Synthesis and Pharmacological Characterization of New H_2-Antagonists Containing NO-Donor Moieties, Endowed with Mixed Antisecretory and Gastroprotective Activities

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Synthesis, structural characterization, and pharmacological profile of a series of H_2-antagonists able to release nitric oxide (NO) are reported. These compounds were obtained by using appropriate spacers to join H_2-antagonistic pharmacophoric groups related to lamtidine and tiotidine to different NO-donor moieties such as esters of HNO_3, nitrosothio groups, and benzenesulfonyl-substituted furoxans. All of the compounds were tested for their NO-donor properties. Furthermore, the hybrid structures synthesized, together with some selected reference compounds, were tested for their H_2-antagonistic properties, both *in vitro* and *in vivo*, and for their gastroprotective effects. Only the hybrid compounds were able both to antagonize histamine effects on guinea-pig papillary muscle and to display *in vivo* antisecretory and gastroprotective action. The best results were obtained with the lamtidine/furoxan hybrid structure.

1. Introduction. – H_2-Antagonists are a well-established class of drugs used in the management of peptic ulcer and gastric-acid-related disorders [1]. Since H_2-receptors were found to be involved not only in histamine response, but also in those of gastrin and acetylcholine, blocking of H_2-receptors causes an inhibition of acid secretion even if other stimuli are applied to the parietal cells [2]. Another interesting application of these drugs is their use for suppressing gastric-acid production during administration of non-steroidal anti-inflammatory drugs (NSAIDs). In this regard, however, proton-pump inhibitors are generally preferred, either for their stronger acid-suppressing capacity or for their additional gastroprotective effects [3]. The presence of the latter property is controversial in H_2-receptor antagonists. Although some studies indicate that they display gastroprotective effects in a number of ulceration models, they fail to trigger protection against gastric damage induced by NSAIDs [4][5]. Nitric oxide (NO), a recently discovered second messenger, displays a number of important roles in different compartments of the body [6]. At the level of the gastric tract, it is involved in muscle relaxation, hemodynamic control, and cytoprotection of the gastric lining [7]. Based on these facts, we thought it would be useful to combine the antisecretory activity of H_2-receptor antagonists with the NO-dependent gastroprotective effect in the same molecule. This can be achieved by joining a H_2-antagonistic pharmacophoric group to an appropriate NO-donor moiety, according to the general block structure **I** (*Fig. 1*).

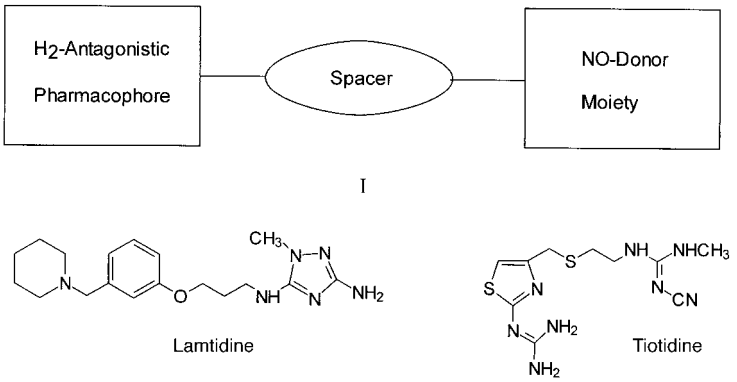


Fig. 1. *General block structure of H_2-antagonists and the reference compounds lamtidine and tiotidine*

In this work, the H_2-antagonistic substructures were derived from lamtidine and tiotidine, respectively, two well-known H_2-receptor antagonists. As NO-releasing moieties, we chose phenylsulfonyl-substituted furoxan (= furazan 2-oxide), esters of HNO_3, and nitrosothio functions. All the structures prepared are collected in *Fig. 2*. As control, the derivatives lacking the NO-releasing moieties were also studied. Synthesis and characterization of **29** were discussed in a preliminary report [8]; nevertheless, a few properties of this compound are also considered in the present paper for comparison.

2. Results and Discussion. – 2.1. *Synthesis of the Nitrates* **8**–**11**. Synthesis of the hybrid models **8**, **9**, **10**, and **11**, containing nitroxy groups, was realized according to the pathway shown in *Scheme 1*. Diphenyl *N*-cyanocarbonimidate (**3**) was used as starting material. This compound shows a very high reactivity towards nucleophiles such as amines [9]. Displacement of the first PhO group occurs under mild conditions, even with poor nucleophiles. Intermediates **4** and **5** were obtained in good yields simply on stirring **3**, at room temperature in MeCN, with nitric-acid esters **1** and **2**. Longer reaction times were necessary for the displacement of the second PhO group by amines **6** and **7**, which led to the formation of **8**–**11** in high yields. ^{13}C-NMR Data are in agreement with the proposed structures (see *Exper. Part*). In particular, signals in the range 70–79 ppm are attributable to nitroxy-substituted C-atoms.

2.2. *Synthesis of the Nitrosothio Derivatives* **20** *and* **21**. Unlike the esters of nitric acid, structures containing nitrosothio groups are characterized by high instability and can only rarely be isolated and purified [10]. One exception is *S*-nitroso-*N*-acetylpenicillamine (SNAP) (= *N*-acetyl-3-(nitrosothio)valine), which was chosen for our experiments. The unusual stability of this compound allowed an X-ray study of its structure to be carried out and also stimulated the preparation of some analogues [11][12]. We planned a sequential approach to the final products **20** and **21**, in which the nitrosothio group was introduced in the last step of the reaction sequence (*Scheme 2*). At room temperature, the reaction of an excess of ethylenediamine (**14**) with **13** afforded **16**. Compound **15** was similarly obtained from **12** according to the procedure reported in [13]. These two intermediates, treated with thietan-2-one **17** in MeOH solution, led to the expected *N*-acetylpenicillaminyl derivatives **18** and **19** in good

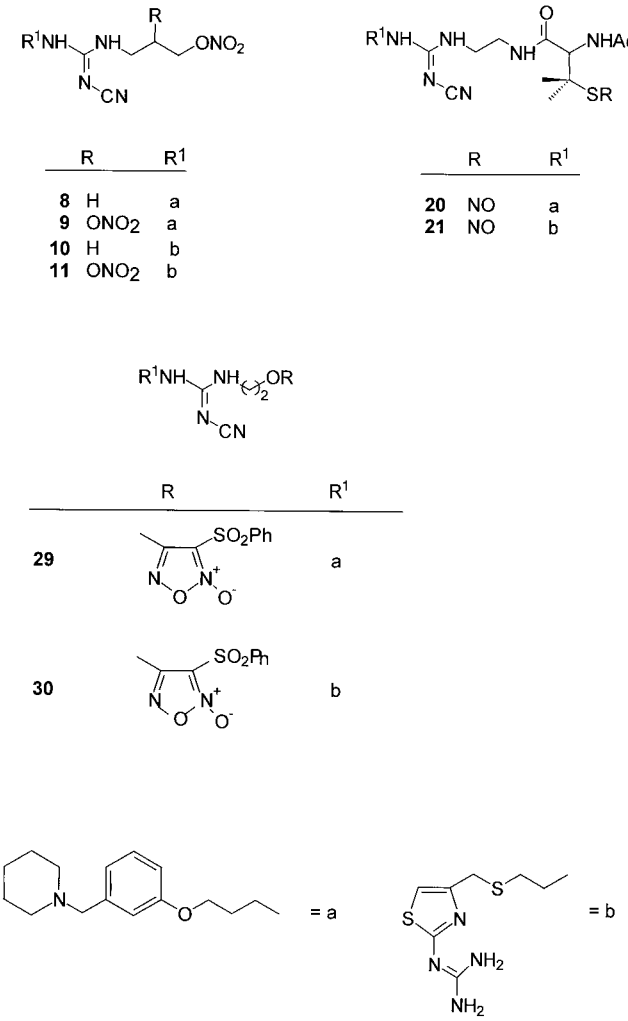
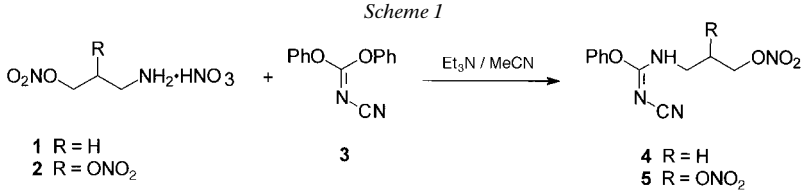


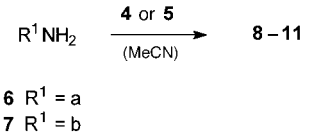
Fig. 2. *Summary of synthesized hybrids containing H_2-antagonistic and NO-donor moieties*

yields. Subsequent nitrosation of these compounds was satisfactorily achieved with *t*-BuONO in AcOH, yielding **20** and **21**, respectively. The final products were sufficiently stable to be isolated by lyophilization of the frozen reaction mixture and to be characterized by routine spectroscopic techniques. ^{13}C-NMR Data were in agreement with the proposed structures (see *Exper. Part*). *S*-Nitrosation led to a significant downfield-shift (13 ppm) of the resonances of the quaternary C-atoms linked to the S-atom.

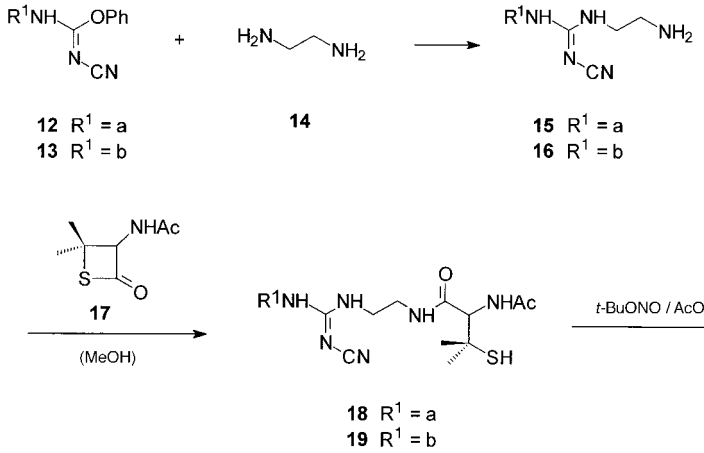
2.3. *Synthesis of the Furoxan and Furazan Derivatives.* Synthesis of the hybrid furoxan model compounds **29** and **30**, and of the related furazan derivative **32** was realized by the pathway depicted in *Scheme 3*. The PhO group present in the intermediates **12** and **13** was displaced at room temperature by the amino alcohols **22**

Scheme 1





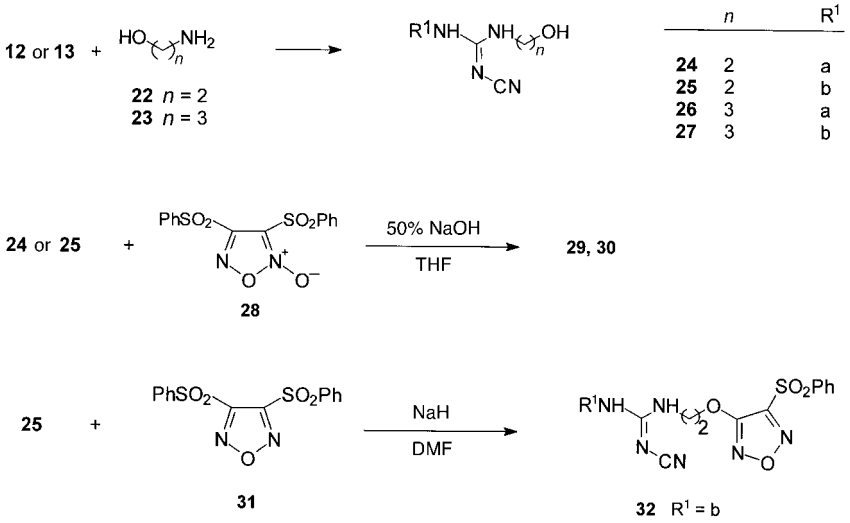
Scheme 2



and **23** to yield H_2-antagonistic pharmacophores **24**–**27** that bear hydroxyalkyl spacers on the terminal amino group of the cyanoguanidine moiety. Compounds **24** and **25**, dissolved in THF (THF/DMF in the case of **25**) in the presence of 50% aqueous NaOH solution, were able to react with 3,4-bis(phenylsulfonyl)furoxan (**28**) to yield the final products **29** and **30**. It is known that **28**, dissolved in THF, reacts with EtOH under basic conditions to give 4-ethoxy-3-(phenylsulfonyl)furoxan [14]. Based on this observation, **29** and **30** were expected to be the products of an analogous reaction of **28** with **24** and **25**, respectively. This assumption was also confirmed by ^{13}C-NMR data (see *Exper. Part*). In particular, the signals at 110.7 and 158.9 ppm, detected for both compounds, are characteristic for C(3) and C(4), respectively, of a 4-alkoxy-3-(phenylsulfonyl)-substituted furoxan ring [15]. The furazan analogue **32** was synthesized in a similar fashion from **25** and **31** in DMF in the presence of NaH.

2.4. *Detection of Nitrite and Determination of Initial NO-Release Rate.* Nitrite is the major oxidation products of NO in aqueous systems, and their detection serves as a basis for the determination of the original NO concentration. However, it is known that

Scheme 3



in the presence of thiols, besides this oxidation, a variety of other reactions can occur. To examine spontaneous NO release, each hybrid compound was dissolved (10^{-4} M) in pH-7.4-buffered H_2O and kept at 37° for 1 h. The nitrites formed were detected by the *Griess* reaction [16]. Furthermore, the NO_2^- concentration was determined in the presence of a large excess of cysteine (Cys) (50 : 1) after 1 h of incubation. The results, expressed as % NO_2^- (mol/mol), are summarized in *Table 1*. The initial rates of NO release in the presence of Cys (5-fold molar excess) and, if necessary, in its absence, were determined at selected concentrations for each hybrid, employing a spectrophotometric technique based on the NO-induced oxidation of oxyhemoglobin (HbO_2) to methemoglobin (MetHb) [17]. The reaction itself was monitored by detecting the increase of absorbance (ΔA) at 401 nm. The initial rates of NO release (in nmol ml^{-1} min^{-1}) were calculated from the measured rates (in ΔA min^{-1}) using a molar extinction coefficient $\Delta\varepsilon = \varepsilon_{401}(\text{MetHb}) - \varepsilon_{401}(HbO_2) = 39.9$ (± 1.4) mM^{-1} cm^{-1}. All results are reported in *Table 1*.

Hybrids **8** and **10**, bearing one nitroxy function, behaved as weak NO-donors only in the presence of cysteine. The extent of NO release at the applied concentration (10^{-4} M) was too small to allow the determination of the initial rate of the process. When two nitroxy functions were present (structures **9** and **11**), the extent of release increased, but, again, it was impossible to determine the initial rate. However, working with cells, tissues, or *in vivo*, not only thiol-induced but also enzymatic production of NO from nitrates should be considered [18].

NO Release by nitrosothio groups was quite complex to follow. Both **20** and **21** led to the formation of nitrite to a similar extent. The initial rates of NO release, determined at 37° in 5×10^{-4} M solutions, were, within the limits of experimental error, identical for the two structures. Analogous measurements in the presence of an excess of cysteine showed a high increase in the amounts of nitrite formed, as well as in the initial rates of NO release. This behavior might be explained by transnitrosation from

Table 1. *NO-Release Characteristics of Hybrids under Different Conditions*[a])

Compound	NO_2^- [%][b]) (−L-cys)	NO_2^- [%][b]) (+L-cys)	NO_2^- [%][b]) (+Cu^+)[d])	NO [nmol ml^{-1} min^{-1}][c]) (−L-cys)	NO [nmol ml^{-1} min^{-1}][c]) (+L-cys)
8	0	0.43 ± 0.11	–	0	0
9	0	6.0 ± 0.3	–	0	0
10	0	0.47 ± 0.11	–	0	0
11	0	4.4 ± 0.1	–	0	0
20	2.1 ± 0.4	28.0 ± 1.0	64.6 ± 2.0	0.989 ± 0.012[e])	0.346 ± 0.025
21	3.8 ± 0.8	50.5 ± 0.6	96.8 ± 1.2	1.015 ± 0.025[e])	0.556 ± 0.030
29	0	56.9 ± 3.4	–	0	0.596 ± 0.077
30	0	31.6 ± 1.5	–	0	1.248 ± 0.085

[a]) Yields are reported in % (mol/mol ± standard error of the mean (SEM), *cf. Exper. Part*). [b]) Nitrite was determined according to the *Griess* procedure; compound: 100 μM; L-cysteine: 5 mM; *cf. Exper. Part.* [c]) The initial NO-release rate was determined by the oxyhemoglobin method; compound: 10 μM; L-cysteine: 50 μM. [d]) [Cu^+]: 10 μM. [e]) With 10 μM Cu^+, immediate oxyhemoglobin oxidation.

the rather stable SNAP analogues **20** and **21** to very unstable *S*-nitrosocysteine [19]. An even more dramatic change in the rate of NO release was observed in the presence of Cu^+ ions (10^{-5} M). Within 1 h, the tiotidine hybrid **21** was 100% denitrosated, and the lamtidine hybrid **20** was about 65% denitrosated. For both compounds, it was impossible to measure the initial rates of NO production since the reactions were too fast. Efficient metal-ion catalysis in nitrosothio-group decomposition would explain our observations [20][21].

NO Release from furoxan derivatives occurred only in the presence of cysteine. The lamtidine hybrid **29** was a stronger overall NO-donor than the tiotidine hybrid **30**, but the initial rate of release was *ca*. twofold higher for **30** than for **29**. Both the extent and the initial rate of NO release in the two hybrids were high. With respect to biological systems, one should consider that NO might also be released enzymatically from furoxans. However, to the best of our knowledge, this possibility has not been investigated yet.

2.5. *Pharmacological Characterization*. All of the hybrid drugs, as well as the related compounds lacking NO-donor moieties, were screened for their H_2-antagonistic properties on isolated guinea-pig papillary muscle *in vitro*, and on anesthetized rats with a lumen-perfused stomach *in vivo* (*i.v.* administration). In the former case, the activity was expressed as pA_2 (CL 95%), in the latter as ID_{50} (in mg/kg) (CL 95%). Gastroprotective activity was assessed against HCl-induced (0.6N) gastric lesions in conscious rats (*i.g.* administration). The results were expressed as %-reduction of the lesion index compared to a control group. It should be noted that, due to different routes of administration, gastroprotective effects of H_2-receptor antagonists containing NO-donor moieties were observed at doses 3–10 times higher than those usually eliciting antisecretory effects. The results are reported in *Table 2*.

Hybrids **8** and **10**, containing one nitroxy function, exhibited 12- and 3-fold lower potency, respectively, at guinea-pig papillary muscle H_2-receptors than the reference compounds **26** and **27**. The ability of **10** to inhibit gastric-acid secretion was almost comparable to that of reference compound **27**. However, a similar comparison between the two lamtidine derivatives **8** and **26** was not possible. In fact, hybrid **8** reduced

Table 2. *H_2-Antagonistic Properties and Gastroprotective Activity of Hybrids* **8**–**11**, **20**, **21**, **29**, *and* **30**, *and the Related Reference Compounds* **18**, **19**, **24**–**27**, *and* **32**

	H_2-antagonistic activity		Gastroprotective activity
	Guinea-pig papillary muscle pA_2 (CL 95%)	Rat lumen-perfused stomach ID_{50} [mg/kg] (CL 95%)	Rat stomach % reduction of lesion index mean ± SEM
8	5.18 (4.29–6.07)[a])[b])	–[c])	44 ± 18 (30 mg/kg)[d]) 56 ± 7 (100 mg/kg)[d])
9	6.16 (5.99–6.33)[a])	0.35 (0.16–0.79)	62 ± 8 (30 mg/kg)[d]) 97 ± 1 (100 mg/kg)[e])
10	5.95 (5.36–6.54)[a])	0.60 (0.32–1.18)	35 ± 20 (30 mg/kg)
11	6.59 (5.9–7.28)[a])	1.01 (0.90–1.14)	–[c])
18	5.34 (5.09–5.59)[a])	0.68 (0.28–1.64)	inactive up to 100 mg/kg
19	5.83 (5.13–6.52)[a])	0.26 (0.08–1.00)	inactive up to 100 mg/kg
20	5.43 (5.28–5.59)[a])	0.94 (0.45–1.96)	36 ± 12 (3 mg/kg)[d])
21	5.62 (5.04–6.19)[a])	0.42 (0.18–0.88)	50 ± 7 (0.03 mg/kg)[d])
24	6.95 (6.44–7.46)[a])	0.02 (0.005–0.12)	inactive up to 100 mg/kg
26	6.25 (5.88–6.62)	0.26 (0.14–0.45)	inactive up to 100 mg/kg
27	6.40 (5.83–6.97)[a])	0.34 (0.20–0.55)	inactive up to 100 mg/kg
29	5.78 (5.65–5.91)[b])	0.40 (0.20–0.76)[b])	20 ± 6 (3 mg/kg) 80 ± 6 (10 mg/kg)[e]) 90 ± 2 (30 mg/kg)[e])
30	6.71 (6.62–6.81)[f])	0.20 (0.15–0.26)	65 ± 8 (30 mg/kg)[d])
32	6.30 (4.84–7.76)[f])	0.11 (0.06–0.20)	inactive up to 100 mg/kg

[a]) pA_2 was calculated only for two surmountable concentrations of the antagonist. [b]) $P < 0.05$ *vs.* the related reference compound (*t*-test for unpaired data). [c]) See text. [d]) $P < 0.05$ *vs.* the vehicle-treated group (ANOVA followed by *Newman-Keuls* test). [e]) $P < 0.01$ *vs.* the vehicle-treated group (ANOVA followed by *Newman-Keuls* test). [f]) pA_2 was calculated only for one surmountable concentration of the antagonist.

histamine response by approximately 50% when tested at a dose of 1.55 mg/kg, but higher doses were toxic for the animals, thus preventing us from evaluating the ID_{50} value. Compounds **8** and **10** showed gastroprotective activity, which was not the case for the related compounds devoid of NO-donor moieties. For **8**, the observed effect did not vary significantly for doses of 30 and 100 mg/kg, respectively, leading in both cases to a *ca*. 50% reduction of the lesion index. Due to the low solubility of **10**, only 30 mg/kg-doses could be evaluated; they were found to provide 35% protection. Compound **9**, containing two nitroxy functions, displayed good H_2-antagonistic activity both *in vitro* and *in vivo*. In addition, it was a better gastroprotective agent than the mononitroxy structure **8**. This is in keeping with its better NO-donor properties. The tiotidine hybrid **11** was a more potent H_2-receptor blocker *in vitro*, but *in vivo*, it was less effective than **10**. The gastroprotective action of **11** was not evaluated because of its low solubility.

The nitrosothio-substituted hybrids **20** and **21** were as effective as the reference compounds **18** and **19** on papillary muscle and in inhibiting gastric-acid secretion. In both tests, the tiotidine structures were more potent than the lamtidine analogues. As expected, the reference compounds were devoid of any gastroprotective effect, while the nitrosothio-substituted hybrids showed a rather complex behavior: structure **20**

caused a 36% reduction of the lesion index at a dose of 3 mg/kg, higher doses, however, were ineffective. Experiments with compound **21** led to very ambiguous results: in some cases, protective effects were observed, in other cases, **21** led to gastric lesions, and sometimes it did not trigger any action at all. However, at very low doses (0.03 mg/kg), we were able to show a statistically significant, partially protective effect ($50 \pm 7\%$). This contradictory behavior might be caused by individual differences in tissue availability of cysteine and/or of those metal ions which strongly influence NO release from nitrosothio-substituted compounds.

The tiotidine-furoxan hybrid **30** was a more potent H_2-receptor antagonist both *in vitro* and *in vivo* than the corresponding lamtidine hybrid **29**, and it showed properties similar to the furazan analogue **32**. Compound **29** was less active than the related compound **24**, but it still displayed a good H_2-antagonistic profile. It caused a dose-dependent reduction of gastric damage, resulting in complete protection at a dose of 30 mg/kg. This compound appears to be a well-balanced hybrid endowed with both H_2-antagonistic activity and gastroprotective effect *in vivo*. The tiotidine-derived structure **30** was only a partial gastroprotector, causing a 65% reduction of the lesion index at 30 mg/kg, which was, due to problems of solubility, the maximum dose tested. As expected, neither of the two reference compounds **24** and **32** displayed this kind of effect as they lack the necessary NO-donor moieties.

In conclusion, all of the hybrids tested are endowed with both antisecretory and gastroprotective activity the lamtidine-derived structures showing the most promising properties. In this series, the hybrid behavior was optimised by the presence of a (phenylsulfonyl)furoxan substructure. These compounds could be the prototypes of a new class of drugs, which may be useful in the therapy of inflammatory disorders and in the management of conditions involving gastric hypersecretion.

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Experimental Part

General. Compounds **1** [22], **2** [23], **3** [9], **6** [24], **7** [25], **12** [26], **13** [27], **15** [13], **17** [12], **24** [13], **28** [28], **29** [8], and **31** [29] were prepared according to the reported methods. Flash chromatography (FC): silica gel *60* (*Merck* 230–400 mesh ASTM). M.p.: *Büchi 530* apparatus, uncorrected. Dec. temp. was detected by introducing the sample into the bath approx. 10° below dec. started, heating rate 3°/min. Some compounds were amorphous solids without defined m.p. For some of these structures, the initial point of dec. detected by differential scanning calorimetry (*Perkin-Elmer DSC 7*, heating rate 10°/min) is reported. All the products were characterized by routine spectroscopy. IR Spectra: *Shimadzu FT-IR8101 M*. ^{1}H- and ^{13}C-NMR spectra: *Bruker AC-200*, at 200 and 50 MHz, resp.; δ in ppm rel. to $SiMe_4$ as internal standard, coupling constants J in Hz; ^{13}C-NMR spectra were fully decoupled. Elemental analyses (C, H, N) were performed by *Redox* (*Cologno M.*)

3-[[(Cyanoimino)(phenoxy)methyl]amino]propyl Nitrate (**4**). To a stirred soln. of **1** (4.00 g, 21.8 mmol) and Et_3N (3.47 ml, 24.9 mmol) in MeCN (22 ml), **3** (5.19 g, 21.8 mmol) was added, and the mixture was stirred for 30 min at r.t. The solvent was evaporated under reduced pressure, the oily residue was diluted with CH_2Cl_2 (20 ml) and washed with H_2O (3×20 ml). The org. phase was dried ($MgSO_4$) and evaporated under reduced pressure to give a viscous oil which was purified by FC (silica gel; AcOEt/CH_2Cl_2 1:40 (300 ml) and AcOEt/CH_2Cl_2 1:4 (500 ml)) to afford **4** (5.00 g, 87%). White solid. M.p. 77° (EtOH). ^{1}H-NMR ($CDCl_3$): 8.09 (*s*, NH); 7.07–7.45 (*m*, 5 arom. H); 4.57 (*t*, $J = 6.1$, CH_2ONO_2); 3.53 (*m*, CH_2NH); 2.08 (*m*, CH_2). ^{13}C-NMR ($CDCl_3$): 164.49; 151.48; 130.08; 127.15; 121.86; 116.16; 70.73; 39.35; 27.15. Anal. calc. for $C_{11}H_{12}N_4O_4$ (264.2): C 50.00, H 4.58, N 21.20; found: C 50.04, H 4.56, N 21.08.

3-{[(Cyanoimino)(phenoxy)methyl]amino}propane-1,2-diyl Dinitrate (**5**). To a stirred soln. of **2** (2.50 g, 10.2 mmol) and Et_3N (1.62 ml, 11.6 mmol) in MeCN (10 ml), **3** (2.57 g, 10.7 mmol) was added, and the mixture was stirred vigorously for 30 min at r.t. The solvent was evaporated under reduced pressure, the residue was diluted with CH_2Cl_2 and washed with H_2O (1 × 20 ml) and brine (2 × 20 ml). The org. phase was dried ($MgSO_4$) and evaporated under reduced pressure to afford a crude product which was purified by FC (silica gel; CH_2Cl_2 (300 ml) and AcOEt/CH_2Cl_2 1:10 (500 ml)) to yield **5** (2.19 g, 66%). White solid. M.p. 93° (i-PrOH). ^{1}H-NMR ($CDCl_3$): 8.37 (*t*, *J* = 5.8, NH); 7.47–7.08 (*m*, 5 arom. H); 5.44 (*m*, $CHONO_2$); 4.74 (*m*, CH_2ONO_2); 3.64–3.92 (*m*, 2 H, CH_2NH). ^{13}C-NMR ($CDCl_3$): 164.11; 150.17; 129.59; 126.91; 121.16; 115.25; 76.70; 68.89; 40.48. Anal. calc. for $C_{11}H_{11}N_5O_7$ (325.2): C 40.62, H 3.41, N 21.53; found: C 40.47, H 3.37, N 21.45.

3-(2-Cyano-3-{3-[3-(piperidinomethyl)phenoxy]propyl}guanidino)propyl Nitrate (**8**). Compound **3** (1.00 g, 3.67 mmol) was added portionwise during 2 h to vigorously stirred **6** (2.46 g, 7.67 mmol). The resulting mixture was stirred for 24 h at r.t., and the crude product was purified by FC (silica gel; MeOH/CH_2Cl_2 1:10) to afford **8** (1.54 g, 97%). Pale-yellow oil. The product was characterized as the oxalate (EtOH/Et_2O). ^{1}H-NMR ((D$_6$)DMSO): 6.98–7.38 (*m*, 6 H); 4.53 (*t*, *J* = 6.1, 2 H); 4.14 (*s*, 2 H); 4.02 (*m*, 2 H); 3.85 (*t*, *J* = 5.6, 2 H); 3.21–3.41 (*m*, 4 H); 2.98 (*m*, 4 H); 1.82–1.95 (*m*, 4 H); 1.71 (*m*, 4 H); 1.52 (*m*, 2 H). ^{13}C-NMR ((D$_6$)DMSO): 165.04; 159.42; 158.71; 132.59; 129.86; 123.11; 118.11; 116.78; 115.28; 71.59; 65.37; 59.48; 52.04; 39.95; 37.76; 28.64; 26.29; 22.71; 21.80. Anal. calc. for $C_{20}H_{30}N_6O_4 \cdot C_2H_2O_4 \cdot 0.5\ H_2O$ (517.5): C 51.06, H 6.43, N 16.24; found: C 51.30, H 6.81, N 16.58.

3-(2-Cyano-3-{3-[3-(piperidinomethyl)phenoxy]propyl}guanidino)propane-1,2-diyl Dinitrate (**9**). To a stirred soln. of **6** (2.43 g, 7.56 mmol) in MeCN (3 ml), **5** (1.81 g, 5.56 mmol) was added, and the mixture was stirred for 20 h at r.t. The solvent was evaporated under reduced pressure, and the residue was purified by FC (silica gel; MeOH/CH_2Cl_2 1:10) to afford **9** (2.59 g, 97%). Pale-yellow oil. The product was characterized as oxalate. M.p. 130–132° (DSC) (MeOH/Et_2O). ^{1}H-NMR ((D$_6$)DMSO): 6.98–7.48 (*m*, 6 H); 5.56 (*m*, 1 H); 4.66–4.98 (*m*, 2 H); 4.14 (*s*, 2 H); 4.02 (*m*, 2 H); 3.29–3.60 (*m*, 4 H); 3.00 (*m*, 4 H); 1.95 (*m*, 2 H); 1.72 (*m*, 4 H); 1.52 (*m*, 2 H). ^{13}C-NMR ((D$_6$)DMSO): 164.65; 159.60; 158.68; 132.11; 129.88; 123.19; 117.64; 116.83; 115.38; 78.30; 70.44; 65.30; 59.30; 51.90; 39.78; 38.61; 28.53; 22.54; 21.70. Anal. calc. for $C_{20}H_{29}N_7O_7 \cdot C_2H_2O_4$ (569.5): C 46.40, H 5.49, N 17.22; found: C 46.62, H 5.56, N 17.06.

3-{2-Cyano-3-[2-({2-[(diaminomethylidene)amino]thiazol-4-yl}methylthio)ethyl]guanidino}propyyl Nitrate (**10**). To a stirred suspension of **7** · 2 HCl (4.60 g, 15.1 mmol) and Et_3N (11.5 ml, 82.5 mmol) in MeCN (66 ml), **4** (2.00 g, 7.57 mmol) was added, and the mixture was stirred for 72 h at r.t. The liquid was decanted, and the solid residue was dissolved in 2N NaOH soln. and extracted with AcOEt (3 × 50 ml). The combined org. layers were dried ($MgSO_4$) and evaporated under reduced pressure, yielding a white solid. The crude product was purified by FC (silica gel; MeOH/CH_2Cl_2 1:10) affording **10** (1.54 g, 54%). Yellow oil. The product was characterized as the oxalate (MeOH/Et_2O). ^{1}H-NMR (MeOD): 7.06 (*s*, 1 H); 4.57 (*t*, *J* = 6.2, 2 H); 3.83 (*s*, 2 H); 3.33–3.47 (*m*, 4 H); 2.72 (*t*, *J* = 6.9, 2 H); 2.01 (*m*, 2 H). ^{13}C-NMR (MeOD): 167.06; 162.06; 161.39; 156.55; 150.91; 120.01; 111.18; 72.39; 42.28; 39.57; 32.21; 32.04; 28.10. Anal. calc. for $C_{14}H_{21}N_9O_7S_2$ (491.5): C 34.21, H 4.31, N 25.65; found: C 34.36, H 4.34, N 25.74.

3-{2-Cyano-3-[2-({2-[(diaminomethylidene)amino]thiazol-4-yl}methylthio)ethyl]guanidino}propane-1,2-diyl Dinitrate (**11**). To a stirred suspension of **7** · 2 HCl (3.80 g, 12.5 mmol) and Et_3N (9.25 ml, 66.3 mmol) in MeCN (10 ml), a soln. of **5** (2.04 g, 6.27 mmol) in MeCN (4 ml) was added and the reaction mixture stirred vigorously for 70 h at r.t. The solvent was evaporated under reduced pressure, the residue dissolved in 2N NaOH soln. and extracted with AcOEt (3 × 50 ml). The dried ($MgSO_4$) org. phase was evaporated under reduced pressure and the residue purified by FC (silica gel, MeOH/CH_2Cl_2 1:10) to give **11** (1.64 g, 56%) as colorless oil. The product was characterized as the oxalate. M.p. 210° (dec.) (DSC) (MeOH). ^{1}H-NMR ((D$_6$)DMSO): 7.82 (*m*, 4 H); 7.39 (*m*, 2 H); 6.81 (*s*, 1 H); 5.54 (*m*, 1 H); 4.84 (*m*, 2 H); 3.70 (*s*, 2 H); 3.31–3.59 (*m*, 4 H); 2.60 (*m*, 2 H). ^{13}C-NMR ((D$_6$)DMSO): 168.16; 166.10; 159.45; 155.92; 148.06; 117.55; 107.21; 78.27; 70.44; 40.85; 39.77; 30.85; 30.16. Anal. calc. for $C_{12}H_{18}N_{10}O_6S_2 \cdot 0.5\ C_2H_2O_4$ (507.5): C 30.77, H 3.77, N 27.60; found: C 31.18, H 3.82, N 27.25.

1-(2-Aminoethyl)-3-[2-({2-[(diaminomethylidene)amino]thiazol-4-yl}methylthio)ethyl]guanidino-2-carbonitrile (**16**). To powdered **13** (6.00 g, 16.0 mmol), *ethane-1,2-diamine* (**14**) (4.8 ml, 71.7 mmol) was added keeping the temp. at 20°. The resulting viscous mixture was stirred for 3 h at r.t., excess **14** was partially removed under high vacuum, and the oily residue was purified by FC (silica gel; MeOH/CH_2Cl_2 1:1 (1000 ml) + 32% aq. NH_4OH (13 ml)) to afford **16** (4.78 g, 88%). Highly hygroscopic solid foam. ^{1}H-NMR ((D$_6$)DMSO): 6.88 (*m*, 4 H); 6.50 (*s*, 1 H); 3.62 (*s*, 2 H); 3.31 (*t*, *J* = 7.1, 2 H); 3.09 (*t*, *J* = 5.8, 2 H); 2.56–2.67 (*m*, 4 H). ^{13}C-NMR ((D$_6$)DMSO): 175.59; 159.82; 157.10; 147.80; 118.29; 104.58; 44.57; 41.07; 40.67; 31.39; 30.46. The product was not further characterized, but converted to **19**.

2-Acetamido-N-*{2-cyano-3-[3-[3-(piperidinomethyl)phenoxy]propyl}guanidino)-3-mercapto-3-methylbutanamide* (**18**). To a soln. of **15** (1.58 g, 4.41 mmol) in MeOH (30 ml) N-*(2,2-dimethyl-4-oxothietan-3-yl)acetamide* (**17**) (0.76 g, 4.41 mmol) was added, and the mixture was stirred for 11 h at r.t. The solvent was evaporated under reduced pressure, and the crude product was purified by FC (silica gel; MeOH/CH_2Cl_2 1:5) affording **18** (1.90 g, 81%). White solid. The product was characterized as the oxalate. M.p. 79° (MeOH/Et_2O). ^{1}H-NMR ((D_6)DMSO): 8.34 (*s*, 1 H); 8.05 (*d*, *J* = 9.3, 1 H); 7.34 (*s*, 1 H); 6.97–7.19 (*m*, 4 H); 4.45 (*d*, *J* = 9.3, 1 H); 4.07 (*s*, 2 H); 4.01 (*m*, 2 H); 3.21–3.37 (*m*, 6 H); 2.94 (*m*, 4 H); 2.79 (*s*, 1 H); 1.93 (*m*, 3 H); 1.70 (*m*, 4 H); 1.51 (*m*, 2 H); 1.36 (*s*, 3 H); 1.33 (*s*, 3 H). ^{13}C-NMR ((D_6)DMSO): 169.75; 169.45; 165.07; 159.45; 158.68; 132.90; 129.79; 122.98; 118.06; 116.67; 115.12; 65.34; 60.99; 59.65; 52.11; 45.99; 40.70; 38.50; 38.20; 30.01; 29.22; 28.61; 22.85; 22.57; 21.95. Anal. calc. for $C_{26}H_{41}N_7O_3S \cdot C_2H_2O_4 \cdot 0.2\ Et_2O$ (636.5): C 54.34, H 7.13, N 15.40; found: C 54.34, H 7.49, N 15.06.

2-Acetamido-N-*{2-cyano-3-[2-({2-[(diaminomethylidene)amino]thiazol-4-yl}methylthio)ethyl]guanidino}-3-mercapto-3-methylbutanamide* (**19**). To a soln. of **16** (2.00 g, 5.86 mmol) in MeOH (12 ml), **8** (1.02 g, 5.86 mmol) was added, and the mixture was stirred for 2 h at r.t. Evaporation of the solvent under reduced pressure and FC (silica gel; MeOH/CH_2Cl_2 3:20 (300 ml) and MeOH/CH_2Cl_2 3:10 (400 ml)) afforded **19** (2.72 g, 90%). Pale-yellow solid. The product was characterized as the oxalate (MeOH/Et_2O). ^{1}H-NMR ((D_6)DMSO): 8.28 (*s*, 1 H); 8.00 (*d*, *J* = 9.3, 1 H); 7.90 (*m*, 4 H); 6.99 (*s*, 1 H); 6.85 (*s*, 1 H); 4.49 (*d*, *J* = 9.3, 1 H); 3.71 (*s*, 2 H); 3.11–3.45 (*m*, 4 H); 2.75 (*s*, 1 H); 2.58 (*m*, 2 H); 1.92 (*s*, 3 H); 1.36–1.38 (*s*, 6 H). ^{13}C-NMR ((D_6)DMSO): 169.78; 169.52; 167.32; 165.67; 159.33; 155.77; 148.15; 117.96; 107.49; 60.94; 46.05; 40.23; 38.15; 30.74; 30.16; 30.04; 29.18; 22.58. Anal. calc. for $C_{18}H_{30}N_{10}O_2S_3 \cdot 0.5\ C_2H_2O_4 \cdot H_2O \cdot 0.3\ Et_2O$ (600.0): C 40.44, H 6.05, N 23.35; found: C 40.75, H 5.89, N 23.16.

2-Acetamido-N-*{2-cyano-3-[3-[3-(piperidinomethyl)phenoxy]propyl}guanidino)-3-methyl-3-(nitrosothio)butanamide* (**20**). To a soln. of **18** (1.00 g, 1.88 mmol) in AcOH (50 ml) in a light-protected vessel, a soln. of *t*-BuONO (0.22 g, 2.16 mmol) in AcOH (5 ml) was added dropwise. After 30 min at r.t., the mixture was frozen and the solvent lyophilized to leave a green gum which was triturated at −10° with dry Et_2O (2 × 20 ml), decanted, and dried (high vacuum) to afford **20** (1.01 g, 86%). Pale-green solid. The product was characterized as the oxalate (MeOH/Et_2O). ^{1}H-NMR ((D_6)DMSO): 8.61 (*s*, 1 H); 8.47 (*d*, *J* = 9.6, 1 H); 7.39–7.00 (*m*, 5 H); 5.18 (*d*, *J* = 9.6, 1 H); 4.20 (*s*, 2 H); 4.02 (*m*, 2 H); 3.05 (*m*, 4 H); 3.20–3.47 (*m*, 6 H); 1.98 (*s*, 3 H); 1.93 (*s*, 3 H); 1.86 (*s*, 3 H); 1.73 (*m*, 4 H); 1.54 (*m*, 2 H). ^{13}C-NMR ((D_6)DMSO): 169.52; 168.79; 164.86; 132.08; 129.93; 123.24; 118.08; 116.97; 115.38; 65.44; 59.07; 58.91; 51.96; 40.35; 39.10; 28.39; 26.59; 24.90; 22.59; 22.43; 21.73. Anal. calc. for $C_{26}H_{40}N_8O_4S \cdot C_2H_2O_4$ (650.8): C 51.68, H 6.51, N 17.22; found: C 52.04, H 7.01, N 16.78.

2-Acetamido-N-*{2-cyano-3-[2-({2-[(diaminomethylidene)amino]thiazol-4-yl}methylthio)ethyl]guanidino}-3-methyl-3-(nitrosothio)butanamide* (**21**). To a soln. of **19** (1.00 g, 1.94 mmol) in AcOH (50 ml) in a light-protected vessel, *t*-BuONO (0.25 ml, 2.14 mmol) was added dropwise. After 30 min of stirring at r.t., the mixture was frozen, and the solvent was lyophilized to leave a green-red gum which was dissolved in 5% aq. AcOH soln. (15 ml) and extracted with AcOEt (3 × 10 ml). AcOH (15 ml) was added to the aq. phase, and the mixture was frozen and lyophilized again. The green-red residue was triturated at −10° with dry Et_2O (2 × 20 ml), decanted, and dried (high vacuum) to afford **21** (0.78 g, 67%). Cream-colored solid. ^{1}H-NMR ((D_6)DMSO): 8.60 (*m*, 1 H); 8.46 (*d*, *J* = 9.6, 1 H); 7.64 (*m*, 4 H); 7.20 (*m*, 1 H); 7.06 (*m*, 1 H); 6.82 (*s*, 1 H); 5.17 (*d*, *J* = 9.6, 1 H); 3.70 (*s*, 2 H); 3.20–3.31 (*m*, 6 H); 2.60 (*m*, 2 H); 1.85–1.98 (*s*, 12 H). ^{13}C-NMR ((D_6)DMSO): 172.21; 169.52; 168.72; 167.81; 159.28; 155.78; 148.13; 131.63; 117.93; 107.28; 59.63; 59.18; 40.75; 40.58; 38.33; 30.74; 30.18; 26.81; 24.87; 22.40; 21.16. Anal. calc. for $C_{18}H_{29}N_{11}O_3S_3 \cdot 2\ AcOH \cdot 3\ H_2O$ (717.8): C 36.81, H 6.04, N 21.48; found: C 36.38, H 5.54, N 21.63.

1-[2-({2-[(Diaminomethylidene)amino]thiazol-4-yl}methylthio)ethyl]-3-(hydroxyethyl)guanidine-2-carbonitrile (**25**). To a stirred suspension of **13** (3.00 g, 8.00 mmol) in MeOH (36 ml), 2-aminoethanol (**22**) (12.5 ml, 207 mmol) was added, and the mixture was stirred for 4 h at r.t. The solvent was evaporated under reduced pressure, and the crude product was purified by FC (silica gel; CH_2Cl_2 (300 ml) and MeOH/CH_2Cl_2 1:10 (500 ml)) to afford **25** (2.41 g, 88%). White solid. The product was characterized as the oxalate. M.p. 130–131° (dec.) (DSC) (H_2O/i-PrOH). ^{1}H-NMR ((D_6)DMSO): 8.39 (*m*, 4 H); 7.16 (*m*, 1 H); 7.01 (*s*, 1 H); 6.94 (*m*, 1 H); 3.76 (*s*, 2 H); 3.48 (*t*, *J* = 5.7, 2 H); 3.14–3.36 (*m*, 4 H); 2.60 (*m*, 2 H). ^{13}C-NMR ((D_6)DMSO): 165.59; 163.49; 159.62; 155.19; 148.39; 118.11; 108.86; 59.70; 44.00; 40.70; 30.50; 30.30. Anal. calc. for $C_{11}H_{18}N_8OS_2 \cdot C_2H_2O_4$ (432.5): C 36.10, H 4.66, N 25.91; found: C 35.92, H 4.66, N 25.90.

1-(3-Hydroxypropyl)-3-{3-[3-(piperidinomethyl)phenoxy]propyl}guanidine-2-carbonitrile (**26**). Finely powdered **12** (2.00 g, 5.09 mmol) was dissolved in *3-aminopropan-1-ol* (**23**; 12 ml, 157 mmol) and stirred for 1 h at r.t. The mixture was poured into H_2O (20 ml) and extracted with AcOEt (4 × 30 ml). The org. phase was dried ($MgSO_4$) and evaporated under reduced pressure yielding a brown oil, which was purified by FC (silica

gel; MeOH/CH_2Cl_2 1:10) to afford **26** (1.90 g, quant.) as pale-yellow oil. The product was characterized as the oxalate. M.p. 121–122° (dec.) (DSC) (MeOH/Et_2O). ^{1}H-NMR ((D$_6$)DMSO): 7.63 (*s*, 1 H); 7.00–7.39 (*m*, 6 H); 4.21 (*s*, 2 H); 4.02 (*m*, 2 H); 3.05–3.47 (*m*, 8 H); 1.93 (*m*, 2 H); 1.52–1.73 (*m*, 8 H). ^{13}C-NMR ((D$_6$)DMSO): 164.48; 159.58; 158.75; 131.52; 129.94; 123.36; 118.33; 116.99; 115.85; 65.44; 59.10; 58.46; 51.81; 38.73; 38.43; 32.02; 28.76; 22.38; 21.56. Anal. calc. for $C_{20}H_{31}N_5O_2 \cdot C_2H_2O_4 \cdot 0.5\ H_2O$ (472.5): C 56.39, H 7.31, N 14.94; found: C 55.92, H 7.25, N 14.82.

1-[2-({2-[(Diaminomethylidene)amino]thiazol-4-yl}methylthio)ethyl]-3-(hydroxypropyl)guanidine-2-carbonitrile (**27**). Finely powdered **13** (1.02 g, 2.98 mmol) was dissolved in **23** (1.9 ml, 24.9 mmol) and stirred vigorously for 1 h at r.t. FC (silica gel; MeOH/CH_2Cl_2 1:10) afforded **27** (0.95 g, 90%). Colorless oil. The product was characterized as the oxalate (MeOH/Et_2O). ^{1}H-NMR ((D$_6$)DMSO): 7.93 (*m*, 4 H); 7.02 (*m*, 2 H); 6.83 (*s*, 1 H); 3.70 (*s*, 2 H); 3.43 (*m*, 2 H); 3.32 (*m*, 2 H); 3.15 (*m*, 2 H); 2.58 (*m*, 2 H); 1.82 (*m*, 2 H). ^{13}C-NMR ((D$_6$)DMSO): 167.41; 165.96 159.38; 155.83; 148.20; 118.17; 107.41; 58.45; 40.86; 39.75; 31.91; 30.78; 30.33. Anal. calc. for $C_{12}H_{20}N_8OS_2 \cdot 0.5\ C_2H_2O_4 \cdot H_2O \cdot 0.3\ Et_2O$ (441.7): C 38.61, H 5.93, N 25.37; found: C 38.59, H 5.52, N 25.78.

1-[2-({2-[(Diaminomethylidene)amino]thiazol-4-yl}methylthio)ethyl]-3-{2-[2-oxido-3-(phenylsulfonyl)furazan-4-yloxy]ethyl}guanidine-2-carbonitrile (**30**). To a stirred soln. of **25** (1.00 g, 2.9 mmol) and *3,4-bis(phenylsulfonyl)furazan 2-oxide* (**28**; 1.28 g, 3.5 mmol) in DMF/THF 1:20 (25 ml), 50% (*w*/*w*) aq. NaOH soln. (0.46 g, 5.75 mmol) was added at r.t., keeping the temp. at 20–25°. The mixture was reacted for 40 min, the solvent was removed under reduced pressure, and the residue was treated with sat. NaCl soln. (30 ml) and extracted with AcOEt (6 × 40 ml). The org. layers were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by FC (silica gel; MeOH/AcOEt 1:10) yielding an orange oil, which was immediately converted to the oxalate by treatment with a soln. of oxalic acid in acetone. The collected solid was washed with boiling EtOH to afford **30** (1.05 g, 60%). Cream-colored solid. M.p. 173–174° (dec.) (DSC). ^{1}H-NMR ((D$_6$)DMSO): 8.22 (*m*, 4 H); 7.74–8.13 (*m*, 5 H); 7.28 (*m*, 2 H); 6.98 (*s*, 1 H); 4.47 (*m*, 2 H); 3.75 (*s*, 2 H); 3.58 (*m*, 2 H); 3.33 (*m*, 2 H); 2.82 (*m*, 2 H). ^{13}C-NMR ((D$_6$)DMSO): 167.66; 165.52; 159.38; 158.95; 155.77; 148.10; 138.61; 136.28; 117.84; 110.75; 107.40; 69.48; 40.88; 39.80; 30.83; 30.19. Anal. calc. for $C_{19}H_{22}N_{10}O_5S_3 \cdot C_2H_2O_4$ (656.7): C 38.41, H 3.68, N 21.31; found: C 38.70, H 3.83, N 21.33.

1-[2-({2-[(Diaminomethylidene)amino]thiazol-4-yl}methylthio)ethyl]-3-{2-[3-(phenylsulfonyl)furazan-4-yloxy]ethyl}guanidine-2-carbonitrile (**32**). NaH (80% in mineral oil; 0.10 g, 3.36 mmol) was washed with abs. Et_2O (2 × 5 ml), decanted, and the solid was dissolved in dry DMF (2 ml). A soln. of **25** (1.00 g, 2.92 mmol) in dry DMF (4 ml) was slowly added to the NaH soln. and, after evolution of gas ceased, *3,4-bis(phenylsulfonyl)furazan* (**31**) (1.07 g, 3.07 mmol) was added. The mixture was stirred for 3 h at r.t., then poured into crushed ice and filtered. The deliquescent yellow solid was purified by FC (silica gel; MeOH/CH_2Cl_2 1:10) to afford **32** (1.01 g, 62%). White foam. The product was characterized as the oxalate. M.p. 128° (dec.) (DSC) (MeCN). ^{1}H-NMR ((D$_6$)DMSO): 8.05–7.52 (*m*, 9 H); 7.50 (*m*, 2 H); 6.83 (*s*, 1 H); 4.50 (*m*, 2 H); 3.71 (*s*, 2 H); 3.60 (*m*, 2 H); 3.34 (*m*, 2 H); 2.63 (*m*, 2 H). ^{13}C-NMR ((D$_6$)DMSO): 164.83; 164.52; 161.07; 159.34; 155.21; 148.86; 148.29; 137.00; 136.26; 117.74; 108.55; 71.99; 40.82; 39.91; 30.60; 30.18. Anal. calc. for $C_{19}H_{22}N_{10}O_4S_3 \cdot C_2H_2O_4$ (640.7): C 39.37, H 3.78, N 21.86; found: C 39.43, H 3.81, N 21.59.

Quantitative Nitrite Detection. A soln. of the appropriate compound (20 µl) in DMSO was added to 2 ml of 50 mM phosphate buffer (pH 7.4), or to 2 ml of 50 mM phosphate buffer containing 5 mM L-cysteine, respectively. The final concentration of the drug was 10^{-4} M. After 1 h at 37°, 1 ml of this mixture was treated with 250 µl of *Griess* reagent (4 g sulfanilamide, 0.2 g *N*-naphthylethylenediamine bis(hydrochloride), 10 ml 85% phosphoric acid in distilled H_2O (final volume: 100 ml)). After 10 min at r.t., absorbance was measured at 540 nm; 10–80 nmol/ml $NaNO_2$ standard solns. were used for the calibration curve. The nitrite yield was expressed in % NO_2^- (mol/mol) ± standard deviation.

Kinetics of NO Release. The rate of NO release was determined by a spectrophotometric technique based on the oxidation of oxyhemoglobin (HbO_2) to methemoglobin (MetHb). The formation of MetHb was followed by recording the increase of absorbance (ΔA) at 401 nm on a *Perkin-Elmer-λ5* spectrophotometer in a thermostated (37°C) cuvette. The reaction was started by adding the drugs, dissolved in DMSO, to a 4 µM HbO_2 soln. in 50 mM phosphate buffer (pH 7.4), containing L-cysteine (5-fold molar excess) and, if necessary, in its absence (final drug concentration 10^{-5} M). HbO_2 was prepared according to the method previously described [30]. The increase of absorbance (ΔA) was recorded over the first 3 min. The initial rates were calculated from the slope of the straight-line portion of each curve. Every NO-releasing rate is the average of at least three determinations. The molar extinction coefficient $\Delta\varepsilon = \varepsilon_{401}(\text{MetHb}) - \varepsilon_{401}(HbO_2) = 39.9\ (\pm 1.4)\ \text{mM}^{-1}\ \text{cm}^{-1}$ was used [30].

Guinea-Pig Papillary Muscle. The procedure previously described was followed [31]. After removing the heart, left papillary muscles were set up at 37° in a 10-ml organ bath filled with oxygenated (95% O_2 and 5% CO_2) *Ringer* soln. of the following composition (mM): NaCl 153.98; $NaHCO_3$ 5.95; KCl 5.63; $CaCl_2 \cdot 2\,H_2O$ 2.16; glucose 5.55. Two Pt electrodes, inserted into the ventricular basis of the papillary muscle, were used to drive the tissue electrically. Square-wave pulses of 2 Hz frequency, 1 ms duration and 20% above-threshold voltage were applied. Changes in force of contraction were recorded by an isometric transducer connected to a plotter. Before new compounds were applied, the tissue was allowed to equilibrate for *ca.* 60 min under a load of 1 g. Then, cumulative concentration-response curves (CRC) to histamine were constructed by increasing the doses by 0.5 log units after each response had reached a plateau (3–4 min). For antagonist studies, the first CRC to histamine was followed by incubation of antagonist for 20 min before a second CRC was obtained. For antagonists which produced surmountable antagonism, linear regression analysis was applied, and pA_2 values (95% confidence limits) and the slope of the *Schild* plot were obtained [32]. For this purpose, at least three antagonist concentrations were used. For compounds which caused surmountable antagonism only at one or two concentrations, pA_2 values were estimated by *Gaddum*'s equation: $pA_2 = -\log [B] + \log [CR - 1]$, where [B] represents the concentration of the antagonist and CR is the concentration ratio at the EC_{50} level. pA_2 Values were reported as geometric means with 95% confidence limits.

Lumen-Perfused Stomachs of Anesthetized Rats. The technique developed by *Bertaccini et al.* [33] was followed. Male rats (*Wistar*, 200–250 g) were used after 18 h fasting. After urethane anesthesia (1.25 g/kg, *i.p.* administration), the stomach was perfused at constant volume (60 ml/h) with saline (0.9% NaCl) at 37° through an esophageal cannula. The perfusion fluid was collected *via* a duodenal cannula. The acid in the perfusate was estimated every 10 min by titration to pH 7 with 10 mM NaOH with an automatic titrator system (*Radiometer*, *Copenhagen*). Gastric-acid secretion was stimulated by a continuous infusion (6 ml/h) of histamine (20 μmol kg^{-1} h^{-1}). Inhibitory compounds were administered in separate experiments by bolus injection at the plateau of acid secretion induced by histamine. Acid-secretory responses were expressed as mean values ± SEM in μequiv. HCl kg^{-1} min^{-1}. The inhibitory effects were expressed as % inhibition in comparison with plateau levels of acid output considered as 100%. The ID_{50} values were calculated from inhibitory dose-response curves and reported as geometric means with 95% confidence limits.

Gastroprotection. Male *Wistar* rats (180–200 g) were used after 24 h fasting. The new compounds or saline were administered intragastrically in a 10-ml/kg volume; 30 min later, 0.6N HCl (1 ml/rat), was given intragastrically. The animals were killed by cervical dislocation 30 min after HCl administration. Stomachs were opened along the lesser curvature and examined under a stereomicroscope. Each individual hemorrhagic lesion was measured along its greatest length (<1 mm: rating of 1; 1–2 mm: rating of 2; >2 mm: rating according to length in mm). The overall sum was designed as 'lesion index'. The results were expressed as % reduction of lesion index compared to controls.

Statistics. Statistical significance was determined by the *Student*'s t-test for unpaired data, or ANOVA followed by the *Newman-Keuls* test.

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