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Synthesis and α_1-antagonist activity of new prazosin- and benextramine-related tetraamine disulfides†

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Summary — Tetraamine disulfides **1**–**10** were designed by combining the structural features of benextramine, an irreversible α_1/α_2-adrenoceptor antagonist, and prazosin, a selective competitive α_1-antagonist. Their biological profile was assessed by functional and binding assays. In rat vas deferens functional experiments, tetraamine disulfides **1**–**10** displayed a marked selectivity at α_1-adrenoceptors. Furthermore, they acted as competitive antagonists at α_1-adrenoceptors and weak noncompetitive (irreversible) antagonists at α_2-adrenoceptors. In binding assays, performed at α_1-adrenoceptors of rat liver (α_{1B}) and submaxillary gland (α_{1A}), compound **5** showed an 11-fold selectivity for α_{1B}-adrenoceptors in contrast to both prazosin and benextramine, which were not selective or selective for the α_{1A}-subtype respectively.

α-adrenoceptor antagonist / α_1-adrenoceptor subtype affinity / benextramine / diaminoquinazoline / prazosin / tetraamine disulfide

Introduction

Adrenoceptors are members of a wide family of G-protein receptors which are subclassified into three types, α_1-, α_2- and β-adrenoceptors, with an evidenced multiplicity within each class.

α_1-Adrenoceptors can be classified into at least three subtypes, namely α_{1A}, α_{1B} and α_{1D} [1, 2]. The α_{1A} subtype has high affinity for antagonists such as WB 4101, 5-methylurapidil and (+)-niguldipine and is insensitive to inactivation by chloroethylclonidine (CEC) [3]. The α_{1B} subtype displays lower affinity for the above antagonists, but is preferentially inactivated by the alkylating agent CEC [3], whereas the α_{1D} subtype has high affinity for the antagonist BMY7378 [4]. 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 α_{1A}-adrenoceptor, whereas rat liver and spleen [9] are considered α_{1B}-adrenoceptor preparations and the α_{1D}-adrenoceptor mediates the contraction in rat aorta [10, 11]. 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 [12–15]. The recombinant α_{1a}-adrenoceptor (formerly designated as α_{1c}) [13, 16], corresponds to the native α_{1A}-adrenoceptor, the recombinant α_{1b} to the native α_{1B} and the α_{1d} (formerly designated as $\alpha_{1a/d}$ in some publications) to the native α_{1D}-adrenoceptor recently characterized in rat aorta. Thus, α_1-adrenoceptors are now classified as α_{1A} (α_{1a}), α_{1B} (α_{1b}) and α_{1D} (α_{1d}), with upper and lower case subscripts being used to designate native or recombinant receptors, respectively [1, 2, 17, 18].

Benextramine and prazosin are prototypes of two structurally and pharmacologically different classes of α_1- and α_2-adrenoceptor antagonists.

Tetraamine disulfides, the prototype of which is benextramine and whose main structural feature is a cystamine moiety carrying amino alkyl substituents on the nitrogen atoms, represent a class of non-competitive antagonists of both α_1- and α_2-adrenoceptors [19, 20]. Their α-adrenoceptor inhibition is the result of covalent bond formation between a receptor target thiol and the disulfide bridge of the antagonist through a disulfide–thiol interchange reaction [19–22]. The optimal activity at α_1-adrenoceptors is dependent on the chain length separating the inner from the outer nitrogens and the type of substituent on the terminal nitrogens [19, 20].

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†Some preliminary pharmacological results, relative to the low homologues of the series, were presented at *X Convegno Nazionale della Divisione di Chimica Farmaceutica*, Società Chimica Italiana, Siena, 16–20 September, 1991.

Prazosin is a selective competitive α_1-adrenoceptor antagonist, belonging to the 2,4-diamino-6,7-dimethoxyquinazolines, which is widely used both as a pharmacological tool for α_1-adrenoceptor characterization and as an effective agent in the management of hypertension [23, 24]. Its antihypertensive activity, due to a peripheral vasodilatation mediated by a postjunctional α_1-adrenoceptor blockade, is particularly interesting because of its lack of unfavorable side effects connected with presynaptic α_2-adrenoceptor blockade [25, 26] and because of its improvement of the plasma lipids profile [27].

On the basis of the effects of certain substitutions and the finding that noradrenaline, at a relatively low concentration (30 μM), afforded complete protection of aortic α_1-adrenoceptors against benextramine, it was hypothesized that the terminal positive nitrogen atoms of benextramine might interact with the adrenoceptor anionic site recognized by the neurotransmitter, while the 2-methoxybenzyl substituent would bind to an accessory area [28]. In agreement with this view, a catechol-bearing tetraamine disulfide, that is a molecular combination of noradrenaline and benextramine, proved as active as benextramine at rat vas deferens α_1-adrenoceptors [29].

It has been suggested that prazosin also binds to the noradrenaline binding site of α_1-adrenoceptors [30]. This model focuses on the importance of charge-reinforced hydrogen bonding between protonated agonists and antagonists and a common negatively charged carboxylate counterion. Both the neurotransmitter and the 2,4-diamino-6,7-dimethoxyquinazoline moiety of prazosin is thought to interact with the receptor ground state in a similar fashion. However, the antagonist would not be able to promote the same conformational change induced by the agonist, which leads to receptor activation [30].

Rationale

If the above hypotheses were correct, then the protonated nitrogen of noradrenaline, the N_1 of prazosin and the outer ones of benextramine could interact with the same anionic receptor site. To investigate this aspect we thought it of interest to study new tetraamine disulfides by combining the structural features of both benextramine and prazosin. These derivatives might acquire relevance not only in elucidating the binding site of tetraamine disulfides and 2,4-diaminoquinazolines but also in α-adrenoceptor subtype characterization.

Benextramine proved to be relatively selective for the α_{1A}-adrenoceptor subtype [31], which may suggest a pivotal role for the tetraamine backbone at the recognition stage. Since the four amine functions of tetraamine disulfides, as well as the length of alkane chain, have already been shown to be essential for α_1-adrenoceptor-blocking activity [20], the relatively low affinity of benextramine and its higher homologues, for α_1-adrenoceptors might be due to a poor fit of the benzyl moiety with the accessory binding site. Considering that the quinazoline moiety of prazosin is essential for high affinity toward α_1-adrenoceptors [30], the replacement of the benzyl moiety of benextramine and homologues with a quinazolinyl nucleus might improve affinity while, hopefully, retaining selectivity for α_1-adrenoceptor subtypes.

This design strategy finds support in the observation that the piperazine moiety of prazosin may not be essential for activity at α_1-adrenoceptors and can be replaced by an α,ω-alkanediamine chain [32]. The finding that activity and selectivity depend on the length of alkane chain and that the most potent compound of this series was the one bearing a 1,6-disubstituted hexanediamine moiety, allowed us to hypothesize the presence of a lipophilic area in the α_1-adrenoceptor, located between the binding sites for the quinazoline and the furan rings of prazosin and which is able to accommodate optimally a 1,6-disubstituted hexanediamine moiety [32]. Since benextramine also has a 1,6-hexanediamine residue, separating the inner from the outer nitrogens of the structure, it is possible that this alkane chain interacts with the same lipophilic pocket where prazosin analogues bind. On the basis of these considerations, we studied a series of new tetraamine disulfides **1–10** as shown in figure 1.

Chemistry

Compounds **1–10** were synthesized as tetrahydrochloride salts by standard methods following the steps shown in scheme 1. Reaction of the 3-(ω-aminoalkyl)thiazolidines **11–20**, with 4-amino-2-chloro-6,7-dimethoxyquinazoline [33] gave the 2,4-diaminoquinazoline derivatives **21–30** which, upon oxidation with 0.1 N I_2 solution [34], yielded the tetraamine disulfides **1–10**. The 3-(ω-aminoalkyl)thiazolidines **11–20** were prepared by two different procedures. The lower homologues **11–13** were prepared by cyclization, with aqueous formaldehyde, of appropriate 2-[(ω-aminoalkyl)amino]ethanthiols **31–33** [35, 36], following the method used for the thiazolidine **13** [34] (scheme 2).

The other 3-(ω-aminoalkyl)thiazolidines **14–16** and the *N*-methylamino derivatives **17–20** were synthesized as shown in scheme 3. The controlled substitution of the α,ω-alkanediol ditosylated compounds **34–37** [37] by thiazolidine gave the intermediates **38–41** that afforded products in two different ways.

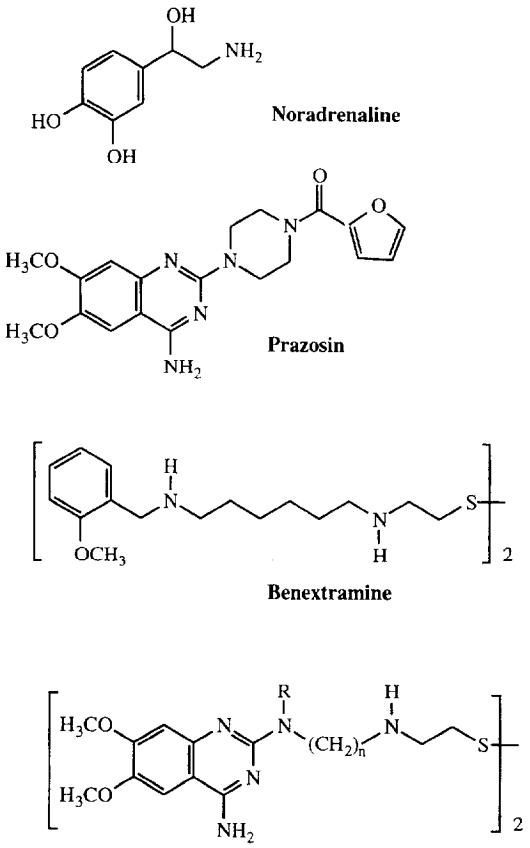
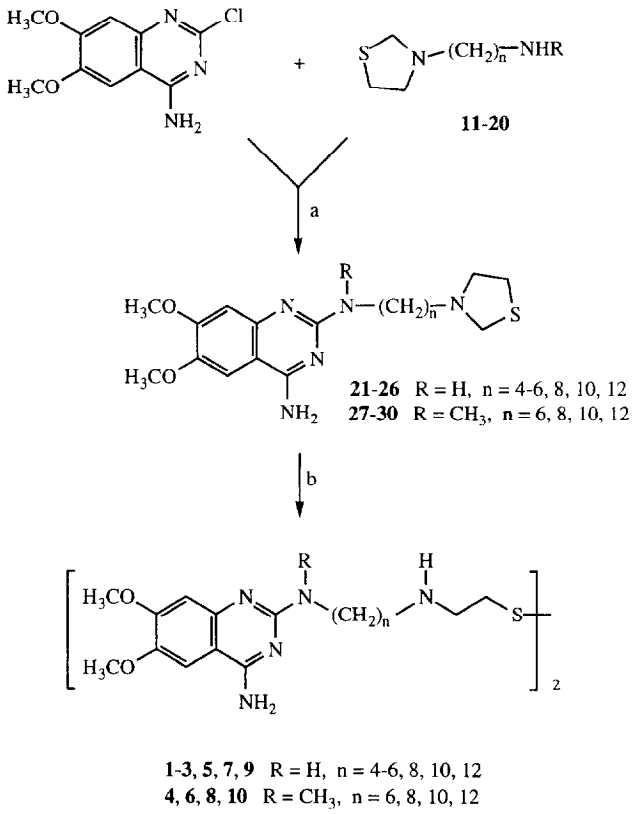


Fig 1. Structures of noradrenaline, prazosin, benextramine and hybrid tetraamine disulfides **1–10**.

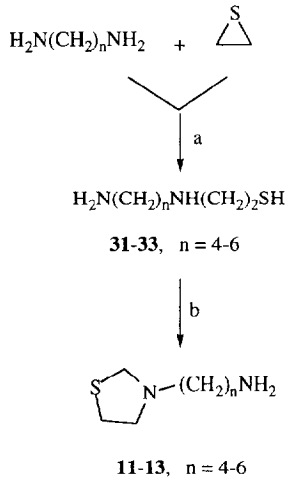


Scheme 1. Reagents: (a) *i*-AmOH, reflux; (b) 0.1 N I_2.

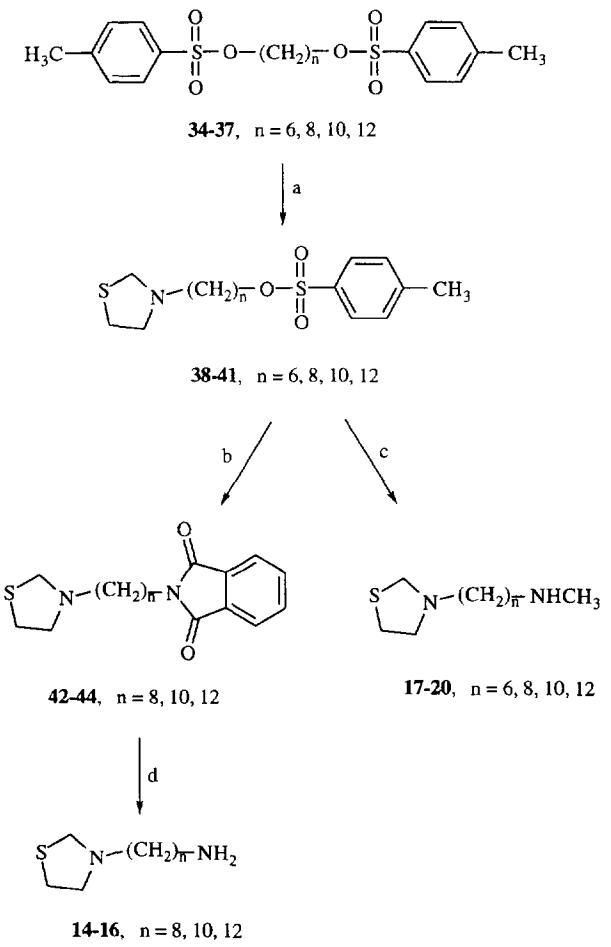
The reaction of the corresponding tosylated compounds with potassium phthalimide gave derivatives **42–44** which were cleaved with the methylamine/ethanol method [38] affording the 3-(ω-aminoalkyl)thiazolidines **14–16**, whereas direct alkylation of methylamine with the monotosylates **38–41** yielded the corresponding 3-(ω-methylaminoalkyl) thiazolidines **17–20**.

Pharmacology

The biological profile of tetraamine disulfides **1–10** was assessed both with functional studies at α_1- and α_2-adrenoceptors of isolated rat vas deferens [39, 40] and with binding experiments at native α_{1A}- and α_{1B}-adrenoceptors subtypes of rat submaxillary gland and liver, respectively [3]. Results are reported in tables I



Scheme 2. Reagents: (a) benzene, reflux; (b) 37% CH_2O, H_2O, H^+.



Scheme 3. Reagents: (a) thiazolidine, CH_3CN; (b) potassium phthalimide, DMF; (c) CH_3NH_2, benzene/EtOH; (d) CH_3NH_2, EtOH, 5 min rt then 2.5 h reflux.

and II. In order to allow comparison of the results, prazosin, benextramine and the α_{1B}-selective antagonist spiperone [3] were used as standard compounds.

α_1-Adrenoceptor-blocking activity was assessed by antagonism of (–)-noradrenaline-induced contractions of the epididymal portion of the vas deferens. α_2-Adrenoceptor-blocking activity was determined by antagonism of the clonidine-induced depression of the twitch responses of the field-stimulated prostatic portion of the vas deferens. The non-competitive (irreversible) α_1- or α_2-antagonism was determined after a 30-min incubation followed by 30 min of washings. The decrease in maximum response was expressed as a percentage of the control value and used to estimate the IC_{50} values from graphical plots of percent inhibition vs log molar concentration. The potency of irreversible inhibitors was expressed as pIC_{50} values. In contrast, the potency of the competitive antagonists was expressed as pA_2 values, calculated according to the method of Arunlakshana and Schild [41], from the plots of dose ratios at the EC_{50} values of the agonists (–)-noradrenaline or clonidine.

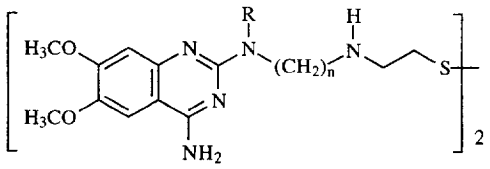
Binding studies of selected tetraamine disulfides **3** and **5–9** were performed on α_{1A}- and α_{1B}-adrenoceptors of rat submaxillary gland and liver, respectively [3], using [^{3}H]prazosin as radioligand. The potency of compounds in the competition with [^{3}H]prazosin was evaluated by incubating 0.1 nM concentrations of the radioligand in the presence or absence of various concentrations of considered drugs. The affinity was expressed as pK_i values, calculated by the Cheng–Prusoff method [42], using the IC_{50} values determined as the x intercept on a Hill plot. Two-site models were compared to one-site models to determine whether the increase of goodness of fit was significantly more than would be expected on the basis of chance alone using a partial F test [43].

Results and discussion

Surprisingly, none of the hybrid tetraamine disulfides, **1–10**, unlike benextramine, irreversibly inhibited α_1-adrenoceptors; rather, they competitively antagonized noradrenaline-induced responses like prazosin. In contrast, compounds **1–4** inhibited irreversibly, although with a significantly lower potency than benextramine, the clonidine-induced responses at α_2-adrenoceptors. This remarkable difference in the observed antagonism at α_1-adrenoceptors could have two main sources: a) hybrid tetraamine disulfides **1–10** and benextramine may react with two distinct sets of sites; or b) they may bind with the same set of sites, but the presence of the 4-amino-6,7-dimethoxyquinazolin-2-yl moiety, instead of the 2-methoxybenzyl group, would not be able to unmask the buried receptor thiol. This would prevent the disulfide–thiol interchange reaction, which is responsible for the irreversible inactivation of the receptor [19–22]. Unfortunately, at this moment, it is not yet possible to distinguish between these two possibilities. However, work is in progress to investigate this aspect in depth.

Compounds **1–10** displayed a significant α_1-adrenoceptor antagonism, albeit 5–80-fold lower than prazosin, whereas, again in contrast with benextramine, they showed only a modest, if any, affinity for α_2-adrenoceptors. As a consequence, these hybrid tetraamine disulfides displayed a marked selectivity toward α_1- with respect to α_2-adrenoceptors (up to three orders of magnitude) whereas benextramine showed a significant α_2-selectivity (about tenfold).

Table I. Antagonist potency of tetraamine disulfides **1–10** and reference compounds benextramine and prazosin at α_1- and α_2-adrenoceptors of isolated rat vas deferens tissues.



Compound	*n*	*R*	pA_2[a] (α_1)	pIC_{50}[b] (α_2)
1	4	H	6.76 ± 0.01	4.17 ± 0.00
2	5	H	7.32 ± 0.04	4.80 ± 0.01
3	6	H	7.38 ± 0.01	5.75 ± 0.02
4	6	Me	7.81 ± 0.01	5.34 ± 0.05
5	8	H	7.32 ± 0.03	ND[c]
6	8	Me	7.65 ± 0.07	NA[d]
7	10	H	6.64 ± 0.10	NA[e]
8	10	Me	7.91 ± 0.02	NA[e]
9	12	H	6.65 ± 0.09	NA[d]
10	12	Me	7.45 ± 0.17	NA[d]
Benextramine			5.12 ± 0.04[b]	6.09 ± 0.04
Prazosin			8.54 ± 0.05	5.43 ± 0.13[a]

[a]pA_2 values ± SEM, determined by Schild plots [41] and constraining [44] the slope to –1, were calculated at three antagonist concentrations, each tested four times. [b]pIC_{50} values, expressing the irreversible blockade, are defined as the concentrations producing 50% inhibition of the maximal response to noradrenaline (α_1) or clonidine (α_2). [c]ND = not determinable since the inhibition was lower than 50% up to a concentration of 100 μM. [d]NA = not active up to a concentration of 50 μM. [e]NA = not active up to a concentration of 100 μM.

Table II. Binding affinities of selected drugs **2**, **3**, **5–9** and reference compounds, benextramine, prazosin and spiperone at α_{1A}- and α_{1B}-adrenoceptors of rat submaxillary gland and liver, respectively.

Compound	pK_i[a]/pIC_{50}[b]		*Selectivity ratio*[c]
	α_{1A}	α_{1B}	α_{1B}/α_{1A}
2	7.63 ± 0.07	8.08 ± 0.04	3
3	7.30 ± 0.08	8.15 ± 0.13[d]	7
5	7.10 ± 0.12	8.14 ± 0.07	11
6	7.91 ± 0.20	8.19 ± 0.07[d]	2
7	7.08 ± 0.09	7.73 ± 0.08	4
8	7.91 ± 0.07	7.75 ± 0.11	0.7
9	7.20 ± 0.10	7.51 ± 0.03[d]	2
Benextramine	7.01 ± 0.12 [b]	6.23 ± 0.05[b]	0.2
Prazosin	9.07 ± 0.15	9.14 ± 0.12	1.2
Spiperone	7.45 ± 0.08	8.80 ± 0.08	22

[a]pK_i for inhibition of [^{3}H]prazosin binding to homogenate membranes. The values, derived using the Cheng–Prusoff equation [42], are the mean ± SE of at least three separate experiments performed in triplicate. Nonspecific binding was assessed in the presence of 10 μM phentolamine. Hill numbers (nH) were not significantly different from unity ($P > 0.05$), unless otherwise specified. [b]pIC_{50} values, instead of pK_i, given the presence of a kinetic term in the irreversible interaction to the [^{3}H]prazosin binding sites. [c]The α_{1B}/α_{1A} selectivity ratio is the antilog of difference between pK_i (or pIC_{50}) values, respectively, at α_{1B}- and α_{1A}-adrenoceptors. [d]Hill number significantly different from unity.

Clearly, the replacement of the 2-methoxybenzyl group of benextramine with a quinazoline nucleus affording tetraamines **1–10** significantly alters the biological profile in comparison to benextramine.

Concerning the chain length effects, it emerged that optimal potency at α_1-adrenoceptors was associated with a six-carbon chain as in **3**, although the homologues **2** and **5** retained the same level of activity. However, at α_2-adrenoceptors the chain length effects were more pronounced since a carbon chain either shorter or longer than six methylenes caused a significant drop in activity. Interestingly, *N*-methylation of the outer nitrogens of **3**, **5**, **7** and **9** affording the corresponding analogues **4**, **6**, **8** and **10** caused a slight increase in affinity (2–20 fold) for α_1- but not for α_2-adrenoceptors. This finding parallels the results observed for a series of prazosin-related compounds bearing a secondary or a tertiary amine function at position 2 of the quinazoline moiety [32]. Compounds **4**, **6**, **8** and **10** proved almost equiactive to each other, suggesting that the chain length separating the inner from the outer nitrogens may not play an important role in affinity. The tetraamine disulfide **8** was the most interesting compound of the series, being more selective than prazosin at α_1-adrenoceptors (α_1/α_2 selectivity ratio $\geq$ 8000 vs 1300) although four times less potent (pA_2 = 7.91 vs 8.54).

The affinity data of compounds **2**, **3** and **5–9** for α_{1A}- and α_{1B}-adrenoceptor subtypes (table II) indicate that, with the exception of **8**, the investigated new tetraamine disulfides, although being slightly more active at α_{1B}-adrenoceptors, were, like prazosin, not able to discriminate markedly between these two α_1-adrenoceptors subtypes. The greatest selectivity was displayed by tetraamine disulfide **5** with 11-fold higher affinity for α_{1B}- than for α_{1A}-adrenoceptors. This finding does not parallel the α_{1A}-selectivity shown by tetraamine disulfide benextramine (α_{1A}/α_{1B} selectivity ratio 6 or 15 [31]) indicating that the 2-methoxybenzyl and quinazolinyl groups play a different role in selectivity, as confirmed from the 2-quinazolinyl derivative cyclazosin where the fusion of the piperazine nucleus of prazosin with a cyclohexyl ring confers α_{1B} vs α_{1A} selectivity [45]. Interestingly, spiperone, which is considered a selective α_{1B}-adrenoceptor antagonist [2] showed only a fourfold higher affinity for α_{1B}-adrenoceptors and only a slightly higher α_{1B}/α_{1A} selectivity than **5** (two fold). However, spiperone may not represent a useful tool for the characterization of α_{1B}-adrenoceptors because of the lack of receptor specificity owing to its moderate/high affinity for α_2-, 5-HT_{1A} and dopamine D_2 receptors [45].

The slopes of the competition curves obtained with new tetraamine disulfides were not significantly different from unity, indicating that the sites labelled with [^{3}H]prazosin in rat submaxillary gland (α_{1A}-adrenoceptors) and liver (α_{1B}-adrenoceptors) membranes were essentially homogeneous. Nevertheless, in liver homogenates, in the case of displacements with compounds **3**, **6** and **9**, the slopes were significantly different from unity ($P < 0.05$). In fact, Hill numbers of 1.19, 1.35, and 0.58, respectively, were calculated and the data were best fitted to a two-site model, revealing a possible heterogeneity of [^{3}H]prazosin binding sites. However, this aspect deserves a deeper investigation before giving particular conclusions.

Experimental protocols

Chemistry

Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin-Elmer 297 and Varian Gemini 200 instruments, respectively. Although the IR spectra data are not included (because of the lack of unusual features), they were obtained for all the compounds reported and were consistent with the assigned structures. The elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Mass spectra were performed with a Hewlett Packard instrument model 5890 A for the separation section and model 5971 A for the mass section. Chromatographic separations were performed on silica-gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. R_f values were determined with silica gel TLC plates (Kieselgel 60 F_{254}, layer thickness 0.25 mm, Merck). The composition and volumetric ratio of eluting mixtures were: A, methylene chloride/ethyl acetate (16:0.5); B, cyclohexane/ethyl acetate (5:2); C, chloroform/petroleum ether/methanol/28% ammonia (12.5:7.5:2:0.2); D, chloroform/petroleum ether/methanol/28% ammonia (12.5:7.5:2:0.1); E, ethyl acetate/cyclohexane/chloroform/methanol (7:3:2:1); F, methanol/1.75% ammonia (9.4:0.6); G, chloroform/petroleum ether/methanol/28% ammonia (10:10:3:0.3); H, ethyl acetate/cyclohexane/chloroform/methanol (7:3:2:4); I, ethyl acetate/cyclohexane/methanol/20% ammonia (5:5:1:0.1); J, ethyl acetate/*n*-hexane/methanol/20% ammonia (5:15:1:0.05). Petroleum ether refers to the fraction with a boiling point of 40–60 °C. The term 'dried' refers to the use of anhydrous sodium sulfate. Compounds were named following IUPAC rules as applied by AUTONOM, a PC software for systematic names in organic chemistry, Beilstein-Institut and Springer-Verlag.

The physical data of new tatraamine disulfides **1–10** and of intermediates products **11**, **12**, **14–20**, and **21–30** are listed in tables III–VI. Those of other intermediates are given as examples of each structural type.

*General procedure for the synthesis of ω-(thiazolidin-3-yl)-alkylamines **11** and **12***

A mixture of the corresponding 2-[(ω-(aminoalkyl)amino]-ethanethiol dihydrochloride (30 mmol) and 37% formaldehyde (36 mmol) in water (5 mL) was left overnight at room temperature then basified with 6 N NaOH and extracted with chloroform. The dried solvent was evaporated to give a liquid that was purified by distillation (table III).

Table III. Physical properties of compounds **11**, **12** and **14–20** (see scheme 1 for structures).

Compound[a,b]	*R*	*n*	*Yield (%)*	R_f [c] *or bp* (mmHg)*	*^{1}H-NMR $CDCl_3$ (δ ppm)*
11	H	4	75	85–86* (0.5)	1.20–1.52 (m, 4H, $NCH_2(CH_2)_2$), 1.58 (s, br, 2H, NH_2 exchangeable), 2.36 (t, J = 7.12 Hz, 2H, CH_2-N_{thiaz}), 2.61 (t, J = 6.78 Hz, 2H, CH_2NH_2), 2.79–2.90 (m, 2H, CH_2S), 2.98–3.06 (m, 2H, NCH_2), 4.06 (s, 2H, NCH_2S)
12	H	5	60	84–85* (0.2)	1.21–1.50 (m, 6H, $NCH_2(CH_2)_3$), 1.70 (s, br, 2H, NH_2 exchangeable), 2.32 (t, J = 7.50 Hz, 2H, CH_2-N_{thiaz}), 2.63 (t, J = 6.75 Hz, 2H, CH_2NH_2), 2.82–2.90 (m, 2H, CH_2S), 3.06–3.12 (m, 2H, NCH_2), 4.08 (s, 2H, NCH_2S)
14	H	8	78	0.27	1.26–1.56 (m, 14H, $NCH_2(CH_2)_6$ and NH_2 exchangeable), 2.35 (t, J = 7.45 Hz, 2H, CH_2-N_{thiaz}), 2.68 (t, J = 6.70 Hz, 2H, CH_2NH_2), 2.84–2.92 (m, 2H, CH_2S), 3.04–3.12 (m, 2H, NCH_2), 4.06 (s, 2H, NCH_2S)
15	H	10	83	0.32	1.22–1.56 (m, 16H, $NCH_2(CH_2)_8$), 1.62 (s, br, 2H, NH_2 exchangeable), 2.35 (t, J = 7.27 Hz, 2H, CH_2-N_{thiaz}), 2.68 (t, J = 6.79 Hz, 2H, CH_2NH_2), 2.82–2.90 (m, 2H, CH_2S), 3.04–3.10 (m, 2H, NCH_2), 4.07 (s, 2H, NCH_2S)
16	H	12	81	0.34	1.22–1.70 (m, 22H, $NCH_2(CH_2)_{10}$ and NH_2 exchangeable), 2.36 (t, J = 7.33 Hz, 2H, CH_2-N_{thiaz}), 2.76–2.93 (m, 4H, CH_2NH_2 and CH_2S), 3.04–3.13 (m, 2H, NCH_2), 4.07 (s, 2H, NCH_2S)
17	CH_3	6	67	0.29	1.24–1.59 (m, 8H, $NCH_2(CH_2)_4$), 2.00 (s, br, 1H, NH exchangeable), 2.21 (s, 3H, CH_3), 2.36 (t, J = 7.55 Hz, 2H, CH_2-N_{thiaz}), 2.61 (t, J = 6.41 Hz, 2H, CH_2NH), 2.83–2.92 (m, 2H, CH_2S), 3.05–3.10 (m, 2H, NCH_2), 4.08 (s, 2H, NCH_2S)
18	CH_3	8	72	0.31	1.23–1.61 (m, 12H, $NCH_2(CH_2)_6$), 2.35 (t, J = 7.72 Hz, 2H, CH_2-N_{thiaz}), 2.46 (s, 3H, CH_3), 2.50 (s, br, 1H, NH exchangeable), 2.61 (t, J = 6.70 Hz, 2H, CH_2NH), 2.83–2.90 (m, 2H, CH_2S), 3.03–3.10 (m, 2H, NCH_2), 4.06 (s, 2H, NCH_2S)
19	CH_3	10	63	0.37	1.21–1.58 (m, 16H, $NCH_2(CH_2)_8$), 1.84 (s, br, 1H, NH exchangeable), 2.36 (t, J = 7.83 Hz, 2H, CH_2-N_{thiaz}), 2.46 (s, 3H, CH_3), 2.60 (t, J = 6.52 Hz, 2H, CH_2NH), 2.82–2.92 (m, 2H, CH_2S), 3.04–3.12 (m, 2H, NCH_2), 4.08 (s, 2H, NCH_2S)
20	CH_3	12	86	0.41	1.19–1.40 (m, 16H, $N(CH_2)_2(CH_2)_8$), 1.37–1.51 (m, 4H, $NCH_2CH_2(CH_2)_8CH_2$), 1.88 (s, br, 1H, NH exchangeable), 2.31 (t, J = 7.80 Hz, 2H, CH_2-N_{thiaz}), 2.40 (s, 3H, CH_3), 2.53 (t, J = 6.30 Hz, 2H, CH_2NH), 2.80–2.87 (m, 2H, CH_2S), 3.00–3.08 (m, 2H, NCH_2), 4.03 (s, 2H, NCH_2S)

[a]Except for **11** and **12**, which were liquids, all compounds were obtained as oils. [b]The mass spectrum revealed a proper molecular ion [M^+] for all componds. [c]Eluting mixture, G.

Table IV. Physical properties of compounds **21–30** (see scheme 1 for structures).

Compound	*R*	*n*	R_f *(eluent)*	*^{1}H-NMR $CDCl_3$ (δ ppm)*
21	H	4	0.18 (I)	1.57–1.75 (m, 4H, $ArNHCH_2(CH_2)_2$), 2.42 (t, J = 7.50 Hz, 2H, CH_2-N_{thiaz}), 2.84–2.90 (m, 2H, CH_2S), 3.04–3.10 (m, 2H, NCH_2), 3.47 (q, J_1 = 13.50 Hz, J_2 = 6.75 Hz, 2H, $ArNHCH_2$), 3.92 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.08 (s, 2H, NCH_2S), 4.80 (s, br, 1H, NH exchangeable), 5.13 (s, br, 2H, NH_2 exchangeable), 6.78 (s, 1H, Ar), 6.90 (s, 1H, Ar)
22	H	5	0.14 (J)	1.45–1.71 (m, 6H, $ArNHCH_2(CH_2)_3$), 2.46 (t, J = 7.67 Hz, 2H, CH_2-N_{thiaz}), 2.84–2.89 (m, 2H, CH_2S), 3.04–3.09 (m, 2H, NCH_2), 3.45 (q, J_1 = 13.80 Hz, J_2 = 6.59 Hz, 2H, $ArNHCH_2$), 3.90 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.05 (s, 2H, NCH_2S), 4.72 (s, br, 1H, NH exchangeable), 5.12 (s, br, 2H, NH_2 exchangeable), 6.80 (s, 1H, Ar), 6.89 (s, 1H, Ar)
23	H	6	0.17 (I)	1.29–1.70 (m, 8H, $ArNHCH_2(CH_2)_4$), 2.35 (t, J = 7.32 Hz, 2H, CH_2-N_{thiaz}), 2.82–2.91 (m, 2H, CH_2S), 3.03–3.09 (m, 2H, NCH_2), 3.45 (q, J_1 = 13.28 Hz, J_2 = 6.80 Hz, 2H, $ArNHCH_2$), 3.92 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.06 (s, 2H, NCH_2S), 4.82 (s, br, 1H, NH exchangeable), 5.23 (s, br, 2H, NH_2 exchangeable), 6.78 (s, 1H, Ar), 6.90 (s, 1H, Ar)
24	H	8	0.27 (H)	1.21–1.70 (m, 12H, $ArNHCH_2(CH_2)_6$), 2.35 (t, J = 7.26 Hz, 2H, CH_2-N_{thiaz}), 2.83–2.92 (m, 2H, CH_2S), 3.02–3.12 (m, 2H, NCH_2), 3.36–3.50 (m, 2H, $ArNHCH_2$), 3.95 (s, 6H, OCH_3), 4.06 (s, 2H, NCH_2S), 5.82 (s, br, 3H, NH_2 and NH, exchangeable), 6.81 (s, br, 1H, Ar), 6.98 (s, br, 1H, Ar)
25	H	10	0.35 (H)	1.18–1.69 (m, 16H, $ArNHCH_2(CH_2)_8$), 2.35 (t, J = 7.82 Hz, 2H, CH_2-N_{thiaz}), 2.83–2.91 (m, 2H, CH_2S), 3.04–3.12 (m, 2H, NCH_2), 3.34–3.48 (m, 2H, $ArNHCH_2$), 3.96 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.07 (s, 2H, NCH_2S), 6.20 (s, br, 3H, NH_2 and NH, exchangeable), 6.77 (s, br, 1H, Ar), 7.11 (s, br, 1H, Ar)
26	H	12	0.37 (H)	1.14–1.69 (m, 20H, $ArNHCH_2(CH_2)_{10}$), 2.35 (t, J = 7.35 Hz, 2H, CH_2-N_{thiaz}), 2.83–2.91 (m, 2H, CH_2S), 3.04–3.13 (m, 2H, NCH_2), 3.34–3.50 (m, 2H, $ArNHCH_2$), 3.95 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.07 (s, 2H, NCH_2S), 6.35 (s, br, 3H, NH_2 and NH, exchangeable), 6.74 (s, br, 1H, Ar), 7.18 (s, br, 1H, Ar)
27	CH_3	6	0.22 (H)	1.31–1.70 (m, 8H, $ArNCH_2(CH_2)_4$), 2.36 (t, J = 7.56 Hz, 2H, CH_2-N_{thiaz}), 2.82–2.91 (m, 2H, CH_2S), 3.02–3.10 (m, 2H, NCH_2), 3.19 (s, 3H, CH_3), 3.65 (t, J = 7.11 Hz, 2H, CH_3NCH_2), 3.92 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.06 (s, 2H, NCH_2S), 5.35 (s, br, 2H, NH_2 exchangeable), 6.86 (s, br, 1H, Ar), 7.00 (s, br, 1H, Ar)
28	CH_3	8	0.35 (H)	1.24–1.68 (m, 12H, $ArNCH_2(CH_2)_6$), 2.36 (t, J = 7.50 Hz, 2H, CH_2-N_{thiaz}), 2.84–2.92 (m, 2H, CH_2S), 3.03–3.12 (m, 2H, NCH_2), 3.19 (s, 3H, CH_3), 3.66 (t, J = 7.14 Hz, 2H, CH_3NCH_2), 3.94 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.07 (s, 2H, NCH_2S), 5.16 (s, br, 2H, NH_2 exchangeable), 6.83 (s, br, 1H, Ar), 6.98 (s, br, 1H, Ar)
29	CH_3	10	0.43 (H)	1.18–1.70 (m, 16H, $ArNCH_2(CH_2)_8$), 2.35 (t, J = 7.56 Hz, 2H, CH_2-N_{thiaz}), 2.82–2.91 (m, 2H, CH_2S), 3.02–3.11 (m, 2H, NCH_2), 3.19 (s, 3H, CH_3), 3.65 (t, J = 7.11 Hz, 2H, CH_3NCH_2), 3.93 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.08 (s, 2H, NCH_2S), 5.20 (s, br, 2H, NH_2 exchangeable), 6.87 (s, br, 1H, Ar), 7.01 (s, br, 1H, Ar)
30	CH_3	12	0.47 (H)	1.15–1.38 (m, 16H, $ArN(CH_2)_2(CH_2)_8$), 1.42–1.68 (m, 4H, $ArNCH_2CH_2$ and CH_2CH_2-N_{thiaz}), 2.34 (t, J = 7.73 Hz, 2H, CH_2-N_{thiaz}), 2.83–2.90 (m, 2H, CH_2S), 3.04–3.10 (m, 2H, NCH_2), 3.18 (s, 3H, CH_3), 3.63 (t, J = 7.42 Hz, 2H, CH_3NCH_2), 3.92 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.06 (s, 2H, NCH_2S), 5.35 (s, br, 2H, NH_2 exchangeable), 6.82 (s, br, 1H, Ar), 7.04 (s, br, 1H, Ar)

Table V. Physical properties of compounds **1**–**10**•4HCl•xH_2O (see fig 1 for structures).

Compound	*R*	*n*	*Mp (°C)*	*Solvent*[a]	R_f[b]	*Yield (%)*	*Formula*[c]
1	H	4	225 dec	A	0.09	62	$C_{32}H_{52}O_4N_{10}S_2Cl_4$•$5H_2O$
2	H	5	230 dec	B	0.10	42	$C_{34}H_{56}O_4N_{10}S_2Cl_4$•$5H_2O$
3	H	6	155 dec	A	0.11	60	$C_{36}H_{60}O_4N_{10}S_2Cl_4$•$6H_2O$
4	CH_3	6	202 dec	B	0.12	19	$C_{38}H_{64}O_4N_{10}S_2Cl_4$•$5H_2O$
5	H	8	193 dec	C	0.10	10	$C_{40}H_{68}O_4N_{10}S_2Cl_4$•$6H_2O$
6	CH_3	8	227–230	B	0.17	31	$C_{42}H_{72}O_4N_{10}S_2Cl_4$•$4H_2O$
7	H	10	215–216	B	0.17	23	$C_{44}H_{76}O_4N_{10}S_2Cl_4$•$1.5H_2O$
8	CH_3	10	174–176	B	0.20	25	$C_{46}H_{80}O_4N_{10}S_2Cl_4$•$4H_2O$
9	H	12	215 dec	D	0.21	29	$C_{48}H_{84}O_4N_{10}S_2Cl_4$•$3H_2O$
10	CH_3	12	203–205	E	0.23	16	$C_{50}H_{88}O_4N_{10}S_2Cl_4$•$1H_2O$

[a]Recrystallization solvent: A, abs EtOH; B, EtOH; C, EtOH/MeOH 2:1; D, EtOH/MeOH 3:1; E, EtOH/MeOH 4:1. [b]Eluting mixture: F. [c]Compounds were analyzed for C, H, N, and S, and results agreed to ± 0.4% of calculated values.

General procedure for the synthesis of ω-(thiazolidin-3-yl)-alkylamines ***14–16***

A solution of 5 M CH_3NH_2 in ethanol (250 mmol) was added to a cooled (–15 °C) and stirred solution of the corresponding 2-(ω-thiazolidin-3-yl-alkyl)isoindole-1,3-dione **42–44**, (2.5 mmol) in ethanol (30 mL); the mixture was then left to rise to room temperature. Following reflux for 2.5 h, the solution was cooled, acidified with conc HCl and the solvent evaporated. Water was added to the residue, the resulting mixture washed with chloroform and then basified with 2 N NaOH. Extraction with ether and removal of the dried solvent gave a crude product that was purified by column chromatography eluting with mixture D (table III).

General procedure for the synthesis of methyl[ω-(thiazolidin-3-yl)alkyl]amines ***17–20***

A 5 M ethanol solution of CH_3NH_2 (100 mmol) was added to a cooled solution (0 °C) of the corresponding toluen-4-sulfonic acid ω-(thiazolidin-3-yl)alkyl esters **38–41** (5 mmol) dissolved in benzene/ethanol 1:1 (100 mL) and then the solution was stirred at room temperature for 96 h. Removal of the solvent gave a residue that was purified by column chromatography eluting with mixture C (table III).

General procedure for the synthesis of toluen-4-sulfonic acid ω-(thiazolidin-3-yl)alkyl esters ***38–41***

A mixture of the appropriate α,ω-alkandiol ditosylated **34–37** (20 mmol) and dry K_2CO_3 (24 g) in acetonitrile (200 mL) was added under stirring and dropwise within 1 h to a solution of thiazolidine (16.8 mmol) in acetonitrile (20 mL). After refluxing for 24 h, the mixture was filtered and the solution evaporated to give a residue that was purified by column chromatography eluting with mixture A. Compounds were obtained as oil (R_f = 0.21–0.27) in 15–39% yield.

Physical data of the example toluen-4-sulfonic acid 8-(thiazolidin-3-yl)octyl ester **39**: Yield 24%, R_f = 0.27, ^{1}H-NMR ($CDCl_3$) δ 1.20–1.38 (m, 8H, $N(CH_2)_2$ (CH_2)$_4$), 1.40–1.54 (m, 2H, NCH_2CH_2), 1.56–1.71 (m, 2H, CH_2CH_2O), 2.30–2.39 (m, 2H, CH_2-N_{thiaz}), 2.46 (s, 3H, CH_3), 2.84–2.93 (m, 2H, CH_2S), 3.04–3.12 (m, 2H, NCH_2), 4.03 (t, J = 6.41 Hz, 2H, CH_2CH_2O), 4.08 (s, 2H, NCH_2S), 7.32–7.40 (m, 2H, Ar), 7.76–7.82 (m, 2H, Ar).

General procedure for the synthesis of 2-(ω-thiazolidin-3-yl-alkyl)isoindole-1,3-dione ***42–44***

A mixture of the corresponding toluen-4-sulfonic acid ω-(thiazolidin-3-yl)alkyl ester **38–41**, (8 mmol) and potassium phthalimide (12.8 mmol) in *N,N*-dimethylformamide (60 mL) was refluxed for 7 h. After cooling, water (180 mL) was added and the mixture extracted with chloroform. Removal of the dried solvent gave a residue that was purified by column chromatography eluting with mixture B. Compounds were obtained as oil (R_f = 0.23–0.34) in 72–90% yield.

Physical data of the example 2-(8-thiazolidin-3-yl-octyl)isoindole-1,3-dione **42**: Yield 90%, R_f = 0.23, ^{1}H-NMR ($CDCl_3$) δ: 1.24–1.40 (m, 8H, $(CH_2)_4(CH_2)_2$-N_{thiaz}), 1.41–1.56 (m, 2H, CH_2CH_2-N_{thiaz}), 1.58–1.75 (m, 2H, CH_2CH_2-N_{phth}), 2.29–2.38 (m, 2H, CH_2-N_{thiaz}), 2.83–2.91 (m, 2H, CH_2S), 3.02–3.11 (m, 2H, CH_2N), 3.68 (t, J = 7.66 Hz, 2H, CH_2-N_{phth}), 4.05 (s, 2H, NCH_2S), 7.67–7.91 (m, 4H, Ar).

General procedure for the synthesis of 6,7-dimethoxy-N_2-(ω-thiazolidin-3-yl)alkyl)quinazoline-2,4-diamines ***21–26*** and *6,7-dimethoxy-N_2-methyl-N_2-(ω-thiazolidin-3-yl)alkyl) quinazoline-2,4-diamines* ***27–30***

Compounds **21–23** were synthesized by refluxing in isoamyl alcohol equimolecular amounts of 4-amino-2-chloro-6,7-dimethoxyquinazoline and the appropriate ω-(thiazolidin-3-yl)alkylamine (**11–13**) for 72 h. After distillation in vacuo, the residue was basified with 2 N NaOH and extracted with chloroform. Removal of the dried solvent gave crude products which were purified by column chromatography. Compounds were obtained as oil or hygroscopic solid in 54–73% yield (table IV).

Compounds **24–26** and **27–30** were prepared by a slightly modified procedure. A mixture of 4-amino-2-chloro-6,7-dimethoxyquinazoline (2 mmol), triethylamine (10 mmol) and the appropriate ω-(thiazolidin-3-yl)alkylamine (**14–16**) or methyl-[ω-(thiazolidin-3-yl)alkyl]amine (**17–20**) (2.4 mmol) in isoamyl alcohol (30 mL) was stirred under reflux for 72 h. After distillation in vacuo, the residue was purified by column chromatography, eluting with mixture E. Compounds were obtained as oil or hygroscopic solid in 48–64% yield and used in the next step without further purification (table IV).

Table VI. ^{1}H-NMR data of compounds **1**–**10**·4HCl·xH$_2$O.

Compound	*DMSO-d_6 (δ ppm)*
1	1.60–1.85 (m, 8H, ArNHCH$_2$(CH_2)$_2$, 2.94–3.06 (m, 4H, CH$_2$S), 3.07–3.35 (m, 8H, CH_2NHCH_2), 3.58–3.78 (m, 4H, ArNHCH_2), 3.86 (s, 6H, OCH$_3$), 3.90 (s, 6H, OCH$_3$), 6.98 (s, br, 2H, Ar), 7.78 (s, br, 2H, Ar), 8.01 (s, br, 2H, NH exchangeable), 8.72 (s, br, 2H, NH exchangeable), 8.91 (s, br, 2H, NH exchangeable), 9.38 (s, br, 4H, NH exchangeable), 12.55 (s, br, 2H, NH exchangeable)
2	1.33–1.52 (m, 4H, ArNH(CH$_2$)$_2$CH_2), 1.52–1.84 (m, 8H, ArNHCH$_2$CH_2CH$_2$CH_2), 2.88–3.08 (m, 4H, CH$_2$S), 3.10–3.20 (m, 8H, CH_2NHCH_2), 3.50–3.71 (m, 4H, ArNHCH_2), 3.87 (s, 6H, OCH$_3$), 3.92 (s, 6H, OCH$_3$), 6.91 (s, br, 2H, Ar), 7.89 (s, br, 2H, Ar), 8.02 (s, br, 2H, NH exchangeable), 8.72 (s, br, 2H, NH exchangeable), 8.87 (s, br, 2H, NH exchangeable), 9.23 (s, br, 4H, NH exchangeable), 12.40 (s, br, 2H, NH exchangeable)
3	1.23–1.46 (m, 8H, ArNH(CH$_2$)$_2$(CH_2)$_2$), 1.52–1.76 (m, 8H, ArNHCH$_2$CH_2(CH$_2$)$_2$CH_2), 2.82–3.04 (m, 4H, CH$_2$S), 3.06–3.30 (m, 8H, CH_2NHCH_2), 3.58–3.74 (m, 4H, ArNHCH_2), 3.84 (s, 6H, OCH$_3$), 3.91 (s, 6H, OCH$_3$), 6.92 (s, br, 2H, Ar), 7.75 (s, br, 2H, Ar), 8.01 (s, br, 2H, NH exchangeable), 8.60 (s, br, 2H, NH exchangeable), 8.89 (s, br, 2H, NH exchangeable), 9.38 (s, br, 4H, NH exchangeable), 12.08 (s, br, 2H, NH exchangeable)
4	1.27–1.50 (m, 8H, ArN(CH$_2$)$_2$(CH_2)$_2$), 1.52–1.78 (m, 8H, ArNCH$_2$CH_2(CH$_2$)$_2$CH_2), 2.82–3.02 (m, 4H, CH$_2$S), 3.06–3.30 (m, 14H, CH_2NHCH_2 and NCH$_3$), 3.63–3.80 (m, 4H, ArNCH_2), 3.85 (s, 6H, OCH$_3$), 3.90 (s, 6H, OCH$_3$), 7.60 (s, br, 2H, Ar), 7.76 (s, br, 2H, Ar), 8.60 (s, br, 2H, NH exchangeable), 8.85 (s, br, 2H, NH exchangeable), 9.30 (s, br, 4H, NH exchangeable), 11.80 (s, br, 2H, NH exchangeable)
5	1.18–1.49 (m, 16H, ArNH(CH$_2$)$_2$(CH_2)$_4$), 1.50–1.75 (m, 8H, ArNHCH$_2$CH_2(CH$_2$)$_4$CH_2), 2.82–3.04 (m, 4H, CH$_2$S), 3.06–3.32 (m, 8H, CH_2NHCH_2), 3.33–3.50 (m, 4H, ArNHCH_2), 3.85 (s, 6H, OCH$_3$), 3.90 (s, 6H, OCH$_3$), 6.95 (s, br, 2H, Ar), 7.74 (s, br, 2H, Ar), 8.02 (s, br, 2H, NH exchangeable), 8.62 (s, br, 2H, NH exchangeable), 8.84 (s, br, 2H, NH exchangeable), 9.20 (s, br, 4H, NH exchangeable), 12.40 (s, br, 2H, NH exchangeable)
6	1.18–1.46 (m, 16H, ArN(CH$_2$)$_2$(CH_2)$_4$), 1.50–1.74 (m, 8H, ArNCH$_2$CH_2(CH$_2$)$_4$CH_2), 2.84–3.01 (m, 4H, CH$_2$S), 3.02–3.31 (m, 14H, CH_2NHCH_2 and NCH$_3$), 3.62–3.78 (m, 4H, ArNCH_2), 3.85 (s, 6H, OCH$_3$), 3.91 (s, 6H, OCH$_3$), 7.55 (s, br, 2H, Ar), 7.75 (s, br, 2H, Ar), 8.56 (s, br, 2H, NH exchangeable), 8.83 (s, br, 2H, NH exchangeable), 9.20 (s, br, 4H, NH exchangeable), 11.72 (s, br, 2H, NH exchangeable)
7	1.22–1.42 (m, 24H, ArNH(CH$_2$)$_2$(CH_2)$_6$), 1.48–1.72 (m, 8H, ArNHCH$_2$CH_2(CH$_2$)$_6$CH_2), 2.84–3.00 (m, 4H, CH$_2$S), 3.04–3.28 (m, 8H, CH_2NHCH_2), 3.30–3.50 (m, 4H, ArNHCH_2), 3.85 (s, 6H, OCH$_3$), 3.90 (s, 6H, OCH$_3$), 6.95 (s, br, 2H, Ar), 7.76 (s, br, 2H, Ar), 8.00 (s, br, 2H, NH exchangeable), 8.60 (s, br, 2H, NH exchangeable), 8.84 (s, br, 2H, NH exchangeable), 9.20 (s, br, 4H, NH exchangeable), 12.15 (s, br, 2H, NH exchangeable)
8	1.20–1.42 (m, 24H, ArN(CH$_2$)$_2$(CH_2)$_6$), 1.50–1.72 (m, 8H, ArNCH$_2$CH_2(CH$_2$)$_6$CH_2), 2.82–3.00 (m, 4H, CH$_2$S), 3.04–3.20 (m, 14H, CH_2NHCH_2 and NCH$_3$), 3.62–3.78 (m, 4H, ArNCH_2), 3.87 (s, 6H, OCH$_3$), 3.92 (s, 6H, OCH$_3$), 7.62 (s, br, 2H, Ar), 7.78 (s, br, 2H, Ar), 8.58 (s, br, 2H, NH exchangeable), 8.89 (s, br, 2H, NH exchangeable), 9.27 (s, br, 4H, NH exchangeable), 11.82 (s, br, 2H, NH exchangeable)
9	1.15–1.44 (m, 32H, ArNH(CH$_2$)$_2$(CH_2)$_8$), 1.48–1.74 (m, 8H, ArNHCH$_2$CH_2(CH$_2$)$_8$CH_2), 2.85–3.00 (m, 4H, CH$_2$S), 3.08–3.12 (m, 8H, CH_2NHCH_2), 3.33–3.52 (m, 4H, ArNHCH_2), 3.86 (s, 6H, OCH$_3$), 3.92 (s, 6H, OCH$_3$), 6.96 (s, br, 2H, Ar), 7.78 (s, br, 2H, Ar), 8.01 (s, br, 2H, NH exchangeable), 8.66 (s, br, 2H, NH exchangeable), 8.91 (s, br, 2H, NH exchangeable), 9.30 (s, br, 4H, NH exchangeable), 12.08 (s, br, 2H, NH exchangeable)
10	1.20–1.40 (m, 32H, ArN(CH$_2$)$_2$(CH_2)$_8$), 1.52–1.76 (m, 8H, ArNCH$_2$CH_2(CH$_2$)$_8$CH_2), 2.84–3.00 (m, 4H, CH$_2$S), 3.07–3.30 (m, 14H, CH_2NHCH_2 and NCH$_3$), 3.63–3.78 (m, 4H, ArNCH_2), 3.86 (s, 6H, OCH$_3$), 3.91 (s, 6H, OCH$_3$), 7.60 (s, br, 2H, Ar), 7.78 (s, br, 2H, Ar), 8.55 (s, br, 2H, NH exchangeable), 8.86 (s, br, 2H, NH exchangeable), 9.35 (s, br, 4H, NH exchangeable), 11.85 (s, br, 2H, NH exchangeable)

*General procedure for the synthesis of N-(4-amino-6,7-dimethoxyquinazolin-2-yl)-N'-{2-[2-(ω-(4-amino-6,7-dimethoxyquinazolin-2-yl-amino)alkylamino)ethyl disulfanyl]ethyl}alkane-α,ω-diamines tetrahydrochlorides **1–3**, **5**, **7**, **9** and N-(4-amino-6,7-dimethoxyquinazolin-2-yl), N-methyl-N'-{2-[2-(ω-(4-amino-6,7-dimethoxyquinazolin-2-yl-methylamino)alkylamino)ethyldisulfanyl]ethyl}alkane-α,ω-diamines tetrahydrochlorides **4**, **6**, **8**, **10***

A 0.1 N solution of I_2 (1.5 meq) was added dropwise to a vigorously stirred solution of the corresponding 6,7-dimethoxy-N_2-(ω-thiazolidin-3-yl)alkyl) quinazoline-2,4-diamine (**21–23**) (1.5 mmol) dissolved in chloroform (30 mL). After 2 h of stirring, the mixture was basified with 2 N NaOH and extracted with chloroform. The organic phases were collected and dried and then evaporated to give a residue that was transformed into the corresponding hydrochloride salt **1–3**, and purified by crystallization (tables V and VI).

Compounds **4–10** were prepared following a slightly modified procedure. The appropriate 6,7-dimethoxy-N_2-(ω-thiazolidin-3-yl)alkyl)quinazoline-2,4-diamine (**24–26**) or 6,7-dimethoxy-N_2-methyl-N_2-(ω-thiazolidin-3-yl)alkyl)quinazoline-2,4-diamine (**27–30**) was oxidized by slow addition, under stirring, of an equivalent amount of 0.1 N solution of I_2 in MeOH/H_2O (95:5) as solvent, then the reaction mixture was left overnight in a refrigerator. The precipitate was filtered and triturated with cold 1 N NaOH. The crude solids were filtered and washed with water. Compounds **4–7** and **9** were obtained by direct transformation into the hydrochloride salts, whereas **8** and **10** were previously purified by column chromatography eluting with mixture F. All products were crystallized by appropriate solvents (tables V and VI).

Pharmacology

Functional antagonism of agonist-induced contraction in isolated rat vas deferens

The α_1- and α_2-adrenoceptor antagonist activity of new tetraamine disulfides **1–10**, and reference compounds benextramine and prazosin, dissolved in 10–30% (v/v) DMSO/H_2O, was determined on isolated rat vas deferens tissues. Male albino rats (Crl: CD, BR), 175–200 g, were killed by a sharp blow to the head and both vasa deferentia were isolated, freed from adhering connective tissue and transversely bisected. Prostatic, 12 mm in length, and epididymal portions, 14 mm in length, were prepared and mounted individually in baths of 10 mL working volume containing Krebs solution at pH 7.4. In the experiments at α_1-adrenoceptors the Krebs solution was of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.52; $MgCl_2$, 2.52; KH_2PO_4, 1.65; $NaHCO_3$, 12.5; glucose, 11.7. When the twitch response to field stimulation was studied, in the above solution the concentration of KH_2PO_4 was reduced to 1.2 mM, whereas the concentration of $NaHCO_3$ was increased to 25 mM. In addition, 0.6 mM $MgSO_4$ heptahydrate was used, instead of 2.52 mM $MgCl_2$. The medium was maintained at 37 °C and gassed with 95% O_2/5% CO_2. The loading tension used to assess α_1- or α_2-blocking activities was 0.4 *g* or 0.5–0.8 *g*, respectively, and contractions were recorded by means of force transducers connected to a two-channel Gemini 7070 polygraph. The tissues were allowed to equilibrate for at least 1 h before addition of any drug. Parallel experiments, in which tissues did not receive any antagonist, were run in order to correct for time-dependent changes in agonist sensitivity [46]. Field stimulation of the tissue was carried out by means of two platinum electrodes, placed near the top and bottom of the vas deferens, at 0.1 Hz using square pulses of 3 ms duration at a voltage of 10–35 V. The stimulation voltage was fixed throughout the experiments. Propranolol hydrochloride (1 μM) and cocaine hydrochloride (10 μM) were present in the Krebs solution throughout the experiments outlined below to block β-adrenoceptors and neuronal uptake mechanisms, respectively.

The α_1-adrenoceptor blocking activity was determined on the epididymal portion of the vas deferens using (–)-noradrenaline as agonist, whereas the agonist clonidine was employed for assessing the α_2-adrenoceptor blocking activity on the prostatic portion.

Given the structural analogy between the novel tetraamine disulfides and the prototype benextramine, compounds **1–10** were first checked for their potential irreversible antagonism on α_1- and α_2-adrenoceptors evaluating the action of a few concentrations of each compound after 30 min incubation followed by 30 min washing of tissues. It emerged that compounds **1–10**, unlike benextramine, were not able to inhibit α_1-adrenoceptors irreversibly, rather they showed, like prazosin, a competitive antagonism. However, at α_2-adrenoceptors an irreversible antagonism was observed.

When assessing α_1-adrenoceptor antagonism, the (–)-noradrenaline dose–response curves were obtained cumulatively, the first being discarded and the second taken as a control. A third dose–response curve was then obtained after incubation with the antagonist for 60 min. All compounds were tested at three different concentrations and each concentration was investigated four times. The competitive antagonist potency was expressed as pA_2 values estimated by Schild plots [41] constrained [44] to slope –1.0. When this method was applied, it was always verified that the experimental data generated a line whose slope was not significantly different from unity ($P > 0.05$). The antagonist potency of benextramine was expressed by the negative logarithm of concentration that causes 50% inhibition of agonist action (pIC_{50}).

The α_2-adrenoceptor blocking activity was assessed by the antagonism to the clonidine-induced depression of the twitch responses of the field-stimulated tissues [47, 48]. 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. Thus, after incubation with the antagonist for 30 min, and washing with physiological solution for 30 min, a dose–response curve was obtained and compared with the control. Each compound was tested at three different concentrations and each concentration was investigated at least four times. The antagonist potency of new compounds, if any, was expressed by the negative logarithm of concentration that causes 50% inhibition of agonist action (pIC_{50}), whereas the prazosin activity was expressed as a pA_2 value.

All data are presented as the mean ± SE of *n* experiments. Differences between mean values were tested for significance by Student's *t*-test.

Radioligand binding studies

Livers and submaxillary glands, obtained from male Sprague–Dawley rats, 250–300 g, were dissected and frozen at –80 °C up to 1 month before assay. For the assay, tissues were separately homogenized in 10 vol (w/v) of 50 mM Tris-HCl, 5 mM EDTA homogenizing buffer (pH 7.4, 4 °C), using an Ultra-Turrax homogenizer (three 10 s bursts). The homogenate was filtered through a double layer of surgical gauze and centrifuged twice at 500 x *g* for 10 min (4 °C) with inter-

mediate resuspension in 2 vol of fresh buffer. The supernatants were combined and centrifuged at 43 000 x *g* for 12 min at 4 °C. Pellets were washed twice with ice cold 50 mM Tris-HCl, 0.5 mM EDTA assay buffer (pH 7.4, 4 °C) and then resuspended in fresh assay buffer. Protein concentration was evaluated on aliquots of the suspension according to Lowry et al [49], using bovine serum albumin as standard. Binding studies were performed in duplicate by incubating membranes (400 μg of liver protein and 500 μg of submaxillary gland protein) in the same buffer with [^{3}H]Prazosin (DuPont NEN, specific activity 24 Ci/mmol), alone or with compounds **2**, **3**, **5–9** and references for 1 h at 25 °C. Following incubation, membranes were collected on Whatman GF/B filters under vacuum and rapidly washed with ice-cold buffer (3 x 5 mL) and the radioactivity retained on filters determined by liquid scintillation spectrometry. Filters were presoaked in 0.3% polyethylenimine to decrease non-specific binding to the filter.

Saturation curves were determined by incubating membranes with increasing concentrations of [^{3}H]Prazosin (0.005–2 nM) and analyzing the data according to Scatchard [50]. In the rat liver, [^{3}H]Prazosin K_D was 98 ± 11 pM and B_{max} was 72.2 ± 8 fmol/μg protein whereas in rat submaxillary gland K_D was 267 ± 33 pM and B_{max} was 253 ± 13 fmol/μg protein (values are the mean ± standard error; n = 3). In both tissue homogenates, the Scatchard plots best fitted to a one site model (data not shown). The potency of compounds in competing for the specific [^{3}H]Prazosin binding sites was determined by incubating 0.1 nM of radioligand in the presence or absence of eight concentrations of the competing drugs. Non-specific binding was evaluated in the presence of 10 μM phentolamine; specific binding represented 65–80% of the total binding at the K_D. The IC_{50} values were determined as the x intercept on a Hill plot, and K_i values were calculated by the Cheng–Prusoff method [42]. Two-site models were compared to one-site models to determine whether the increase of goodness of fit was significantly more than could be expected on the basis of chance alone using a partial F test [43]. The P values less than 0.05 were considered significant. This allowed for a more precise determination of the affinities of the competitors at each of the one or more putative binding sites.

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