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[3-(1*H*-Imidazol-4-yl)propyl]guanidines Containing Furoxan Moieties: A New Class of H_3-Antagonists Endowed with NO-Donor Properties

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Abstract—Synthesis and pharmacological characterisation of a series of products obtained by coupling the H_3-antagonist SKF 91486 through appropriate spacers with the NO-donor 3-phenylfuroxan-4-yloxy and 3-benzenesulfonylfuroxan-4-yloxy moieties, as well as with the corresponding furazan substructures, devoid of NO-donating properties, are reported. All the products were tested for their H_3-antagonistic and H_2-agonistic properties on electrically-stimulated guinea-pig ileum segments and guinea-pig papillary muscle, respectively. The whole series of compounds displayed good H_3-antagonist behaviour and feeble partial H_2-agonist activity. Among furoxan derivatives, the benzenesulfonyl hybrid **28**, a good NO-donor, triggered a dual NO-dependent muscle relaxation and H_3-antagonistic effect on guinea-pig intestine.

Introduction

The synthesis of molecular hybrids is a strategy to design novel products endowed with multiple pharmacological properties. In recent years, several classes of hybrid compounds, obtained combining appropriate pharmacophoric groups with NO-releasing functions, have been described.[1] A number of them, such as NO-aspirin,[2] NO-steroids[3] and NO-ursodeoxycholic acid[4] are now under clinical investigations. There is strong evidence that the furoxan system (1,2,5-oxadiazole 2-oxide) is able to release NO under the action of thiol cofactors.[5] By introducing appropriate substituents at the ring, it is possible to modulate the rate and amount of NO-production.[6] The overall reaction mechanism is complex, and different NO-redox forms could be involved. In our laboratories furoxan moieties have been used to create different classes of hybrids, such as ibuprofen-like anti-inflammatory drugs[7] or lamtidine-like H_2 receptor antagonists,[8] obtaining prototypical compounds which combine anti-inflammatory or gastric antisecretory activities with NO-dependent gastroprotective effects. As further development in the field of hybrid design we now propose a series of NO–H_3-antagonists. Histamine, through H_3 receptors,[9–11] and NO[12] display a variety of effects in different body compartments including central and peripheral nervous system. While the role of H_3 receptors in the periphery has still to be established, their role in different brain areas, such as the hippocampus and the hypothalamus, has been demonstrated. Accordingly, a number of potential therapeutic applications for H_3 receptor antagonists in several CNS pathologies, including memory and learning disorders, have been suggested.[10] Since NO too is implicated in learning and memory formation,[13] the simultaneous block of histamine H_3 receptors and NO release in specific areas of the brain could synergistically contribute to a curative effect in such pathological

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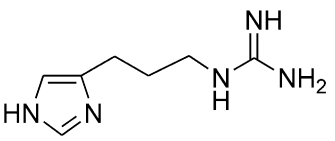
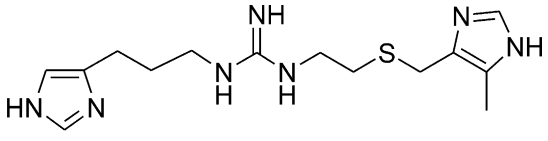
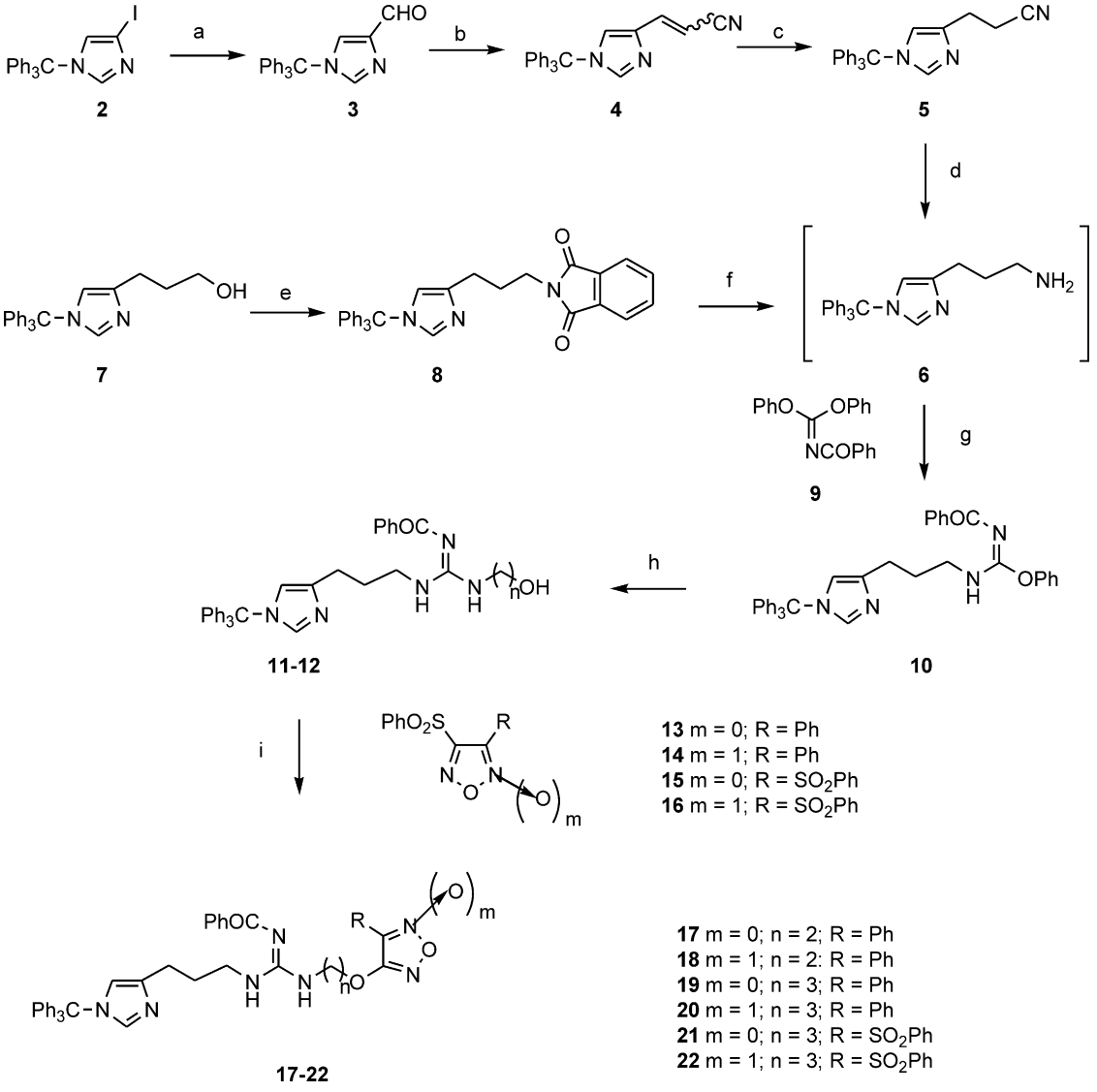


1 SKF 91486



Impromidine

conditions. In this paper we discuss synthesis and preliminary 'in vitro' pharmacological characterisation of novel hybrids obtained by coupling, through appropriate spacers, furoxan groups (3-phenylfuroxan-4-yloxy and 3-benzenesulfonylfuroxan-4-yloxy) to the compound SKF 91486. Such compound is the [3-(1*H*-imidazol-4-yl)propyl]guanidino portion of the histamine H_2-agonist/H_3-antagonist impromidine, endowed with feeble activity at the histamine H_2 receptor.[14] The use of SKF 91486 as a pharmacophore could allow the generation of novel H_3 receptor ligands able to release NO (der.s **24**, **26**, **28**). The corresponding furazan models (der.s **23**, **25**, **27**), devoid of NO-donating properties, were also synthesised and tested as control compounds. The combined NO-donating properties and the activity at histamine H_3 receptors of all the synthesised compounds were evaluated 'in vitro' using the isolated, electrically-driven, guinea-pig ileum, while the activity



Scheme 1. (a) EtMgBr, DMF, rt; (b) $NaNH_2$, $(EtO)_2P(O)CH_2CN$, THF, reflux; (c) H_2, Pd/C, THF; (d) $LiAlH_4/AlCl_3$, Et_2O/THF/(2:1), rt; (e) PPh_3, Phtalimide, DIAD, THF, 0 °C; (f) 40% aq CH_3NH_2, EtOH, rt; (g) **9**, CH_2Cl_2; (h) 2-aminoethanol or 3-aminopropan-1-ol, THF, reflux; (i) 50% aq NaOH, 20–25 °C.

at histamine H_2 receptors was evaluated on the isolated guinea-pig papillary muscle.

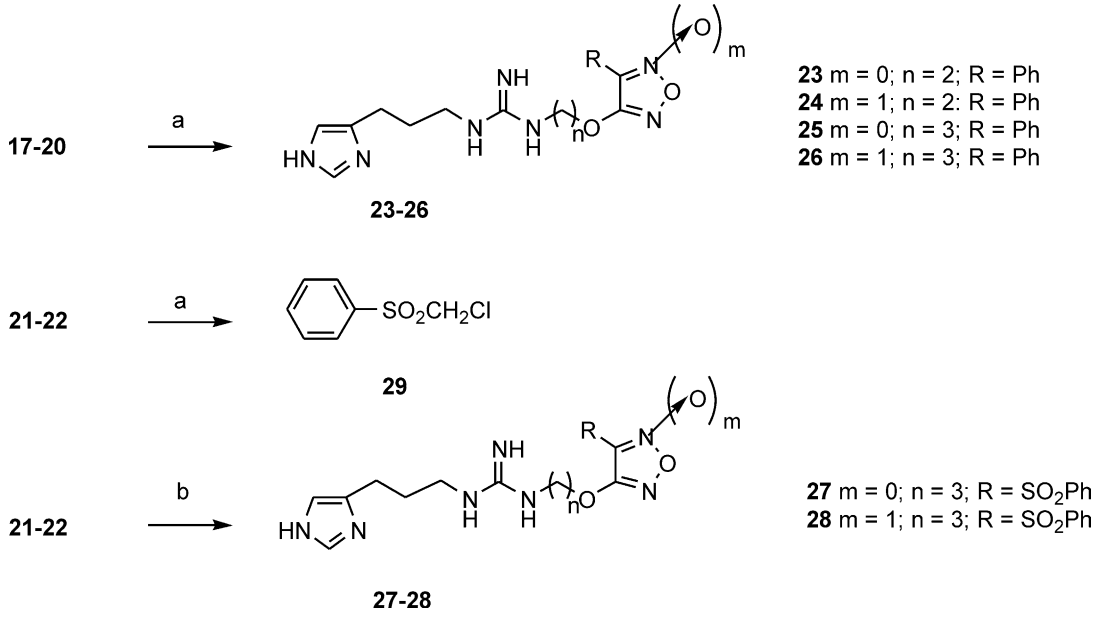
Results

Chemistry

According to our strategy, synthesis of the final products **23**–**28** requires the preparation of the common intermediate *N*-benzoyl-*O*-phenylisourea **10** (Scheme 1). This compound can be easily obtained by nucleophilic displacement at room temperature in CH_2Cl_2 solution of one of the two phenoxy groups present in *N*-benzoyldiphenylimidocarbonate (**9**), under the action of 3-(1-tritylimidazol-4-yl)propylamine (**6**). Preparation of **6** is achieved by the two different approaches depicted in Scheme 1. The first implies synthesis of the phtalimido derivative **8** by reaction of phtalimide, triphenylphosphine, diisopropylazodicarboxylate (DIAD) and **7** in THF at 0 °C. Product **8** dissolved in ethanol is easily converted at room temperature into **6** by aqueous 40% methylamine. The second route starts from trityl-protected iodoimidazole **2**. Transformation of this product into the aldehyde **3** was previously achieved by action of *n*-butylithium at −78 °C in dry THF followed by rapid quenching of the reaction mixture with DMF.[15] We modified this procedure using ethylmagnesium bromide in CH_2Cl_2 at room temperature. The aldehyde **3** provides α,β-unsaturated nitrile **4** by means of a modified Wittig approach using cyanomethyl diethylphosphonate, according to the procedure described in literature.[16] Also subsequent reduction of **4** over 10% Pd/C to give **5** was obtained according to literature.[16] Finally, action on **5** of a mixture of $LiAlH_4/AlCl_3$ in 2:1 Et_2O/THF solution at room temperature affords in good yields the expected amino derivative **6**. This product was not isolated and purified but used directly for further transformation. Nucleophilic substitution of the phenoxy group in **10** by the appropriate aminoalcohol in THF at reflux gave the intermediate alcohols **11**, **12**. It is known that bis(benzenesulfonyl)furoxan (**16**) when treated with ethanol under basic conditions in distilled THF at room temperature, selectively affords 3-benzenesulfonyl-4-ethoxyfuroxan.[17] Under the same conditions, also the nucleophilic displacement of 4-benzenesulfonyl group from the 3-phenylfuroxan **14** occurs. Related furazans **13**, **15** behave similarly. Therefore, to prepare the protected derivatives **17**–**22** we allowed the appropriate furoxan and furazan derivative to react with alcohols **11**, **12** in the above reported conditions. ^{13}C and ^{1}H NMR spectra of the products **21**, **22** indicate that, in this case too, selective displacement of 4-benzenesulfonyl group occurs. Subsequent deprotection of **17**–**20** in boiling 5 N HCl gives the final products **23**–**26** (Scheme 2). Surprisingly, this procedure does not work with benzenesulfonyl derivatives **21**, **22**. In fact, extensive decomposition occurs with formation of a mixture of products among which we isolated, in the case of **22**, benzenesulfonylmethyl chloride (**29**) in quantitative yield. The mechanism of this transformation remains to be cleared. Final derivatives **27**, **28** were obtained in good yield by treating derivatives **21**, **22** with 3 M *p*-TsOH at 95 °C for 72 h.

Detection of nitrite

To evaluate the thiol-induced NO-generation the appropriate furoxan derivative was dissolved in pH 7.4 buffered water and incubated for 1 h at 37 °C in the presence of a large excess of L-cysteine (1:50). Nitrite, which is an important oxidation product of NO in aerobic solution, was determined by the Griess reaction



Scheme 2. (a) 5 N HCl, reflux; (b) 3 M *p*-TsOH, reflux.

Table 1. NO-release characteristics, H_3 receptor antagonism and H_2-agonistic properties of synthesised compounds **23**–**28**, reference **1**, and histamine

Compd	NO_2^- (%)[a] (+L-cys)	H_3-antagonism[b] Guinea-pig ileum	H_2-agonistic activity[c] Guinea-pig papillary muscle	
		$pA_2 \pm SEM$	($pD_2 \pm SEM$)	(i.a.)[d]
1	—	7.24±0.07	4.49±0.07 (0.52)	0.52
23	—	8.19±0.07	4.34±0.04 (0.41)	0.41
24	3.8±0.1	7.82±0.09	4.54±0.07 (0.61)	0.61
25	—	8.49±0.03	5.70±0.04 (0.40)	0.40
26	4.0±0.06	8.25±0.07	5.90±0.06 (0.59)	0.59
27	—	7.42±0.03	5.88±0.07 (0.53)	0.53
28	2.67±3.4	7.02±0.03 7.05±0.05[e]	5.28±0.012 (0.63)	0.63
Histamine	—	—	6.15±0.02 (1.00)	1.00[d]

— not determined.
[a]Yields are reported in% [mol/mol±standard error of the mean (SEM)].
[b]pA_2 was calculated only for one surmountable concentration (1 μM) of the antagonist using the Gaddum's equation $pA_2 = -\log [B] + \log [CR-1]$; data are the mean±SEM of 5–11 observations.
[c]$pD_2 = -\log EC_{50}$; data are the mean±SEM of 4–6 observations.
[d]i.a., intrinsic activity; histamine = 1.
[e]Determined in the presence of ODQ 1 μM.

according to a previously reported procedure.[6] The results expressed as a percentage of NO_2^- (mol/mol) are reported in Table 1. It is worthy of note that nitrite production by furoxan is strongly dependent on the medium, the concentration and the nature of the thiol employed; therefore, it only gives a trend of the NO production which might occur in the cellular environment; it does not give information about the NO-redox form(s) involved in the release.

Pharmacology

The H_3-antagonistic activity of the products was assessed by their ability at 1 μM concn to antagonise the concentration-dependent inhibitory effect of (*R*)-α-methylhistamine (MHA) on electrically evoked twitches of isolated guinea-pig ileum segments.[18] pA_2 values were calculated using the Gaddum equation ($pA_2 = -\log [B] + \log [CR-1]$) and are reported in Table 1. The H_2-agonistic activity of the products was investigated by their ability to induce changes in the contraction force of electrically stimulated guinea-pig papillary muscle according to the reported method.[19] Potencies were expressed as pD_2 values ($pD_2 = -\log EC_{50}$) and reported in Table 1.

Discussion

All the products display quite good antagonistic activity at H_3 receptor. In fact they are able to inhibit, at 1 μM concentration, the concentration-dependent inhibitory effect of (*R*)-α-methylhistamine (MHA) on electrically evoked contractile response (acetylcholine release) of guinea-pig ileum preparations. Analysis of pA_2 values of the furazan derivatives **23**, **25**, **27** shows that they display more potent or similar antagonistic activity with

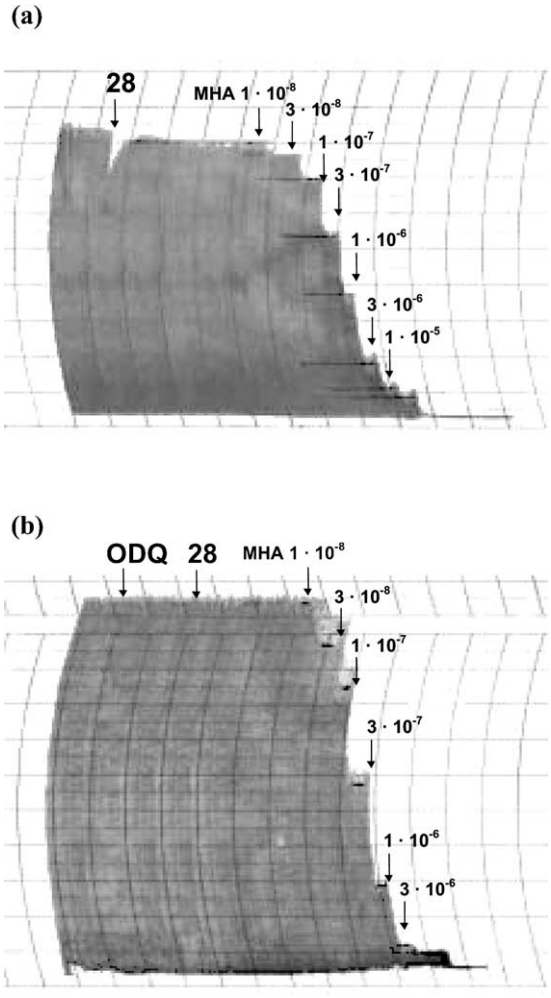


Figure 1. NO-dependent effect of compound **28** on electrically stimulated guinea-pig ileum (panel a); experiment repeated in the presence of ODQ (panel b); MHA reported as M concentration.

respect to lead **1** (SKF 91486). Furoxan derivatives **24** and **26**, which are feeble NO-donors, behave in a strictly similar manner as the furazan analogues **23** and **25**, unable to release NO. Different behaviour is presented by the furoxan **28**, a potent NO-donor. In fact, when the product was added to the bath at 1 μM concentration after the electrical contraction of the tissue, a partial relaxation (~20%) of the preparation was observed (Fig. 1a). This effect was transient and disappeared after 5 min.

The phenomenon was absent when the experiment was repeated in the presence of 1 μM ODQ (1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one), a selective inhibitor of soluble guanilate cyclase (sGC) (Fig. 1b), thus suggesting the involvement of NO as the final mediator of this effect. Accordingly, relaxing effect on guinea-pig ileum induced by NO-donors was earlier observed.[20] Morover other furoxan derivatives tested in our laboratory display similar behaviour, as observed in preliminary experiments.[21] The calculated pA_2 value for derivative **28** was 7.02 (±0.03) close to those of the parent furazan **27** and of the lead **1**.

Since lead **1** shows weak agonistic properties at the H_2 receptor, we further characterised compounds **23**–**28** for their ability to interact with the H_2 receptor.

The data reported in Table 1 show that all the synthesised compounds induce a concentration-dependent increase in the contractile force when tested on electrically stimulated guinea-pig papillary muscle. Unlike histamine they are unable to produce the maximal response evoked by H_2 receptor stimulation on this tissue. The positive inotropic effect of the compounds **23**–**28**, was antagonised by the presence of 1 μM Famotidine, a well-known H_2-antagonist. The pD_2 values of compounds **23**–**28** suggest that the activity at H_2 receptors occurs at concentrations 100–500 times higher than those required to block H_3 receptors. Therefore all the synthesised products behave, similarly to the lead **1**, as weak partial agonists at H_2 receptor, their efficacy lying between 40 and 63% of that of histamine. Development of the present work will address structural modulation of this new class of hybrids in order to obtain products endowed with suitable brain penetrating properties,[22] and to evaluate their ability to interfere with different H_3 receptor subtypes which occur in central neurons.[11]

Conclusion

The results show that conjugation of the substituted 1,2,5-oxadiazole moiety or of its 2-oxide analogue with the [3-(1*H*-imidazol-4-yl)propyl]guanidino pharmacophore is not detrimental to the H_3-antagonistic activity, most of the derivatives being more active than lead compound **1**. The response evoked by our compounds at the H_3 receptor is remarkably higher than the response evoked at the H_2 receptor. Compound **28** displays a dual NO-dependent muscle relaxation and H_3-antagonistic effect. The described class of NO-donor-H_3 antagonists could be an interesting starting point for the design of further hybrids.

Experimental

Melting points were determined with a capillary apparatus (Büchi 530) and are uncorrected or by differential scanning calorimetry (Perkin-Elmer DSC 7, heating rate 10 °C/min). All the compounds were routinely checked by IR (Shimadzu FT-IR 8101 M). ^1H and ^{13}C NMR spectra were obtained on a Bruker AC-200 at 200 and 50 MHz resp. and on a Bruker Avance 300, at 300 and 75 MHz resp.; δ in ppm rel. to $SiMe_4$ as internal standard; coupling constants *J* in Hz; ^{13}C NMR spectra were fully decoupled. Mass spectra were recorded on Finnigan-Mat TSQ-700. Flash chromatography (FC): silica gel (Merck Kieselgel 60) 70–230 mesh ASTM. Anhydrous magnesium sulfate ($MgSO_4$) was used as the drying agent of organic phases unless otherwise stated. Analysis (C, H, N) of the new compounds was performed by REDOX (Monza). Structures **2**,[15] **4**, **5**,[16] **7**,[23] **9**,[24] **13**,[25] **14**,[26] **15**,[17] **16**[27] were synthesised according to reported methods. Following abbreviations are used: DMF, *N,N*-dimethylformamide; Et_2O, diethyl ether; THF, tetrahydrofuran; DIAD, diisopropylazodicarboxylate; PE, petroleum ether 40–60 °C; AcOEt, ethyl acetate.

[(1-Triphenylmethyl)imidazol-4-yl]carbaldehyde (3). To a stirred solution of **2** (1.50 g, 3.44 mmol) in 14 mL of dry CH_2Cl_2 a solution of ethylmagnesium bromide 3 M in Et_2O (1.27 mL) was added at room temperature and the reaction mixture was stirred for 30 min. After this time, dry DMF (0.29 mL, 3.78 mmol) was added and the mixture stirred at room temperature for another 30 min. The mixture was treated with half-saturated NH_4Cl solution and the organic layer washed with water (3×20 mL), dried and evaporated under reduced pressure to give a yellow solid which was purified by FC (silica gel; PE/AcOEt 6:4) to afford **3** (0.96 g, 82%) as white solid. MS (EI): *m/z* (M^+): 338; mp 199–201 °C (*i*PrOH). ^1H NMR ($CDCl_3$) δ 9.89 (s, 1H); 7.62 (s, 1H); 7.54 (s, 1H); 7.41–7.31 (m, 9H); 7.16–7.08 (m, 6H). ^{13}C NMR ($CDCl_3$): 186.43; 141.38; 140.71; 140.47; 129.50; 128.24; 127.88; 116.88; 76.75. Analysis for $C_{23}H_{18}N_2O$ (338.4), calcd C, 81.63; H, 5.36; N, 8.28; found: C, 81.36; H, 5.34; N, 8.25.

***N*-{3-[(1-Triphenylmethyl)imidazol-4-yl]propyl}phtalimide (8).** To a stirred solution of **6** (4.00 g, 10.9 mmol) in dry THF (100 mL) at 0 °C phtalimide (2.39 g, 16.3 mmol) and triphenylphosphine (4.27 g, 16.3 mmol) were added. The mixture was kept at 0 °C and a solution of DIAD (4.71 g, 23.3 mmol) in dry THF (120 mL) was added dropwise during 1.5 h. The reaction mixture was concentrated under reduced pressure to precipitate a solid which was filtered and washed with THF (2×20 mL) to yield **8** (4.97 g, 92%) as a white solid. CIMS (isobutane) *m/z* 498 (MH^+); mp 217–219 °C (*i*PrOH); ^1H NMR ($CDCl_3$) δ 7.80–7.69 (m, 4H); 7.32–7.26 (m, 10H); 7.17 (s, 1H); 7.15–7.05 (m, 6H); 6.58 (s, 1H); 3.73 (t, *J* = 7.2, 2H); 2.61 (t, *J* = 7.2, 2H); 1.90 (m, 2H). ^{13}C NMR

($CDCl_3$): 168.35; 142.57; 140.59; 138.35; 133.78; 132.19; 129.8; 127.98; 127.92; 123.12; 118; 75.07; 37.65; 28.28; 25.96. Analysis for $C_{33}H_{27}N_3O_2$ (497.57), calcd C, 79.66; H, 5.47; N, 8.44; found: C, 79.30; H, 5.49; N, 8.32.

3-[(1-Triphenylmethyl)imidazol-4-yl]propylamine (6) and 1-benzoyl-2-{3-[(1-triphenylmethyl)imidazol-4-yl]propyl}-*O*-phenylisourea (10). Method A. To a stirred suspension of **8** (4.00 g, 8.04 mmol) in EtOH (100 mL), methylamine 40% aq solution (10 mL, 120 mmol) was added. After 3 h of stirring at room temperature, the mixture was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (30 mL), and **9** (2.55 g, 8.04 mmol) was added. After 30 min at room temperature, the solvent was evaporated and the crude product purified by FC (silica gel; CH_2Cl_2/AcOEt 8.5:1.5) to obtain **10** as white foam (2.94 g, 62%). The product showed identical data when compared with a sample synthesised according to method B. **Method B**. To a stirred suspension of $LiAlH_4$ (0.25 g, 6.60 mmol) in dry Et_2O (12.5 mL) a solution of $AlCl_3$ (0.88 g, 6.60 mmol) in dry Et_2O (12.5 mL) was added and, after 10 min of stirring at room temperature, **5** (2.00 g, 5.50 mmol) in dry THF (12 mL) was added carefully. Reduction was completed in 1 h, the reaction mixture was treated with H_2O (1.5 mL), NaOH 10% (2.5 mL) and again H_2O (2.5 mL). The precipitate was filtered and washed thoroughly with THF (200 mL) and CH_2Cl_2 (300 mL). The organic phase was dried (K_2CO_3) and evaporated under reduced pressure to afford **6** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.35–7.28 (m, 10H); 7.18–7.10 (m, 6H); 6.6 (s, 1H); 2.71 (t, $J=7.0$, 2H); 2.58 (t, $J=7.5$, 2H); 2.02 (br s, 2H); 1.79 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 142.36; 141.16; 138.12; 129.58; 127.79 (two overlapping carbons); 117.59; 74.87; 41.54; 33.01; 25.56. The product **6** was not further characterised but directly converted to **10** by addition of *N*-benzoyldiphenylimidocarbonate (**9**) (2.54 g, 7.94 mmol) to a solution of **6** (2.02 g, 5.49 mmol) in CH_2Cl_2 (50 mL). After 30 min of stirring at room temperature the solvent was evaporated under reduced pressure and the oily residue purified by FC (silica gel; CH_2Cl_2/AcOEt 9:1) to yield **10** (2.79 g, 86%) as white foam. Mp 144–145 °C (cold acetone/H_2O). ^{1}H NMR ($CDCl_3$) δ 10.2 (br s, 1H); 7.8 (m, 2H); 7.37–7.14 (m, 24H); 6.6 (s, 1H); 3.59 (br m, 2H); 2.71 (br m, 2H); 2.08 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 177.63; 162.61; 151.82; 142.51; 140.35; 138.59; 137.34; 131.55; 129.71; 129.23; 128.99; 127.98; 127.79; 127.79; 125.60; 121.68; 118.05; 75.08; 41.14; 29.34; 25.65. Analysis for $C_{39}H_{34}N_4O_2$ (590.69), calcd C, 79.30; H, 5.80; N, 9.48.

***N*-Benzoyl-*N′*-(2-hydroxyethyl)-*N″*-{3-[(1-triphenylmethyl)-imidazol-4-yl]propyl}guanidine (11).** To a stirred solution of **10** (1.41 g, 2.35 mmol) in dry THF (10 mL), 2-aminoethanol (1.50 mL, 24.7 mmol) was added and the mixture was refluxed for 40 min. After cooling the mixture was diluted with H_2O (10 mL), the solvent was removed under reduced pressure and the aqueous residue extracted with CH_2Cl_2 (5×20 mL). The organic layer was dried and the solvent evaporated under reduced pressure to give a solid which was purified by FC (silica gel; CH_2Cl_2/MeOH 9.5:0.5) yielding **11** (0.92 g, 70%) as white foam. Mp 164–165 °C (MeOH/H_2O). ^{1}H NMR ($CDCl_3$) δ 10.6 (br s, exchangeable signal not always observed); 8.18 (m, 2H); 7.43–7.34 (m, 13H); 7.18–7.11 (m, 6H); 6.61 (s, 1H); 3.80–3.67 (m, 4H); 3.41 (m, 2H); 2.62 (t, $J=6.2$, 2H); 1.95 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 178.81; 161.91; 142.10; 140.27; 138.61; 137.81; 130.68; 129.57; 128.58; 128 (two overlapping carbons); 127.75; 118.43; 72.75; 64.43; 44.19; 39.78; 29.30; 23.13. Analysis for $C_{35}H_{35}N_5O_2$ (557.7), calcd C, 75.38; H, 6.33; N, 12.56; found: C, 75.48; H, 6.45; N, 12.33.

***N*-Benzoyl-*N′*-(3-hydroxypropyl)-*N″*-{3-[(1-triphenylmethyl)imidazol-4-yl]propyl}guanidine (12).** The reaction was carried out as described above using **10** (1.41 g, 2.35 mmol) and 3-aminopropan-1-ol (1.89 mL, 24.7 mmol). (Yield: 61%). White foam; mp 123–124 °C (MeOH/H_2O). ^{1}H NMR (DMSO-d_6) recorded at 55 °C δ 10.25 (s, 1H, observed at 25 °C); 8.07 (m, 2H); 7.40–7.36 (m, 13H); 7.10 (m, 6H); 6.64 (s, 1H); 4.23 (s, 1H); 3.51 (m, 2H); 3.36 (m, 4H); 2.56–2.50 (m, 2H); 1.85 (m, 2H); 1.72 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 175.03; 160.34; 142.64; 140.71; 139.68; 137.91; 130.44; 129.37; 128.58; 128.26; 128.13; 127.7; 117.68; 74.71; 58.53; 38.03; 32.57; 30.71; 29; 25.33. Analysis for $C_{36}H_{37}N_5O_2{\cdot}0.3H_2O$ (577.13), calcd C, 74.92; H, 6.47; N, 12.14; found: C, 74.94; H, 6.51; N, 12.1.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N′*-{2-[(4-phenylfurazan-3-yl)oxy]ethyl}guanidine dihydrochloride (23).** To a stirred solution of **11** (1.38 g, 2.47 mmol) and **13** (0.85 g, 2.97 mmol) in distilled THF (30 mL), 50% (w/w) aq NaOH solution (0.8 g, 9.9 mmol) was added maintaining the temperature at 20–25 °C. The reaction mixture was stirred vigorously at room temperature for 5.5 h, then diluted with H_2O (20 mL) and the organic solvent was evaporated under reduced pressure (25 °C). The aqueous residue was extracted with AcOEt (3×30 mL), the org. phase dried and evaporated to afford a gummy solid which was purified by FC (silica gel; CH_2Cl_2/AcOEt 7:3) to give the protected derivative **17** (1.24 g, 72%). The intermediate was readily hydrolysed with 68 mL of 5 N HCl at reflux for 16 h. After cooling the mixture was filtered through a sinter, the aqueous phase extracted with Et_2O (3×30 mL) and then evaporated under reduced pressure (60 °C). The semisolid residue was treated with benzene (3×20 mL), evaporated and dried in a desiccator over P_2O_5/NaOH/paraffin under high vacuum to obtain **23** (0.66 g, 63% from **11**) as white amorphous solid. Mp 192–197 °C (MeOH/Et_2O). ^{1}H NMR (DMSO-d_6) δ 14.61 (br s, 2H); 9.03 (s, 1H); 8.27 (m, 2H); 8.01 (m, 2H); 7.83 (s, 2H); 7.65–7.56 (m, 3H); 7.44 (m, 10H); 4.54 (t, $J=4.5$, 2H); 3.8 (m, 2H); 3.26–3.17 (m, 2H); 2.71 (t, $J=7.4$, 2H); 1.83 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 163.34; 156.24; 145.27; 133.59; 132.44; 131.15; 129.42; 127.55; 124.22; 115.81; 71.20; 40.08; 39.97; 23.42; 21.04. Analysis for $C_{17}H_{21}N_7O_2{\cdot}2HCl{\cdot}0.5H_2O{\cdot}0.2\ C_4H_{10}O$ (452.16), calcd C, 47.28; H, 5.80; N, 21.68; found: C, 47.17; H, 5.76; N, 21.46.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N′*-{2-[(3-phenylfuroxan-4-yl)oxy]ethyl}guanidine dihydrochloride (24).** To a stirred solution containing **11** (1.00 g, 1.79 mmol) and **14** (0.76 g, 2.50 mmol) in distilled THF (30 mL), 50% (w/w) aq NaOH solution (0.56 g, 7.02 mmol) was added

maintaining the temperature at 20–25 °C. After 3 h of vigorous stirring at room temperature, the mixture was diluted with H_2O (20 mL) and the organic solvent was evaporated under reduced pressure (25 °C). The aqueous residue was extracted with CH_2Cl_2 (3×30 mL) and the extracts dried and evaporated under reduced pressure to afford a pale yellow oil. The crude product was purified by FC (silica gel; CH_2Cl_2/AcOEt 6:4) to obtain the protected intermediate **18** (1.22 g, 95%) as a white solid. Derivative **18** was immediately hydrolysed by refluxing in 5 N HCl (66 mL) for 16 h. The cooled mixture was filtered through a sinter, extracted with Et_2O (3×30 mL) and the aqueous layer evaporated under reduced pressure (60 °C). After drying under high vacuum over P_2O_5/NaOH/paraffin **24** (0.64 g, 78% from **11**) was obtained as white amorphous solid. Mp 162–169 °C (dec.) (dry MeOH/Et_2O). ^{1}H NMR (DMSO-d_6) δ 14.53 (br s, 2H); 9.04 (s, 1H); 8.33 (br m, 2H); 8.09 (m, 2H); 7.87 (br m, 2H); 7.64–7.53 (m, 3H); 7.44 (s, 1H); 4.57 (br s, 2H); 3.81 (m, 2H); 3.23 (m, 2H); 2.71 (t, J = 7, 2H); 1.83 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 162.25; 156.28; 133.54; 132.47; 130.75; 129.18; 126.46; 121.86; 115.84; 107.87; 69.22; 39.9 (two overlapping carbons); 27.50; 21.08. Analysis for $C_{17}H_{21}N_7O_3$·2HCl·0.6H_2O (455.1), calcd C, 44.86; H, 5.36; N, 21.54; found: C, 44.63; H, 5.64; N, 21.35.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N′*-{3-[(4-phenylfurazan-3-yl)oxy]propyl}guanidine dihydrochloride (25).** To a stirred solution of **12** (1.00 g, 1.75 mmol) and **13** (0.70 g, 2.45 mmol) in distilled THF (30 mL), 50% (w/w) aq NaOH solution (0.56 g, 7.02 mmol) was added at room temperature and the reaction stirred vigorously for 6 h. The mixture was diluted with H_2O (20 mL) and the organic solvent evaporated under reduced pressure (25 °C). The aqueous residue was extracted with CH_2Cl_2 (4×30 mL), the organic phase dried and evaporated under reduced pressure to give a pale yellow oil, which was purified by FC (silica gel; CH_2Cl_2/AcOEt 6:4) to obtain **19** (0.92 g, 74%) as a white solid. This intermediate was deprotected by refluxing with 5 N HCl (50 mL) for 16 h. The cooled reaction mixture was filtered, extracted with Et_2O (3×30 mL), CH_2Cl_2 (1×30 mL), and the aqueous layer was evaporated under reduced pressure (60°). The semisolid product was dried in a desiccator under high vacuum over P_2O_5/NaOH/paraffin to afford **25** (0.47 g, 61% from **12**) as a white amorphous solid. Mp 170–174 °C (dec.) (dry MeOH/Et_2O). ^{1}H NMR (DMSO-d_6) δ 14.7 (br s, 2H); 9.06 (s, 1H); 8.1 (m, 2H); 7.93 (m, 2H); 7.70 (m, 2H); 7.68 (m, 3H); 7.47 (s, 1H); 4.54 (t, J = 5.6, 2H); 3.42–3.18 (m, 4H); 2.72 (t, J = 7.2, 2H); 2.10 (m, 2H); 1.84 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 163.39; 156.08; 145.30; 133.55; 132.51; 131.18; 129.46; 127,44; 124.46; 115.67; 70.47; 39.95; 37.80; 28.13; 27.52; 21.10. Analysis for $C_{18}H_{23}N_7O_2$·2HCl·0.6H_2O (453.15), calcd C, 47.71; H, 5.83; N, 21.64; found: C, 47.67; N, 5.87; N, 21.29.

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N′*-{3-[(3-phenylfuroxan-4-yl)oxy]propyl}guanidine dihydrochloride (26).** To a stirred solution of **12** (1.00 g, 1.75 mmol) and 3-phenyl-4-benzenesulfonylfuroxan (**14**) (0.74 g, 2.45 mmol) in distilled THF (75 mL), 50% (w/w) aq NaOH solution (0.56 g, 7.02 mmol) was added at room temperature; after 3 h of stirring the mixture was diluted with H_2O (25 mL) and the organic solvent evaporated under reduced pressure (25 °C). The aqueous residue was extracted with CH_2Cl_2 (3×30 mL), the extracts dried and evaporated to obtain the crude product which was purified by FC (silica gel, CH_2Cl_2/AcOEt 6:4) to obtain pure **20** (1.00 g; 78%) as a pale yellow foam. The protected intermediate **20** was refluxed in 5 N HCl for 16 h; after cooling the mixture was filtered and extracted with Et_2O (3×30 mL) and the aqueous phase evaporated to dryness under reduced pressure (60 °C). The product was dried over P_2O_5/NaOH/paraffin under high vacuum to yield **26** (0.63 g; 78% from **12**) as white amorphous solid. An analytical sample was obtained as trifluoroacetate. Mp 184 °C (DSC) (MeOH/Et_2O). ^{1}H NMR (DMSO-d_6) δ a few exch. protons not observed; 9.01 (s, 1H); 8.07 (m, 4H); 7.61 (m, 4H); 7.45 (s, 1H); 4.57 (t, J = 5.6, 2H); 3.4 (m, 2H); 3.2 (m, 2H); 2.72 (t, J = 7, 2H); 2.11 (m, 2H); 1.87 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 162.28; 156.04; 134.20; 133.60; 132.58; 130.75; 129.12; 126.31; 122.04; 115.66; 107.03; 68.43; 39.99; 37.77; 27.91; 27.50; 21.13. Analysis for $C_{18}H_{23}N_7O_3$·1.5 CF_3COOH·1.5H_2O (583.5), calcd C, 43.23; H, 4.75; N, 16.8; found: C, 43.43; H, 4.94; N, 16.67.

***N*-[3-(1H-Imidazol-4-yl)propyl]-*N′*-{3-[(4-benzenesulfonyl-furazan-3-yl)oxy]propyl}guanidine ditosylate (27).** To a solution of **12** (1.17 g, 2.04 mmol) and bis(benzenesulfonyl)furazan (**15**) (1.00 g, 2.85 mmol) in distilled THF (10 mL), 50% (w/w) aq NaOH solution (0.65 g, 8.16 mmol) was added and the mixture stirred vigorously at room temperature for 2 h. The reaction was diluted with water (10 mL) and the solvent evaporated under reduced pressure (25 °C). The aqueous residue was treated with brine (10 mL) and extracted with CH_2Cl_2 (3×30 mL), the organic phase was dried and evaporated under reduced pressure to give a pale yellow oil. The product was purified by FC [silica gel, CH_2Cl_2/AcOEt 8:2 (300 mL) then CH_2Cl_2/AcOEt 1:1 (400 mL)] to obtain pure **21** (1.43 g, 90%) as a white foam. Intermediate **21** was hydrolysed by heating at 95 °C with *p*-TsOH 3 M (75 mL) for 72 h; after cooling the mixture was diluted with water (40 mL), extracted with Et_2O (5×40 mL) and the aqueous phase evaporated to dryness (60 °C). The residue was dried thoroughly in a desiccator over P_2O_5/NaOH/paraffin under high vacuum for 48 h. The obtained cream-coloured solid was triturated with Et_2O (6×100 mL) to eliminate excess acid and the residue was crystallised from dry MeOH/Et_2O to obtain **27** (1.09 g, 75% from **12**) as a white amorphous solid. Mp 134 °C (dec.) (DSC). ^{1}H NMR (DMSO-d_6) δ 9.01 (s, 1H); 8.10 (m, 2H); 7.96–7.74 (m, 4H); 7.60–7.44 (m, 10H); 7.13 (m, 4H); 4.42 (t, J = 5.8, 2H); 3.22 (m, 4H); 2.68 (t, J = 7.4, 2H); 2.30 (s, 6H); 1.96 (m, 2H); 1.83 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 161; 155.66; 148.63; 145.11; 138.18; 136.88; 136.33; 134.01; 132.62; 130.32; 128.78; 128.29; 125.53; 115.73; 71.39; 40.18; 37.46; 27.78; 27.30; 21.20; 20.87. Analysis for $C_{18}H_{23}N_7O_4S$·2 $C_7H_8O_3$·H_2O·0.2 $C_4H_{10}O$ (810.7), calcd C, 48.59; H, 5.35; N, 12.09; found: C, 48.8; H, 5.14; N, 12.06.

***N*-[3-(1H-Imidazol-4-yl)propyl]-*N'*-{3-[(3-benzenesulfonyl-furoxan-4-yl)oxy]propyl}guanidine ditosylate (28).** To a solution of **12** (1.00 g, 1.75 mmol) and bis(benzenesulfonyl)furoxan (**16**) (0.90 g; 2.45 mmol) in dry THF (8 mL), 50% (w/w) aq NaOH solution (0.56 g, 7.0 mmol) was added dropwise and the reaction mixture stirred vigorously at room temperature for 1 h. The mixture was diluted with H_2O (10 mL), the organic solvent evaporated under reduced pressure (25 °C). The obtained residue was treated with brine (10 mL) and extracted with CH_2Cl_2 (3×30 mL). The extracts were dried and evaporated under reduced pressure to afford the crude product which was purified by FC [silica gel, CH_2Cl_2/AcOEt 7:3 (400 mL) then CH_2Cl_2/AcOEt 1:2 (300 mL)] to give the protected intermediate **22** as a white foam (1.23 g, 88%). The protected product **22** (0.60 g, 0.75 mmol) was suspended in 3 M *p*-TsOH (35 mL) and heated at 95 °C for 72 h. After cooling the mixture was filtered, extracted with Et_2O (5×20 mL) and the aqueous phase evaporated under reduced pressure (45 °C). The residue was dried under high vacuum, then washed with Et_2O (7×50 mL). The semisolid residue was dissolved in water (10 mL) and extracted with AcOEt (3×20 mL), the aqueous layer was evaporated under reduced pressure (45 °C) to obtain a white solid which was crystallised from dry MeOH/Et_2O to give **28** (0.48 g, 70% from **12**) as a white amorphous solid. Mp 144–153 °C (dec.) (DSC) (MeOH/Et_2O). ^{1}H NMR (DMSO-d_6) δ few exch. protons not observed; 9.02 (s, 1H); 8.04 (m, 2H); 7.96–7.72 (m, 4H); 7.62–7.40 (m, 9H); 7.14 (m, 4H); 4.46 (t, J = 5.9, 2H); 3.49–3.19 (m, 4H); 2.69 (t, J = 7.4, 2H); 2.30 (s, 6H); 2.02 (m, 2H); 1.84 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 158.93; 155.67; 145.16; 138.13; 137.11; 136.3; 134; 132.59; 130.16; 128.48; 128.29; 125.54; 115.73; 110.68; 68.82; 40.22; 37.54; 27.66; 27.29; 21.29; 20.87. Analysis for $C_{18}H_{23}N_7O_5S{\cdot}2\ C_7H_8O_3S{\cdot}H_2O{\cdot}0.2\ C_4H_{10}O$ (826.7), calcd C, 47.65; H, 5.24; H, 11.86; found: C, 47.85; H, 5.01; N, 11.51.

Attempted hydrolysis of 22 with 5 N HCl: synthesis of benzenesulfonylmethyl chloride (29). The protected intermediate **22** (0.45 g, 0.56 mmol) was refluxed with 5 N HCl (35 mL) for 16 h. The cooled reaction mixture was filtered and extracted with Et_2O (5×15 mL). The ethereal phase was washed with 10% $NaHCO_3$ (3×10 mL), dried and evaporated under reduced pressure to give a pale orange oil which was purified by FC (silica gel, PE/CH_2Cl_2 1:1) to afford the title product **29** (0.10 g, 98%) as white solid. Mp 53–54 °C (EtOH/H_2O) (lit.[28] 52–53 °C). ^{1}H NMR ($CDCl_3$) δ 7.97 (m, 2H); 7.84–7.72 (m, 3H); 5.33 (s, 2H). ^{13}C NMR (DMSO-d_6) δ 136; 134.82; 129.57; 128.87; 57.48. Analysis for $C_7H_7ClO_2S$ (190.6), calcd C, 44.1; H, 3.70; found: C, 43.94; H, 3.73.

Quantitative nitrite detection. A solution of the appropriate compound (20 μL) in DMSO was added to 2 mL of 50 mM phosphate buffer (pH 7.4) containing 5 mM L-cysteine. The final concentration of the drug was 10^{-4} M. After 1 h at 37 °C, 1 mL of this mixture was treated with 250 μL of Griess reagent [4 g sulfanilamide, 0.2 g *N*-naphtylethylenediamine bis(hydrochloride), 10 mL 85% phosphoric acid in distilled H_2O (final volume: 100 mL)]. After 10 min at room temperature, absorbance was measured at 540 nm; 10–80 nmol/mL $NaNO_2$ standard solutions were used for the calibration curve. The nitrite yield was expressed in % NO_2^- (mol/mol) ± standard error.

Isolated guinea-pig papillary muscle

Left papillary muscles were dissected from guinea pig hearts and suspended under 0.5–1 g of passive load in 10-mL organ baths, containing Ringer solution, at 37 °C and gassed with 95% O_2 and 5% CO_2. Two platinum electrodes, implanted into the ventricular basis, were used to drive electrically the tissues. Square wave pulses (2 Hz, 1 ms, 20% above threshold voltage) were continuously delivered and changes in contraction force of papillary muscles were recorded by an isometric transducer, connected with a pen recorder. After 60–90 min equilibration period, cumulative concentration–response curves to histamine and to H_2 receptor agonists were constructed by increasing doses by half-log units after each response had reached a plateau (1–2 min). Up to three reproducible concentration–response curves could be constructed in the same preparation at 1-h intervals, without any sign of desensitisation. When H_2 receptor antagonists were tested, the curve to histamine or to other agonists was repeated in the same tissue after a 20-min incubation with the antagonist.

Isolated guinea-pig ileum

All compounds were tested for H_3-antagonistic effects in vitro using the guinea pig ileum (a portion of the small intestine 20–50 cm proximal to the ileocaecal valve)[18] isolated from male albino guinea-pigs weighing 250–350 g. The tissues were mounted under 1 g tension (isotonic transducer) and placed in Krebs–bicarbonate buffer (composition in mM: NaCl 111.2; KCl 5.0; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.0; $NaHCO_3$ 12; glucose 11.1; pH = 7.4) at 37 °C and gassed with 95% O_2–5% CO_2. After equilibration of 2 h with washings every 20 min, the muscle segments were stimulated with square wave pulses delivered by a Grass Stimulator S-44 (10 V, 0.1 Hz, 0.5 ms). After 30 min of stimulation, cumulative concentration–response curves (half-log increments) of the histamine H_3 agonist (*R*)-α-methylhistamine (MHA) were recorded until no change in response was found. To avoid the desensitisation phenomena, a single concentration-response curve to the H_3 agonist MHA was carried out in the same preparation. From each animal four segments of ileum were prepared: one was used as a control and the others were incubated with the antagonist for 20 min before the generation of concentration–response curves with MHA. All the experiments were carried out in the presence of H_1 and H_2 receptor blockers (Pyrilamine 1 μM and Ranitidine 1 μM) to prevent the activation of these receptors by the highest concentration of MHA.[29] In order to check eventual effect by NO on the concentration–response curve of MHA, preliminary experiments were carried out. The concentration–response curve for MHA was recorded in the presence of the furoxan NO-donor CHF 2363 (0.1 μM)[30] without observing any significant change in the EC_{50}

value. Similarly no change in the affinity of the reference H_3-antagonist Tioperamide was observed when the experiment was repeated in the presence of CHF 2363.

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