238–240 °C; (IR) (KBr) 1660, 1625, 1500, 1450, 1425 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.65 (s, 2 H), 7.18–7.66 (m, 8 H),, 7.78 (s, 1 H), 8.07 (s, br, NH), 8.23 (d, J = 8.0 Hz, 1 H), 8.41 (s, 1 H), 11.56 (s, indole 1 H); MS (CI, CH_4), 274 (M + 1). Anal. ($C_{18}H_{15}N_3 \cdot HCl \cdot {}^1/_8EtOH$) C, H, N.

Acknowledgment. We wish to thank Liesl Schindler and Adiga Godi for excellent technical assistance and Anju Gupta for preparation of this manuscript. We also wish to thank the NIMH (Grant MH 36644) and NIH (Grant NS 22287) for generous financial support. The 500-MHz NMR spectrometer was purchased via grants from the NIH–BRSG program and the graduate school (UWM).

Registry No. **2**, 114819-74-8; **2** (free base), 91985-82-9; **3**, 114819-73-7; **3** (free base), 91985-81-8; **4**, 114819-75-9; **4** (free base), 91985-83-0; **5**, 91985-80-7; **6**, 114819-76-0; **6** (free base), 114819-77-1; **7**, 114819-79-3; **8**, 114819-72-6; **8** (free base), 73834-77-2; **8** (dihydrochloride), 114819-78-2; **9**, 91985-78-3; **13**, 114819-81-7; **13** (free base), 114819-80-6; **18**, 106613-33-6; benzaldehyde, 100-52-7.

Structure–Activity Relationships among Benextramine-Related Tetraamine Disulfides. Chain Length Effect on α-Adrenoreceptor Blocking Activity[1]

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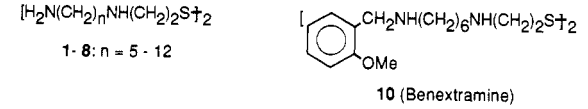
Several N′-substituted N,N''-(dithiodi-2,1-ethanediyl)bis(1,ω-alkanediamines) were prepared and evaluated for their blocking activity on α-adrenoreceptors in the isolated rat vas deferens and human blood platelets. The results were compared with those obtained for benextramine (N,N''-(dithiodi-2,1-ethanediyl)bis[N'-[(2-methoxyphenyl)-methyl]-1,6-hexanediamine], **10**). Bendotramine (N,N''-(dithiodi-2,1-ethanediyl)bis[N'-[(2-methoxyphenyl)-methyl]-1,12-dodecanediamine], **16**) proved to be as active as **10** on α_1-adrenoreceptors, showing that optimum activity is associated with two carbon chain lengths separating inner from outer nitrogens of tetraamine disulfides. On the other hand, **16** had no activity up to 20 μM at α_2-adrenoreceptors. The optimum activity at this receptor subtype was associated with a six to eight carbon chain (**10–12**). Furthermore, **10** proved to be more selective toward α_2-adrenoreceptors whereas **16** was a selective α_1-antagonist. The tetraamine disulfides were shown also to be potent inhibitors of human platelet aggregation induced by ADP or epinephrine. The potency increased with the carbon chain length. However, the results on platelets did not parallel those found in the rat vas deferens, indicating that differences exist between the α-adrenoreceptor subtypes investigated. In conclusion, **10** may be a useful tool in characterizing α_2-adrenoreceptors whereas **16** might help in investigating α_1-adrenoreceptors.

The development of polymethylene tetraamines, whose main feature is a cystamine moiety carrying aminoalkyl substituents on the nitrogens, as α-antagonists started from the observation that the linear tetraamine disulfide **1** (n = 5), originally developed as a radioprotective agent,[2] showed a relatively weak but apparently selective and irreversible α-blocking activity. Extensive structure–activity relationships were carried out with the aim of improving the potency of **1** and elucidating the active-site topography in the region of the target thiol.[3,4] These studies led to the discovery of compounds such as benextramine (**10**),[4a] pyrextramine,[4g] or **4** (n = 8)[4a] displaying an α_1-blocking activity about 2 orders of magnitude higher than that of the parent compound **1**.



10 (Benextramine)

It was shown that α_1-adrenoreceptor inhibition by tetraamine disulfides 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.[3,4a] It was also found that optimum activity is associated with two different carbon chain lengths separating the inner from the outer nitrogens and depends on the type of substituents on the terminal nitrogens. Thus, optimum α_1-blocking activity in the series with benzyl-type substituents on the terminal nitrogens is associated with a six-carbon chain, as in **10**, on rat vas deferens[4a,g] and rabbit aorta[4d] and left atrium[5] whereas in the unsubstituted series it is associated with an eight-carbon chain, as in **4**, on rat vas deferens[4a] and a seven-carbon chain, as in **3**, on rabbit aorta.[4d]

The prototype of tetraamine disulfides **10** has been fairly well investigated in both functional studies and binding experiments.[4a,g,6–27] Compound **10** irreversibly blocked

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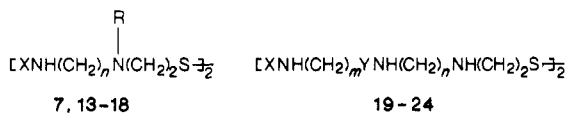
Table I. α-Adrenoreceptor Blocking Activity in the Isolated Rat Vas Deferens and Inhibition of Human Blood Platelet Aggregation[a]

$[ArCH_2NHYNR(CH_2)_2S]_2$

antagonist[b]		rat vas deferens			human blood platelet	
no.	Y	α_1 IC_{50}, μM, against norepinephrine	α_2 IC_{50}, μM, against clonidine	α_2/α_1 selectivity ratio[c]	IC_{50}, μM, against epinephrine	IC_{50}, μM, against ADP
9	$(CH_2)_5$	55.80 ± 4.1	25.80 ± 7.10	2.16	46.0 ± 3.6	87.0 ± 8.8
10	$(CH_2)_6$	7.55 ± 0.68	0.82 ± 0.086	9.20	31.3 ± 2.5	97.0 ± 12.9
11	$(CH_2)_7$	55.20 ± 9.1	1.04 ± 0.21	53.08	20.6 ± 2.6	41.3 ± 0.9
12	$(CH_2)_8$	98.50 ± 16.5	1.22 ± 0.12	80.74	5.4 ± 0.2	22.7 ± 1.8
13	$(CH_2)_9$	68.00 ± 9.8	inactive[e]	<3.4	2.3 ± 0.5	6.0 ± 0.1
14	$(CH_2)_{10}$	62.80 ± 11.3	inactive[e]	<3.14	2.2 ± 0.4	2.8 ± 0.3
15	$(CH_2)_{11}$	51.30 ± 10.0	inactive[e]	<2.57	1.7 ± 0.1	2.1 ± 0.4
16	$(CH_2)_{12}$	8.23 ± 1.47	inactive[e]	<0.41	1.7 ± 0.1	2.1 ± 0.1
17	$(CH_2)_{12}$	7.30 ± 5.4	inactive[e]	<0.37		
18	$(CH_2)_{12}$	46.90 ± 0.9	inactive[e]	<2.35		
21	$(CH_2)_2CONH(CH_2)_8$	inactive[d]				
22	$(CH_2)_5CONH(CH_2)_6$	inactive[d]				
23	$(CH_2)_3NH(CH_2)_8$	inactive[d]				
24	$(CH_2)_6NH(CH_2)_6$	inactive[d]				
adenosine					1.8 ± 0.3	2.5 ± 0.4

[a] IC_{50} values are expressed as the mean ± standard error of at least four independent observations. [b] **9–16, 21–24**: Ar = 2-$MeOC_6H_4$, R = H. **17**: Ar = 2-pyrrolyl, R = H. **18**: Ar = 2-$MeOC_6H_4$, R = Me. [c] The α_2/α_1 selectivity ratio is a measure of the selectivity and is the antilog of the difference between the −log IC_{50} values at α_2- and α_1-adrenoceptors. [d] Inactive up to a concentration of 20 μM (see the Experimental Section). [e] Inactive up to a concentration of 20 μM (see the Experimental Section).

Table II. Physical Characteristics of Polyamine Disulfides



7, 13–18 **19–24**

no.	X	R	Y	*n*	*m*	mp,[a] °C	purifn solvent[b]	formula[c]
7	H	H		11		289–291	A	$C_{26}H_{58}N_4S_2 \cdot 4HCl$
13	2-$MeOC_6H_4CH_2$	H		9		166–168	A/B	$C_{38}H_{66}N_4O_2S_2 \cdot 4HCl \cdot H_2O$
14	2-$MeOC_6H_4CH_2$	H		10		195–197	C	$C_{40}H_{70}N_4O_2S_2 \cdot 4HCl \cdot H_2O$
15	2-$MeOC_6H_4CH_2$	H		11		198–200[d]	A–B	$C_{42}H_{74}N_4O_2S_2 \cdot 4HCl \cdot H_2O$
16	2-$MeOC_6H_4CH_2$	H		12		192–194	A–B	$C_{44}H_{78}N_4O_2S_2 \cdot 4HCl \cdot 2H_2O$
17	pyrrol-2-ylmethyl	H		12		indef[e]	D	$C_{38}H_{72}N_6S_2 \cdot 4H_2C_2O_4 \cdot H_2O$
18	2-$MeOC_6H_4CH_2$	Me		12		149–151[f]	A–B	$C_{46}H_{82}N_4O_2S_2 \cdot 4H_2C_2O_4$
19	H		CO	8	2	225–227	A–D	$C_{26}H_{56}N_6O_2S_2 \cdot 4HBr$
20	H		CO	6	5	192–197	C	$C_{28}H_{60}N_6O_2S_2 \cdot 4HBr$
21	2-$MeOC_6H_4CH_2$		CO	8	2	191–192	A–D	$C_{42}H_{72}N_6O_4S_2 \cdot 4H_2C_2O_4 \cdot H_2O$
22	2-$MeOC_6H_4CH_2$		CO	6	5	186–188	A–D	$C_{44}H_{76}N_6O_4S_2 \cdot 4H_2C_2O_4 \cdot 1.5H_2O$
23	2-$MeOC_6H_4CH_2$		CH_2	8	2	237–239	A	$C_{42}H_{76}N_6O_2S_2 \cdot 6HCl \cdot 1.5H_2O$
24	2-$MeOC_6H_4CH_2$		CH_2	6	5	235–237	C	$C_{44}H_{80}N_6O_2S_2 \cdot 6HCl \cdot 3H_2O$

[a] All compounds were crystallized at least three times and decolorized with charcoal when necessary. Their purity was uniformly checked by TLC (silica). IR and NMR spectra of all compounds were in agreement with the expected structures. The heating rate was 1 °C/min for melting point determinations. [b] A, methanol; B, 2-propanol; C, absolute ethanol; D, water. [c] Analyses for C, H and N were within ±0.4% of the theoretical value required. [d] Decomposition began at 180 °C. [e] Compound **17** has indefinite melting point. Decomposition began at 110 °C. [f] It crystallizes also with two water molecules: mp, 128–131 °C.

both α_1- and α_2-adrenoreceptors.[4,11] However, nothing is known about the structural requirements for optimum occupancy of α_2-adrenoreceptors by tetraamine disulfides. To this end we investigated the effect of the chain length separating the inner from the outer nitrogens of tetraamine disulfides on the α_2-blocking activity of isolated rat vas deferens and human blood platelets. Since at α_1-adrenoreceptors only tetraamine disulfides **9–12** having five to eight methylenes between the nitrogens were investigated,[4a] we extended that study to compounds **13–16** in order to achieve a better understanding of the chain length effect on α_1-blocking activity. Since **16** (n = 12) turned out to be as active as **10** (n = 6), some derivatives were designed with the aim of correlating the binding sites of **16** with those of **10** or alternatively **4**. Thus, hexaamine disulfides **23** and **24** were investigated to verify whether **16** may bind with two additional receptor anionic sites situated at an

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appropriate distance from the binding sites for **10** or **4**. In fact, **23** and **24** have a chain length separating the inner from the outer nitrogens that is similar to that in **16** while bearing additional nitrogens inserted in the carbon chain at the same position of the outer nitrogens of **10** and **4**, respectively. N-methylation of the inner nitrogens of **10** and **4** resulted in a different effect on activity, suggesting that **10** and **4** can hardly interact with the same set of anionic sites.[4a,28,29] Thus, a similar structural modification was performed on the inner nitrogens of **16** yielding the *N,N''*-dimethyl analogue **18** to gain information on its binding sites. To the same end, the effect of terminal nitrogen substitution of **16** affording **17** was investigated.

Chemistry. Benextramine analogues were synthetized by standard procedures and their structure is shown in Table II.

The previously unreported tetraamine disulfide **7** (n = 11) was obtained through the reaction of thiazolidine and *N*-(11-bromoundecyl)phthalimide,[30] affording *N*-[11-(1,3-thiazolidin-3-yl)undecyl]phthalimide. Oxidative ring opening and dimerization by treatment with iodine followed by removal of the phthalimido protecting group by hydrazine treatment gave tetraamine disulfide **7**. The substituents on the terminal nitrogens were easily introduced by condensation of **1**–**8**[4a] with the appropriate aromatic aldehyde and subsequent reduction of the intermediate Schiff bases affording **9**–**17**. Substituted tetraamine disulfides **13**–**17** were previously unreported.

3-(12-Aminododecyl)-1,3-thiazolidine was obtained by the procedure already reported for other analogues[31] and was converted to *N,N''*-(dithiodi-2,1-ethanediyl)bis[*N'*-(benzyloxycarbonyl)-1,12-dodecanediamine] by reaction with benzyloxycarbonyl chloride followed by oxidative ring opening and dimerization with iodine. Methylation of the inner nitrogens was carried out by the Eschwiler–Clarke method[32] followed by removal of the benzyloxycarbonyl protecting group to give *N,N''*-(dithiodi-2,1-ethanediyl)-*N,N''*-dimethylbis(1,12-dodecanediamine), which was transformed into **18** as described for **9**–**17**.

3-(6-Aminohexyl)- and 3-(8-aminooctyl)-1,3-thiazolidines[31] were N-acylated with *N*-(benzyloxycarbonyl)-6-aminohexanoic acid or *N*-(benzyloxycarbonyl)-3-aminopropionic acid. Oxidative ring opening and dimerization with iodine followed by acid hydrolysis of the benzyloxycarbonyl protecting group gave tetraamine diamide disulfides **22** and **21**. Terminal substitution was carried out as already described for **9**–**17**. Finally, reduction of tetraamine diamide disulfides **21** and **22** followed by oxidation gave hexaamine disulfides **23** and **24**.

Pharmacology. The biological profile of hexaamine and tetraamine disulfides at α-adrenoreceptors was assessed on isolated rat vas deferens and human blood platelets (Table I and Figure 1).

α_1-Adrenoreceptor blocking activity was assessed by antagonism of (−)-norepinephrine-induced contractions of the epididymal portion of the vas deferens. α_2-Adrenoreceptor blocking activity was determined by antagonism of the clonidine-induced depression of the twitch responses of the field-stimulated prostatic portion of vas deferens.

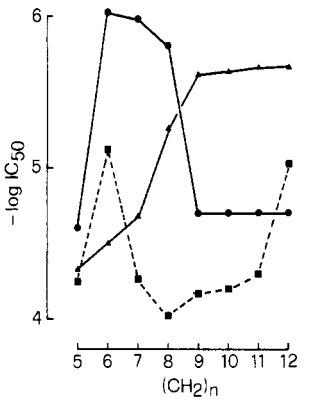

Figure 1. Comparative potencies of the tetraamine disulfides **10**–**16** at rat vas deferens α_1- (■) and α_2-adrenoreceptors (●), and human blood platelet α-adrenoreceptors (▲) vs length of the carbon chain [$(CH_2)_n$] separating inner from outer nitrogens. The results are expressed as −log IC_{50} values calculated from Table I. Note that the values for **13**–**16** at α_2-adrenoreceptors were calculated assuming IC_{50} values of 20 μM (see table I).

In order to allow comparison of the results, we used the same techniques and statistical evaluation of the bioassays as for other tetraamine disulfides.[4a,g] Thus, the noncompetitive (irreversible) α_1- or α_2-antagonism was determined at three different concentrations after a 30-min incubation followed by 30 min of washing. The decrease in maximum response was expressed as a percentage of the control value.

α-Adrenoreceptors of human platelets are often said to be similar to α_2-adrenoreceptors, but there are differences.[17,33] Thus, tetraamine disulfides **9**–**16** were investigated on human platelet aggregation to verify whether there is a correlation with the potency displayed at rat vas deferens. To this end the ability of **9**–**16** to inhibit epinephrine-induced as well as ADP-induced platelet aggregation, in comparison with that of adenosine, was measured in vitro in human platelet-rich plasma.

The potency for each compound is expressed as IC_{50} values, the concentrations that produce 50% inhibition of the agonist maximal response.

Results and Discussion

The biological results obtained in the present investigation are assembled in Table I and are graphically shown in Figure 1. It is evident that benextramine analogues **9**–**18** have a significant and irreversible α_1-blocking activity whereas compounds **21**–**24** have no activity up to a concentration of 20 μM. It was previously found that with terminal 2-methoxybenzyl substituents optimum activity is strongly associated with a six-carbon chain as in **10**.[4a,28,29] Present results show that a 12-carbon chain is as effective as a six-carbon one. In fact, bendotramine (**16**) was as active as benextramine (**10**) (Figure 1). It was advanced that substituted and unsubstituted tetraamine disulfides, exemplified by **10** and **4**, respectively, bind with two distinct sets of sites disposed like a cross while sharing a common target thiol.[4c,28,29,34] This hypothesis was based on the observation that N-methylation of the common cystamine segments of **10** and **4** had a significant effect on the activity (decrease) of only one (**10**) of the two prototypes and that the unsymmetrical tetraamine disulfide which results from the combination of half of **10** with half

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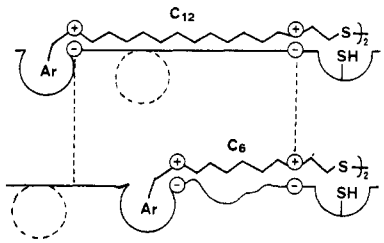

Figure 2. Hypothetical conformational change in the rat vas deferens α_1-adrenoreceptor induced by terminal *N*-(2-methoxy)benzyl (Ar) substitution and chain length separating the nitrogens of tetraamine disulfides bendotramine (**16**) and benextramine (**10**).

of **4** retained the same activity as the prototypes.[4c] Furthermore, on the basis of protection experiments and substituent effects we have advanced the idea that the terminal nitrogens of **10** may interact with the anionic site recognized by the neurotransmitter while the 2-methoxybenzyl substituent would bind to an accessory area.[28,29] Present results raise the question of whether the receptor binding sites recognized by the tetraamine disulfide **16** are identical with those for **10** and **4**, nonidentical but connected, or unconnected.

As preliminary remarks, we note that **4** can be excluded because of the observation that N-methylation of the inner nitrogens of **16**, giving **18**, had the same effect on activity (decrease) (Table I) as in **10** (in contrast to **4**),[4c] suggesting that the inner nitrogens of **16** and those of **4** can hardly interact with the same set of sites. Furthermore, the finding that the anionic sites for the terminal nitrogens of **4** do not accept benzyl-type substituents[4] eliminates also the possibility of a common binding site for the terminal nitrogens of **16** and **4**.

We will limit our discussion to the first two possibilities because our data is not sufficient to confirm or exclude the third one. The simplest explanation of the results obtained with **10** and **16** would be provided either of the following possibilities: (a) the two antagonists bind with a single set of sites that adapts conformationally at either one of the two structures or (b) the terminal nitrogens of **16** bind with two additional receptor anionic sites whereas the cystamine moiety interacts with the same sites for **10** (inner nitrogens). The latter hypothesis was ruled out by the observation that the hexaamine disulfide **24**, bearing two additional cationic nitrogens at the same distance as in **16** (outer nitrogens), had no activity at a concentration (20 μM) (Table I) at which both **10** and **16** inhibit completely rat vas deferens α_1-adrenoreceptors (not shown). In favor of possibility a, we observe that the substitution of 2-methoxybenzyl groups of **16** for pyrrol-2-ylmethyl groups, giving **17**, resulted in a slight increase of activity as in **10**. Taken together, the parallel effects observed by substituting the inner and outer nitrogens of **10** and **16** point to a common set of sites (hypothesis a). That the binding sites be identical requires the distance separating the receptor anionic sites, when **16** is the ligand, must be shortened by six bond lengths. This could be accomplished by a receptor conformational change induced by the different chain length of **10** (Figure 2).

Table I and Figure 1 clearly show that the structural requirements for optimum α-adrenoreceptor occupancy by tetraamine disulfides are different at the two receptor subtypes. Compounds **9–12** have an irreversible α_2-blocking activity that is significantly higher than that observed at the α_1-adrenoreceptor. In contrast, tetraamine disulfides **13–18** have no activity up to 20 μM concentration. Thus, optimum activity at rat vas deferens α_2-adrenoreceptors is now associated with a six to eight carbon chain length separating inner and outer nitrogens. The finding that **16** has no α_2-blocking activity up to 20 μM is of interest as this is a concentration that completely inhibits α_1-adrenoreceptors. This clearly indicates that **16** is a selective and irreversible α_1-adrenoreceptor antagonist whereas **10** is more selective toward α_2-adrenoreceptors having IC_{50} values of 7.55 and 0.82 μM for α_1- and α_2-adrenoreceptors, respectively. The higher selectively of **10** for α_2-adrenoreceptors has also been confirmed by binding experiments.[9,10] However, tetraamine disulfides **11** and **12** bearing a seven- and an eight-carbon chain, respectively, proved to be the most selective toward α_2-adrenoreceptors with an α_2/α_1 selectivity ratio of about 2 orders of magnitude while being as active as **10** (Table I). The lack of activity of **16** at α_2-adrenoreceptors may be relevant for delineating differences between active-site topographies in the region of the target thiol of α_1- and α_2-adrenoreceptors. If **16** interacted with α_1-adrenoreceptors according to the hypothesis outlined in Figure 2, its inactivity toward α_2-adrenoreceptors would indicate that this receptor carries four complementary anionic sites around a target thiol for the binding of **10** but is unable to accomodate **16** by way of a conformational change.

Tetraamine disulfides **9–16** were effective inhibitors of human platelet aggregation induced by ADP and epinephrine (Table I). The results clearly indicate that there is a strict correlation between inhibitory activity and carbon chain length. As to the ADP-induced platelet aggregation, compounds **9** (n = 5, IC_{50} = 87 μM) and **10** (n = 6, IC_{50} = 95 μM) are about 45 times less effective than **16** (n = 12, IC_{50} = 2.1 μM), the inhibitory activity of compounds **14–16** being approximately the same. Also in the case of epinephrine-induced aggregation the inhibitory potency correlates with carbon chain length. Thus, compound **9** (IC_{50} = 46 μM) is about 30 times less active than **15** and **16** (IC_{50} = 1.7 μM). It is worth noting that benextramine analogues gave a completely different pattern at platelet α_2-adrenoreceptors when compared to that observed at rat vas deferens α_1- and α_2-adrenoreceptors.

In a previous study it was observed that the biological profile of **10** differs from that of phentolamine, which has a much higher α-adrenoreceptor blocking potency in platelets. In fact, it has been shown that[17] phentolamine causes 51% inhibition of the epinephrine effect in platelets in a concentration of 100 nM, whereas it only slightly affects the ADP-induced aggregation. In the present study we conclude that the analogues of **10** antagonize aggregation induced by ADP in concentrations slightly higher than those that inhibit aggregation induced by epinephrine. It is also seen that the IC_{50} values for compounds **14–16** are similar to those for adenosine both in ADP- and in epinephrine-induced aggregation.

In conclusion, the present investigation has confirmed and extended the view that tetraamine disulfides are effective antagonists of rat vas deferens α-adrenoreceptors and human platelet aggregation induced by epinephrine. However, the structural requirements for optimum occupancy were shown to be different at the receptor subtypes investigated.

Experimental Section

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 EM-390 instruments, respectively. Although the IR and NMR spectral 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 microanalyses were performed by the Microanalytical Laboratory of our department, and the elemental compositions of the compounds agreed to within ±0.4%

of the calculated value. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.063–0.200 mm, Merck). The term "dried" refers to the use of anhydrous sodium sulfate.

***N*-[11-(1,3-Thiazolidin-3-yl)undecyl]phthalimide.** A solution of *N*-(11-bromoundecyl)phthalimide[30] (3.8 g, 10.0 mmol) and an excess of thiazolidine (3.0 g) in absolute EtOH (40 mL) was heated under reflux for 40 h under nitrogen. The solvent was removed under reduced pressure, and the residue was taken up in 1 N NaOH (25 mL) and extracted with chloroform (3 × 50 mL). Extracts were dried and then evaporated to give an oil, which was purified by column chromatography by eluting with cyclohexane–chloroform–ethyl acetate (7:2:1), to give 1.25 g of desired compound, which was used in the next step without further purification.

***N*,*N*″-(Dithiodi-2,1-ethanediyl)bis(1,11-undecanediamine) Tetrahydrochloride (7).** A solution of *N*-[11-(1,3-thiazolidin-3-yl)undecyl]phthalimide (1.25 g, 3.22 mmol) in chloroform was treated dropwise with a solution of 0.1 N iodine in 2.5% potassium iodide (32.2 mL) with stirring. The reaction mixture was treated with 2 N NaOH (25 mL) and extracted with chloroform (3 × 50 mL). Removal of dried solvents gave an oil, which was dissolved in absolute EtOH (25 mL) and treated with hydrazine monohydrate (0.17 g). The solution was heated under reflux for 24 h. Removal of solvent gave a residue, which was taken up in 6 N HCl (10 mL) and heated at 50 °C for 15 min. The reaction mixture was diluted with warm water (200 mL), and the precipitate was filtered off and washed with water. The solution was washed with chloroform (30 mL) and then made basic with 6 N NaOH (10 mL) and extracted with chloroform (4 × 30 mL). Extracts were dried and then evaporated to give 0.63 g of **7**, which was purified by crystallization of the tetrahydrochloride salt (Table II).

***N*,*N*″-(Dithiodi-2,1-ethanediyl)bis[*N*′-[(2-methoxyphenyl and pyrrol-2-yl)methyl]-1,ω-alkyldiamine] Tetrahydrochlorides (13–16) and Tetraoxalate (17).** These compounds were synthetized from the unsubstituted tetraamine disulfide[4a] (**5–8**) and the appropriate aldehyde (2-methoxybenzaldehyde or pyrrole-2-carboxaldehyde) following the procedure described for benextramine (**10**) and analogues.[4a,g] Compounds **13–16** were purified by crystallization of the tetrahydrochloride salts whereas **17** was characterized as tetraoxalate salt (Table II). Yields were in the range of 55–70%.

3-(12-Aminododecyl)-1,3-thiazolidine Dihydrochloride. This compound was synthetized in 82% yield from *N*-(12-aminododecyl)cysteamine dihydrochloride[4a] via the procedure described for 3-(6-aminohexyl)-1,3-thiazolidine:[31] mp 135–139 °C (from 2-PrOH). Anal. ($C_{15}H_{36}Cl_2N_2S$) C, H, N.

***N*,*N*″-(Dithiodi-2,1-ethanediyl)bis[*N*′-(benzyloxycarbonyl)-1,12-dodecanediamine].** Benzyl chloroformate (1.25 g, 7.34 mmol) was added dropwise to a stirred and cooled solution of 3-(12-aminododecyl)-1,3-thiazolidine as the free base (2.0 g, 7.34 mmol) and Et_3N (0.75 g, 7.41 mmol) in chloroform (100 mL). After the mixture stood for 1 h, the solvent was removed under reduced pressure to give a residue, which was taken up in EtOAc–cyclohexane (1:1). The precipitate was removed by filtration. Removal of solvents gave an oil, which was purified by column chromatography by eluting with ethyl acetate–cyclohexane (14:86), to give 1.1 g (37%) of a while solid (mp 59–60 °C). This solid (2.71 mmol) was dissolved in chloroform (100 mL) and treated with a solution of 0.1 N iodine in 2.5% KI (27.1 mL) with stirring. After 1 h, the reaction mixture was made basic with 2 N NaOH (25 mL) and was extracted with chloroform (3 × 50 mL). Extracts were dried and then evaporated to give 0.9 g (84%) of desired compound (mp 80–82 °C), which was used in the next step without further purification.

***N*,*N*″-(Dithiodi-2,1-ethanediyl)-*N*,*N*″-dimethylbis(1,12-dodecanediamine).** *N*,*N*″-(Dithiodi-2,1-ethanediyl)bis[*N*′-(benzyloxycarbonyl)-1,12-dodecanediamine] (0.9 g, 1.14 mmol) was treated with HCOOH (5 mL) and 40% HCHO (5 mL) for 8 h at reflux temperature, and, after cooling, the mixture was made basic with 40% NaOH (20 mL) and was extracted with chloroform (3 × 50 mL). Extracts were dried and then evaporated to give a residue, which was dissolved in AcOH (4 mL) and treated with HBr gas for 10 min with stirring. After 1 h at room temperature, ether was added to the solution, which was kept overnight at 0 °C. The precipitate was filtered and then dissolved in water; the resulting solution was made basic with 2 N NaOH (5 mL), saturated with NaCl, and extracted with chloroform (3 × 30 mL). Extracts were dried and then evaporated to give 0.29 g (50%) of desired compound as an oil, which was used without further purification in the next step.

***N*,*N*″-(Dithiodi-2,1-ethanediyl)-*N*,*N*″-dimethylbis[*N*′-[(2-methoxyphenyl)methyl]-1,12-dodecanediamine] Tetraoxalate (18), *N*,*N*″-(Dithiodi-2,1-ethanediyl)bis[*N*′-[3-[[(2-methoxyphenyl)methyl]amino]propanoyl]-1,8-octanediamine] and *N*,*N*″-(Dithiodi-2,1-ethanediyl)bis[*N*′-[6-[[(2-methoxyphenyl)methyl]amino]hexanoyl]-1,6-hexanediamine] Tetraoxalates (21 and 22).** Compounds **18**, **21**, and **22** were synthesized from *N*,*N*″-(dithiodi-2,1-ethanediyl)-*N*,*N*″-dimethylbis(1,12-dodecanediamine), **19** and **20**, respectively, and 2-methoxybenzaldehyde following the procedure described for **10**.[4a] Crude **18** was purified as the free base by column chromatography by eluting with concentrated ammonia–MeOH (2:98). It was converted into its oxalate salt by treating an alcoholic solution with 4 equiv of oxalic acid dihydrate. After cooling, the solid was collected, washed with ether, and recrystallized to give pure **18** in 60% yield (Table II).

Crude **21** and **22** were purified in 75% yield by crystallization of the tetraoxalate salts (Table II).

***N*,*N*″-(Dithiodi-2,1-ethanediyl)bis[*N*′-(3-aminopropanoyl)-1,8-octanediamine] and *N*,*N*″-(Dithiodi-2,1-ethanediyl)bis[*N*′-(6-aminohexanoyl)-1,6-hexanediamine] Tetrahydrobromides (19 and 20).** Ethyl chlorocarbonate (2.51 g, 23.0 mmol) was added dropwise to a stirred and cooled (ice) solution of *N*-(benzyloxycarbonyl)-3-aminopropionic acid (5.15 g, 23.0 mmol) and Et_3N (2.34 g, 23.0 mmol) in chloroform (300 mL), followed after 30 min by the addition of a solution of 3-(8-aminooctyl)-1,3-thiazolidine[31] (5.0 g, 23.0 mmol) in chloroform (30 mL). The resulting reaction mixture was stirred overnight at room temperature and then was washed with 2 N HCl, 2 N NaOH, and finally with water. Extracts were dried and then evaporated to give a white solid, which was washed with ethyl acetate and then ether to yield 6.0 g (62%) of a solid (mp 91–92 °C) that was used in the next step without further purification. A solution of 0.1 N iodine in 2.5% KI (142 mL) was added dropwise with stirring to a solution of the compound obtained above (6.0 g, 14.2 mmol) in chloroform (180 mL). After 1 h, the reaction mixture was made basic with 2 N NaOH and extracted with chloroform (3 × 50 mL). Extracts were dried and then evaporated to give 4.2 g of a yellow solid (mp 115–119 °C), which was dissolved in AcOH (15 mL) and treated with HBr gas for 10 min with cooling (ice). After 1 h at room temperature, ether was added to the solution, which was kept overnight at 0 °C. The precipitate was collected and purified by crystallization to give 3.18 g of **19** as the tetrahydrobromide salt.

Compound **20** was similarly obtained starting from 3-(6-aminohexyl)-1,3-thiazolidine[31] and *N*-(benzyloxycarbonyl)-6-aminohexanoic acid (Table II).

***N*,*N*″-(Dithiodi-2,1-ethanediyl)bis[*N*′-[3-[[(2-methoxyphenyl)methyl]amino]propyl]-1,8-octanediamine] and *N*,*N*″-(Dithiodi-2,1-ethanediyl)bis[*N*′-[6-[[(2-methoxyphenyl)methyl]amino]hexyl]-1,6-hexanediamine] (23 and 24).** A solution of 10 M $BH_3CH_3SCH_3$ (0.1 mL) in dry diglyme (5 mL) was added dropwise at room temperature to a solution of **22** (0.43 g, 0.52 mmol) in dry diglyme (30 mL) with stirring under a stream of dry nitrogen with exclusion of moisture. When the addition was completed, the reaction mixture was heated at 120 °C for 8 h. After the mixture was cooled at 0 °C, excess borane was destroyed by cautious dropwise addition of 20 mL of MeOH. The resulting mixture was left at room temperature for 5 h with stirring, treated with HCl gas for 10 min, and then was heated at 120 °C for 4 h. Removal of the solvent under reduced pressure gave a residue, which was crystallized from MeOH, yielding 0.27 g (51%) of the corresponding thiol of **24**. This solid was dissolved in water (1 mL), the pH was adjusted to 8–9 by the addition of 1 N NaOH, and the solution treated with potassium ferricyanide (0.47 g) in water (1 mL). After being allowed to stand 30 min, the reaction mixture was made basic with NaOH pellets (0.5 g) while being cooled in ice and then was saturated with NaCl and extracted with chloroform (3 × 25 mL). Extracts were washed (saturated NaCl), dried, and then evaporated to give 0.1 g (47%)

of **24** (free base) as an oil, which was dissolved in absolute EtOH, and the solution was treated with HCl gas. Removal of the solvent gave **24** as the hexahydrochloride salt.

Compound **23** was similarly obtained in a global yield of 28% starting from **21** (Table II).

Pharmacology. Rat Vas Deferens. Male albino rats (175–200 g) were killed by a sharp blow on 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 20-mL working volume containing physiological salt solution of the following composition (PSS) (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.52; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.1. The $MgSO_4 \cdot 7H_2O$ concentration was reduced to 0.6 mM when twitch response to field stimulation was studied. 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 0.5 g, respectively, and the contractions were recorded by means of force transducers connected to a two-channel Gemini 7070 polygraph.

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 with square pulses of 3-ms duration at a voltage of 10–15 V. The stimulation voltage was fixed throughout the experiments.

Propranolol hydrochloride (1 μM) and cocaine hydrochloride (10 μM) were present in the PSS throughout the experiments outlined below to block β-adrenoreceptors and neuronal uptake mechanisms, respectively.

Postsynaptic α_1-adrenoreceptor blocking activity was determined on the epididymal portion of the vas deferens. The tissues were allowed to equilibrate for at least 1 h before the addition of any drug. Norepinephrine dose–response curves were obtained cumulatively, the first one being discarded and the second one taken as a control. After incubation with the antagonist for 30 min followed by 30-min washing, a third dose–response curve was obtained. Responses were expressed as a percentage of the maximal response obtained in the control curve. Parallel experiments, in which tissues did not receive any antagonist, were run in order to correct for time-dependent changes in agonist sensitivity.[35] It was generally verified that the third dose–response curve was identical with the second because the change in dose–ratio is less than 2, which usually represents a minimal correction.

α_2-Adrenoreceptor blocking activity was assessed on the prostatic portion of the vas deferens with clonidine as the agonist. The procedure was substantially the same as described for unstimulated vasa, with the only exception being that two dose–response curves to clonidine were constructed and the first one was taken as a control.

Each antagonist was tested at three different concentrations and each concentration was investigated at least four times. Compounds **21–24**, however, showed no α_1-adrenoreceptor blocking activity up to a concentration of 20 μM. At higher concentrations they gave only a slight displacement to the right of norepinephrine dose–response curve without affecting the maximum. Similarly compounds **13–18** showed no α_2-adrenoreceptor blocking activity up to 20 μM. They could not be studied at higher concentrations because they markedly depressed the twitch of the field stimulated vas deferens in a dose-dependent fashion. The twitch was abolished at 100 μM (results not shown). However, it was found that it was probably not an α_2-agonistic effect since the depression was not reversed by the addition of a selective antagonist-like idazoxan.

The α-blockade for each compound is expressed as IC_{50} values, which were estimated from graphical plots of percent inhibition vs log molar concentration and are defined as the concentrations that produce 50% inhibition of the maximal response to the agonist.

Human Blood Platelets. Blood was obtained by venipuncture in the forearms of apparently healthy humans and collected in polyethylene tubes containing $^1/_{10}$ final volume of 3.8% trisodium citrate. Platelet-rich plasma (PRP) was prepared by centrifugation of the blood for 10 min at 200*g* and platelet-poor plasma (PPP) was obtained by centrifugation for 20 min at 1500*g*. PRP was adjusted to 350 000 platelets/mL by adding PPP. Platelet aggregation was measured photometrically[36] with a BIO/DATA Platelet aggregation profiler (Model PAP-3) and calculated as the percentage decrease in optical density of the PRP, 100% being that of PPP.

The potential inhibitors were dissolved in 10% dimethyl sulfoxide, which was present in a final concentration of 0.5% or less in all test samples and controls, and had no effect at this concentration on platelet aggregation. ADP and (–)-epinephrine were used as aggregating agents. In a typical experiment, 940 μL of PRP was pipetted into each 1-mL siliconized glass cuvette containing 20 μL of the test compound or control. The cuvette was allowed to incubate at 37 °C for 5 min, after which 10 μL of an aqueous solution of ADP (final concentration 2–6 μM) or epinephrine (final concentration 4–6 μM) was added to induce platelet aggregation.

Inhibition of platelet aggregation by a test compound was calculated by dividing the maximum deflection in the optical density curve by that observed in the control, then multiplying by 100. Each antagonist was tested at three different concentrations and each concentration was investigated at least four times. IC_{50} values for each inhibitor were estimated from graphical plots of % inhibition vs log molar concentration and were defined as the concentrations that produce 50% inhibition of the aggregatory response to ADP or epinephrine. Data were expressed as the mean ± standard error.

Statistical Evaluation. Student's *t* test was used to assess the significance of the experimental results.

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Registry No. **5**, 75575-23-4; **6**, 77799-84-9; **7**, 115483-35-7; **8**, 82414-72-0; **9**, 68535-78-4; **10**, 69790-18-7; **11**, 75575-15-4; **12**, 82414-71-9; **13**, 115483-57-3; **13**·4HCl, 115483-39-1; **14**, 115483-58-4; **14**·4HCl, 115483-40-4; **15**, 115483-59-5; **15**·4HCl, 115483-41-5; **16**, 115483-60-8; **16**·4HCl, 115483-42-6; **17**, 115483-43-7; **17**·$4H_2C_2O_4$, 115483-44-8; **18**, 115483-45-9; **18**·$4H_2C_2O_4$, 115483-46-0; **19**, 115483-61-9; **19**·4HBr, 115483-47-1; **20**, 115483-62-0; **20**·4HBr, 115483-48-2; **21**, 115483-49-3; **21**·$4H_2C_2O_4$, 115483-50-6; **22**, 115483-63-1; **22**·$4H_2C_2O_4$, 115512-51-1; **23**, 115483-64-2; **23**·6HCl, 115483-51-7; **24**, 115483-65-3; **24**·6HCl, 115483-52-8; *N*-(11-bromoundecyl)phthalimide, 115483-33-5; thiazolidine, 504-78-9; *N*-[11-(1,3-thiazolidin-3-yl)undecyl]phthalimide, 115483-34-6; 2-methoxybenzaldehyde, 135-02-4; pyrrole-2-carboxaldehyde, 1003-29-8; 3-(12-aminododecyl)-1,3-thiazolidine dihydrochloride, 115483-36-8; 3-(12-aminododecyl)-1,3-thiazolidine, 115483-37-9; 3-[12-[(benzyloxycarbonyl)amino]dodecyl]-1,3-thiazolidine, 115483-38-0; *N,N''*-(dithio-2,1-ethanediyl)bis[*N'*-(benzyloxycarbonyl)-1,12-dodecanediamine, 115483-53-9; *N,N''*-(dithio-2,1-ethanediyl)-*N,N''*-dimethylbis(1,12-dodecanediamine, 115483-54-0; *N*-(benzyloxycarbonyl)-3-aminopropionic acid, 2304-94-1; 3-(8-aminooctyl)-1,3-thiazolidine, 115483-55-1; *N*-[8-[[3-[(benzyloxycarbonyl)amino]-1-oxopropyl]amino]octyl]-1,3-thiazolidine, 115483-56-2; 3-(6-aminohexyl)-1,3-thiazolidine, 88346-63-8; *N*-(benzyloxycarbonyl)-6-aminohexanoic acid, 1947-00-8; 2-[[-[[6-[(2-methoxybenzyl)amino]hexyl]amino]hexyl]amino]ethanethiol, 115512-52-2.

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