Notes

The Furoxan System as a Useful Tool for Balancing "Hybrids" with Mixed α_1-Antagonist and NO-like Vasodilator Activities

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The design of new vasodilator derivatives in which two different pharmacophoric groups are present in a single molecule has been pursued by substitution of NO-prodrug furoxan moieties for the furanylcarbonyl function in Prazosin, a well-known α_1-receptor antagonist. The aim was to obtain new antihypertensive agents in which two vasodilation mechanisms, α_1-antagonist and NO-mediated, can operate in an appropriate balance. The α_1-antagonist activity was assessed on rat aortic strips in the presence and in the absence of oxyhemoglobin (HbO_2), a well-known scavenger of nitric oxide. The resulting hybrids displayed different pharmacological behaviors. When the 4-furoxanylcarbonyl system, bearing an ester or an amide function at the 3-position, was present (derivatives **7a,b**), hybrids with predominant α_1-antagonist activity were obtained. By contrast, in the derivative **7c**, in which the nitrile function is linked to the 3-position of the furoxan ring, the NO-mediated vasodilating properties are predominant. Finally, the (furoxanylsulfonyl)piperidine derivatives **13a,b** showed NO vasodilation and α_1-antagonist activities in an appropriate balance. For the furoxan derivatives, the NO-dependent vasodilating ability, assessed on the K^+-depolarized aortic strip, and the NO release features under the action of thiol cofactors are also discussed.

Introduction

Recent results have shown that furoxan derivatives are able to activate the soluble guanylate cyclase by releasing nitric oxide under the action of thiol cofactors.[1-5] Since NO is involved in many bioregulatory processes,[6] the furoxan system **1** could be used in the design of a variety of "hybrid" molecules (Chart 1).

We have recently synthesized the furoxans **3a–d**, analogues of the Prazosin **2**, in which the phenyl(or methyl)furoxanylcarbonyl system was substituted for the 2-furanylcarbonyl moiety[7] (Chart 2).

The aim was to obtain new vasodilators capable of displaying the typical NO-dependent effects on the micro- and macrovascular system, mixed with the α_1-antagonist activities. The vasodilating activity of these compounds was assessed on the epididymal portion of rat vas deferens, and the results showed that all of the hybrids were strongly (**3b**) or completely (**3a,c,d**) biased toward α_1-antagonist properties.

In order to have better balanced hybrids, we have now changed the substitution pattern at the furoxan ring. In this paper, we report synthesis and vasodilating activities, assessed on rat aortic strips, of the Prazosin analogues **7a–c** and **13a–c**. We designed these compounds on the basis of the results of previous works on the vasodilating properties of series of furoxan derivatives[1,4] and their synthetic accessibility. By an iterative procedure, we characterized the derivatives **13a,b** as two well-balanced hybrids.

Chemistry

Derivatives **7a–c** were synthesized according to the pathway reported in Scheme 1. 4-Amino-6,7-dimethoxy-2-piperazinylquinazoline (**6**) was treated with the activated ester **5**, prepared *in situ* from **4**, *N*-hydroxysuccinimide (NHS), and 1,3-dicyclohexylcarbodiimide (DCC), to afford the expected compound **7a**. This derivative was transformed into **7b** by ammonia. The amide function of **7b** was easily dehydrated with trifluoroacetic anhydride (TFAA) in dry pyridine, giving **7c**. Phenylfuroxanylsulfonyl isomers **13a,b** and the related furazan **13c** were prepared according to the pathway reported in Scheme 2. The intermediates **10a–c** were obtained from 4-mercaptopiperidine and **8a**, **9**, and **8b**, respectively, working in phase transfer conditions, using Aliquat 336. The compounds were oxidized, with 80% hydrogen peroxide in trifluoroacetic acid (TFA), to the corresponding sulfones **11a–c**, which, by reaction with 4-amino-2-chloro-6,7-dimethoxyquinazoline (**12**), afforded the final products **13a–c**. The latter reaction

Chart 1

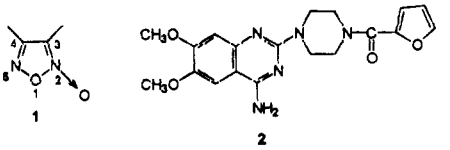
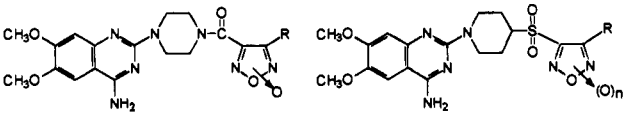


Chart 2



3a R = 3-C_6H_5 **7a** R = 3-COOMe
3b R = 4-C_6H_5 **7b** R = 3-$CONH_2$
3c R = 3-CH_3 **7c** R = 3-CN
3d R = 4-CH_3

13a n = 1 R = 3-C_6H_5
13b n = 1 R = 4-C_6H_5
13c n = 0 R = -C_6H_5

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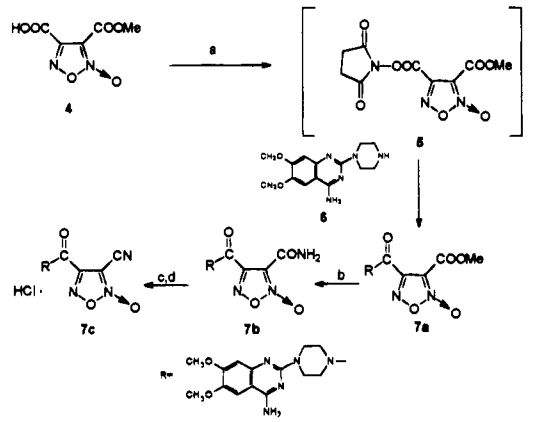
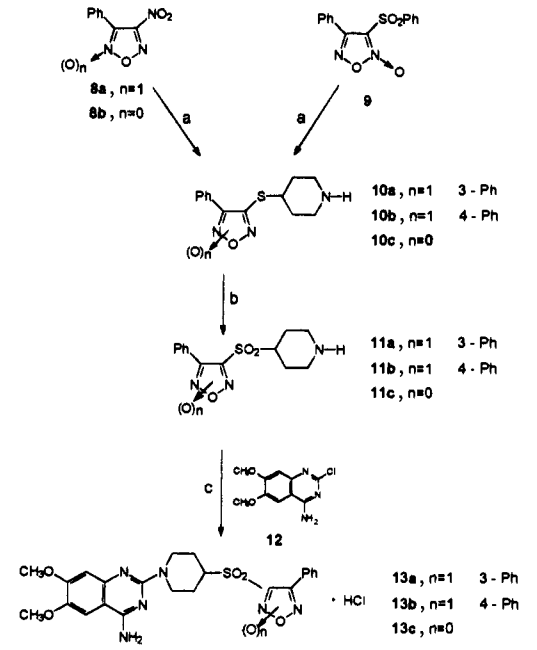
Scheme 1[a]



[a] (a) NHS, DCC; THF; (b) 32% NH_4OH; MeOH/Me_2CO; (c) TFAA; pyridine; (d) Me_2CO/HCl.

Scheme 2[a]



[a] (a) 4-mercaptopyperidine-HCl, Aliquat 336, NaOH; benzene/H_2O; (b) 80% H_2O_2; TFA; (c) **13a**/DMSO, 80 °C; **13b,c**/boiling EtOH.

was run at 80 °C in the appropriate solvent, without the furoxan isomerization occurring (NMR detection).[8]

Biological Evaluation and Discussion

In order to reach our goal, the compound **7b**, in which two amide functions are present on the furoxan ring, was first designed on the basis of the observation that furoxandicarboxamide derivatives are more potent vasodilating agents than the monoamide analogues.[1] The α_1-adrenoceptor blocking activity of **7b** and of its ester precursor **7a** was assessed by antagonism of (−)-norepinephrine-induced contractions of rat aortic strips. These derivatives caused, in the concentration range 3 × 10^{-9} M to 1 × 10^{-7} M, a shift to the right of the cumulative concentration−response curves of (−)-norepinephrine, in a parallel manner, without reduction of the maximal response. At higher concentrations (3 × 10^{-7} M to 1 × 10^{-6} M), a dose-dependent decrease of the maximum effect, reversible with washing, was observed. At these concentrations, the maximal response was restored by the presence of 10 μM oxyhemoglobin (HbO_2), a well-known NO scavenger.[9] This behavior is illustrated, for the derivative **7b**, in Figure 1a in which the curves in the upper panel are obtained in the absence of HbO_2 and those in the lower panel are obtained in the presence of HbO_2. The shift to the right of the curves in which the full effect is restored either in the absence or in the presence of HbO_2 was analyzed according to Schild, and the pA_2 values were calculated (Table 1). The behavior of the tested derivatives indicates that the α_1-antagonist-mediated vasodilation appears at lower concentrations (100-fold lower) than the one mediated by NO.

The following step was the transformation of the lateral amide function present in **7b**, into the cyano group, to obtain compound **7c**. This modification was suggested by the results of our recent work on the vasodilating properties of several NO-releasing phenylfuroxans bearing at the 3- or at the 4-position different kinds of substituents.[4] A quite complex picture of structure−activity relationships was revealed, and the most potent derivatives appeared to be the cyano-substituted furoxans. Derivative **7c** caused, in the whole range of the concentration tested (1 × 10^{-9} M to 1 × 10^{-7} M), a shift to the right of the (−)-norepinephrine curves accompanied by a decrease of the maximum response, reversible with washing (Figure 1b, upper panel). When the experiments were repeated in the presence of 10 μM HbO_2, the maximal response was restored and the concentration−response curves at 1 × 10^{-9} M and 3 × 10^{-9} M overlapped that of the control (Figure 1b, lower panel). The shift to the right of the curves in the presence of HbO_2 was analyzed according to Schild to obtain the pA_2 value (Table 1). Therefore, in **7c**, the NO-dependent vasodilating effects are dominant compared with those due to α_1-antagonist properties.

At this step, the knowledge that sulfonyl-substituted phenylfuroxans display high potency, but less than the corresponding cyano analogues,[4] suggested that a reasonable structural modification could be the substitution of a SO_2 moiety for the nitrile function in **7c**. We discarded this possibility in view of the difficulties connected with the synthesis of such a compound. Previous data reported in the literature[10] indicate that a sulfomethylene (SO_2CH_2) group could mimic the H bond acceptor basicity (β-parameter) as well as the dipolarity/polarizability properties (π^*-parameter) of the amide carbonyl group. On the basis of these observations and in view of their easy accessibility through the pathway reported in Scheme 2, we selected for the next synthesis the isomers **13a,b**. The phenylfuroxanylsulfonyl isomer **13b** at a 3 × 10^{-9} M concentration shifted the (−)-norepinephrine curve to the right, with conservation of the maximal response. At higher concentrations (1 × 10^{-8} M to 3 × 10^{-7} M), a decrease of the maximum response, reversible with washing, was observed. Compound **13a**, in the whole concentration range tested (1 × 10^{-9} M to 1 × 10^{-7} M), shifted the control curve to the right with a reversible decrease of the maximum response (Figure 1c, upper panel). In the presence of 10 μM HbO_2, the behavior of both compounds was that typical of a competitive antagonist (pA_2 values listed in Table 1). Therefore, derivatives **13a,b** are well-balanced hybrids, since both the vasodilating mechanisms operate approximately in the same range

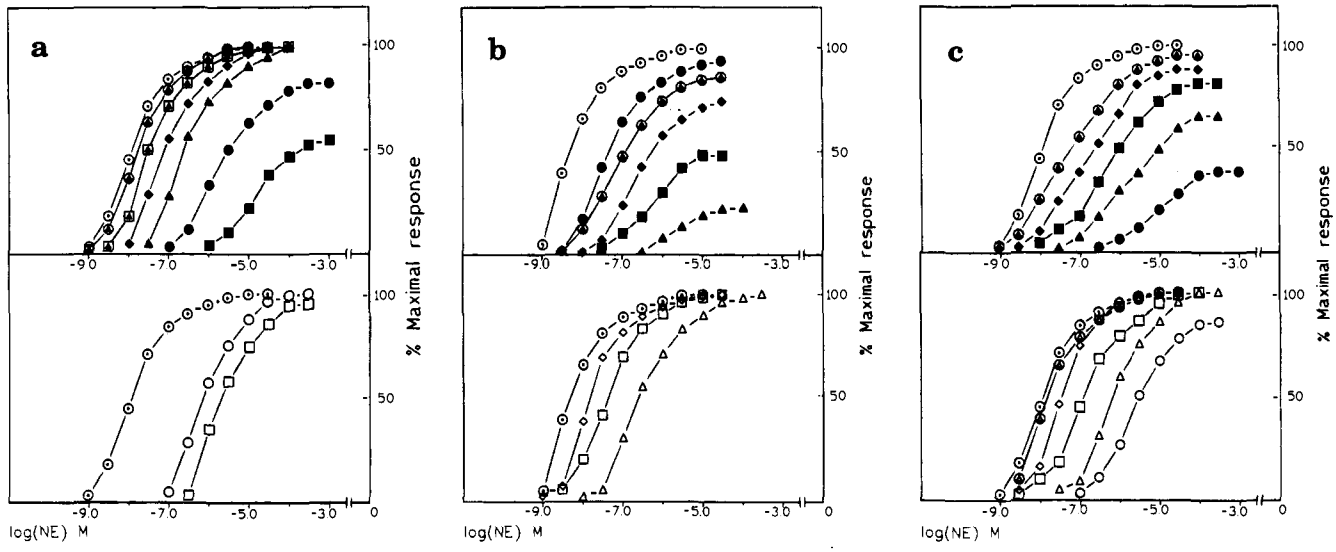


Figure 1. (a) Concentration–response curves induced by (−)-norepinephrine (NE) in the absence and in the presence of different concentrations of **7b**. Upper panel: ⊙, control curve; ▲ inside a circle, 3×10^{-9} M; ▲ inside a square, 1×10^{-8} M; ◆, 3×10^{-8} M; ▲, 1×10^{-7} M; ●, 3×10^{-7} M; ■, 1×10^{-6} M. Lower panel: ⊙, control curve + HbO_2; ○, 3×10^{-7} M + HbO_2; □, 1×10^{-6} M + HbO_2. (b) Concentration–response curves induced by (−)-norepinephrine (NE) in the absence and in the presence of different concentrations of **7c**. Upper panel: ⊙, control curve; ●, 1×10^{-9} M; ▲ inside a circle, 3×10^{-9} M; ◆, 1×10^{-8} M; ■, 3×10^{-8} M; ▲, 1×10^{-7} M. Lower panel: ⊙, results of three different experiments (see text), control curve + HbO_2, 1×10^{-9} M + HbO_2, and 3×10^{-9} M + HbO_2; ◇, 1×10^{-8} M + HbO_2; □, 3×10^{-8} M + HbO_2; △, 1×10^{-7} M + HbO_2. (c) Concentration–response curves induced by (−)-norepinephrine (NE) in the absence and in the presence of different concentrations of **13a**. Upper panel: ⊙, control curve; ▲ inside a circle, 1×10^{-9} M; ◆, 3×10^{-9} M; ■, 1×10^{-8} M; ▲, 3×10^{-8} M; ●, 1×10^{-7} M. Lower panel: ⊙, control curve + HbO_2; △ inside a circle, 1×10^{-9} M + HbO_2; ◇, 3×10^{-9} M + HbO_2; □, 1×10^{-8} M + HbO_2; △, 3×10^{-8} M + HbO_2; ○, 1×10^{-7} M + HbO_2.

Table 1. α_1-Antagonistic Activity and Vasodilating Properties of Compounds **7a–c** and **13a–c**

compound	pA_2 ± 95% CL[a]	slope ± 95% CL[a]	IC_{50} (μM)[b] − HbO_2	IC_{50} (μM)[b] + HbO_2
7a	8.55 ± 0.19	0.99 ± 0.18	0.63 ± 0.06	#
7b	8.25 ± 0.10	1.04 ± 0.12	2.5 ± 0.4	#
7c	8.04 ± 0.14	1.05 ± 0.19	0.0097 ± 0.0011	0.081 ± 0.013
13a	8.96 ± 0.22	1.09 ± 0.23	0.052 ± 0.013	0.61 ± 0.10
13b	8.49 ± 0.19	1.02 ± 0.15	0.095 ± 0.013	0.70 ± 0.09
13c	9.17 ± 0.12	0.92 ± 0.10	–	–
2	9.81 ± 0.27	0.89 ± 0.15	–	–
SNP[c]	–	–	0.015 ± 0.003	0.048 ± 0.007

[a] CL = confidence limits. [b] Mean ± standard error. [c] SNP = sodium nitroprusside; # = not tested.

of concentrations. This balance is particularly well-achieved in the case of derivative **13a**.

The α_1-antagonist properties of the Prazosin analogues designed in this work deserve some comments. All of the derivatives are reversible and competitive antagonists. In the derivatives **7a–c**, as well as in the derivatives **3a–d** studied in our previous paper,[7] the substitution of the furoxan moieties for the furan ring in Prazosin affords compounds less potent, in which the activity is modulated over a narrow range (10-fold). Taking the Prazosin as reference, the derivatives **13a,b** show a potency like that of derivatives **3a,b**.[7] This finding supports our working hypothesis that the sulfonemethylene group could be used as a bioisoster moiety of the amide function in the design of new α_1-antagonist analogues of Prazosin. Derivatives **13a,b** display pA_2 values similar to those of the furazan analogue **13c**, which we synthesized for control purpose since it is unable to release NO. This result parallels the one we found for derivatives **3a–d** and the related furazans.[7] Likely, the small differences between the activity of the furazan and furoxan series are due to some difference in the affinity for the α_1-receptor of the hetero-ring-substituted systems.

For the furoxan derivatives, we also tested their NO-dependent vasodilating ability by the K^+-depolarized aortic strip assay. Table 1 shows IC_{50} values obtained in the test. The potency follows the sequence **7c** > **13a** ≅ **13b** > **7a** > **7b**. Again, the involvement of nitric oxide in the relaxation of the tissue is confirmed by the increase of IC_{50} values when the test was performed in the presence of 10 μM HbO_2. All the compounds were able to react, in phosphate buffer (pH 7.4) under aerobic conditions, with a large excess of L-cysteine to give nitrites, well-known oxidative metabolites of NO. The amount of nitrites, formed after 1 h of incubation, was detected by the Griess reaction according to the sequence **7c** (62 ± 1%) > **13b** (38 ± 0.5%) ≅ **7b** (32 ± 0.1%) > **13a** (3 ± 0.1%) ≅ **7a** (1.7 ± 0.1%). The initial NO formation rates, in the presence of L-cysteine, were measured by a spectrophotometric technique based on the induced NO oxidation of oxyhemoglobin (HbO_2^{2+}) to methemoglobin ($MetHb^{3+}$) according to the equation $HbO_2^{2+} + NO \rightarrow MetHb^{3+} + NO_3^-$, following the procedure reported in the literature,[1] partially modified (see the Experimental Section). Differences among the compounds both in the solubilities and in the NO-releasing rates, prevented the use of these later at the same concentration. Thus, we evaluated for each derivative the concentration necessary to have an initial 0.1 absorbance increase (2.6 μM min^{-1}, NO formation rate) (see the Experimental Section). The sequence obtained **7c** (0.013 mM) < **7a** (0.20 mM) < **7b** (0.72 mM) is in keeping with that of the NO-dependent vasodilating potencies of these compounds as reported above. The very low solubility of the derivatives **13a,b** prevented us from measuring similar figures for them and thereby having a complete picture of the influence of these data

on the NO-dependent vasodilating potency. However, the complex matter of the correlation between the NO release under the action of thiol cofactors from furoxan derivatives and their vasodilating properties is still in need of a thorough investigation. One should note the recent finding that, in contrast to earlier reports, the dilator response of some furoxancarboxamides in isolated rabbit femoral artery and jugular vein is not thiol-dependent.[11] Spontaneous or enzymatic NO production might account for it, as shown for other "nitrovasodilators".[12,13] Work is in progress to investigate this point in greater detail.

In conclusion, derivatives **13a,b** display α_1-antagonist activities similar to that of Prazosin (7- and 21-fold lower, respectively) and NO-vasodilating properties near that of SNP (3.5- and 6-fold lower, respectively). On the whole, these results show that the substituted furoxan ring is a very flexible system in the design of hybrids in which a NO-dependent activity can be mixed with a mutually complementary biological activity.

Experimental Section

Chemistry. Melting points were determined on a Büchi 530 apparatus and are uncorrected. Melting points with decomposition were taken introducing the sample into the bath at a temperature 10 °C lower than the melting point, with a heating rate of 3 °C/min. The derivatives **7c** and **11a** decompose without melting. The decomposition range has been detected by differential scanning calorimetry measurement (DSC 7 Perkin-Elmer instrument). All the compounds were routinely checked by infrared spectrophotometry (IR, Perkin-Elmer Model 781 instrument). ^{1}H and ^{13}C nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded in dimethyl sulfoxide-d_6 (Me_2SO-d_6) at 200 and 50 MHz, respectively, with a Bruker AC-200 spectrometer. The assignments were given on the basis of literature data[7] and DEPT pulse sequence. Thin layer chromatography (TLC) was carried out on 5 × 20 cm plates precoated with Merck silica gel 60 F_{254}, with a layer thickness of 0.25 mm. Anhydrous $MgSO_4$ was used as drying agent. Solvent removal was done under reduced pressure at 35 °C. Analyses of the new compounds were performed by REDOX (Cologno M.), and the results are within ±0.4% of the theoretical values. Intermediates **4**,[14] **6**,[15] **8a**,[16] **8b**,[17] **9**,[17,18] and **12**,[19] were synthesized according to the literature.

1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-[3-(methoxycarbonyl)-4-furoxanylcarbonyl]piperazine (7a). To a stirred solution of **4** (1.21 g, 6.4 mmol) in dry tetrahydrofuran (THF) (60 mL) were added NHS (0.81 g, 7.0 mmol) and DCC (1.44 g, 7.0 mmol). The reaction mixture was allowed to stir for 1 h and then filtered. To the filtrate was added a solution of **6** (1.85 g, 6.4 mmol) in a mixture of dry THF (20 mL) and dry dimethylformamide (DMF) (20 mL). The mixture was concentrated under reduced pressure and the residue treated with ice–water. The yellow precipitate formed was collected by filtration, washed with methanol, and dried to yield pure **7a** (2.52 g, 86%; TLC detection, AcOEt/MeOH, 9.5 /0.5): mp 171 °C dec (DMF/H_2O); ^{1}H NMR δ 7.21 (s, br, 2H, NH_2), 7.46 (s, 1H, H-5), 6.77 (s, 1H, H-8), 3.8–3.5 (m, 8H, piperazine), 3.89, 3.85 (2s, 6H, OCH_3), 3.81 (s, 3H, $COOCH_3$); ^{13}C NMR δ 161.3 (C-4), 158.1 (C-2), 155.6, 155.4, 154.3 (NC=O, OC=O, C-7), 151.9 (*C*CON), 148.7 (C-1a), 145.2 (C-6), 105.3 (C-8), 103.7 (C-5), 103.1 (C-4a, *C*COO overlapped), 55.9, 55.5 (6-OCH_3, 7-OCH_3), 53.6 (COO*C*H_3), 46.2, 43.8, 43.2, 41.9 (piperazine). Anal. ($C_{19}H_{21}N_7O_7$·0.5H_2O) C, H, N.

1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(3-carbamoyl-4-furoxanylcarbonyl)piperazine (7b) Hydrochloride. To a stirred solution of **7a** (1.0 g, 2.2 mmol) in a mixture of MeOH (20 mL) and Me_2CO (40 mL) was added 32% NH_4OH (10 mL). After the reaction mixture was stirred for 5 min at room temperature, it was concentrated under reduced pressure and then cooled in an ice bath. The yellow precipitate formed was collected by filtration, washed with water, and dried to yield pure **7b** (0.81 g, 83%; TLC detection, AcOEt). The product was transformed into the corresponding hydrochloride: mp 242–244 °C dec (EtOH/H_2O); ^{1}H NMR δ 12.5 (s, br, 1H, NH^+), 9.0, 8.7 (2s, br, 2H, 4-NH_2), 8.5, 7.9 (2s, br, 2H, $CONH_2$), 7.77 (s, 1H, H-5), 7.58 (s, 1H, H-8), 4.0–3.7 (m, 8H, piperazine), 3.90, 3.87 (2s, 6H, OCH_3); ^{13}C NMR δ 161.4 (C-4), 156.0, 155.4, 154.9 (NC=O, H_2NC=O, C-7), 152.6 (*C*CON), 151.4 (C-2), 147.0 (C-6), 136.3 (C-1a), 110.4 (*C*CONH$_2$), 105.0 (C-5), 101.8 (C-4a), 99.2 (C-8), 56.3, 56.2 (6-OCH_3, 7-OCH_3), 45.3, 44.7, 43.9, 41.3 (piperazine). Anal. ($C_{18}H_{20}N_8O_6$·HCl·0.5H_2O) C, H, N.

1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(3-cyano-4-furoxanylcarbonyl)piperazine Hydrochloride (7c). To a stirred and ice–water-cooled solution of **7b** (0.44 g, 1.0 mmol) in dry pyridine (8 mL) was added dropwise over 45 min TFAA (0.84 g, 4.0 mmol). The reaction mixture was stirred for an additional 15 min and then poured into ice–water. The yellow precipitate formed was collected by filtration, washed with water, and dried. The crude compound was then dissolved in Me_2CO (10 mL) and the solution saturated with gaseous HCl and concentrated under reduced pressure. The white precipitate formed was collected by filtration, washed with Me_2CO, and dried to yield pure **7c** (0.29 g, 62%; TLC detection, AcOEt): 228–232 °C dec; ^{1}H NMR δ 12.6 (s, br, 1H, NH^+), 9.0, 8.7 (2s, br, 2H, 4-NH_2), 7.79 (s, 1H, H-5), 7.68 (s, 1H, H-8), 4.1–3.7 (m, 8H, piperazine), 3.90, 3.87 (2s, 6H, OCH_3); ^{13}C NMR δ 161.3 (C-4), 155.3, 154.1 (NC=O, C-7), 151.4, 151.3 (*C*CON, C-2), 146.9 (C-6), 136.2 (C-1a), 106.2 (CN), 105.0 (C-5), 101.8 (C-4a), 99.3 (C-8), 98.8 (*C*CN), 56.3, 56.1 (6-OCH_3, 7-OCH_3), 45.5, 44.7, 43.8, 41.9 (piperazine). Anal. ($C_{18}H_{18}N_8O_5$·HCl·H_2O) C, H, N.

3-Phenyl-4-(4-piperidylthio)furoxan (10a) Picrate. To a well-stirred mixture of **8a** (1.03 g, 5.0 mmol) dissolved in benzene (15 mL) and of 4-mercaptopiperidine hydrochloride (0.76 g, 5.0 mmol) dissolved in water (10 mL) containing Aliquat 336 (0.30 g, 0.75 mmol) was added dropwise under N_2 a solution of NaOH (0.40 g, 10 mmol) in water (5 mL). After 1 h, the organic layer was collected, washed twice with water, and extracted several times with 1 N HCl. The combined aqueous layers were adjusted to alkaline pH with $KHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried, filtered, and evaporated in vacuo to give a pure yellow oil (0.27 g, 63%; TLC detection, AcOEt/MeOH/32% NH_4OH, 8.5/1/0.5) which can be used without additional purification. If small amounts of Aliquat 336 are revealed by TLC detection (I_2 reagent), the product is filtered on a short silica gel column (eluent, AcOEt/MeOH/32% NH_4OH, 8.5/1/0.5). The product was characterized as picrate: mp 180–181 °C dec (H_2O/EtOH); ^{1}H NMR (DMSO-d_6) δ 8.71, 8.55 (picrate), 7.94–7.71 (m, 5H, C_6H_5), 4.11–1.95 (m, 9H, piperidine); ^{13}C NMR (DMSO-d_6) δ 161.1, 141.6, 125.5, 121.5 (picrate), 153.2 (C-4), 131.2, 129.2, 127.7, 124.9 (C_6H_5), 114.5 (C-3), 42.5, 40.0, 28.1 (piperidine). Anal. ($C_{19}H_{18}O_9N_6S$) C, H, N.

4-Phenyl-3-(4-piperidylthio)furoxan (10b) Hydrochloride. The title product was prepared from **9** following the procedure used for the synthesis of **10a**. The reaction mixture was stirred well at room temperature for 5 h. The final pure (TLC) residue (80% yield) obtained after solvent removal was characterized as hydrochloride: mp 188–189 °C dec (EtOH); ^{1}H NMR δ 9.26 (s, br, 2H, exchangeable protons), 7.95–7.60 (m, 5H, C_6H_5), 3.67–1.66 (m, 9H, piperidine); ^{13}C NMR δ 158.5 (C-4), 131.6, 129.2, 128.1, 126.0 (C_6H_5), 110.1 (C-3), 42.4, 41.3, 28.9 (piperidine). Anal. ($C_{13}H_{15}N_3O_2S$·HCl) C, H, N.

4-Phenyl-3-(4-piperidylthio)furazan (10c) Hydrochloride. The title product was prepared from **8b** following the procedure used for the synthesis of **10a**. The reaction mixture was stirred well at room temperature for 2 h. The final pure (TLC) residue (67% yield) obtained after solvent removal was characterized as hydrochloride: mp 178–179 °C (iPrOH); ^{1}H NMR δ 9.42 (s, br, 2H, exchangeable protons), 7.84–7.59 (m, 5H, C_6H_5), 4.07–1.92 (m, 9H, piperidine); ^{13}C NMR δ 153.0, 150.1 (C-4, C-3), 131.4, 129.5, 128.2, 124.4 (C_6H_5), 42.3, 41.2, 28.2 (piperidine). Anal. ($C_{13}H_{15}N_3OS$·HCl) C, H, N.

3-Phenyl-4-(4-piperidylsulfonyl)furoxan (11a) Hydrochloride. To a stirred and ice–water-cooled solution of **10a** (2.77 g, 10 mmol) in TFA (6 mL) was added dropwise over 30

min a solution of 80% H_2O_2 (0.86 g, 20 mmol) in TFA (2 mL). The stirring was continued for 1 h at room temperature, and then the reaction mixture was cooled with ice–water. A solution of 80% H_2O_2 (0.86 g, 20 mmol) in TFA (2 mL) was added dropwise over 30 min, the reaction mixture was kept under stirring for 2 h at room temperature, and if necessary (TLC detection), additional H_2O_2 was added. Solvent removal in vacuo gave an oil which, treated with ethanol, afforded a white solid. The precipitate, collected by filtration, was treated with 2 N NaOH and the resulting solution extracted with CH_2Cl_2. The combined organic layers were dried, filtered, and evaporated in vacuo to give a pure white solid (TLC detection, AcOEt/MeOH/32% NH_4OH, 8.5/1/0.5) (1.61 g, 54% yield), which was transformed into the corresponding hydrochloride: 223–227 °C dec (H_2O); ^1H NMR δ 9.20 (s, br, 2H, exchangeable protons), 7.78–7.59 (m, 5H, C_6H_5), 4.01–1.88 (m, 9H, piperidine); ^{13}C NMR δ 155.5 (C-4), 131.4, 130.1, 128.8, 120.7 (C_6H_5), 114.1 (C-3), 58.1, 41.5, 20.9 (piperidine). Anal. ($C_{13}H_{15}N_3O_4S\cdot HCl\cdot 0.5H_2O$) C, H, N.

4-Phenyl-3-(4-piperidylsulfonyl)furoxan (11b) Hydrochloride. The title compound was prepared from **10b** following the procedure used for the synthesis of **11a**. TFA removal gave a yellow solid which was dissolved in 2 N NaOH and extracted with CH_2Cl_2. From the combined organic layers, a pure (TLC) white solid (78% yield) was obtained which was transformed into the corresponding hydrochloride: mp 232–233 °C dec (MeOH); ^1H NMR δ 8.96 (s, br, 2H, exchangeable protons), 7.74–7.55 (m, 5H, C_6H_5), 3.94–1.84 (m, 9H, piperidine); ^{13}C NMR δ 155.8 (C-4), 131.4, 129.8, 128.5, 124.9 (C_6H_5), 115.2 (C-3), 58.5, 41.3, 20.8 (piperidine). Anal. ($C_{13}H_{15}N_3O_4S\cdot HCl$) C, H, N.

4-Phenyl-3-(4-piperydylsulfonyl)furazan (11c) Hydrochloride. The title compound was prepared from **10c** following the procedure used for the synthesis of **11a**. The reaction mixture was poured into ice–water, basified with 2 N NaOH, and extracted with CH_2Cl_2. From the combined organic layers, a pure (TLC) white solid (66% yield) was obtained which was transformed into the corresponding hydrochloride: mp 262–264 °C dec (EtOH); ^1H NMR δ 9.35 (s, br, 2H, exchangeable protons), 7.88–7.57 (m, 5H, C_6H_5), 4.18–1.93 (m, 9H, piperidine); ^{13}C NMR δ 153.4, 153.0 (C-3, C-4), 131.6, 129.9, 128.9, 122.8 (C_6H_5), 58.8, 41.4, 21.1 (piperidine). Anal. ($C_{13}H_{15}N_3O_3S\cdot HCl$) C, H, N.

1-(4-Amino-6,7-dimethoxy-2-quinazolyl)-4-(3-phenyl-4-furoxanylsulfonyl)piperidine Hydrochloride (13a). A mixture of **11a** (0.68 g, 2.2 mmol) and **12** (0.48 g, 2.0 mmol) in DMSO (5 mL) was heated at 80 °C for 10 h and then left at room temperature for one night. The pure precipitate formed (TLC detection, EtOH/MeOH/32% NH_4OH, 8.5/1/0.5) was filtered, washed with water, and dried (0.70 g, 64% yield): mp 228–230 °C dec (EtOH); ^1H NMR δ 12.4 (s, br, 1H, NH^+), 8.9, 8.6 (2s, br, 2H, NH_2), 7.79–7.59 (m, 5H, C_6H_5), 7.77 (s, 1H, H-5), 7.62 (s, 1H, H-8), 4.82–1.73 (m, 9H, piperidine), 3.89, 3.86 (2s, 6H, OCH_3); ^{13}C NMR δ 161.4 (C-4), 155.7, 155.3 (CSO_2, C-7), 151.2 (C-2), 146.8 (C-6), 136.0 (C-1a, br), 131.3, 130.1, 128.7, 120.8 (C_6H_5), 114.2 (CC_6H_5), 105.0 (C-5), 101.8 (C-4a), 99.3 (C-8), 56.3, 56,1 (6-OCH_3, 7-OCH_3), 60.6, 43.2, 23.6 (piperidine). Anal. ($C_{23}H_{24}N_6O_6S\cdot HCl\cdot 1.5H_2O$) C, H, N.

1-(4-Amino-6,7-dimethoxy-2-quinazolyl)-4-(4-phenyl-3-furoxanylsulfonyl)piperidine Hydrochloride (13b). The title compound was prepared from **11b** following the procedure used for the synthesis of **13a**. The reaction mixture in ethanol was refluxed for 30 h. The formed precipitate was filtered, washed with ethanol, and dried (50% yield): mp 225–227 °C dec (EtOH/H_2O); ^1H NMR δ 12.5 (s, br, 1H, NH^+), 8.9, 8.6 (2s, br, 2H, NH_2), 7.78–7.54 (m, 7H, C_6H_5, H-5, H-8), 4.87–1.73 (m, 9H, piperidine), 3.87, 3.85 (2s, 6H, OCH_3); ^{13}C NMR δ 161.4 (C-4), 155.8, 155.2 (CC_6H_5, C-7), 151.2 (C-2), 146.7 (C-6), 136.0 (C-1a, br), 131.4, 129.8, 128.5, 125.0 (C_6H_5), 115.4 (CSO_2), 104.9 (C-5), 101.9 (C-4a), 99.0 (br, C-8), 56.3, 56.1 (6-OCH_3, 7-OCH_3), 60.9, 43.1, 23.4 (piperidine). Anal. ($C_{23}H_{24}N_6O_6S\cdot HCl\cdot H_2O$) C, H, N.

1-(4-Amino-6,7-dimethoxy-2-quinazolyl)-4-(4-phenyl-3-furazanylsulfonyl)piperidine Hydrochloride (13c). The title compound was prepared from **11c** following the procedure used for the synthesis of **13b**. The reaction mixture was refluxed for 37 h (77% yield): mp 255–259 °C dec (MeOH/H_2O); ^1H NMR δ 12.4 (s, br, 1H, NH^+), 8.9, 8.7 (2s, br, 2H, NH_2), 7.89–7.58 (m, 7H, C_6H_5, H-5, H-8), 4.83–1.78 (m, 9H, piperidine), 3.89, 3.86 (2s, 6H, OCH_3); ^{13}C NMR δ 161.4 (C-4), 155.3, 153.3, (C-7, CC_6H_5, CSO_2, two peaks overlapped), 151.2 (C-2), 146.8 (C-6), 136.4 (C-1a), 131.7, 129.9, 128.9, 122.9 (C_6H_5), 105.0 (C-5), 101.8 (C-4a), 99.3 (C-8), 56.4, 56.1 (6-OCH_3, 7-OCH_3), 61.1, 43.2, 23.7 (piperidine). Anal. ($C_{23}H_{24}N_6O_5S\cdot HCl\cdot 0.5H_2O$) C, H, N.

Quantitative Nitrite Detection and Initial Rate of Nitric Oxide Release. A solution of the appropriate furoxan derivative (20 μL) in DMSO was added to 2 mL of 50 mM phosphate buffer (pH 7.4) containing the appropriate amount of the cysteine to have a 5 mM concentration. The final concentration of the derivatives was 10^{-4} M. After 1 h at 37 °C, 1 mL of the reaction mixture was treated with 250 μL of the Griess reagent sulfanilamide (4 g), *N*-(naphthylethylene)-diamine dihydrochloride (0.2 g), and 85% phosphoric acid (10 mL) in distilled water (final volume, 100 mL). The mixture was kept for 10 min at room temperature, and then the absorbance was measured at 540 nm; 10–80 nmol/mL sodium nitrite standard solutions were used for the calibration curve. The yield in nitrite was expressed as % NO_2^- (mol/mol) ± standard error. The initial rate of the NO release was evaluated at 37 °C with an incubation mixture composed of the appropriate compound and 5-fold molar excess L-cysteine in the presence of 4×10^{-6} M HbO_2^{2+} (relative to heme) in 50 mM phosphate buffer (pH 7.4). The absorbance increase versus time was measured at 401 nm. The release was evaluated for each compound at 3–4 different concentrations in the ranges 0.01–0.05 mM (**7a**), 0.25–0.75 mM (**7b**), and 0.075–0.75 mM (**7c**). The reactions finished within 3 min as indicated by a constant absorbance. The initial absorbance increase rates were calculated from the slope of the absorbance readings versus time obtained in the first minute. The concentrations of the compounds able to give 0.1 absorbance increase per minute (2.6 μM min^{-1}, NO formation rate) were intrapolated from the curves concentration versus initial rate of the increasing absorbance.

Pharmacology. Rat Aortic Strip Preparation. Thoracic aorta were isolated from male Wistar rats (200–250 g) anesthetized with CO_2 and sacrified by decapitation. All animals were dealt with in a humane way in accordance with recognized guidelines on animal experimentation.

The vessels were helically cut, the endothelium was removed, and two strips were obtained from each aorta. The tissues were suspended under a tension of 1*g* in organ baths containing 30 mL of Krebs–Heinselet solution of the following composition (mM): NaCl, 137; KCl, 2.68; $MgCl_2$, 0.5; $CaCl_2$, 5.44; NaH_2PO_4, 0.54; $NaHCO_3$, 8.93; and glucose, 8.3. The physiological salt solution for functional α_1-antagonism also contained 0.1 mM ascorbic acid, 1×10^{-7} M desmethylimipramine hydrochloride, 5×10^{-6} M deoxycorticosterone acetate, 5×10^{-6} M propranolol, and 1×10^{-7} M yohimbine hydrochloride to prevent oxidation and neuronal and extraneuronal uptake of (−)-norepinephrine and to block β- and α_2-adrenoceptors, respectively. The medium was maintained at 37 °C and at pH of 7.4 (by gassing with 95% O_2/5% CO_2).

Functional Antagonism at α_1-Adrenoceptors. The aortic strips were allowed to equilibrate for 2 h before the experiments were started. (−)-Norepinephrine-induced contractions were determined cumulatively in the absence or presence of antagonist, which was preincubated for 30 min. One of the two strips cut from each aorta served as a control, while a dose–response curve in the presence of antagonist was performed on the other strip. Effects of oxyhemoglobin (HbO_2) on relaxation were evaluated in a separate series of experiments, by exposing aortic strips to 10 μM HbO_2 (relative to heme) for at least 10 min before addition of the antagonist. No effect of HbO_2 on the norepinephrine response was detected.

Reversibility of α_1-Antagonist Effects. In studies which examined the reversibility of α_1-antagonist effects, aortic strips were exposed to the analogues of Prazosin at the maximal concentration tested, for 30 min. Tissues were washed with Krebs–Heinselet solution at 15 min intervals for 1 h, and thus,

the control concentration–response curve for the (−)-norepinephrine was assessed.

Vasoactivity Determinations. The aortic strips were allowed to equilibrate for 1 h and then were contracted by replacing the bath medium with a Krebs–Henseleit solution containing 137 mM KCl (equimolar substitution of K^+ for Na^+). During this first contraction, the absence of intact endothelium was verified by addition of 1 μM acetylcholine, which was found not to induce relaxation. The preparations were then extensively washed, and a second contraction was evoked. When the response to the agonist plateaued, cumulative concentrations of the vasodilating agent were added. Effects of oxyhemoglobin on relaxation were evaluated in a separate series of experiments, by exposing precontracted aortic strips to 10 μM HbO_2 for at least 15 min before addition of the vasodilating agent.

Preparation of Oxyhemoglobin. Bovine hemoglobin type 1 (H-2500, Sigma Chemical Co.) contains a mixture of oxyhemoglobin and the oxidized derivative methemoglobin. Pure oxyhemoglobin was prepared daily by addition to a solution of commercially available hemoglobin in phosphate saline buffer (pH 7.4) of a 10-fold molar excess of the reducing agent sodium dithionite ($Na_2S_2O_4$) at 4 °C and was then protected from light. Within a few minutes, the mixture was centrifuged at 1000*g* for 10 min, at 4 °C, and the supernatant loaded onto a chromatographic column (Sephadex G-25 medium, Pharmacia, Uppsala, Sweden) and eluted with phosphate buffer. The purity of the oxyhemoglobin solution was determined spectrophotometrically (λ_{max} = 576 and 541 nm; ϵ = 14 600 and 13 800).

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