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SEARCH FOR α_1–ADRENOCEPTOR SUBTYPES SELECTIVE ANTAGONISTS: DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY OF CYSTAZOSIN, AN α_{1D}-ADRENOCEPTOR ANTAGONIST

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Abstract. Two novel quinazolines (**2** and **3**) related to both prazosin and its open analogue **1** were synthesized, and their biological profile at α_1–adrenoceptor subtypes was assessed by functional assays in rat isolated tissues, namely prostatic vas deferens (α_{1A}), spleen (α_{1B}) and aorta (α_{1D}). Furthermore, the binding profile of **3** was assessed at native α_2 and D_2 receptors, and cloned human 5-HT_{1A} receptors, in comparison to prazosin, (+)-cyclazosin, **1** and BMY 7383. It turned out that the cystamine–bearing quinazoline **3** (cystazosin) has a reversed affinity profile relative to (+)-cyclazosin owing to a higher affinity for α_{1D}–adrenoceptors and a significantly lower affinity for the α_{1A} and α_{1B} subtypes. Furthermore, in comparison to BMY 7378, cystazosin (**3**) displays a much better specificity profile since it has lower affinity for D_2 and 5–HT_{1A} receptors.

Pharmacological and binding studies have shown that α_1-adrenoceptors can be classified into at least three subtypes, namely α_{1A}, α_{1B} and α_{1D}.[1] Current evidence indicates that rat submaxillary gland,[2] human liver,[3] and various tissues such as prostatic rat vas deferens,[4] rabbit prostate and prostatic urethra[5] contain predominantly the α_{1A}-adrenoceptor, whereas rat liver and spleen[6] are considered α_{1B}-adrenoceptor preparations and the α_{1D}-adrenoceptor mediates the contraction in rat aorta.[7, 8] Cloning studies have confirmed the existence of three distinct α_1-adrenoceptors, which are now designated as α_{1a}, α_{1b} and α_{1d} subtypes.[1] Thus, α_1-adrenoceptors are now classified as α_{1A} (α_{1a}), α_{1B} (α_{1b}) and α_{1D} (α_{1d}), with upper and lower case subscripts being used to designate native or recombinant receptor, respectively.[1, 9-11]

Although, several so–called selective α_1-adrenoceptor antagonists are available,[12] it should be emphasized, however, that the ideal selective ligand which recognizes only one among multiple receptor subtypes is not available yet for α_{1B} and α_{1D} subtypes and it remains a formidable challenge to medicinal chemists. Thus, pharmacotherapy is still waiting for the possible advantages arising from the identification of the presently known α_1-adrenoceptor subtypes. For example, BMY 7378 is by far the most selective α_{1d}-adrenoceptor antagonist reported to date.[13] It displayed a 75- and 80-fold selectivity for the α_{1d}-adrenoceptor relative to α_{1b} and α_{1a} subtypes, respectively. However, it resulted more potent (14-fold) at 5-HT_{1A} receptors and only 9–fold less potent at D_2 receptors with respect to α_{1d}-adrenoceptors.[12]

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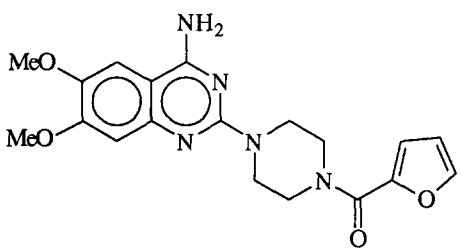
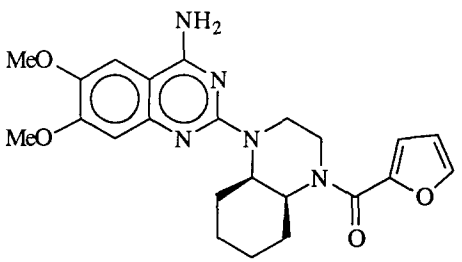
Prazosin is a selective competitive α_1-adrenoceptor antagonist relative to α_2-adrenoceptors and is widely used not only as a pharmacological tool for α_1-adrenoceptor subtypes characterization[11] but also as an effective agent in the management of hypertension. However, the finding that prazosin is a poor selective ligand at α_1-adrenoceptor subtypes stimulated interest in achieving subtype selectivity by modifying its structure. It was shown that the piperazine ring of prazosin is not essential for activity and can be replaced with an α,ω-alkanediamine chain.[14] Among a series of quinazolines bearing a polymethylene chain, compound **1** displayed the highest affinity for rat vas deferens α_1-adrenoceptors being even more potent than prazosin. It was suggested that the hexane chain of **1** might contribute to the binding by interacting with a lipophilic site located between the sites where quinazoline and furan rings interact.[14] More recently, it was demonstrated that the hexane chain can be constrained into a cyclohexyl moiety as in cyclazosin.[15] The (+)-enantiomer of cyclazosin can be considered the first α_{1B}-selective adrenoceptor antagonist in binding assays, being about 100-fold selective versus the native α_{1A} and about 40-fold versus the recombinant α_{1a} and α_{1d} subtypes.[16]

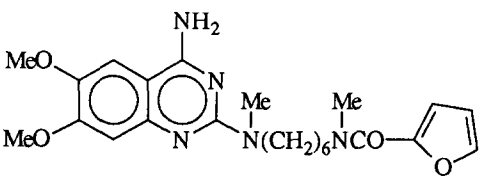
The finding that the affinity profile of prazosin–related quinazolines can depend on the type of moiety linking the two nitrogen atoms of the piperazine ring of prazosin, prompted us to further modify prazosin structure, in an attempt to improve the affinity and selectivity for different α_1-adrenoceptor subtypes. Thus, we describe here the synthesis and the pharmacological profile of the novel quinazolines **2** and **3**, designed by replacing the piperazine ring of prazosin with a cystamine moiety, which is a structural feature of benextramine, an irreversible α-adrenoceptor antagonist.[17]



Prazosin



Cyclazosin

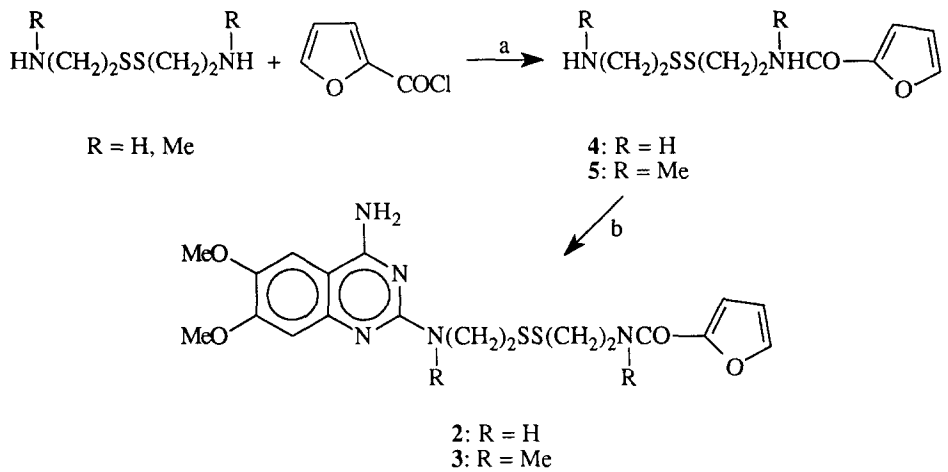


1

Quinazolines **2** and **3** were synthesized as shown in Scheme I.[18] Cystamine and *N,N'*-dimethylcystamine[19] were acylated in the presence of less than 0.5 molar equivalent 48% HBr with 2-furoyl chloride to give a 80–90% yield of amides **4** and **5**,[20] respectively. The reaction of **4** and **5** with 4-ammino-2-chloro-6,7-dimethoxyquinazoline gave a 20–30% yield of **2** and **3**,[21] respectively.

The pharmacological profile of prazosin-related quinazolines **2** and **3** was evaluated at α_1-adrenoceptor subtypes on isolated rat tissues following reported procedures.[14, 22–24] Prazosin and the acyclic analogue **1** were used as the standard compounds. α_1-Adrenoceptor subtypes blocking activity was assessed by antagonism of (–)-norepinephrine-induced contraction of prostatic vas deferens (α_{1A})[22] or thoracic aorta (α_{1D})[23] and by antagonism of (–)-phenylephrine-induced contraction of spleen (α_{1B}).[24] The potencies of the drugs were expressed as pA_2 values.[25]

Scheme I



(a) 48% HBr, EtOH-H_2O, 80 °C, 3 h; (b) 4-amino-2-chloro-6,7-dimethoxyquinazoline, isoamyl alcohol, reflux, 36 h.

Receptor selectivity of **3** was further determined by employing receptor binding assays as previously described.[16] [^{3}H]Rauwolscine and [^{3}H]spiperone were used to label α_2-adrenoceptors in rat cortex and D_2 receptors in rat striatum, respectively, whereas [^{3}H]8-hydroxy-2-(di-n-propylamino)tetraline ([^{3}H]8-OH-DPAT) was the radioligand to label cloned human 5-HT_{1A} receptors which were expressed in HeLa cells.[26] The inhibition of specific binding of the radioligands by the tested drugs was analyzed to estimate the IC_{50} value by using the non-linear curve-fitting program Allfit.[27] The IC_{50} value was converted to an affinity constant (K_i) by using the Cheng–Prusoff equation.[28]

The biological results, expressed as pA_2 and pK_i values, of quinazolines **2** and **3** are shown in Table I together with those of standard compounds prazosin, (+)-cyclazosin, BMY 7378 and **1**, which were included in this study for a comparison. It can be seen that replacing the piperazine ring of prazosin by a cystamine moiety, affording **2** and **3**, altered markedly both affinity and selectivity toward α_1-adrenoceptor subtypes. The replacement of the piperazine ring of prazosin with a *cis*-decahydroquinoxaline moiety afforded (+)-cyclazosin which displayed a 40–90-fold selectivity for the $\alpha_{1B}(\alpha_{1b})$-adrenoceptor relative to $\alpha_{1A}(\alpha_{1a})$ and $\alpha_{1D}(\alpha_{1d})$ subtype.[16] On the contrary, the inclusion of a cystamine moiety instead of a *cis*-decahydroquinoxaline residue afforded cystazosin (**3**) which displayed an interesting selectivity profile in comparison to both (+)-cyclazosin and the carbon

analogue **1**, owing to an higher affinity for α_{1D}-adrenoceptors and a significantly lower affinity for all other α_1-adrenoceptor subtypes so far investigated. Interestingly, it turned out that the unsubstituted analogue **2** is able to discriminate significantly between α_{1D} and α_{1B} relative to α_{1A}–adrenoceptors with a complementary affinity profile compared to **3**. However, a major finding of present investigation is the high receptor specificity displayed by cystazosin in comparison to the α_{1D} selective antagonist BMY 7378. In fact, cystazosin, like (+)-cyclazosin, showed only a weak, if any, affinity for α_2–adrenoceptors, D_2 and 5-HT_{1A} receptors, whereas BMY 7378, beside its high affinity for the α_{1D}–adrenoceptor, resulted also a potent ligand at both D_2 and 5-HT_{1A} receptors. Clearly, cystazosin may represent a valuable tool for the pharmacological identification of α_1–adrenoceptor subtypes in functional assays. Surprisingly enough, however, in binding experiments performed on cloned human α_1–adrenoceptor subtypes expressed in CHO cells,[29] cystazosin did not show the same selectivity observed in functional assays, owing to a substantial increase in affinity for both α_{1a}– and α_{1b}–adrenoceptor subtypes. We are currently investigating on the reason of this discrepancy and the results will be published in due course.

Table I. Antagonist Affinities, Expressed as pA_2 Values, of **1–3** at α_1-Adrenoceptors on Isolated Tissue from the Rat, Namely Prostatic Vas Deferens (α_{1A}), Spleen (α_{1B}), and Thoracic Aorta (α_{1D}), and Affinity Estimates, Expressed as pK_i, of **3** for Cloned Human 5-HT_{1A} Receptors and Native Rat Cortex α_2-Adrenoceptors and Rat Striatum D_2-Receptors in Comparison to Reference Compounds

	pA_2[a]				pK_i[b]		
no.[c]	α_{1A}	α_{1B}	α_{1D}	Affinity profile[d]	α_2	D_2	5-HT_{1A}
PZ	8.60±0.07	8.99±0.01	8.91±0.04	$\alpha_{1B}=\alpha_{1D}=\alpha_{1A}$	6.80±0.03	< 5	< 6
1	9.04±0.02	9.84±0.01	9.19±0.03	$\alpha_{1B}\geq\alpha_{1D}=\alpha_{1A}$	7.00±016	5.63±0.02	< 6
2	5.75±0.03	6.94±0.02	7.34±0.01	$\alpha_{1D}=\alpha_{1B}>\alpha_{1A}$	–[e]	–[e]	–[e]
3	7.53±0.01	7.49±0.01	8.54±0.02	$\alpha_{1D}>\alpha_{1B}=\alpha_{1A}$	6.23±0.07	≤ 5	< 6
BY	6.94±0.08	7.55±0.07	8.34±0.05	$\alpha_{1D}>\alpha_{1B}\geq\alpha_{1A}$	5.98±0.20	7.32±0.04	8.76±0.28
CZ	7.48±0.05[f]	9.16±0.02[f]	7.57±0.01[f]	$\alpha_{1B}>\alpha_{1D}=\alpha_{1A}$	6.13±0.04	5.08±0.07	< 6

[a]pA_2 values ± SE were calculated from Schild plots, constrained to slope -1.0.[25] pA_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 (ED_{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]Values are the mean ± SE of two–three separate experiments, performed in triplicate. The pseudo-Hill coefficients (nH) were not significantly different from unity ($p > 0.05$). Equilibrium dissociation constants (K_i) were derived using the Cheng-Prusoff equation.[28] Scatchard plots were linear or almost linear in all preparations tested. The affinity estimates were derived from displacement of [^{3}H]rauwolscine from α_2-adrenoceptors, [^{3}H]spiperone from D_2 receptors and [^{3}H]8-hydroxy-2-(di-n-propylamino)tetraline ([^{3}H]8-OH-DPAT) from 5-HT_{1A} receptors. [c]PZ, prazosin; CZ, (+)-cyclazosin; BY, BMY 7378. [d]Differences in antagonistic affinities for α_1-adrenoceptor subtypes by a factor of ≤3, >3÷5, and >5, are indicated by =, ≥, and >, respectively. [e]Not determined. [f]pK_i values for binding to animal clones taken from Ref. 16.

An analysis of the results shown in Table I reveals that the replacement of two methylenes of the hexane spacer of **1** with a disulfide moiety leading to cystazosin (**3**) did not affect markedly (less than 5–fold) the affinity for α_{1D}–adrenoceptors while decreasing dramatically (30-200–fold) that for α_{1A} and α_{1B} subtypes. This finding clearly

indicates that, at least in functional assays, the quinazoline and furane systems may interact selectively with different α_1-adrenoceptor subtypes according to the spacer which links these two groups.

In conclusion, we have further demonstrated that an appropriate diamine moiety linking the two aromatic groups of prazosin controls both affinity and selectivity for α_1-adrenoceptor subtypes.

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21. These compounds were purified as the free bases twice by flash silica column chromatography eluting with *n*-hexane–ethyl acetate–isopropanol–30% ammonia (5:4:1:0.15) (**2**) or petroleum ether–chloroform–ethyl acetate–ethanol–30% ammonia (6:4:2:1:0.1) (**3**). They were characterized as the hydrochloride salts. **2**: 25% yield; mp, 150–153 °C (EtOH-diethyl ether); ^{1}H NMR (DMSO-d_6) δ 2.91 (t, 2), 3.02 (t, 2), 3.50 (m, 2), 3.65 (m, 2), 3.82 (s, 3), 3.86 (s, 3), 6.58 (m, 1), 6.95 (br s, 1), 7.10 (d, 1), 7.71 (s, 1), 7.82 (s, 1), 8.02 (br s, 1, exchangeable with D_2O), 8.62 (m, 1, exchangeable with D_2O), 8.65 (br s, 1, exchangeable with D_2O), 8.90 (br s, 1, exchangeable with D_2O), 12.30 (br s, 1, exchangeable with D_2O). Anal ($C_{19}H_{23}N_5O_4S_2$·HCl) C, H, N.
3: 25% yield; mp, 180–184 °C (EtOH-diethyl ether); ^{1}H NMR (DMSO-d_6) 2.91-3.12 (m, 4), 3.20 (m, 3), 3.25 (s, 3), 3.75 (m, 2), 3.95 (s, 2), 3.87 (s, 3), 3.90 (s, 3), 6.58 (s, 1), 7.10 (s, 1), 7.55 (s, 1), 7.77 (s, 1), 7.82 (s, 1), 8.65 (br s, 1, exchangeable with D_2O), 8.90 (br s, 1, exchangeable with D_2O), 11.90 (br s, 1, exchangeable with D_2O). Anal ($C_{21}H_{27}N_5O_4S_2$·HCl) C, H, N.
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