 2H, H_{5-8} perhydroquinoxaline), 2.80 (s, 3H, CH_3), 3.10–3.70 (m, 4H, H_{2-3} perhydroquinoxaline), 3.87 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.70–4.95 (m, 1H, H_{4a} perhydroquinoxaline), 5.10–5.40 (m, 1H, H_{8a} perhydroquinoxaline), 7.80 (s, 1H, arom), 7.85 (s, 1H, arom), 8.78 (s, br, 1H, NH exchangeable with D_2O), 9.10 (s, br, 1H, NH exchangeable with D_2O), 11.20 (s, br, 1H, NH exchangeable with D_2O), 13.06 (s, br, 1H, NH exchangeable with D_2O). *Anal.* ($C_{19}H_{27}N_5O_2 \cdot 2HCl \cdot 3H_2O$) (C, H, N).

5.2. Biology

5.2.1. Functional antagonism in isolated tissues

Male guinea-pigs (250–300 g) and male albino rats (CD, BR, 125–150 g) were used for the experiments at α_1- and α_2-adrenoceptor, respectively. Animals were killed by cervical dislocation, the required organs isolated, freed from adhering connective tissue and set up rapidly, under a suitable tension, in 20 ml organ baths containing physiological salt solution (pH 7.4) 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 ca. 3-fold at each step, with each addition being made only after the response of 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 addition parallel experiments in which tissues did not receive any antagonist were run in order to check any variation in sensitivity.

5.2.2. Guinea-pig spleen

α_1-Adrenoceptor antagonism was determined on the spleen [17]. The tissue was removed and bisected longitudinally into two strips which were suspended in tissue baths containing Krebs solution of the following composition (mM): NaCl, 120; KCl, 5.5; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 20; glucose, 11. This solution contained also 0.1 μM cocaine hydrochloride and 1 μM (±) normetanephrine to prevent the neuronal uptake of (−)-NA and 1 μM (±)-propranolol hydrochloride to block the β-adrenoceptors. The spleen strips were placed under 1 g resting tension and equilibrated for 45 min. The cumulative concentration–response curves to (−)-NA were obtained

at 30 min intervals, the first one being discarded and the second one taken as control. The antagonist was allowed to equilibrate with the tissue for 30 min then a new concentration–response curve to the agonist was obtained. The results were expressed as dissociation constants.

5.2.3. Rat vas deferens

The α_2-adrenoceptor blocking activity was assessed on prostatic portion by antagonism to clonidine, which inhibits the twitch responses of the field-stimulated tissue by acting on the α_2-adrenoceptor [18,19]. A first clonidine dose–response curve, taken as control, was obtained cumulatively avoiding the inhibition of more than 90% of twitch responses, while the concentration of clonidine causing 100% inhibition was deduced from the second dose–response curve obtained from parallel experiments. Under these conditions it was possible to obtain a second dose–response curve, which was not significantly different from the first one. Thus, after incubation with antagonist for 30 min, a dose–response curve was obtained. The results were expressed as dissociation constants.

5.2.4. Radioligand binding assays at cloned receptors

Competition binding assays to cloned human α_1-adrenoceptor subtypes were performed in membrane preparations from CHO cell lines transfected by electroporation with DNA expressing the gene encoding each α_1-adrenoceptor subtypes. Cloning and stable expression of the human α_1-adrenoceptor gene was performed as previously described [23]. CHO cells membranes (30 μg proteins) were incubated in 50 mM Tris–HCl buffer, pH 7.4, with 0.1–0.4 nM [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.

5.2.5. Data analysis

In functional studies responses were expressed as percentage of the maximal contraction observed in the agonist concentration–response curve taken as control. Pharmacological computer programs analyzed the agonist concentration–response curves.

In the α_1-adrenoceptor antagonism, pA_2 values for compounds **7**, **14**, **15** were determined by Schild plots obtained by computer program calculations according to Arunlakshana and Schild [20]. The linear regressional analysis was based on equation: $pK_B = \log(DR-1) - \log[B]$, where B is the antagonist concentration and DR the dose-ratio as ratio of potency of the agonist (EC_{50}) in presence of antagonist and in its absence. DR values were obtained at three antagonist concentrations and each concentration was tested three to six times. Schild plots were constrained to the slope of -1, as requested by theory [21], since the experimental data generated a line with a slope not significantly different from the unity ($P > 0.05$). For all other tested compounds, the slope appeared to be significantly different from unity, therefore an affinity estimate, as pK_B value, was obtained using the above equation according to van Rossum [22]. The lowest active concentration of antagonist was used for this calculation.

Concerning the α_2-adrenoceptor antagonism the majority of compounds were inactive at low concentrations, however, higher concentrations could not be assayed because of the twitch inhibition of the electrically stimulated tissue. Thus, an experimental antagonist activity was evaluated only for compound **2** at its lower active concentration (30 μM). It was investigated four times and the result given as pK_B value according to van Rossum. For all other compounds only an estimated affinity (pK_B) was reported from the analysis of their behavior at tested concentrations. Data from functional assays are presented as the mean $\pm$SE of n experiments. Differences between mean values were tested for significance by Student's t-test. A P value < 0.05 was taken to indicate a statistically significant difference.

Binding data were analyzed using a non-linear curve-fitting program Allfit [31]. 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 an affinity constant (K_i) by the Cheng–Prusoff equation [24]: $K_i = IC_{50}/(1 + L/K_d)$, where L and K_d are the concentration and the equilibrium dissociation constant of the radioligand. Data are expressed as mean of pK_i values $\pm$SE of two to three separate experiments performed in triplicate.

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