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# Design, Synthesis, and Biological Activity of Prazosin-Related Antagonists. Role of the Piperazine and Furan Units of Prazosin on the Selectivity for $\alpha_1$-Adrenoreceptor Subtypes

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Prazosin-related quinazolines **4**−**20** were synthesized, and their biological profiles at $\alpha_1$-adrenoreceptor subtypes were assessed by functional experiments in isolated rat vas deferens ($\alpha_{1A}$), spleen ($\alpha_{1B}$), and aorta ($\alpha_{1D}$) and by binding assays in CHO cells expressing human cloned $\alpha_1$-adrenoreceptor subtypes. The replacement of piperazine and furan units of prazosin (**1**) by 1,6-hexanediamine and phenyl moieties, respectively, affording **3**−**20**, markedly affected both affinity and selectivity for $\alpha_1$-adrenoreceptor subtypes in functional experiments. Cystazosin (**3**), bearing a cystamine moiety, was a selective $\alpha_{1D}$-adrenoreceptor antagonist being 1 order of magnitude more potent at $\alpha_{1D}$-adrenoreceptors (p$A_2$, 8.54 ± 0.02) than at the $\alpha_{1A}$- (p$A_2$, 7.53 ± 0.01) and $\alpha_{1B}$-subtypes (p$A_2$, 7.49 ± 0.01). The insertion of substituents on the furan ring of **3**, as in compounds **4** and **5**, did not improve the selectivity profile. The simultaneous replacement of both piperazine and furan rings of **1** gave **8** which resulted in a potent, selective $\alpha_{1B}$-adrenoreceptor antagonist (85- and 15-fold more potent than at $\alpha_{1A}$- and $\alpha_{1D}$-subtypes, respectively). The insertion of substituents on the benzene ring of **8** affected, according to the type and the position of the substituent, affinity and selectivity for $\alpha_1$-adrenoreceptors. Consequently, the insertion of appropriate substituents in the phenyl ring of **8** may represent the basis of designing new selective ligands for $\alpha_1$-adrenoreceptor subtypes. Interestingly, the finding that polyamines **11**, **16**, and **20**, bearing a 1,6-hexanediamine moiety, retained high affinity for $\alpha_1$-adrenoreceptor subtypes suggests that the substituent did not give rise to negative interactions with the receptor. Finally, binding assays performed with selected quinazolines (**2**, **3**, and **14**) produced affinity results, which were not in agreement with the selectivity profiles obtained from functional experiments. This rather surprising and unexpected finding may be explained by considering neutral and negative antagonism.

## Introduction

It is clear now that $\alpha_1$-adrenoreceptors are comprised of multiple subtypes that can be classified by both pharmacological and binding studies into at least three subtypes, that is $\alpha_{1A}(\alpha_{1a})$, $\alpha_{1B}(\alpha_{1b})$, and $\alpha_{1D}(\alpha_{1d})$, with upper and lower case subscripts being used to designate native or recombinant receptors, respectively.[1−3] However, the situation appears to be more complex as, in addition to $\alpha_{1A}$-, $\alpha_{1B}$-, and $\alpha_{1D}$-adrenoreceptor subtypes, which share a high affinity for prazosin, the existence of additional $\alpha_1$-adrenoreceptors has been proposed. These are called $\alpha_{1L}$-adrenoreceptors and are characterized by a low activity for prazosin. However, these receptors have not been cloned yet and their characterization is still difficult.[4]

The existence of multiple $\alpha_1$-adrenoreceptor subtypes holds the promise of developing new molecules, which target only one receptor while not affecting others. Furthermore, the different localization of these receptor subtypes brought about the possibility of designing drugs that selectively interact with distinct subtypes, thus avoiding the occurrence of possible side effects. Current evidence indicates that rat submaxillary gland,[5] human liver,[6] and various tissues such as prostatic rat vas deferens,[7] rabbit prostate, and prostatic urethra[8] contain predominantly the $\alpha_{1A}$-adrenoreceptor, whereas rat liver and spleen[9] are considered $\alpha_{1B}$-adrenoreceptor preparations, and the $\alpha_{1D}$-adrenoreceptor mediates the contraction in rat aorta.[10,11] As a result, the effort to design agents selective for each of the three $\alpha_1$-adrenoreceptor subtypes has been an active area of research. Several relatively selective ligands for $\alpha_1$-adrenoreceptors are now available.[12] For example, SNAP 5089,[12] KMD-3213,[13] Rec 15/2739,[14] RS-17053,[15] and (−)-mephendioxan[16] are selective for $\alpha_{1A}$-adrenoreceptors, (+)-cyclazosin[17] and L-765,314[18] are selective for the $\alpha_{1B}$-subtype, and BMY-7378[19] and cystazosin[20] are selective for the $\alpha_{1D}$-subtype. Whereas it has been demonstrated that $\alpha_{1A}$-adrenoreceptor antagonists can be useful in the treatment of benign prostatic hyperplasia, a potential therapeutic use for both $\alpha_{1B}$- and $\alpha_{1D}$-subtype antagonists has not been defined yet. Perhaps, the fact that only recently so-called selective $\alpha_1$-adrenoreceptor antagonists have become available has prevented the physiological roles of $\alpha_{1B}$- and $\alpha_{1D}$-adrenoreceptor subtypes in blood pressure control or other physiological functions from being revealed. It should be emphasized

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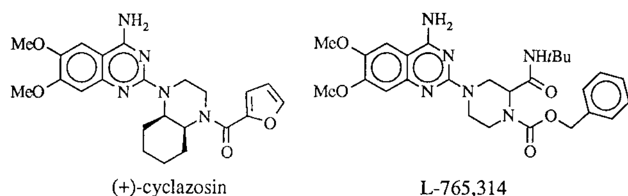
**Chart 1**




as well that the ideal selective ligands, which recognize only one among multiple receptor subtypes, are few and it remains a formidable challenge to medicinal chemists to realize useful drugs of this kind. For example, BMY-7378 is by far the most selective $\alpha_{1d}$-adrenoreceptor antagonist reported to date.[19] It displayed a 75- and 80-fold selectivity for the $\alpha_{1d}$-adrenoreceptor relative to $\alpha_{1b}$ and $\alpha_{1a}$ subtypes, respectively. However, BMY-7378 is more potent (14-fold) at 5-$HT_{1A}$ receptors and only 9-fold less potent at $D_2$ receptors with respect to $\alpha_{1d}$-adrenoceptors.[12]

## Design Rationale

The starting point of this investigation was the observation that modifying the piperazine ring of prazosin (**1**) may afford antagonists, which are able to differentiate among $\alpha_1$-adrenoreceptor subtypes. It was shown that the piperazine ring of prazosin is not essential for activity and can be replaced with an $\alpha,\omega$-alkanediamine chain.[21] Among a series of quinazolines bearing a polymethylene chain, compound **2** displayed the highest affinity for rat vas deferens $\alpha_1$-adrenoceptors, being even more potent than prazosin. It was suggested that the hexane chain of **2** might contribute to the binding by interacting with a lipophilic site located between the sites where quinazoline and furan rings interact.[21] More recently, it was demonstrated that the hexane chain could be constrained into a cyclohexyl moiety as in cyclazosin.[22] The (+)-enantiomer of cyclazosin (Chart 1) can be considered the first $\alpha_{1b}$-selective adrenoreceptor antagonist in binding assays, being about 100-fold selective versus the native $\alpha_{1A}$ and about 40-fold versus the recombinant $\alpha_{1a}$- and $\alpha_{1d}$-subtypes.[16] Following a design rationale similar to that leading to cyclazosin, Patane et al.[18] synthesized, by incorporating new structural elements into the prazosin piperazine moiety, L-765,314 (Chart 1) which turned out to be a selective $\alpha_{1b}$-adrenoreceptor antagonist.

Thus, the finding that the affinity profile of prazosin-related quinazolines can depend on the type of moiety linking the two nitrogen atoms of the piperazine ring of prazosin prompted us to further modify the structure of analogue **2**, in an attempt to improve the affinity and selectivity for different $\alpha_1$-adrenoreceptor subtypes. In particular, two types of structural modifications were performed on the structure of **2**, that is (a) replacement of the hexane spacer with a cystamine moiety and (b) insertion of substituents on the furan ring or its replacement by an (un)substituted phenyl unit. Thus, replacing the hexane chain of **2** by a cystamine moiety, which is a structural feature of benextramine,[23] an irreversible $\alpha$-adrenoreceptor antagonist, afforded cystazosin (**3**) which displayed an interesting selectivity profile in comparison with both (+)-cyclazosin and the carbon analogue **2**, owing to a significantly lower affinity for $\alpha_{1A}$- and $\alpha_{1B}$-adrenoreceptor subtypes relative to the $\alpha_{1D}$-subtype.[20] This observation formed the basis for further structural modifications. We thought that increasing the number of contacts between a ligand and its receptor would hopefully also increase receptor subtype selectivity. To this end we introduced a chloromethyl substituent at position 5 of furan ring of **3** and **2**, affording **4** and **6**, respectively, because it can be easily functionalized as in **5** and **7**. According to its properties, an amine function can be protonated at physiological pH giving rise to a possible additional interaction with a nucleophilic, complementary receptor group, which would increase the possibility to achieve receptor subtype selectivity. Subtle and unpredictable differences in the binding pockets may account for selectivity; thus incorporation of additional structural elements in the structure of a nonselective ligand may well lead to preferential recognition of a particular receptor subtype. Next, the furan ring of **2** was replaced by a phenyl ring affording **8**, which offered us the possibility to incorporate additional structural elements at different positions, yielding **9**−**20**. These 12 compounds allow us to investigate the effect of the substituents not only upon the affinity but also on the selectivity for $\alpha_1$-adrenoreceptor subtypes. The design strategy for our compounds is shown in Figure 1.

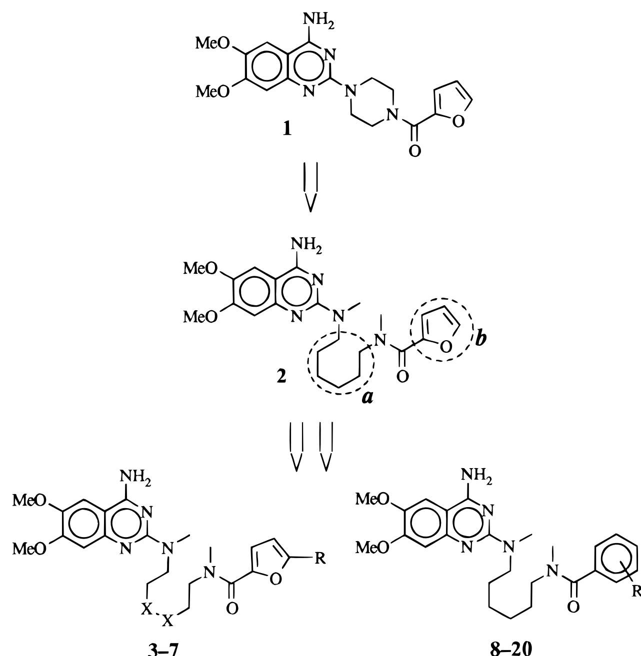



**Figure 1.** Design strategy for the synthesis of quinazolines **3**−**20** by replacing the hexane spacer and/or the furan ring of **2**, an open analogue of prazosin (**1**), by (a) a cystamine moiety and (b) an (un)substituted phenyl ring, respectively.

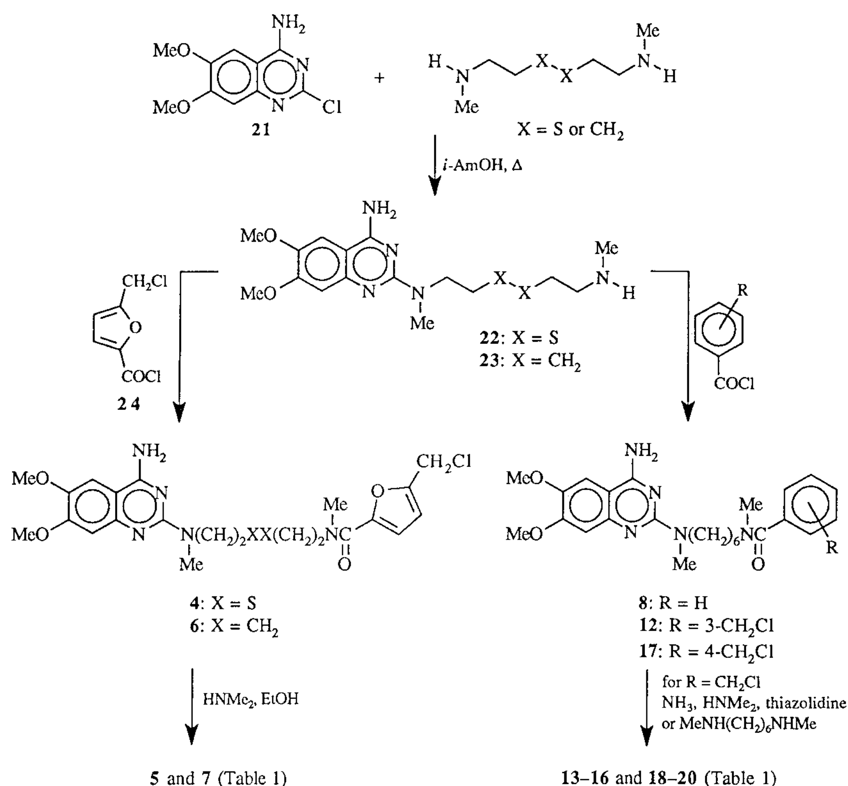
We describe here the synthesis and the pharmacological profile of quinazolines **4**−**20** in functional and binding experiments in comparison with prototypes prazosin (**1**) and its open analogues **2** and **3**.

A preliminary communication dealing with cystazosin (**3**) has been published recently.[20]

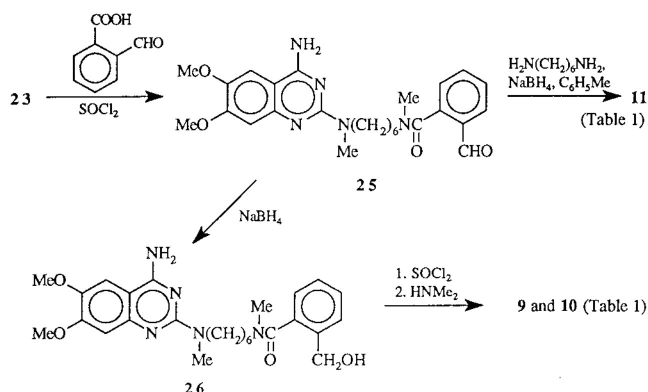
## Chemistry

The new compounds were synthesized by standard methods as illustrated in Schemes 1 and 2 and characterized by $^1H$ NMR, IR, and elemental analysis.

**Scheme 1**




**Scheme 2**




4-Amino-2-chloro-6,7-dimethoxyquinazoline (**21**) was the common starting material. Adapting the procedure reported for **23**,[24] compound **22** was obtained by aromatic nucleophilic substitution of *N,N*-dimethylcystamine[20] on **21**. The reaction of **22** and **23** with 5-chloromethyl-2-furoyl chloride (**24**)[25] afforded **4** and **6**, respectively, which were transformed by reaction with dimethylamine into the corresponding *N,N*-dimethylamino analogues **5** and **7** (Scheme 1). Compound **8** was synthesized by reaction of **23** and benzoyl chloride. Similarly, **12** and **17** were obtained from **23** and 3- or 4-chloromethylbenzoyl chloride, respectively. Chlorides **12** and **17** were reacted with dimethylamine, thiazolidine, or *N,N*-dimethyl-1,6-hexanediamine[26] to give the corresponding amines **13**−**16** and **18**−**20**.

Finally, 2-substituted derivatives **9**−**11** were synthesized following the synthetic pathway shown in Scheme 2. Intermediate **25** was obtained through amidation of **23** with 2-formyl-benzoyl chloride, which was generated in situ by treating 2-formylbenzoic acid with $SOCl_2$. Reductive amination of **25** by 1,6-hexanediamine afforded **11** whereas reduction of **25** with $NaBH_4$ gave the corresponding alcohol **26**. The latter compound was transformed into the chloride **9** which, in turn, was alkylated to the amine **10**.

## Biology

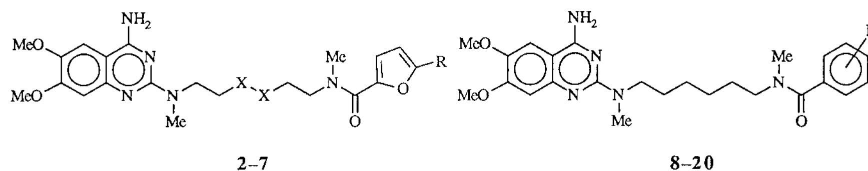
**Functional Studies.** The pharmacological profile of prazosin-related quinazolines **2**−**20** was evaluated at $\alpha_1$-adrenoreceptor subtypes and $\alpha_2$-adrenoreceptors on different isolated tissues using prazosin (**1**) and BMY-7378 as standard compounds. $\alpha_1$-Adrenoreceptor subtypes blocking activity was assessed by antagonism of (−)-noradrenaline-induced contraction of prostatic vas deferens ($\alpha_{1A}$)[27] or thoracic aorta ($\alpha_{1D}$)[28] and by antagonism of (−)-phenylephrine-induced contraction of spleen ($\alpha_{1B}$),[28] while $\alpha_2$-adrenoreceptor blocking activity was determined by antagonism of the clonidine-induced depression of the twitch responses of the field-stimulated prostatic portion of rat vas deferens. The potencies of the drugs were expressed as $pA_2$ values.[29−31]

**Binding Experiments.** Receptor subtype selectivity of few prazosin-related quinazolines was further determined by employing receptor binding assays. [$^3$H]-Prazosin was used to label cloned human $\alpha_1$-adrenoreceptors expressed in Chinese hamster ovary (CHO) cells.[32] Furthermore, [$^3$H]rauwolscine and [$^3$H]spiperone were used to label $\alpha_2$-adrenoreceptors in rat cortex and $D_2$ receptors in rat striatum, respectively, whereas [$^3$H]8-hydroxy-2-(di-*n*-propylamino)tetralin ([$^3$H]8-OH-DPAT) was the radioligand to label cloned human 5-$HT_{1A}$ receptors which were expressed in HeLa cells.[33,34]

## Results and Discussion

The biological activity expressed as $pA_2$ values, at $\alpha_1$-adrenoreceptor subtypes and $\alpha_2$-adrenoreceptors of com-

**Table 1.** Antagonist Affinities, Expressed as p$A_2$, of **2**−**20** at $\alpha_1$- and $\alpha_2$-Adrenoreceptors on Isolated Tissue from the Rat, Namely, Prostatic Vas Deferens ($\alpha_{1A}$ and $\alpha_2$), Spleen ($\alpha_{1B}$), and Thoracic Aorta ($\alpha_{1D}$), in Comparison to Reference Compounds Prazosin (**1**) and BMY-7378




| no | X | R | p$A_2$[a] | | | |
|---|---|---|---|---|---|---|
| | | | $\alpha_{1A}$ | $\alpha_{1B}$ | $\alpha_{1D}$ | $\alpha_2$[b] |
| **1** | (prazosin) | | 8.60 ± 0.07 | 8.99 ± 0.01 | 8.91 ± 0.04 | 5.43 ± 0.13 |
| **2** | $CH_2$ | H | 9.04 ± 0.02 | 9.84 ± 0.01 | 9.19 ± 0.03 | 6.66 ± 0.03 |
| **3** | S | H | 7.53 ± 0.01 | 7.49 ± 0.01 | 8.54 ± 0.02 | <5 |
| **4** | S | $CH_2Cl$ | 7.78 ± 0.07 | 7.37 ± 0.05 | 8.03 ± 0.09 | <5 |
| **5** | S | $CH_2NMe_2$ | 6.70 ± 0.08 | 7.27 ± 0.03 | 8.22 ± 0.06 | <5 |
| **6** | $CH_2$ | $CH_2Cl$ | 8.17 ± 0.02 | 8.97 ± 0.06 | 9.39 ± 0.02 | <5 |
| **7** | $CH_2$ | $CH_2NMe_2$ | 7.41 ± 0.04 | 8.62 ± 0.02 | 8.23 ± 0.01 | <5 |
| **8** | | H | 7.42 ± 0.03 | 9.35 ± 0.02 | 8.16 ± 0.02 | 6.52 ± 0.05 |
| **9** | | 2-$CH_2Cl$ | 6.38 ± 0.05 | 8.73 ± 0.04 | 8.55 ± 0.02 | 5.31 ± 0.01 |
| **10** | | 2-$CH_2NMe_2$ | 7.04 ± 0.01 | 7.88 ± 0.04 | 8.26 ± 0.02 | <5 |
| **11** | | 2-$CH_2NH(CH_2)_6NH_2$ | 7.97 ± 0.01 | 9.17 ± 0.02 | 8.50 ± 0.04 | 5.47 ± 0.01 |
| **12** | | 3-$CH_2Cl$ | 7.10 ± 0.01 | 7.18 ± 0.01 | 7.29 ± 0.05 | <5 |
| **13** | | 3-$CH_2NH_2$ | 7.32 ± 0.01 | 8.61 ± 0.02 | 8.69 ± 0.01 | 5.44 ± 0.01 |
| **14** | | 3-$CH_2NMe_2$ | 7.26 ± 0.08 | 7.43 ± 0.02 | 8.26 ± 0.03 | <5 |
| **15** | | 3-$CH_2N$ (S ring) | 7.82 ± 0.09 | 8.03 ± 0.05 | 8.51 ± 0.06 | 5.94 ± 0.03 |
| **16** | | 3-$CH_2N(Me)(CH_2)_6NHMe$ | 8.05 ± 0.07 | 7.71 ± 0.05 | 7.84 ± 0.04 | <5 |
| **17** | | 4-$CH_2Cl$ | 7.11 ± 0.02 | 9.15 ± 0.04 | 7.71 ± 0.01 | 5.54 ± 0.02 |
| **18** | | 4-$CH_2NMe_2$ | 7.14 ± 0.01 | 8.53 ± 0.01 | 7.86 ± 0.08 | 5.12 ± 0.06 |
| **19** | | 4-$CH_2N$ (S ring) | 7.23 ± 0.10 | 8.01 ± 0.03 | 8.17 ± 0.03 | <5 |
| **20** | | 4-$CH_2N(Me)(CH_2)_6NHMe$ | 6.72 ± 0.04 | 9.21 ± 0.09 | 8.46 ± 0.07 | 5.60 ± 0.03 |
| BMY-7378 | | | 6.94 ± 0.08 | 7.55 ± 0.07 | 8.34 ± 0.05 | <5 |

[a] p$A_2$ values ± SE were calculated from Schild plots,[29] constrained to a slope of −1.0, unless otherwise specified.[30] p$A_2$ is the positive value of the intercept of the line derived by plotting log(DR − 1) vs log [antagonist]. The log(DR − 1) was calculated at least at three different antagonist concentrations, and each concentration was tested from four to six times. Dose−ratio (DR) values represent the ratio of the potency of the agonist ($EC_{50}$) in the presence of the antagonist and in its absence. Parallelism of dose−response curves was checked by linear regression, and the slopes were tested for significance ($p < 0.05$). [b] p$A_2$ values were calculated at only one concentration (10 $\mu$M) according to van Rossum.[31]

pounds used in the present study, is shown in Table 1. To make relevant considerations on structure−activity relationships, prototypes **1** and **2** and the reference compound BMY-7378 were included for comparison. All quinazolines behaved as competitive antagonists as revealed by the slopes of their Schild plots, which were not significantly different from unity ($p > 0.05$) (Table 1). By taking as a starting point the hexane analogue **2** of prazosin, it is possible to observe how affinity and selectivity for $\alpha_1$-adrenoreceptor subtypes can be markedly affected by replacing the hexane moiety and/or the furan ring by a cystamine unit and an (un)substituted phenyl unit, respectively. Interestingly, all prazosin-related compounds of the present investigation displayed a weak, if any, affinity for $\alpha_2$-adrenoreceptors in comparison with $\alpha_1$-adrenoreceptor subtypes.

An analysis of the results shown in Table 1 reveals that the replacement of the hexane spacer or the furan ring of **2** by a cystamine moiety or a phenyl group, affording **3** and **8**, respectively, caused a dramatic effect in the affinity profile for $\alpha_1$-adrenoreceptor subtypes as shown in Figure 2. Clearly, **2** is a very potent $\alpha_1$-adrenoreceptor antagonist but, at the same time, is not selective, displaying only a slight preference for the $\alpha_{1B}$-subtype. Interestingly, the structural modifications performed on **2** did not improve affinity for $\alpha_1$-adrenoreceptor subtypes but, what is more important, gave rise to selectivity. Thus, cystazosin (**3**) resulted in a selective $\alpha_{1D}$-adrenoreceptor antagonist, owing to a slight (4.5-fold) decrease in affinity for the $\alpha_{1D}$-subtype and a large drop in affinity (32- and 224-fold, respectively) for $\alpha_{1A}$- and $\alpha_{1B}$-subtypes in comparison with **2**. On the other

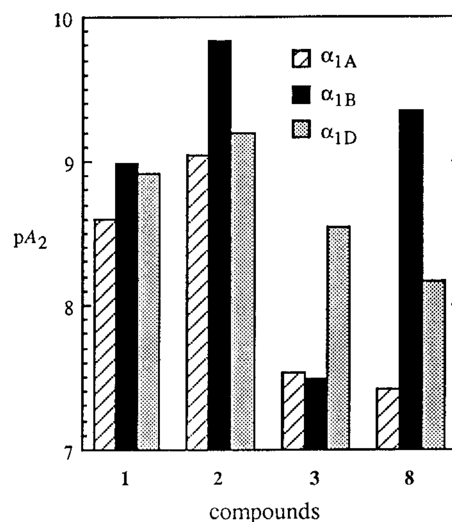



**Figure 2.** Affinity constants (p$A_2$) in rat prostatic vas deferens ($\alpha_{1A}$), spleen ($\alpha_{1B}$), and aorta ($\alpha_{1D}$) $\alpha_1$-adrenoreceptor subtypes of cystazosin (**3**) and **8** in comparison with prazosin (**1**) and **2**.

**Table 2.** Affinity Constants ($pK_i$) of **2**, **3**, and **14** for Cloned $\alpha_1$-Adrenoreceptor Subtypes and 5-$HT_{1A}$ Receptors, Native $\alpha_2$-Adrenoreceptors, and $D_2$ Receptors in Comparison to Reference Compounds[a]

| no. | $pK_i$, human cloned receptors | | | $pK_i$, native receptors (rat) | | |
|---|---|---|---|---|---|---|
| | $\alpha_{1a}$ | $\alpha_{1b}$ | $\alpha_{1d}$ | 5-$HT_{1A}$ | $\alpha_2$ (cerebral cortex) | $D_2$ (striatum) |
| **1** | 9.23 ± 0.07 | 9.39 ± 0.10 | 9.65 ± 0.08 | <6 | 6.80 ± 0.03 | <5 |
| **2** | 9.78 ± 0.04 | 9.96 ± 0.07 | 9.71 ± 0.01 | <6 | 7.00 ± 0.16 | 5.63 ± 0.02 |
| **3** | 9.38 ± 0.05 | 8.97 ± 0.09 | 9.14 ± 0.07 | <6 | 6.23 ± 0.07 | ≤5 |
| **14** | 9.49 ± 0.06 | 9.78 ± 0.06 | 9.61 ± 0.11 | 6.13 ± 0.04 | 7.72 ± 0.25 | 5.71 ± 0.15 |
| BY[b] | 6.36 ± 0.06 | 7.19 ± 0.04 | 8.89 ± 0.01 | 8.76 ± 0.28 | 5.98 ± 0.20 | 7.32 ± 0.04 |

[a] Values are the mean ± SE of at least three separate experiments performed in triplicate. The pseudo-Hill coefficients (nH) were not significantly different from unity ($p > 0.05$). Equilibrium inhibition constants ($K_i$) were derived using the Cheng–Prusoff equation.[40] Scatchard plots were linear or almost linear in all preparations tested. The affinity estimates were derived from displacement of [$^3$H]prazosin from $\alpha_1$-adrenoreceptors, [$^3$H]rauwolscine from $\alpha_2$-adrenoreceptors, [$^3$H]spiperone from $D_2$ receptors, and [$^3$H]8-hydroxy-2-(di-*n*-propylamino)tetraline from 5-$HT_{1A}$ receptors. [b] BY, BMY-7378.

hand, the phenyl analogue **8** displayed a significantly improved $\alpha_{1B}$-selectivity (85- and 15-fold relative to $\alpha_{1A}$- and $\alpha_{1D}$-subtypes, respectively), owing to a much larger decrease in affinity for both $\alpha_{1A}$- and $\alpha_{1D}$-subtypes than for the $\alpha_{1B}$-subtype in comparison with **2** (Figure 2).

The insertion of a 5-chloromethyl or a 5-*N,N*-dimethylaminomethyl substituent on the furan ring of **3**, affording **4** and **5**, respectively, did not improve the selectivity profile. The same structural modification performed on **2** to afford **6** and **7** caused a marked decrease in affinity which was more pronounced for 5-*N,N*-dimethylaminomethyl group. However, compound **6** was slightly more potent than **2** at $\alpha_{1D}$-adrenoreceptors while displaying a significantly lower affinity at $\alpha_{1A}$- and $\alpha_{1B}$-subtypes as revealed by its $pA_2$ values ($\alpha_{1A}$, 8.17 ± 0.02; $\alpha_{1B}$, 8.97 ± 0.06; $\alpha_{1D}$, 9.39 ± 0.02). Clearly, this finding suggests that appropriate substituents on the aromatic moiety may have a role in achieving receptor subtype selectivity.

Next, the insertion of a substituent on the benzene ring of **8**, affording **9**–**20**, affected differently, according to substituent type and position, the affinity and, as a consequence, the selectivity for $\alpha_1$-adrenoreceptor subtypes. The affinity profiles of **9**, **12**, and **17** reveal that a chloromethyl substituent at any position did not improve potency relative to **8**, the exception being **9** with a slightly higher affinity at $\alpha_{1D}$-adrenoreceptors. However, it should be noted that when the substituent is at position 4, as in **17**, both affinity and selectivity profiles were not markedly influenced relative to **8**, whereas at position 2 and 3 the affinity for $\alpha_1$-adrenoreceptor subtypes was affected in such a way that **9**, bearing a 2-substituent, was highly selective (224–148-fold) for $\alpha_{1B}$- and $\alpha_{1D}$-adrenoreceptors versus the $\alpha_{1A}$-subtype, and **12**, having a 3-substituent, resulted a weak and nonselective $\alpha_1$-adrenoreceptor antagonist.

Replacing chlorine atom of **9**, **12**, and **17** with an *N,N*-dimethylamino group afforded **10**, **14**, and **18**, respectively, which were almost as active as **8** at $\alpha_{1A}$- and $\alpha_{1D}$-adrenoreceptors while being significantly less potent at $\alpha_{1B}$-adrenoreceptors. Consequently, the selectivity profile of these analogues was different from that of **8** and parent chlorides. It turned out that amines **10** and **14** had a reversed selectivity profile relative to **8** and **9** or **12**, respectively, being more potent at $\alpha_{1D}$-adrenoreceptors, whereas amine **18** retained a selectivity profile similar to that of **8** and **17** owing to its higher affinity for $\alpha_{1B}$-adrenoreceptors.

The finding that inserting an *N,N*-dimethylaminomethyl moiety onto the phenyl group of **8** resulted into a marked effect on the selectivity profile prompted us to further modify the amine function, as in **13**, **15** and **19**. Thiazolidine analogues **15** and **19** did not display a better affinity profile than prototypes **14** and **18**, whereas **13** was as active as **14** at $\alpha_{1A}$- and $\alpha_{1D}$-adrenoreceptors, while showing a marked increase in affinity for the $\alpha_{1B}$-subtype.

Finally, replacing the chlorine atom of **9**, **12**, and **17** with a substituted 1,6-diaminohexane moiety, affording **11**, **16**, and **20**, respectively, resulted in a marked effect on the affinity for $\alpha_1$-adrenoreceptors. Polyamine **11** was as active as, if not more active than, prototype **8**, whereas analogue **16**, bearing the *N,N'*-dimethyl-1,6-diaminohexane moiety in position 3, turned out to be slightly or markedly less potent at $\alpha_{1D}$- and $\alpha_{1B}$-adrenoreceptors, respectively, while being more potent at the $\alpha_{1A}$-subtype than **8**, and **20** retained a comparable affinity at $\alpha_{1B}$-adrenoreceptors while being markedly less potent at the $\alpha_{1A}$-subtype and more potent at the $\alpha_{1D}$-subtype in comparison with **8**.

A most intriguing finding of the present investigation is the observation that polyamines **11**, **16**, and **20** retained high affinity for $\alpha_1$-adrenoreceptor subtypes, which suggests clearly that a 1,6-diaminohexane chain on the benzene ring did not give rise to negative interactions with the receptor. This observation may have relevance for the development of new quinazolines bearing a polyamine backbone on which additional substituents can be mounted to improve selectivity for $\alpha_1$-adrenoreceptor subtypes. Clearly, the site where the terminal aromatic ring of **8** interacts does not seem to present steric hindrance and particularly stringent requirements.

The binding affinities, expressed as $pK_i$ values, in CHO cells expressing human cloned $\alpha_1$-adrenoreceptor subtypes and HeLa cells expressing human 5-$HT_{1A}$ receptors and in membranes of rat cerebral cortex ($\alpha_2$-adrenoreceptors) and striatum ($D_2$ receptors) of quinazolines **2**, **3**, and **14** are shown in Table 2 in comparison with those of prazosin (**1**) and BMY-7378. As anticipated for cystazosin (**3**),[20] the results obtained in binding experiments did not show the same selectivity profile observed in functional assays. It can be seen that while binding affinities of reference compounds prazosin (**1**), **2**, and BMY-7378 are qualitatively and quantitatively comparable with $pA_2$ values derived from functional experiments, those observed for **3** and **14** are not in agreement at all from both a qualitative and a quantitative point of view with functional affinities. Both compounds were devoid of selectivity for $\alpha_1$-adrenore-

ceptor subtypes in binding assays, owing to a marked increase in affinity of about 2 orders of magnitude for $\alpha_{1a}$- and $\alpha_{1b}$-adrenoreceptors and of about 1 order of magnitude for the $\alpha_{1d}$-subtype. As a matter of fact, the theory states that the affinity of an antagonist assessed in functional assays should not differ from that determined in binding experiments using both native and recombinant receptors. For this reason competitive antagonists are considered better tools for receptor characterization and classification than agonists because for the latter ones, in addition to affinity, other pharmacological parameters must be taken into account.[35,36] Consequently, there is no apparent explanation for the discrepancy observed between our functional and binding results. However, very recently we discussed the possibility that if an antagonist does not adhere perfectly to the concept of neutral antagonism in the interaction with the receptor but behaves as a negative antagonist (inverse agonist), then its affinity may not be, as assumed by theory, system-independent, giving rise to affinity values which might be different according to the system employed for the determination.[35] In other words, as pointed out by Leff,[36] the use of inverse agonists as neutral antagonists may have, like agonists, problems since their estimated affinities are system-dependent. Thus, for inverse agonists the affinity values estimated in functional assays may not necessarily be comparable with those obtained in binding experiments. Interestingly, a survey of literature has revealed that some of the so-called competitive antagonists behave as inverse agonists when tested in the appropriate model. In the field of $\alpha_1$-adrenoreceptor antagonists, prazosin (**1**), WB 4101, and benoxathian were shown to be inverse agonists in a vascular model.[37] Thus, the difference, which is often observed for functional and binding affinities of antagonists, might be explained by the fact that these compounds are inverse agonists, and hence their affinity is system-dependent. Work is in progress to determine the nature of antagonism displayed by **3** and **14** and the other quinazolines of the present investigation.

## Experimental Section

**Chemistry.** Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR, electron impact (EI) mass, and $^1$H NMR spectra were recorded on Perkin-Elmer 297, VG 7070E, and Varian VXR 300 instruments, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), br s (broad singlet), d (doublet), t (triplet), or m (multiplet). Although the IR spectra data are not included (because of the lack of unusual features), they were obtained for all compounds reported and were consistent with the assigned structures. The elemental compositions of the compounds agreed to within ±0.4% of the calculated values. When the elemental analysis is not included, crude compounds were used in the next step without further purification. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. Compounds were named following IUPAC rules as applied by AUTONOM, a PC software for systematic naming in organic chemistry (Beilstein-Institut and Springer-Verlag).

**6,7-Dimethoxy-*N*²-methyl-*N*²-[2-(2-methylaminoethyldisulfanyl)ethyl]quinazoline-2,4-diamine (22).** A mixture of **21** (1.0 g, 4.2 mmol) and *N,N'*-dimethylcystamine[20] (3.5 g, 19.4 mmol) in *i*-AmOH (15 mL) was refluxed for 30 h. Removal of the solvent under reduced pressure gave a residue that was purified by chromatography. Eluting with methylene chloride–methanol–aqueous 30% ammonia (9:1:0.05) afforded 0.4 g (25% yield) of **22** as the free base: $^1$H NMR ($CDCl_3$) $\delta$ 2.03–2.39 (m, 2 + 1 exchangeable with $D_2O$), 2.43 (s, 3), 2.79–3.08 (m, 6), 3.21 (s, 3), 3.93 (s, 3), 3.97 (s, 3), 5.59 (br s, 2, exchangeable with $D_2O$), 6.81 (s, 1), 6.94 (s, 1).

**5-Chloromethyl-2-furoyl Chloride (24).** A solution of 5-hydroxymethylfuran-2-carboxylic acid[25] (0.3 g, 2.11 mmol) and $SOCl_2$ (1.5 mL, 10 mmol) in benzene (10 mL) was refluxed for 1 h. Removal of the solvent under reduced pressure afforded crude **24** in a quantitative yield.

**5-Chloromethylfuran-2-carboxylic Acid (2-{2-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]ethyldisulfanyl}ethyl)methylamide Hydrochloride (4).** A solution of **24** (0.19 g, 1.1 mmol) in THF (20 mL) was added dropwise to a solution of **22** (0.4 g, 1.1 mmol) in THF (15 mL). After the mixture was stirred at room temperature for 3 h, the solvent was removed under reduced pressure to give a residue that was purified by chromatography. Eluting with methylene chloride–methanol–aqueous 30% ammonia (9.5:0.5:0.05) afforded 0.34 g (65% yield) of **4** as the free base that was transformed into the hydrochloride salt: mp 170 °C (from EtOH/ether); $^1$H NMR (free base; $CDCl_3$) $\delta$ 2.96–3.42 (m, 10), 3.82–4.05 (m, 4), 3.93 (s, 3), 3.97 (s, 3), 4.61 (s, 2) 5.32 (br s, 2, exchangeable with $D_2O$), 6.48 (d, 1), 6.85 (m, 1), 6.92–7.07 (m, 2). Anal. ($C_{22}H_{29}Cl_2N_5O_4S$) C, H, N.

**5-Dimethylaminomethylfuran-2-carboxylic Acid (2-{2-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]ethyldisulfanyl}ethyl)methylamide Dioxalate (5).** A 5.6 M ethanolic solution of dimethylamine (4 mL) was added to a solution of **4** (0.08 g, 0.14 mmol) in absolute ethanol (10 mL). After the mixture was stirred at room temperature for 2 h, the solvent was removed under reduced pressure to give a residue that was purified by chromatography. Eluting with methylene chloride–methanol–aqueous 30% ammonia (9.5:0.5:0.04) afforded 0.03 g (40% yield) of **5** as the free base that was transformed into the dioxalate salt and crystallized: mp 106 °C (from EtOH/ether); $^1$H NMR (free base; $CDCl_3$) $\delta$ 2.25 (s, 6), 2.93–3.01 (m, 4), 3.20 (s, 6), 3.50 (s, 2), 3.73–3.93 (m, 10), 5.49 (br s, 2, exchangeable with $D_2O$), 6.28 (d, 1), 6.85–7.01 (m, 3). Anal. ($C_{26}H_{38}N_6O_{12}S_2$) C, H, N.

**5-Chloromethylfuran-2-carboxylic Acid {6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}methylamide Hydrochloride (6).** A solution of **24** (0.3 g, 1.68 mmol) in THF (20 mL) was added dropwise to a solution of **23**[24] (0.58 g, 1.7 mmol) in THF (15 mL). After the mixture was stirred at room temperature for 2 h, the solid was collected by filtration, washed with ether, and purified by chromatography. Eluting with methylene chloride–methanol–aqueous 30% ammonia (9.5:0.5:0.03) gave 0.16 g (20% yield) of **6** as the free base that was transformed into the hydrochloride salt and crystallized: mp 130 °C (from EtOH/ether); $^1$H NMR (free base; $CDCl_3$) $\delta$ 1.18–1.69 (m, 8), 2.95–3.29 (m, 7), 3.42–3.71 (m, 5), 3.87 (s, 3), 3.92 (s, 3), 4.56 (s, 2), 5.49 (br s, 2, exchangeable with $D_2O$), 6.33–6.43 (m, 1), 6.81–7.03 (m, 3). Anal. ($C_{24}H_{33}Cl_2N_5O_3$) C, H, N.

**5-Dimethylaminomethylfuran-2-carboxylic Acid {6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}methylamide Dihydrochloride (7).** This was synthesized from **6** and dimethylamine following the procedure described for **5** and purified by chromatography. Eluting with methylene chloride–methanol–aqueous 30% ammonia (9.5:0.5:0.03) afforded 0.08 g (80% yield) of **7** as the free base that was transformed into the dihydrochloride salt and crystallized: mp 145 °C (from EtOH/ether); $^1$H NMR (free base; $CDCl_3$) $\delta$ 1.18–1.69 (m, 8), 2.25 (s, 6), 3.01–3.22 (m, 7), 3.42–3.54 (m, 5), 3.66 (t, 2), 3.89 (s, 3), 3.94 (s, 3), 5.51 (br s, 2, exchangeable with $D_2O$), 6.27 (d, 1), 6.82–7.01 (m, 3). Anal. ($C_{26}H_{40}Cl_2N_6O_4$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-*N*-methylbenzamide Hydrochloride (8).** A solution of benzoyl chloride (0.06 mL, 0.5 mmol) in dioxane (10 mL) was added dropwise to a solution of **23**[24] (0.17 g, 0.5 mmol) in dioxane (15 mL). After the mixture was stirred at

room temperature for 4 h, the solid was filtered and washed with ether affording **8**: 90% yield; mp 133−135 °C (from EtOH/ether); $^1$H NMR (DMSO-$d_6$) δ 1.03−1.68 (m, 8), 2.86−2.94 (m, 3), 3.17−3.21 (m, 4), 3.32−3.44 (m, 1), 3.53−3.80 (m, 2), 3.84 (s, 3), 3.89 (s, 3), 7.31−7.43 (m, 5), 7.48 (s, 1), 7.72 (s, 1), 8.42 (br s, 1, exchangeable with $D_2O$), 11.85 (br s, 1, exchangeable with $D_2O$). Anal. ($C_{25}H_{34}ClN_5O_3$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-2-formyl-*N*-methylbenzamide (25).** 2-Formylbenzoyl chloride was synthesized in a quantitative yield from 2-formylbenzoic acid following the procedure described for **24**. A solution of this chloride (0.12 g, 0.72 mmol) in dioxane (5 mL) was added dropwise to a solution of **23** (0.25 g, 0.72 mmol) and triethylamine (0.1 mL, 0.72 mmol) in dioxane (10 mL). After the mixture was stirred at room temperature for 24 h, the solvent was removed under reduced pressure to give a residue that was purified by chromatography. Eluting with methylene chloride−methanol−aqueous 30% ammonia (9.5:0.5:0.04) afforded 0.3 g (87% yield) of **25** as the free base: $^1$H NMR ($CDCl_3$) δ 1.20−1.82 (m, 8), 3.15−3.22 (m, 6), 3.86−3.93 (m, 4), 4.03 (s, 3), 4.10 (s, 3), 5.82 (br s, 2, exchangeable with $D_2O$), 7.19−7.15 (m, 2), 7.30−7.38 (m, 1), 7.89−7.95 (m, 2), 8.07 (d, 1), 10.11 (s, 1).

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-2-hydroxymethyl-*N*-methylbenzamide Hydrochloride (26).** Sodium borohydride (0.04 g, 1.0 mmol) was added portionwise to a cooled (0 °C) and stirred solution of **25** (0.5 g, 1.0 mmol) in absolute ethanol (18 mL). After the mixture was stirred and cooled for 3 h, water was added to the solution, and the solvent was removed under reduced pressure to give a residue that was purified by chromatography. Eluting with methylene chloride−methanol−aqueous 30% ammonia (9.5:0.5:0.04) afforded 0.37 g (79% yield) of **26** as the free base that was transformed into the hydrochloride salt: mp 172 °C (from EtOH/ether); $^1$H NMR (free base; $CDCl_3$) δ 1.20−1.79 (m, 8), 3.07−3.21 (m, 6), 3.52−3.74 (m, 4 + 1 exchangeable with $D_2O$), 3.84 (s, 3), 3.92 (s, 3), 4.52 (s, 2), 5.38 (br s, 2, exchangeable with $D_2O$), 6.77−6.92 (m, 2), 7.31−7.44 (m, 4).

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-2-chloromethyl-*N*-methylbenzamide Hydrochloride (9).** A mixture of **26** (0.25 g, 0.52 mmol) and $SOCl_2$ (0.1 mL, 1.5 mmol) in anhydrous chloroform (20 mL) was refluxed for 2 h. Removal of the solvent gave 0.26 g of **9**: mp 155 °C (from EtOH/ether); $^1$H NMR (DMSO-$d_6$) δ 1.10−1.97 (m, 8), 2.86−2.97 (m, 3), 3.10−3.24 (m, 3), 3.35−3.48 (m, 2), 3.61−3.81 (m, 2), 3.91 (s, 3), 3.97 (s, 3), 4.85 (s, 2), 7.28−7.45 (m, 5), 7.81 (s, 1), 8.58 (br s, 1, exchangeable with $D_2O$), 8.85 (br s, 1, exchangeable with $D_2O$), 11.85 (br s, 1, exchangeable with $D_2O$). Anal. ($C_{26}H_{35}Cl_2N_5O_3$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-2-dimethylaminomethyl-*N*-methylbenzamide Dihydrochloride (10).** This was synthesized from **9** (0.20 g, 0.37 mmol) following the procedure described for **5** and purified by chromatography. Eluting with methylene chloride−ethanol−aqueous 30% ammonia (9.2:0.8:0.04) gave 0.14 g (75% yield) of **10** as the free base that was transformed into the dihydrochloride: mp 158 °C (from EtOH/ether); $^1$H NMR ($CD_3OD$) δ 1.08−1.80 (m, 8), 2.86 (s, 6), 3.01−3.15 (m, 3), 3.22−3.27 (m, 3), 3.60−3.81 (m, 4), 3.92 (s, 3), 3.98 (s, 3), 4.27 (s, 2), 7.29 (s, 1), 7.50−7.68 (m, 5), 8.58 (br s, 2, exchangeable with $D_2O$). Anal. ($C_{28}H_{42}Cl_2N_6O_3$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-2-[(6-aminohexylamino)methyl]-*N*-methylbenzamide Trihydrochloride (11).** A solution of **24** (0.1 g, 0.2 mmol) and 1,6-hexanediamine (0.23 g, 2.0 mmol) in toluene (50 mL) was refluxed, and the water formed was continuously removed for 24 h. The cooled mixture was filtered and the filtrate evaporated to give the corresponding Schiff base that was dissolved in ethanol (10 mL) and treated with $NaBH_4$ (0.07 g). The mixture was stirred at room temperature for 3 h, acidified with 2 N HCl (6 mL), made basic with 2 N NaOH, and finally extracted with chloroform. Removal of dried ($Na_2SO_4$) solvents gave a residue that was purified by chromatography. Eluting with methylene chloride−ethanol−aqueous 30% ammonia (8:2:0.2) afforded 0.08 g (70% yield) of **11** as the free base that was transformed into the trihydrochloride: mp 148 °C (from EtOH/ether); $^1$H NMR ($CD_3OD$) δ 1.10−1.89 (m, 16), 3.14−3.31 (m, 7), 3.38−3.47 (m, 3), 3.80−4.01 (m, 4), 4.10 (s, 3), 4.19 (s, 3), 4.38−4.42 (m, 2), 5.33 (br s, 2, exchangeable with $D_2O$), 7.50−7.57 (m, 1), 7.67−7.89 (m, 5). Anal. ($C_{32}H_{52}Cl_3N_7O_3$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-3-chloromethyl-*N*-methylbenzamide Hydrochloride (12).** This was synthesized from **23** (0.80 g, 2.3 mmol) and 3-chloromethylbenzoyl chloride (0.32 mL, 2.3 mmol) following the procedure described for **8**: 1.1 g (90% yield); mp 145 °C (from 2-PrOH/dioxane); $^1$H NMR (DMSO-$d_6$) δ 1.03−1.94 (m, 8), 2.83−2.95 (m, 3), 3.17−3.22 (m, 4), 3.33−3.45 (m, 1), 3.58−3.80 (m, 2), 3.84 (s, 3), 3.90 (s, 3), 4.80 (s, 2), 7.30−7.45 (m, 5), 7.71 (s, 1), 8.56 (br s, 1, exchangeable with $D_2O$), 8.81 (br s, 1, exchangeable with $D_2O$), 11.57 (br s, 1, exchangeable with $D_2O$). Anal. ($C_{26}H_{35}Cl_2N_5O_3$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-3-aminomethyl-*N*-methylbenzamide Dihydrochloride (13).** A solution of **12** (0.05 g, 0.09 mmol) in ethanol (10 mL) was saturated with gaseous $NH_3$ at −10 °C. After the mixture was stirred at room temperature for 48 h, the solvent was removed under reduced pressure affording a residue that was purified by chromatography. Eluting with methylene chloride−methanol−aqueous 30% ammonia (9:1:0.1) gave **13** as the free base that was transformed into the dihydrochloride salt and crystallized: 0.04 g (80% yield); mp 150 °C (from EtOH/ether); $^1$H NMR ($CD_3OD$) δ 1.10−1.83 (m, 8), 2.91−3.10 (m, 3), 3.17−3.36 (m, 4), 3.44−3.81 (m, 3), 3.92 (s, 3), 3.98 (s, 3), 4.18 (s, 2), 7.19−7.31 (m, 1), 7.39−7.63 (m, 5). Anal. ($C_{26}H_{38}Cl_2N_6O_3$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-3-dimethylaminomethyl-*N*-methylbenzamide Dihydrochloride (14).** This was synthesized from **12** (0.30 g, 0.56 mmol) following the procedure described for **5** and purified by chromatography. Eluting with methylene chloride−ethanol−aqueous 30% ammonia (9.2:0.8:0.04) afforded 0.15 g (87% yield) of **14** as the free base that was transformed into the dihydrochloride salt and crystallized: mp 165 °C (from EtOH/ether); $^1$H NMR (DMSO-$d_6$) δ 1.03−1.73 (m, 8), 2.68 (s, 3), 2.70 (s, 3), 2.90−2.97 (m, 3), 3.21−3.25 (m, 4), 3.41−3.53 (m, 1), 3.62−3.80 (m, 2), 3.86 (s, 3), 3.90 (s, 3), 4.31−4.35 (m, 2), 7.38−7.63 (m, 5), 7.79 (s, 1), 8.56 (br s, 1, exchangeable with $D_2O$), 8.80 (br s, 1, exchangeable with $D_2O$), 11.01 (br s, 1, exchangeable with $D_2O$), 11.93 (br s, 1, exchangeable with $D_2O$); EI MS *m/z* 508 ($M^+$). Anal. ($C_{28}H_{42}Cl_2N_6O_3$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-*N*-methyl-3-thiazolidin-3-ylmethylbenzamide Dioxalate (15).** A mixture of **12** (0.3 g, 0.56 mmol), thiazolidine (0.05 mL, 0.62 mmol), triethylamine (0.09 mL, 0.62 mmol), and KI (few crystals) in absolute ethanol (15 mL) was refluxed for 27 h. Removal of the solvent gave a residue that was purified by chromatography. Eluting with methylene chloride−methanol−aqueous 30% ammonia (9.5:0.5:0.04) afforded 0.16 g (52% yield) of **15** as the free base that was transformed into the dioxalate salt and crystallized: mp 101−103 °C (from (EtOH/ether); $^1$H NMR (free base; $CDCl_3$) δ 1.19−1.70 (m, 8), 2.95 (s, 3), 3.06 (s, 3), 3.16−3.25 (m, 4), 3.56−3.72 (m, 6), 3.89 (s, 3), 3.95 (s, 3), 4.03 (s, 2), 6.24 (br s, 1, exchangeable with $D_2O$), 6.60 (br s, 1, exchangeable with $D_2O$), 7.10−7.21 (m, 2), 7.25−7.46 (m, 4). Anal. ($C_{33}H_{44}N_6SO_4$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-*N*-methyl-3-{[methyl-(6-methylaminohexyl)amino]methyl}benzamide Trioxalate (16).** A solution of **12** (0.13 g, 0.26 mmol) and *N,N'*-dimethyl-1,6-hexanediamine[26] (0.38 g, 2.6 mmol) in ethanol (15 mL) was stirred at 60 °C for 1 h and at room temperature for 24 h. Removal of the solvent under reduced pressure gave a residue that was purified by chromatography. Eluting with methylene chloride−methanol−aqueous 30% ammonia (9:1:0.1) afforded 0.11 g (70% yield) of

**16** as the free base that was transformed into the trioxalate salt: mp < 50 °C (from EtOH/ether); $^1$H NMR (free base; $CDCl_3$) $\delta$ 1.15−1.83 (m, 16), 2.16−2.30 (m, 5), 2.46 (s, 3), 2.54−2.66 (m, 2), 2.85−3.04 (m, 3), 3.10−3.27 (m, 4), 3.38−3.77 (m, 5), 3.90 (s, 3), 3.94 (s, 3), 5.55 (br s, 2, exchangeable with $D_2O$), 6.87−7.01 (m, 2), 7.19−7.38 (m, 5). Anal. ($C_{40}H_{59}N_7O_{15}$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-4-chloromethyl-*N*-methylbenzamide Hydrochloride (17).** This was synthesized from **23** (0.8 g, 2.3 mmol) and 4-chloromethylbenzoyl chloride (0.43 g, 2.3 mmol) following the procedure described for **8** and purified by chromatography. Eluting with methylene chloride−ethanol−aqueous 30% ammonia (9.5:0.5:0.03) afforded 0.45 g (40% yield) of **17** as the free base that was transformed into the hydrochloride salt and crystallized: mp 162−164 °C (from EtOH/ether); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.03−1.94 (m, 8), 2.85−2.93 (m, 3), 3.17−3.22 (m, 4), 3.37−3.44 (m, 1), 3.58−3.78 (m, 2), 3.83 (s, 3), 3.87 (s, 3), 4.79 (s, 2), 7.31−7.42 (m, 2), 7.43−7.50 (m, 2), 7.61−7.66 (m, 1) 7.75 (s, 1), 8.56 (br s, 1, exchangeable with $D_2O$), 8.50−8.62 (br s, 1, exchangeable with $D_2O$), 8.86 (br s, 1, exchangeable with $D_2O$), 11.82 (br s, 1, exchangeable with $D_2O$). Anal. ($C_{26}H_{35}Cl_2N_5O_3$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-4-dimethylaminomethyl-*N*-methylbenzamide Dihydrochloride (18).** This was synthesized from **17** (0.11 g, 0.2 mmol) following the procedure described for **5** and purified by chromatography. Eluting with methylene chloride−ethanol−aqueous 30% ammonia (9.2:0.8:0.04) afforded 0.09 g (88% yield) of **18** as the free base that was transformed into the dihydrochloride salt and crystallized: mp 162−164 °C (from EtOH/ether); $^1$H NMR (free base; $CDCl_3$) $\delta$ 1.03−1.71 (m, 8), 2.21 (s, 6), 2.90−3.27 (m, 7), 3.40 (s, 2), 3.43−3.60 (m, 3), 3.83 (s, 3), 3.92 (s, 3), 5.52 (br s, 2, exchangeable with $D_2O$), 6.88−6.93 (m, 2), 7.31 (s, 4). Anal. ($C_{28}H_{42}Cl_2N_6O_3$) C, H, N.

***N*-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-*N*-methyl-4-thiazolidin-4-ylmethylbenzamide Dihydrochloride (19).** This was synthesized from **17** (0.1 g, 0.19 mmol) following the procedure described for **15** and purified by chromatography. Eluting with methylene chloride−methanol−aqueous 30% ammonia (9.5:0.5:0.04) afforded 0.06 g (50% yield) of **19** as the free base that was transformed into the dihydrochloride salt and crystallized: mp 162 °C (from (EtOH/ether); $^1$H NMR (free base; $CDCl_3$) $\delta$ 1.18−1.73 (m, 8), 2.92−3.30 (m, 10), 3.49−3.70 (m, 6), 3.89 (s, 3), 3.95 (s, 3), 4.03 (s, 2), 5.50 (br s, 1, exchangeable with $D_2O$), 6.86 (s, 1), 6.90−7.11 (m, 1), 7.38 (s, 4). Anal. ($C_{29}H_{42}Cl_2N_6O_3$) C, H, N.

**N-{6-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]hexyl}-*N*-methyl-4-{[methyl-(6-methylaminohexyl)amino]methyl}benzamide Trioxalate (20).** This was synthesized from **17** (0.14 g, 0.28 mmol) and *N,N'*-dimethyl-1,6-hexanediamine[26] following the procedure described for **16** and purified by chromatography. Eluting with methylene chloride−methanol−aqueous 30% ammonia (9:1:0.1) afforded 0.09 g (57% yield) of **20** as the free base that was transformed into the trioxalate salt: mp < 60 °C; $^1$H NMR (free base; $CDCl_3$) $\delta$ 1.04−1.79 (m, 16), 2.10−2.33 (m, 5), 2.51 (s, 3), 2.58−2.68 (m, 2), 2.87−3.09 (m, 3), 3.13−3.32 (m, 4), 3.40−3.78 (m, 5), 3.93 (s, 3), 3.96 (s, 3), 5.60 (br s, 2, exchangeable with $D_2O$), 6.91−7.04 (m, 2), 7.30−7.41 (m, 5). Anal. ($C_{40}H_{59}N_7O_{15}$) C, H, N.

**Biology. Functional Antagonism in Isolated Rat Tissues.** Male Sprague Dawley rats (Charles River, Italy) were killed by cervical dislocation under ketamine anesthesia and the organs required were isolated, freed from adhering connective tissue, and set up rapidly under a suitable resting tension in 15 mL organ baths containing physiological salt solution kept at appropriate temperature (see below) and aerated with 5% $CO_2$:95% $O_2$ at pH 7.4. Concentration−response curves were constructed by cumulative addition of agonist. The concentration of agonist in the organ bath was increased approximately 5-fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Contractions were recorded by means of a force displacement transducer (FT.03 Grass and 7003 Basile) connected to a four-channel pen recorder (Battaglia-Rangoni KV 380). In addition, parallel experiments in which tissues did not receive any antagonist were run in order to check any variation in sensitivity.

**Vas Deferens Prostatic Portion.** This tissue (from rats of 200−230 g) was used to assess $\alpha_{1A}$-adrenergic antagonism.[27] Prostatic portions of 2 cm length were mounted under 400−450 g of tension at 37 °C in Tyrode solution of the following composition (mM): NaCl, 130.0; KCl, 2.0; $CaCl_2 \cdot 2H_2O$, 1.8; $MgCl_2$, 0.89; $NaHCO_3$, 25.0; $NaH_2PO_4 \cdot 2H_2O$, 0.42; glucose, 5.6. Desipramine hydrochloride (0.01 $\mu$M) was added to prevent the neuronal uptake of (−)-noradrenaline. The preparations were equilibrated for 45−60 min. During this time the bathing solution was changed every 10 min. Concentration−response curves for isotonic contractions in response to (−)-noradrenaline were obtained at 30 min intervals: the first one being discarded and the second one was taken as control. The antagonist was allowed to equilibrate with the tissue for 30 min, and then a new concentration−response curve to the agonist was obtained. (−)-Noradrenaline solutions contained 0.05% $Na_2S_2O_5$ to prevent oxidation.

$\alpha_2$-Adrenoreceptor antagonist potency was determined also on vas deferens prostatic portions of 1.5−2 cm length which were set up in an organ bath containing Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.52; $MgSO_4$, 0.6; $KH_2PO_4$, 1.2; $NaHCO_3$, 25.0; glucose, 11.1. (±)-Propranolol hydrochloride (1 $\mu$M) and desipramine (0.01 $\mu$M) were present in the Krebs solution throughout the experiments to block $\beta$-adrenoreceptor and neuronal uptake mechanisms, respectively. The medium was maintained at 37 °C. Field stimulation of the tissues was carried out by means of two platinum electrodes placed near the top and bottom of the vas deferens using 0.1 Hz square pulses of 3 ms duration at voltage of 20−40 V. The tissues were allowed to equilibrate for at least 1 h under a resting tension of 0.35 g before addition of any drug. A first clonidine concentration−response curve, taken as control, was obtained cumulatively, avoiding the inhibition of more than 90% of twitch. Under these conditions it was possible to obtain a second concentration−response curve not significantly different from the first one. The antagonist was allowed to equilibrate with the tissue for 30 min before a second cumulative concentration−response curve with agonist was made. Parallel experiments without any antagonist were run in order to determine the concentration of agonist causing 100% inhibition of twitch response.

**Spleen**. This tissue (from rats of 250−300 g) was employed to determine $\alpha_{1B}$-adrenoreceptor antagonist potency.[28] The spleen was removed and bisected longitudinally into two strips which were suspended in tissue baths containing Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 1.9; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; $NaH_2PO_4$, 1.2; glucose, 11.7. Desipramine hydrochloride (0.01 $\mu$M) and (±)-propranolol hydrochloride (1 $\mu$M) were added to prevent the neuronal uptake of (−)-phenylephrine and to block $\beta$-adrenoreceptors, respectively. The spleen strips were placed under 1 g of resting tension and equilibrated for 1 h. The cumulative concentration−response curves to phenylephrine were measured isometrically and obtained at 30 min intervals, the first one being discarded and the second one taken as control. The antagonist was allowed to equilibrate with the tissue for 30 min, and then a new concentration−response curve to the agonist was constructed.

**Aorta.** This tissue (from rats of 250−300 g) was used to assess $\alpha_{1D}$-adrenoreceptor antagonist potency.[28] Thoracic aorta was cleaned from extraneous connective tissue and placed in Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 1.9; $MgSO_4$ 1.2; $NaHCO_3$, 25.0; $NaH_2PO_4$, 1.2; glucose, 11.7. Desipramine hydrochloride (0.01 $\mu$M) and (±)-propranolol hydrochloride (1 $\mu$M) were added to prevent the neuronal uptake of (−)-noradrenaline and to block $\beta$-adrenoreceptors, respectively. Two helicoidal strips (15 mm

× 3 mm) were cut from each aorta beginning from the end most proximal to the heart. The endothelium was removed by rubbing with filter paper: the absence of acetylcholine-induced relaxation was taken as an indicator that vessel was denuded successfully. Vascular strips were then tied with surgical thread and suspended in a jacketed tissue bath containing Tyrode solution. Strips were secured at one end to Plexiglas hooks and connected to a transducer for monitoring changes in isometric contraction. After at least a 1 h equilibration period under an optimal tension of 1 g, cumulative (−)-noradrenaline concentration−response curves were recorded at 30 min intervals, the first two being discarded the third one taken as control. The antagonist was allowed to equilibrate with the tissue for 30 min before the generation of the fourth cumulative concentration−response curve to (−)-noradrenaline.

**Radioligand Binding Assays**. **Native Receptors.** Binding studies on native $\alpha_2$-adrenoreceptors and $D_2$ receptors were carried out in membranes of rat cerebral cortex and striatum, respectively. Male Sprague Dawley rats (200−300 g, SD Harlan/Nossan, Italy) were killed by cervical dislocation, and different tissues were excised and immediately frozen and stored at −70 °C until use. For $\alpha_2$ membrane preparations, cerebral cortices were homogenized (2 × 20 s) in 50 volumes of cold Tris-HCl buffer, pH 7.4, using a Politron homogenizer (speed 7). Homogenates were centrifuged at 49000*g* for 10 min, resuspended in 50 volumes of the same buffer, incubated at 37 °C for 15 min, and centrifuged and resuspended 2 more times. The final pellets were suspended in 100 volumes of Tris-HCl buffer, pH 7.4, containing 10 $\mu$M pargiline and 0.1% ascorbic acid. Membranes were incubated in a final volume of 1 mL for 30 min at 25 °C with 0.5−1.5 nM [$^3$H]rauwolscine, in absence or presence of competing drugs. For $D_2$ membrane preparations, rat striata were homogenized (2 × 20 s) in 30 volumes of cold Tris-HCl buffer, pH 7.4, using a Politron homogenizer (speed 7) and centrifuged at 49000*g* for 10 min. The final pellets were suspended in 200 volumes of Tris-HCl incubation buffer containing 10 $\mu$M pargiline, 0.1% ascorbic acid, and the following saline concentrations: NaCl, 120 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1 mM. The membranes were then incubated for 15 min at 37 °C with 0.2−0.6 nM [$^3$H]-spiperone. Nonspecific binding was determined in the presence of 10 $\mu$M phentolamine ($\alpha_2$-adrenoreceptors) and 1 $\mu$M (+)-butaclamol ($D_2$ receptors). The incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through 0.2% polyethyleneimine pretreated Whatman GF/B or Schleicher & Schuell GF52 filters. The filters were then washed with ice-cold buffer, and the radioactivity retained on the filters was counted by liquid scintillation spectrometry.

**Cloned Receptors.** Binding to cloned human $\alpha_1$-adrenoreceptor subtypes was performed in membranes from CHO cells transfected by electroporation with DNA expressing the gene encoding each $\alpha_1$-adrenoreceptor subtype. Cloning and stable expression of the human $\alpha_1$-adrenoreceptor gene was performed as previously described.[32] CHO cell membranes (30 $\mu$g proteins) were incubated in 50 mM Tris-HCl, pH 7.4, with 0.1−0.4 nM [$^3$H]prazosin, in a final volume of 1.02 mL for 30 min at 25 °C, in absence or presence of competing drugs (1 pM−10 $\mu$M). Nonspecific binding was determined in the presence of 10 $\mu$M phentolamine. The incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through 0.2% polyethyleneimine pretreated Whatman GF/B or Schleicher & Schuell GF52 filters. Genomic clones G-21 coding for the human 5-$HT_{1A}$ receptor are stably transfected in a human cell line (HeLa).[33] HeLa cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum, gentamicin (100 $\mu$g/mL), and 5% $CO_2$ at 37 °C. Cells were detached from the growth flask at 95% confluence by a cell scraper and were lysed in ice-cold Tris 5 mM and EDTA 5 mM buffer (pH 7.4). Homogenates were centrifuged at 40000*g* for 20 min, and pellets were resuspended in a small volume of ice-cold Tris 5 mM and EDTA 5 mM buffer (pH 7.4) and immediately frozen and stored at −70 °C until use. On the experimental day, cell membranes were resuspended in binding buffer of 50 mM Tris-HCl (pH 7.4), 2.5 mM $MgCl_2$, and 10 $\mu$M pargiline.[34] Membranes were incubated in a final volume of 1 mL for 30 min at 30 °C with 0.7−1.4 nM [$^3$H]8-OH-DPAT, in the absence or presence of competing drugs. Nonspecific binding was determined in the presence of 10 $\mu$M 5-HT. The incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through 0.2% polyethyleneimine pretreated Whatman GF/B or Schleicher & Schuell GF52 filters.

**Data Analysis.** The affinity constants (p$A_2$ values, Table 1) were determined by Schild plots[29] obtained from the dose ratios at the $EC_{50}$ values of the agonists calculated at three antagonist concentrations. Each concentration was tested five times, and Schild plots were constrained to a slope of −1.[30] The compounds were tested at only one concentration when determining $\alpha_2$-adrenoreceptor blocking activity because of their low affinity for this receptor. In these cases, p$A_2$ (−log $K_B$) values were calculated according to van Rossum.[31] Functional data were analyzed by pharmacological computer programs[38] and are presented as the mean ± SE of *n* experiments. Differences between mean values were tested for significance by Student's t-test.

Binding data were analyzed using the nonlinear curve-fitting program Allfit.[39] Scatchard plots were linear in all preparations. All pseudo-Hill coefficients (nH) were not significantly different from unity ($p > 0.05$). Equilibrium inhibition constants ($K_i$) were derived from the Cheng−Prusoff equation,[40] $K_i = IC_{50}/(1 + L/K_d)$, where $L$ and $K_d$ are the concentration and the equilibrium dissociation constant of the radioligand. p$K_i$ values (Table 2) are the mean ± SE of three separate experiments performed in triplicate.

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## References


(1) (a) Bylund, D. B.; Eikenberg, D. C.; Hieble, J. P.; Langer, S. Z. Lefkowitz, R. J.; Minneman, K. P.; Molinoff, P. B.; Ruffolo, R. R., Jr.; Trendelenburg, U. IV. International union of pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.* **1994**, *46*, 121−136. (b) Hieble, J. P.; Bylund, D. B.; Clarke, D. E.; Eikenburg, D. C.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Ruffolo, R. R. International union of pharmacology X. Recommendation for nomenclature of $\alpha_1$-adrenoceptors: consensus update. *Pharmacol. Rev.* **1995**, *47*, 267−270.

(2) Faure, C.; Pimoule, C.; Arbilla, S.; Langer, S. Z.; Graham, D. Expression of $\alpha_1$-adrenoceptor subtypes in rat tissues: implications for $\alpha_1$-adrenoceptor classification. *Eur. J. Pharmacol. (Mol. Pharmacol. Section)* **1994**, *268*, 141−149.

(3) Ford, A. P. D. W.; Williams, T. J.; Blue, D. R.; Clarke, D. E. $\alpha_1$-Adrenoceptor classification: sharpening Occam's razor. *Trends Pharmacol. Sci.* **1994**, *15*, 167−170.

(4) Testa, R.; Guarneri, L.; Angelico, P.; Poggesi, E.; Taddei, C.; Sironi, G.; Colombo, D.; Sulpizio, A. C.; Naselsky, D. P.; Hieble, J. P.; Leonardi, A. Pharmacological characterization of the uroselective *alpha*-1 antagonist Rec 15/2739 (SB 216469): role of the *alpha*-1L adrenoceptor in tissue selectivity, Part II. *J. Pharmacol. Exp. Ther.* **1997**, *281*, 1284−1293.

(5) Michel, A. D.; Loury, D. N.; Whiting, R. L. Identification of a single $\alpha_1$-adrenoceptor corresponding to the $\alpha_{1A}$-subtype in rat submaxillary gland. *Br. J. Pharmacol.* **1989**, *98*, 883−889.

(6) García-Sáinz, J. A.; Romero-Avila, Ma. T.; Tórres-Márquez, Ma. E. Characterization of the human liver $\alpha_1$-adrenoceptors: predominance of the $\alpha_{1A}$-subtype. *Eur. J. Pharmacol. (Mol. Pharmacol. Section)* **1995**, *289*, 81−86.

(7) Eltze, M.; Boer, R.; Sanders, K. H.; Kolassa, N. Vasodilation elicited by $5HT_{1A}$ receptor agonists in constant pressure perfused kidney is mediated by blockade of $\alpha_{1A}$-adrenoceptors. *Eur. J. Pharmacol.* **1991**, *202*, 33−44.

(8) Testa, R.; Guarneri, L.; Ibba, M.; Strada, G.; Poggesi, E.; Taddei, C.; Simonazzi, I.; Leonardi, A. Characterization of $\alpha_1$-adrenoceptor subtypes in prostate and prostatic urethra of rat, rabbit, dog and man. *Eur. J. Pharmacol.* **1993**, *249*, 307−315.

(9) Han, C.; Abel, P. W.; Minneman, K. P. $\alpha_1$-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular $Ca^{2+}$ in smooth muscle. *Nature* **1987**, *329*, 333−335.

(10) Aboud, R.; Shafii, M.; Docherty, J. R. Investigation of the subtypes of $\alpha_1$-adrenoceptor mediating contractions of rat aorta, vas deferens and spleen. *Br. J. Pharmacol.* **1993**, *109*, 80−87.

(11) Testa, R.; Destefani, C.; Guarneri, L.; Poggesi, E.; Simonazzi, I.; Taddei, C.; Leonardi, A. The $\alpha_{1d}$-adrenoceptor subtype is involved in the noradrenaline-induced contractions of rat aorta. *Life Sci.* **1995**, *57*, PL 159−163.
(12) (a) Kenny, B.; Ballard, S.; Blagg, J.; Fox, D. Pharmacological options in the treatment of benign prostatic hyperplasia. *J. Med. Chem.* **1997**, *40*, 1293−1315. (b) Leonardi, A.; Testa, R.; Motta, G.; De Benedetti, P. G.; Hieble, P.; Giardinà, D. $\alpha_1$-Adrenoceptors: subtype- and organ-selectivity of different agents. In *Perspective in Receptor Research*; Giardinà, D., Piergentili, A., Pigini, M., Eds.; Elsevier: Amsterdam, 1996; pp 135−152. (c) Ruffolo, R. R., Jr.; Bondinell, W.; Hieble, J. P. α- and β-Adrenoceptors. From the gene to the clinic. 2. Structure−activity relationships and therapeutic applications. *J. Med. Chem.* **1995**, *38*, 3681−3716.
(13) Shibata, K.; Foglar, R.; Horie, K.; Obika, K.; Sakamoto, A.; Ogawa, S.; Tsujimoto, G. KMD-3213, a novel, potent, $\alpha_{1a}$-adrenoceptor-selective antagonist: characterization using recombinant human $\alpha_1$-adrenoceptors and native tissues. *Mol. Pharmacol.* **1995**, *48*, 250−258.
(14) Testa, R.; Guarneri, L.; Taddei, C.; Poggesi, E.; Angelico, P.; Sartani, A.; Leonardi, A.; Gofrit, O. N.; Meretyk, S.; Caine, M. Functional antagonistic activity of Rec 15/2739, a novel *alpha*-1 antagonist selective for the lower urinary tract, on noradrenaline-induced contraction of human prostate and mesenteric artery. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 1237−1246.
(15) Ford, A. P. D. W.; Arredondo, N. F.; Blue, D. R.; Bonhaus, D. W.; Jasper, J.; Kawa, M. S.; Lesnick, J.; Pfister, J. R.; Shieh, I. A.; Vimont, R. L.; Williams, T. J.; McNeal, J. E.; Stamey, T. A.; Clarke, D. E. RS-17053 (N-[2-(2-cyclopropyl-methoxyphenoxy)-ethyl]-5-chloro-α,α-dimethyl-*1H*-indole-3-ethanamine hydrochloride), a selective $\alpha_{1a}$-adrenoceptor antagonist, displays low affinity for functional $\alpha_1$-adrenoceptors in human prostate: implications for adrenoceptor classification. *Mol. Pharmacol.* **1996**, *49*, 209−215.
(16) Quaglia, W.; Pigini, M.; Tayebati, S. K.; Piergentili, A.; Giannella, M.; Leonardi, A.; Taddei, C.; Melchiorre, C. Synthesis, absolute configuration, and biological profile of the enantiomers of *trans*-[2-(2,6-dimethoxyphenoxy)ethyl][(3-*p*-tolyl-2,3-dihydro-1,4-benzodioxin-2-yl)methyl]amine (mephendioxan), a potent competitive $\alpha_{1A}$-adrenoreceptor antagonist. *J. Med. Chem.* **1996**, *39*, 2253−2258.
(17) Giardinà, D.; Crucianelli, M.; Romanelli, R.; Leonardi, A.; Poggesi, E.; Melchiorre, C. Synthesis and biological profile of the enantiomers of [4-(4-amino-6,7-dimethoxyquinazolin-2-yl)-*cis*-octahydroquinoxalin-1-yl]furan-2-ylmethanone (cyclazosin), a potent competitive $\alpha_{1B}$-adrenoceptor antagonist. *J. Med. Chem.* **1996**, *39*, 4602−4607.
(18) Patane, M. A.; Scott, A. L.; Broten, T. P.; Chang, R. S. L.; Ransom, R. W.; DiSalvo, J.; Forray, C.; Bock, M. G. 4-Amino-2-[4-[1-(benzyloxycarbonyl)-2(*S*)-[[(1,1-dimethylethyl)amino]carbonyl]-piperazinyl]-6,7-dimethoxyquinazoline (L-765, 314): a potent and selective $\alpha_{1b}$ adrenergic receptor antagonist. *J. Med. Chem.* **1998**, *41*, 1205−1208.
(19) Goetz, A. S.; King, H. K.; Ward, S. D. C.; True, T. A.; Rimele, T. J.; Saussy, D. L., Jr. BMY-7378 is a selective antagonist of the D subtype of $\alpha_1$-adrenoceptor. *Eur J. Pharmacol.* **1995**, *272*, R5−R6.
(20) Minarini, A.; Budriesi, R.; Chiarini, A.; Leonardi, A.; Melchiorre, C. Search for $\alpha_1$-adrenoceptor subtypes selective antagonists: design, synthesis and biological activity of cystazosin, an $\alpha_{1D}$-adrenoceptor antagonist. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1353−1358.
(21) Giardinà, D.; Brasili, L.; Gregori, M.; Massi, M.; Picchio, M. T.; Quaglia, W.; Melchiorre, C. Structure−activity relationships among prazosin-related compounds. Effect of piperazine ring replacement by an alkanediamine moiety on $\alpha_1$-adrenoreceptor blocking activity. *J. Med. Chem.* **1989**, *32*, 50−55.
(22) Giardinà, D.; Gulini, U.; Massi, M.; Piloni, M. G.; Pompei, P.; Rafaiani, G.; Melchiorre, C. Structure−activity relationships in prazosin-related compounds. 2. Role of the piperazine ring on α-blocking activity. *J. Med. Chem.* **1993**, *36*, 690−698.
(23) Melchiorre, C. Tetramine disulfides: a new tool in α-adrenergic pharmacology. *Trends Pharmacol. Sci.* **1981**, *2*, 209−211.
(24) Giardinà, D.; Crucianelli, M.; Marucci, G.; Melchiorre, C.; Polidori, C.; Pompei, P.; Massi, M. Pharmacological evaluation of prazosin- and doxazosin-related compounds with modified piperazine ring. *Arzneim.-Forsch./Drug Res.* **1996**, *46* (II), 1054−1059.
(25) El Hajj, T.; Masrona, A.; Martin, J. C.; Descotes, G. Synthesis of 5-(hydroxymethyl)furan-2-carboxaldehyde and its derivative by acid treatment of sugars on ion-exchange resins. *Bull. Soc. Chim. Fr.* **1987**, 855−860.
(26) Devinsky, F.; Lacko, I.; Krasnec, L. A convenient synthesis of secondary *N,N*-dimethylalkanediamines. *Synthesis* **1980**, 303−305.
(27) Eltze, M.; Boer, R.; Sanders, K. H.; Kolossa, N. Vasodilatation elicited by 5-$HT_{1A}$ receptor agonists in constant-pressure-perfused rat kidney is mediated by blockade of $\alpha_{1A}$-adrenoreceptors. *Eur. J. Pharmacol.* **1991**, *202*, 33−44.
(28) Ko, F. N.; Guh, J. H.; Yu, S. M.; Hou, Y. S.; Wu, Y. C.; Teng, C. M. (−)-Discretamine, a selective $\alpha_{1D}$-adrenoreceptor antagonist, isolated from *Fissistigma glaucescens*. *Br. J. Pharmacol.* **1994**, *112*, 1174−1180.
(29) Arunlakshana, O.; Schild, H. O. Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* **1959**, *14*, 48−58.
(30) Tallarida, R. J.; Cowan, A.; Adler M. W. p$A_2$ and receptor differentiation: a statistical analysis of competitive antagonism. *Life Sci.* **1979**, *25*, 637−654.
(31) Van Rossum, J. M. Cumulative dose−response curves. II. Technique for the making of dose−response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn.* **1963**, *143*, 299−330.
(32) Testa, R.; Taddei, C.; Poggesi, E.; Destefani, C.; Cotecchia, S.; Hieble, J. P.; Sulpizio, A. C.; Naselsky, D.; Bergsma, D.; Ellis, S.; Swift, A.; Ganguly, S.; Ruffolo, R. R.; Leonardi, A. Rec 15/2739 (SB 216469): a novel prostate selective $\alpha_1$-adrenoceptor antagonist. *Pharmacol. Commun.* **1995**, *6*, 79−86.
(33) Fargin, A.; Raymond, J. R.; Regan, J. W.; Cotecchia, S.; Lefkowitz, R. J.; Caron, M. G. Effector coupling mechanisms of the cloned 5-$HT_{1A}$ receptor. *J. Biol. Chem.* **1989**, *284*, 14848−14852.
(34) Fargin, A.; Raymond, J. R.; Lohse, M. J.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. The genomic clone G-21 which resembles a β-adrenergic receptor sequence encodes the 5-$HT_{1A}$ receptor. *Nature* **1988**, *335*, 358−360.
(35) Melchiorre, C.; Bolognesi, M. L.; Budriesi, R.; Chiarini, A.; Giardinà, D.; Minarini, A.; Quaglia, W.; Leonardi, A. Search for selective antagonists at $\alpha_1$-adrenoreceptors: neutral or negative antagonism? *Il Farmaco* **1998**, *53*, 278−286.
(36) Leff, P. The two-state model of receptor activation. *Trends Pharmacol. Sci.* **1995**, *16*, 89−97.
(37) Noguera, M. A.; Ivorra, I. D.; D'Ocon, P. Functional evidence of inverse agonism in vascular smooth muscle. *Br. J. Pharmacol.* **1996**, *119*, 158−164.
(38) Tallarida, R. J.; Murray, R. B. Manual of pharmacologic calculations with computer programs. Springer-Verlag: New York, 1991; Version 2.
(39) De Lean, A.; Munson, P. J.; Rodbard, D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose−response curves. *Am. J. Physiol.* **1978**, *235*, E97−E102.
(40) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant ($K_i$) and the concentration of inhibitor which causes 50% inhibition ($I_{50}$) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

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