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Furoxan analogues of the histamine H_3-receptor antagonist imoproxifan and related furazan derivatives

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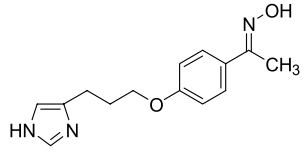
Abstract—Synthesis and pharmacological characterisation of a series of compounds in which the oxime substructure present in imoproxifan was constrained in the pentatomic NO-donor furoxan ring, as well as their structurally related furazan analogues devoid of NO-donating properties, are described. The whole series of products displayed reversible histamine H_3-antagonistic activity on guinea-pig ileum. 4-(4-(3-(1*H*-Imidazol-4-yl)propoxy)phenyl)furoxan-3-carbonitrile **16** was also able to induce partial relaxation when added to the bath after electrical contraction of the guinea-pig ileum during the study of its H_3-antagonistic properties. This phenomenon seems to be dependent on NO-mediated sGC activation. The lipophilic–hydrophilic balance of all the products was investigated.

1. Introduction

In our continuing efforts toward the design of hybrid structures in which an appropriate pharmacophoric group is joined to a nitric oxide (NO) releasing moiety,[1] we have recently designed new selective histamine H_3-receptor antagonists endowed with NO-donor properties. Part of these products was obtained by coupling the H_3-antagonist SKF 91486, a (3-(1*H*-imidazol-4-yl)propyl)guanidine, with differently substituted NO-donor furoxan substructures.[2] Other selective NO-donor H_3-antagonists, characterised by a lipophilic–hydrophilic balance more appropriate to enter the central nervous system, were obtained by joining the 3-(1*H*-imidazol-4-yl)propoxy moiety of the proxifan series[3] with furoxan and nitrooxy NO-donor moieties.[4] The analogue compounds containing in the side chain a S atom or a SO_2 moiety in place of the oxygen atom displayed H_3-antagonist activity too, but they were also partly active at the muscarinic receptors.[4] Here, we describe the synthesis and the study of the lipophilic–hydrophilic balance as well as the preliminary pharmacological characterisation of a series of products in which the oxime substructure present in imoproxifan (**1**, 4-(3-(1*H*-imidazol-4-yl)propoxy)phenylethanone oxime), which exists in an (*E*)-configuration,[5] was constrained in the appropriately substituted pentatomic NO-donor furoxan ring (compounds **13**–**16**). The corresponding furazan derivatives, unable to release NO, are also described (**13a**–**16a**).



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2. Chemistry

The synthetic approach to the final products involves the preparation of a series of phenol derivatives bearing at the *p*-position the appropriate furoxan and furazan moieties. These compounds were obtained according

Keywords: Histamine; H_3-antagonists; Furoxans; Furazans.
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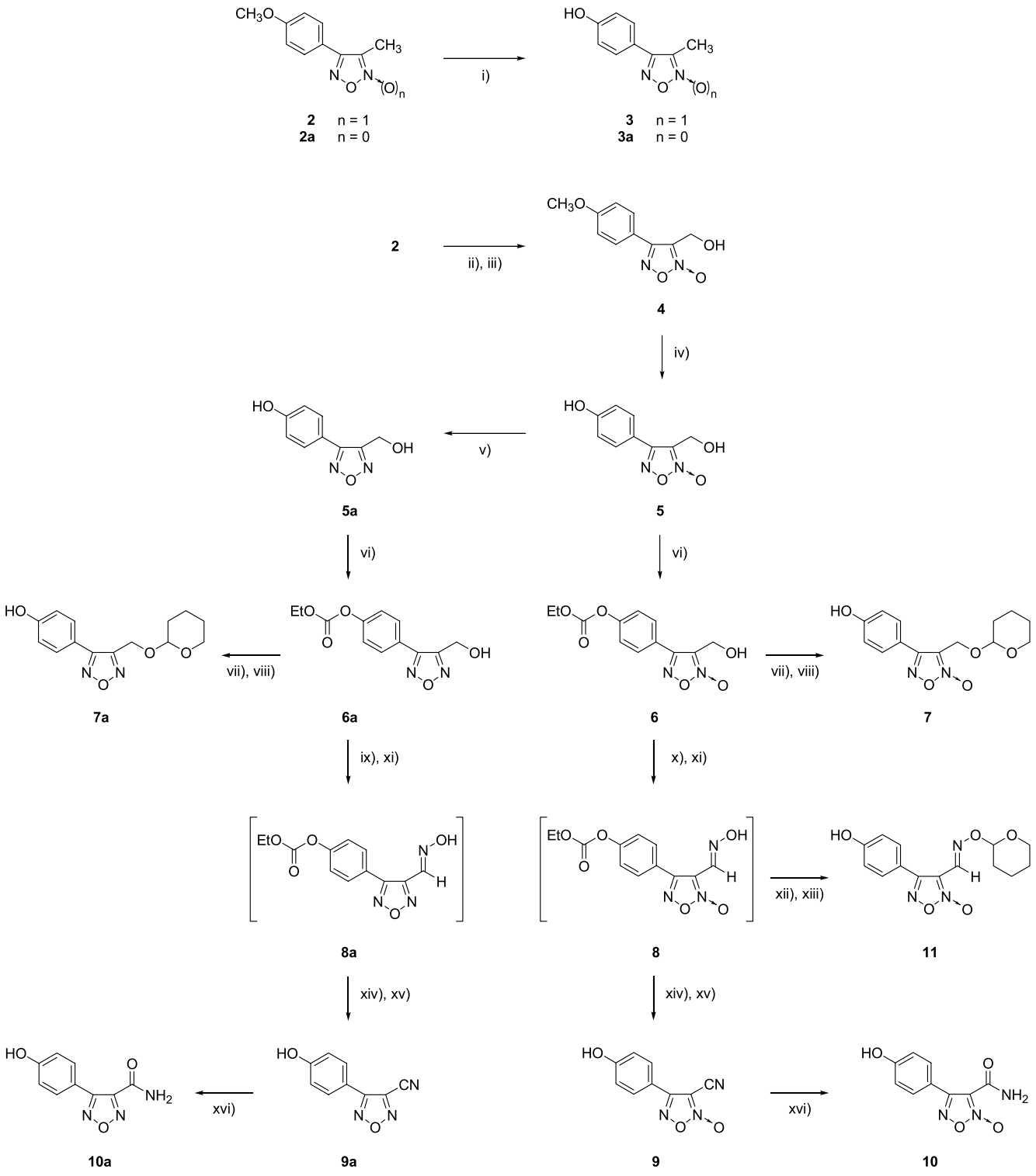
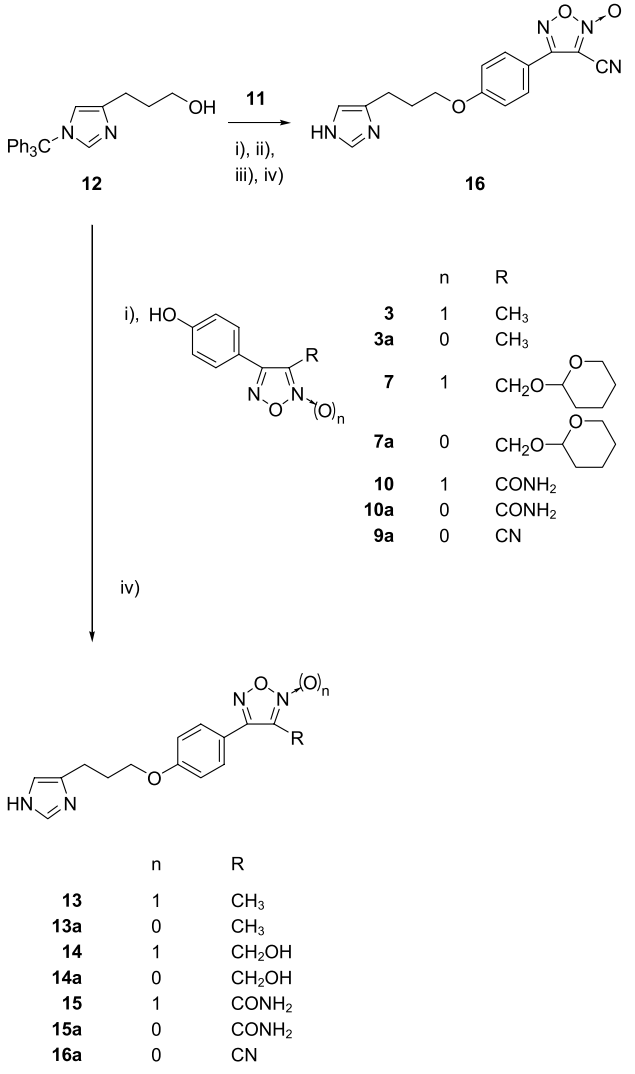


Scheme 1. Reagents and conditions: (i) CH_3SO_2OH, DL-Met, 60 °C; (ii) CCl_4, NBS, cat. benzoyl peroxide, reflux; (iii) dioxane, H_2O, $CaCO_3$, reflux; (iv) CH_2Cl_2, $AlCl_3$, sealed flask, 60 °C; (v) $(CH_3O)_3P$, reflux; (vi) Et_2O, ethyl chloroformate, Et_3N, −15 °C; (vii) acetone, DHP, cat. CH_3SO_2OH, 0 °C → rt; (viii) acetone, H_2O, NaOH, 0 °C; (ix) dioxane, MnO_2, reflux; (x) CH_2Cl_2, MnO_2, rt; (xi) CH_3CN, $NH_2OH{\cdot}HCl$, dry pyridine, 60 °C; (xii) acetone, DHP, cat. CH_3SO_2OH, reflux; (xiii) acetone, H_2O, NaOH, 0 °C; (xiv) H_2O, NaOH, 0 °C; (xv) DMF, $SOCl_2$, 0 °C; (xvi) acetone, H_2O, K_2CO_3, rt.

to the pathway reported in Scheme 1. The 4-(4-methoxyphenyl)-3-methylfuroxan **2** and the furazan analogue **2a** were transformed into the corresponding phenol derivatives **3** and **3a** by means of DL-methionine in methanesulfonic acid solution. Compound **2** was also used as the starting material to prepare the phenols **5** and **5a**.

The furoxanic methyl of this product underwent free radical bromination with *N*-bromosuccinimide in refluxing CCl_4 using benzoyl peroxide as initiator. The intermediate bromomethyl derivative was immediately hydrolysed to the corresponding hydroxymethyl derivative **4** in a dioxane/water solution in the presence of $CaCO_3$. The methyl aryl ether **4** was transformed into the corresponding phenol **5** under the action of $AlCl_3$ in CH_2Cl_2, heating at 60 °C in a sealed vessel suitable for high-pressure reactions. The latter was reduced to the corresponding furazan **5a** in refluxing trimethyl phosphite. The phenol groups of **5** and **5a** were protected as ethyl carbonate by means of ethyl chloroformate in the presence of triethylamine to give **6** and **6a** which, by acid-catalysed reaction with 3,4-dihydro-2*H*-pyran (DHP) followed by alkaline deprotection of the phenol groups, afforded the corresponding tetrahydropyranyl ethers **7**, **7a**. The protected phenols **6** and **6a** were also transformed into the oximes **8**, **8a** through MnO_2 oxidation, followed by treatment with hydroxylamine. The oximes **8**, **8a** were not isolated and characterised but they were immediately deprotected in NaOH solution and dehydrated to the cyano derivatives **9**, **9a** with $SOCl_2$ in DMF. The furoxan and furazancarboxamides **10**, **10a** were obtained by hydrolysis of the parent cyano derivatives in acetone/water solution in the presence of K_2CO_3. The final products were prepared by Mitsunobu coupling of 3-(1-trityl-1*H*-imidazol-4-yl)-1-propanol **12** with the appropriate phenol derivatives, according to Scheme 2. The reaction was run in THF in the presence of Ph_3P and diisopropylazodicarboxylate (DIAD). The products thus obtained were deprotected to the final compounds with trifluoroacetic acid (TFA) in CH_2Cl_2. When the Mitsunobu coupling was attempted with cyanofuroxan **9**, extended decomposition of the starting material occurred. Better results were obtained using in this reaction the tetrahydropyranyl derivative **11** of oxime **8**, which was prepared as the analogues **7**, **7a**. Selective hydrolysis of the tetrahydropyranyl moiety by means of *p*-toluenesulfonic acid in methanol, followed by $SOCl_2$-mediated dehydration and trityl group removal, afforded the final product **16**.



Scheme 2. Reagents and conditions: (i) dry THF, Ph_3P, DIAD, 0 °C → rt; (ii) MeOH, *p*-TsOH, rt; (iii) DMF, $SOCl_2$, −40 °C → 18 °C; (iv) 10% TFA in CH_2Cl_2, rt.

3. Results and discussion

3.1. Detection of nitrite

The ability of furoxan derivatives to produce nitrite, which is the most important oxidation product of NO in aerobic water solution, was evaluated by the Griess reaction.[6] The assay was performed in a pH 7.4 buffered solution at 37 °C under the action of an excess of cysteine (1:50), according to a previously reported procedure;[6] the results are reported in Table 1. The NO release ranks in the order **16** > **15** > **14** ≈ **13**, in line with the finding that the thiol-induced release from furoxans is enhanced by the presence of electron-withdrawing substituents at the ring.[7]

Table 1. H_3-antagonism and NO-release properties of the compounds under study

Compound	H_3-antagonism $pA_2 \pm$ SE[a]	NO_2^- (%)[b](+ L-cys)
1[c]	8.42 ± 0.05	—
13	7.34 ± 0.09	0
13a	7.99 ± 0.04	—
14	6.41 ± 0.07	<1
14a	6.59 ± 0.08	—
15	6.10 ± 0.05	3.3 ± 0.2
15a	6.62 ± 0.11	—
16	—[d]	
	(6.39 ± 0.11)[e]	18.7 ± 0.7
16a	7.10 ± 0.07	—

—, not determined.

[a] pA_2 values were estimated at the concentrations reported in Section 4.

[b] Yields are reported in % [mol/mol] ± SEM.

[c] $pK_i = 9.58 \pm 0.05$ (Ref. 5).

[d] It was impossible to estimate pA_2 value in the absence of ODQ because at 1 μM concentration it determined 100% relaxation of electrically stimulated guinea-pig ileum.

[e] In the presence of 1 μM ODQ.

Table 2. Dissociation constants and lipophilicity descriptors in octanol/water of the compounds under study

Compound	$pK_a \pm SD$[a]	$\log P^N \pm SD$[b]	$\log P^I \pm SD$[c]	$\log D^{7.4}$[d]
13	7.18 ± 0.01	2.40 ± 0.02	−0.05 ± 0.02	2.19
13a	7.18 ± 0.01	2.92 ± 0.02	0.43 ± 0.05	2.71
14	7.16 ± 0.01	2.03 ± 0.02	−0.32 ± 0.01	1.83
14a	7.14 ± 0.01	2.15 ± 0.01	−0.24 ± 0.02	1.96
15	7.16 ± 0.02	1.57 ± 0.03	−0.47 ± 0.02	1.37
15a	7.15 ± 0.01	1.79 ± 0.03	−0.30 ± 0.02	1.61
16	7.16 ± 0.01	3.19 ± 0.02	0.72 ± 0.03	2.99
16a	7.14 ± 0.01	3.24 ± 0.02	0.78 ± 0.03	3.04

[a] Dissociation constants determined by aqueous titrations in the presence of methanol as cosolvent (20–50 wt%) according to Yasuda–Shedlovsky procedure.

[b] $\log P^N_{oct}$ of the neutral form obtained with Sirius GLpK_a.

[c] $\log P^I_{oct}$ of the ionised form obtained by the shake-flask technique at pH 4.0.

[d] $\log D$ at pH 7.4 calculated from the following equation:

$$D = P^N \cdot \left(\frac{1}{1 + 10^{pK_a - pH}}\right) + P^I \cdot \left(\frac{10^{pK_a - pH}}{1 + 10^{pK_a - pH}}\right)$$

and validated by the shake-flask technique.

3.2. Lipophilic–hydrophilic balance

Potentiometric titrations of the final products **13**–**16** and **13a**–**16a** were performed with a Sirius GLpK_a automated potentiometric system. Owing to the low aqueous solubility of the products, the titrations were carried out using methanol in different ratios as a cosolvent.[8] The aqueous pK_a values were determined by extrapolation to 0% methanol according to the Yasuda–Shedlovsky procedure. The pK_a values obtained are listed in Table 2. They lie, as expected, in the narrow 7.14–7.18 range, according to the presence in the structures of an imidazole ring. Partition coefficients (expressed as $\log P$) between *n*-octanol and water were measured by dual-phase titration using various amounts of water-saturated *n*-octanol.[9] Distribution coefficients (expressed as log*D*) were calculated for each compound at pH 7.4 from its pK_a, $\log P^N$ (partition coefficient of the neutral form) and $\log P^I$ (partition coefficient of the ionised form); all values are listed in Table 2. Frequently, $\log D$ values give better correlations with pharmacokinetic parameters than $\log P^N$, probably because the influence of ionisation is taken into account. The most lipophilic compounds appear to be the cyano derivatives, while the least are the carboxamide derivatives. Log *D* values range from 1.37 to 3.04. Hansch suggested an optimum $\log P_{oct}$ value of about 2 ± 0.5 for a compound to diffuse into the central nervous system.[10] However, a more careful treatment of this aspect should include the evaluation of additional molecular descriptors such as $\Delta\log P(\log P_{oct} - \log P_{cyc})$[11] or solvatochromic parameters.[12]

3.3. Pharmacology

The H_3-antagonist activity of the products was assessed by their ability to antagonise the concentration-dependent inhibitory effect of (*R*)-α-methylhistamine (MHA) on electrically evoked twitches of isolated guinea-pig ileum segments.[13] pA_2 values were calculated using the Gaddum equation ($pA_2 = -\log[B] + \log[CR - 1]$) and they are reported in Table 1. Some experiments were assessed in the presence of 1 μM ODQ (1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one), a well-known inhibitor of the soluble guanylate cyclase (sGC). The analysis of Table 1 shows that all the products display antagonist activity at the H_3-receptors, even if lower than that of imoproxifan. Furoxan derivatives **13**, **14** and **15**, which are feeble NO-donors, behave similarly to the furazan analogues, being just a little less potent. The most active products bear the methyl substituent and the least active ones the carbamoyl substituent. The cyano substituted furoxan derivative **16**, which is a potent NO-donor, behaves in a particular manner. In fact, when the product was added to the bath at 1 μM concentration, after the electrical contraction of the tissue, a complete annulment of the contraction was observed. When the experiments were repeated in the presence of ODQ, the contraction of the tissue to the electrical stimulus was fully restored, suggesting that the tissue relaxation was NO-dependent. The pA_2 of this product, evaluated at 1 μM concentration, was 6.39, indicating a lower affinity of the compound for the H_3-receptor with respect to the furazan analogue **16a**.

In conclusion, in this paper we describe new H_3-antagonists in which the oxime moiety present in imoproxifan is constrained in differently substituted furoxan and furazan rings. All the products display lesser affinity for the H_3-receptor than the lead. The cyano substituted furoxan **16** is able, at the concentration used in the assay, to relax electrically contracted guinea-pig ileum with a NO-dependent mechanism and to display H_3-antagonist activity.

4. Experimental

4.1. Chemistry

All compounds were purified by recrystallisation before characterisation. Melting points were determined with a capillary apparatus (Büchi B-540) and are uncorrected; the recrystallisation solvent is reported in parentheses. All the products were routinely checked by IR (Shimadzu FT-IR 8101 M). ^1H and ^{13}C NMR spectra were obtained on a Bruker Avance 300, at 300 and 75 MHz, respectively; δ in ppm rel to $SiMe_4$ as the internal standard; coupling constants *J* in Hz. ^{13}C NMR spectra were fully decoupled. The following abbreviations are used: s, singlet; d, doublet; t, triplet; qt, quartet; qn, quintet; br, broad; Fx, furoxan; Fz, furazan; THP, tetrahydropyran; *, uncertain assignment. Mass spectra were recorded on a Finnigan-Mat TSQ-700. Flash chromatography (FC) was performed on BDH silica gel (particle size 40–63 μm). When not otherwise specified, anhydrous magnesium sulfate ($MgSO_4$) was used as the drying agent of organic phases. Analysis (C, H and N) of the new compounds was performed by REDOX (Monza). Structures **1**,[5] **2**,[14] **2a**[15] and **12**[16] were synthesised according to reported methods.

4.2. 4-(3-Methylfuroxan-4-yl)phenol (3)

Compound **2** (5.00 g, 24.2 mmol), DL-methionine (5.42 g, 36.3 mmol) and methanesulfonic acid (25 mL) were stirred at 60 °C under N_2 for 4 h. After cooling, the reaction mixture was poured into cold water (500 ml) and extracted with Et_2O (4 × 50 mL). The combined organic phase was washed with brine (2 × 25 mL) and then extracted with 1 N NaOH (3 × 50 mL). The combined ethereal layers were washed with brine (25 mL), dried and evaporated to recover the unreacted starting material. The aqueous phase was acidified with concd HCl and extracted with Et_2O (4 × 50 mL); the combined organic extracts were washed with a buffer solution (0.750 M Na_2CO_3, 0.375 M $NaHCO_3$; 6 × 25 mL), then with brine (25 mL), dried and evaporated yielding **3** (2.93 g, 63%) as a white solid. CIMS (isobutane): *m/z* 193 (MH^+); mp 162–163 °C (1,2-dichloroethane). ^{1}H NMR (DMSO-d_6): δ, 10.17 (s, 1H, OH), 7.61 (d, 2H, J = 8.6 Hz, Ph-2,6-H), 6.97 (d, 2H, J = 8.6 Hz, Ph-3,5-H), 2.29 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ, 160.8 (Ph-4-C), 157.9 (Fx-4-C), 129.9 (Ph-2,6-C), 117.8 (Ph-1-C), 116.9 (Ph-3,5-C), 113.6 (Fx-3-C), 9.9 (CH_3). Anal. Calcd for $C_9H_8N_2O_3$ (192.17): C, 56.25; H, 4.20; N, 14.58. Found: C, 56.30; H, 4.20; N, 14.50.

4.3. 4-(4-Methylfurazan-3-yl)phenol (3a)

Compound **2a** (5.00 g, 26.3 mmol), DL-methionine (5.89 g, 39.4 mmol) and methanesulfonic acid (25 mL) were stirred at 60 °C under N_2 for 4 h. The reaction was worked up as for **3** obtaining **3a** (3.10 g, 67%) as a white solid. CIMS (isobutane): *m/z* 177 (MH^+); mp 93.5–94 °C (*n*-hexane/1,2-dichloroethane). ^{1}H NMR (DMSO-d_6): δ, 10.09 (s, 1H, OH), 7.65 (d, 2H, J = 8.6 Hz, Ph-2,6-H), 6.96 (d, 2H, J = 8.6 Hz, Ph-3,5-H), 2.54 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ, 160.4 (Ph-4-C), 154.5 (Fz-4-C), 151.1 (Fz-3-C), 130.5 (Ph-2,6-C), 116.9 (Ph-3,5-C), 116.8 (Ph-1-C), 10.2 (CH_3). Anal. Calcd for $C_9H_8N_2O_2$ (176.17): C, 61.36; H, 4.58; N, 15.90. Found: C, 61.53; H, 4.52; N, 15.92.

4.4. (4-(4-Methoxyphenyl)furoxan-3-yl)methanol (4)

Compound **2** (30.0 g, 145 mmol) was suspended in CCl_4 (300 mL), then NBS (33.0 g, 185 mmol) was added, followed by benzoyl peroxide (1.00 g, 4.13 mmol). The reaction mixture was refluxed under vigorous stirring for 12 h, then benzoyl peroxide (1.00 g, 4.13 mmol) was added and the reflux was continued for 12 more hours. After cooling, the reaction mixture was stirred for 12 h with powdered $Na_2S_2O_3$ (30 g) then the solution was filtered on a celite pad. The filtrate was evaporated obtaining a yellow oil, which was dissolved in dioxane (75 mL); H_2O (75 mL) and $CaCO_3$ (72.5 g, 725 mmol) were added and then the reaction mixture was refluxed under stirring for 12 h. After cooling, the solvent was evaporated and the residue was treated with concd HCl until CO_2 ceased evolving, then the precipitate was collected on a Buchner funnel and washed with water. The crude product was dissolved in boiling MeOH/water (1:2) decanting the insoluble dark oil; upon cooling, **4** precipitated (22.6 g, 70%) as a white solid. CIMS (isobutane): *m/z* 223 (MH^+); mp 95.5–97 °C (*n*-hexane/$CHCl_3$). ^{1}H NMR (DMSO-d_6): δ, 7.86 (d, 2H, J = 8.8 Hz, Ph-2,6-H), 7.15 (d, 2H, J = 8.8 Hz, Ph-3,5-H), 5.96 (t, 1H, J = 5.8 Hz, OH), 4.52 (d, 2H, J = 5.8 Hz, CH_2), 3.85 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ, 162.4 (Ph-4-C), 157.7 (Fx-4-C), 130.1 (Ph-2,6-C), 119.3 (Ph-1-C), 115.9 (Fx-3-C), 115.6 (Ph-3,5-C), 56.3 (CH_3), 53.0 (CH_2). Anal. Calcd for $C_{10}H_{10}N_2O_4$ (222.20): C, 54.05; H, 4.54; N, 12.61. Found: C, 54.09; H, 4.50; N, 12.60.

4.5. (4-(4-Hydroxyphenyl)furoxan-3-yl)methanol (5)

Compound **4** (16.0 g, 72.0 mmol) was suspended in anhydrous CH_2Cl_2 (160 mL) in a glass vessel suitable for high-pressure reactions; $AlCl_3$ (48.0 g, 360 mmol) was carefully added, then the vessel was tightly closed, heated at 60 °C and kept under vigorous stirring for 24 h. After cooling, the vessel was opened and the reaction mixture was slowly poured into cold 1 N HCl (500 mL) and extracted with Et_2O (4 × 50 mL). The combined organic phases were extracted with 1 N NaOH (3 × 50 mL) and then discarded; the aqueous phase was acidified with concd HCl and then extracted again with Et_2O (4 × 50 mL). The combined ethereal layers were washed with a buffer solution (0.750 M Na_2CO_3, 0.375 M $NaHCO_3$; 6 × 25 mL), then with brine (25 mL), dried and evaporated yielding **5** (12.4 g, 83%) as a white solid. EIMS: *m/z* 208 (M^+); mp 147–148 °C (1,2-dichloroethane). ^{1}H NMR (DMSO-d_6): δ, 10.18 (s, 1H, Ph-OH), 7.75 (d, 2H, J = 8.6 Hz, Ph-2,6-H), 6.97 (d, 2H, J = 8.6 Hz, Ph-3,5-H), 5.93 (s, 1H, CH_2-O*H*), 4.51 (s, 2H, CH_2-OH); ^{13}C NMR (DMSO-d_6): δ, 161.0 (Ph-4-C), 157.8 (Fx-4-C), 130.1 (Ph-2,6-C), 117.6 (Ph-1-C), 116.9 (Ph-3,5-C), 115.8 (Fx-3-C), 53.0 (CH_2). Anal. Calcd for $C_9H_8N_2O_4$ (208.17): C, 51.93; H, 3.87; N, 13.46. Found: C, 51.95; H, 3.89; N, 13.36.

4.6. (4-(4-Hydroxyphenyl)furazan-3-yl)methanol (5a)

Compound **5** (4.00 g, 19.2 mmol) was refluxed with trimethylphosphite (40 mL) for 18 h. After cooling, the reaction mixture was poured into cold 1 N HCl (400 mL) and extracted with Et_2O (4 × 50 mL). The combined ethereal layers were extracted with 1 N NaOH (3 × 50 mL) and then discarded. The aqueous phase was acidified with concd HCl and extracted with Et_2O (4 × 50 mL); the combined organic extracts were washed with a buffer solution (0.750 M Na_2CO_3, 0.375 M $NaHCO_3$; 6 × 25 mL), then with brine (25 mL), dried and evaporated to afford **5a** (2.36 g, 64%) as a white solid. CIMS: *m/z* 193 (MH^+); mp 116.5–117.5 °C (*n*-hexane/1,2-dichloroethane). ^{1}H NMR (DMSO-d_6): δ, 10.10 (s, 1H, Ph-OH), 7.79 (d, 2H, J = 8.6 Hz, Ph-2,6-H), 6.95 (d, 2H, J = 8.6 Hz, Ph-3,5-H), 5.90 (t, 1H, J = 5.1 Hz, CH_2–O*H*), 4.80 (d, 2H, J = 5.1 Hz, CH_2–OH); ^{13}C NMR (DMSO-d_6): δ, 160.6 (Ph-4-C), 154.5* (Fz-4-C), 154.1* (Fz-3-C), 130.8 (Ph-2,6-C), 116.9 (Ph-3,5-C), 116.6 (Ph-1-C), 53.4 (CH_2). Anal. Calcd for $C_9H_8N_2O_3$ (192.17): C, 56.25; H, 4.20; N, 14.58. Found: C, 56.26; H, 4.15; N, 14.48.

4.7. Ethyl 4-(3-(hydroxymethyl)furoxan-4-yl)phenyl carbonate (6)

Compound **5** (11.4 g, 54.5 mmol) was dissolved in Et_2O (300 mL) and cooled to −15 °C, then Et_3N (8.44 mL, 60.0 mmol) was added. A solution of ethyl chloroformate (5.44 mL, 57.2 mmol) in Et_2O (50 mL) was added dropwise under stirring over 1 h. The reaction mixture was washed with 1 N HCl (2 × 100 mL), H_2O (100 mL), 10% $NaHCO_3$ (50 mL) and brine (25 mL), dried and evaporated to yield crude **6** (11.8 g, 78%), pure enough to be used as an intermediate. An analytical sample was obtained by FC (eluent: petroleum ether in gradient from 20% to 30% of EtOAc), white solid. CIMS: *m*/*z* 281 (MH^+); mp 86–87 °C (*n*-hexane/*i*Pr_2O). ^1H NMR (DMSO-d_6): δ, 7.97 (d, 2H, J = 8.7 Hz, Ph-2,6-H), 7.50 (d, 2H, J = 8.7 Hz, Ph-3,5-H), 5.98 (t, 1H, J = 5.7 Hz, OH), 4.55 (d, 2H, J = 5.7 Hz, CH_2–OH), 4.29 (qt, 2H, J = 7.1 Hz, CH_3–CH_2–O), 1.32 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ, 157.3 (Ph-4-C), 153.6* (C═O), 153.4* (Fx-4-C), 130.1 (Ph-2,6-C), 124.9 (Ph-1-C), 123.2 (Ph-3,5-C), 115.9 (Fx-3-C), 65.8 (CH_3–CH_2–O), 53.0 (CH_2–OH), 14.8 (CH_3). Anal. Calcd for $C_{12}H_{12}N_2O_6$ (280.24): C, 51.43; H, 4.32; N, 10.00. Found: C, 51.47; H, 4.31; N, 9.96.

4.8. Ethyl 4-(4-(hydroxymethyl)furazan-3-yl)phenyl carbonate (6a)

Compound **5a** (7.42 g, 38.6 mmol) was dissolved in Et_2O (250 mL) and cooled to −15 °C, then Et_3N (5.92 mL, 42.5 mmol) was added. A solution of ethyl chloroformate (3.86 mL, 40.5 mmol) in Et_2O (50 mL) was added dropwise under stirring over 1 h. The reaction was worked up as for **6** to yield crude **6a** (9.22 g, 90%), pure enough to be used as an intermediate. An analytical sample was obtained by FC (eluent: petroleum ether in gradient from 20% to 30% of EtOAc), white solid. CIMS: *m*/*z* 265 (MH^+); mp 73–74.5 °C (*n*-hexane/*i*Pr_2O). ^1H NMR (DMSO-d_6): δ, 8.02 (d, 2H, J = 8.4 Hz, Ph-2,6-H), 7.48 (d, 2H, J = 8.4 Hz, Ph-3,5-H), 5.97 (t, 1H, J = 5.6 Hz, OH), 4.85 (d, 2H, J = 5.6 Hz, CH_2–OH), 4.29 (qt, 2H, J = 7.1 Hz, CH_3–CH_2–O), 1.32 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ, 154.4* (Ph-4-C), 154.2* (Fz-4-C), 153.5* (Fz-3-C), 153.3* (C═O), 130.8 (Ph-2,6-C), 124.0 (Ph-1-C), 123.1 (Ph-3,5-C), 65.8 (CH_3–CH_2–O), 53.3 (CH_2–OH), 14.8 (CH_3). Anal. Calcd for $C_{12}H_{12}N_2O_5$ (264.24): C, 54.55; H, 4.58; N, 10.60. Found: C, 54.66; H, 4.58; N, 10.53.

4.9. 4-(3-((Tetrahydro-2*H*-pyran-2-yloxy)methyl)-furoxan-4-yl)phenol (7)

Compound **6** (2.00 g, 7.14 mmol) was dissolved in acetone (8 mL) and cooled to 0 °C; 3,4-dihydro-2*H*-pyran (3.26 mL, 35.7 mmol) was added, followed by methanesulfonic acid (3 drops). After 1 h, the cooling bath was removed and the reaction mixture was stirred for 1 h at rt. It was cooled again to 0 °C and acetone (8 mL) was added, followed by 10% NaOH (6 mL). After 30 min, the reaction mixture was diluted with H_2O (120 mL) and washed with Et_2O (3 × 20 mL). The aqueous phase was cooled to 0 °C and adjusted to pH 5 with acetic acid, then extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were washed with 10% $NaHCO_3$ (20 mL), brine (20 mL), dried and evaporated to yield a crude oil. The latter was purified by FC (eluent: CH_2Cl_2) obtaining **7** (1.83 g, 88%) as a white solid. CIMS: *m*/*z* 293 (MH^+); mp 109.5–110.5 °C (*n*-hexane/$CHCl_3$). ^1H NMR (DMSO-d_6): δ, 10.21 (s, 1H, OH), 7.70 (d, 2H, J = 8.6 Hz, Ph-2,6-H), 6.98 (d, 2H, J = 8.6 Hz, Ph-3,5-H), 4.74–4.59 (m, 3H, THP-CH–O, Fx-CH_2–O), 3.70–3.63 (m, 1H, THP-CH_2–O), 3.43–3.38 (m, 1H, THP-CH_2–O), 1.64–1.46 (m, 6H, THP-CH_2); ^{13}C NMR (DMSO-d_6): δ, 161.0 (Ph-4-C), 157.9 (Fx-4-C), 130.1 (Ph-2,6-C), 117.4 (Ph-1-C), 117.0 (Ph-3,5-C), 114.1 (Fx-3-C), 99.4 (THP-CH–O), 62.4 (THP-CH_2–O), 58.2 (Fx-CH_2–O), 30.7 (THP-CH_2), 25.6 (THP-CH_2), 19.6 (THP-CH_2). Anal. Calcd for $C_{14}H_{16}N_2O_5 \cdot 0.1H_2O$ (294.09): C, 57.18; H, 5.55; N, 9.53. Found: C, 57.10; H, 5.48; N, 9.54.

4.10. 4-(4-((Tetrahydro-2*H*-pyran-2-yloxy)methyl)-furazan-3-yl)phenol (7a)

Compound **6a** (3.68 g, 13.9 mmol) was dissolved in acetone (15 mL) and cooled to 0 °C; 3,4-dihydro-2*H*-pyran was added (6.34 mL, 69.5 mmol), followed by methanesulfonic acid (3 drops). After 1 h, the cooling bath was removed and the reaction mixture was stirred for 1 h at rt. It was cooled again to 0 °C and acetone (15 mL) was added, followed by 10% NaOH (10 mL). After 30 min, the reaction was worked up as for **7** obtaining **7a** (3.30 g, 86%) as a white solid. CIMS: *m*/*z* 277 (MH^+); mp 73.5–74.5 °C (*n*-hexane/$CHCl_3$). ^1H NMR ($CDCl_3$): δ, 7.77 (d, 2H, J = 8.2 Hz, Ph-2,6-H), 6.95 (d, 2H, J = 8.2 Hz, Ph-3,5-H), 6.20 (s, 1H, OH), 4.99–4.82 (m, 3H, THP-CH–O, Fz-CH_2–O), 3.91–3.85 (m, 1H, THP-CH_2–O), 3.62–3.59 (m, 1H, THP-CH_2–O), 1.79–1.58 (m, 6H, THP-CH_2); ^{13}C NMR ($CDCl_3$): δ, 158.0 (Ph-4-C), 154.0 (Fz-4-C), 150.2 (Fz-3-C), 130.3 (Ph-2,6-C), 117.7 (Ph-1-C), 116.2 (Ph-3,5-C), 98.5 (THP-CH–O), 62.6 (THP-CH_2–O), 57.7 (Fz-CH_2–O), 30.3 (THP-CH_2), 25.2 (THP-CH_2), 19.0 (THP-CH_2). Anal. Calcd for $C_{14}H_{16}N_2O_4$ (292.29): C, 60.86; H, 5.84; N, 10.14. Found: C, 60.85; H, 5.84; N, 10.13.

4.11. Procedure for the synthesis of derivatives 9, 11. Synthesis of the common intermediate ethyl 4-(3-((E)-(hydroxyimino)methyl)furoxan-4-yl)phenyl carbonate (8)

A solution of **6** (15.5 g, 55.4 mmol) in CH_2Cl_2 (100 mL) was stirred for 12 h with activated MnO_2 (48.2 g, 554 mmol). After filtration on a celite pad, the solvent was evaporated and the residue was dissolved in CH_3CN (100 mL). $NH_2OH{\cdot}HCl$ (4.23 g, 60.9 mmol) was added, followed by dry pyridine (4.92 mL, 60.9 mmol), then the reaction mixture was stirred at 60 °C for 1 h. After cooling, the solvent was evaporated, the residue was taken up in 1 N HCl (100 mL) and extracted with Et_2O (4 × 50 mL). The combined organic layers were washed with brine (20 mL), dried and evaporated to yield **8** as a crude product. ^1H NMR

(DMSO-d_6): δ, 12.49 (s, 1H, OH), 7.93 (s, CH=N), 7.84 (d, 2H, J = 8.6 Hz, Ph-2,6-H), 7.46 (d, 2H, J = 8.6 Hz, Ph-3,5-H), 4.29 (qt, 2H, J = 7.1 Hz, CH_2), 1.32 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ, 160.0 (Ph-4-C), 155.2 (Fx-4-C), 152.6 (C=O), 135.0 (C=N), 130.3 (Ph-2,6-C), 123.7 (Ph-1-C), 121.8 (Ph-3,5-C), 111.5 (Fx-3-C), 64.9 (CH_2), 13.9 (CH_3). Owing to its instability, **8** was not further characterised, but readily reacted in the following step.

4.12. 4-(4-Hydroxyphenyl)furoxan-3-carbonitrile (9)

Compound **8** was dissolved in 10% ice-cold NaOH. After 10 min, the reaction mixture was washed with Et_2O (50 mL), then adjusted to pH 5 with acetic acid and extracted with Et_2O (4 × 50 mL). The combined ethereal extracts were washed with 10% $NaHCO_3$ (3 × 25 mL), brine (25 mL), dried and evaporated to yield a crude solid. The latter was dissolved in DMF (40 mL) and cooled to 0 °C, then $SOCl_2$ (8.10 mL, 111 mmol) was added. After 30 min, the reaction mixture was poured into ice/water (400 mL) and extracted with Et_2O (4 × 50 mL). The combined ethereal layers were washed with 10% $NaHCO_3$ (6 × 25 mL), brine (25 mL), dried and evaporated to yield a crude oil, which was purified by FC (eluent: petroleum ether/EtOAc 9:1) obtaining **9** (4.61 g, 41% from **6**) as a yellow solid. CIMS: m/z 204 (MH^+); mp 134–135 °C (n-hexane/iPr$_2$O). ^{1}H NMR (DMSO-d_6): δ, 10.49 (s, 1H, OH), 7.74 (d, 2H, J = 9.6 Hz, Ph-2,6-H), 7.04 (d, 2H, J = 9.6 Hz, Ph-3,5-H); ^{13}C NMR (DMSO-d_6): δ, 162.1 (Ph-4-C), 155.7 (Fx-4-C), 129.6 (Ph-2,6-C), 117.4 (Ph-3,5-C), 115.2 (Ph-1-C), 108.5 (CN), 98.8 (Fx-3-C). Anal. Calcd for $C_9H_5N_3O_3 \cdot 0.1H_2O$ (204.96): C, 52.74; H, 2.56; N, 20.50. Found: C, 53.00; H, 2.51; N, 20.25.

4.13. 4-(4-Hydroxyphenyl)furoxan-3-carbaldehyde *O*-(tetrahydro-2*H*-pyran-2-yl)oxime (11)

Compound **8** was dissolved in acetone (100 mL), 3,4-dihydro-2*H*-pyran (10.1 mL, 111 mmol) was added, followed by methanesulfonic acid (5 drops), then the reaction mixture was refluxed for 2 h. After cooling at 0 °C, acetone (100 mL) was added, followed by 10% NaOH (50 mL). After 1 h, the reaction mixture was poured into ice/water (500 mL) and washed with Et_2O (4 × 50 mL), then adjusted to pH 5 with acetic acid and extracted with Et_2O (4 × 50 mL). The combined ethereal extracts were washed with 10% $NaHCO_3$ (2 × 50 mL), brine (25 mL), dried and evaporated to yield a crude oil, which was purified by FC (eluent: petroleum ether in gradient from 10% to 20% of EtOAc) obtaining **11** (8.32 g, 49% from **6**) as a white solid. CIMS: m/z 306 (MH^+); mp 141–142 °C (n-hexane/iPr$_2$O). ^{1}H NMR (DMSO-d_6): δ, 10.20 (s, 1H, OH), 8.11 (s, CH=N), 7.66 (d, 2H, J = 8.4 Hz, Ph-2,6-H), 6.93 (d, 2H, J = 8.4 Hz, Ph-3,5-H), 5.29 (s, 1 H, THP-CH–O), 3.76 (m, 1H, THP-CH_2–O), 3.54 (m, 1H, THP-CH_2–O), 1.74–1.53 (m, 6H, THP-CH_2); ^{13}C NMR (DMSO-d_6): δ, 161.0 (Ph-4-C), 156.6 (Fx-4-C), 138.1 (C=N), 131.2 (Ph-2,6-C), 116.8 (Ph-1-C), 116.4 (Ph-3,5-C), 111.4 (Fx-3-C), 101.90 (THP-CH–O), 62.6 (THP-CH_2–O), 28.88 (THP-CH_2), 25.41 (THP-CH_2), 19.56 (THP-CH_2). Anal. Calcd for $C_{14}H_{15}N_3O_5$ (305.29): C, 55.08; H, 4.95; N, 13.76. Found: C, 55.18; H, 5.03; N, 13.68.

4.14. 4-(4-Hydroxyphenyl)furazan-3-carbonitrile (9a)

A solution of **6a** (15.1 g, 57.2 mmol) in dioxane (100 mL) was stirred under reflux for 6 h with activated MnO_2 (49.7 g, 572 mmol). After cooling, the reaction mixture was filtered on a celite pad, the solvent was evaporated and the residue was dissolved in CH_3CN (100 mL). $NH_2OH \cdot HCl$ (4.37 g, 62.9 mmol) was added, followed by dry pyridine (5.09 mL, 62.9 mmol), then the reaction mixture was stirred at 60 °C for 1 h. After cooling, the solvent was evaporated, the residue was taken up in 1 N HCl (100 mL) and extracted with Et_2O (4 × 50 mL). The combined ethereal layers were cooled to 0 °C, extracted with ice-cold 10% NaOH (3 × 50 mL) and then discarded. The combined aqueous phase was washed with Et_2O (50 mL), then adjusted to pH 5 with acetic acid and extracted with Et_2O (4 × 50 mL). The combined ethereal extracts were washed with 10% $NaHCO_3$ (3 × 25 mL), brine (25 mL), dried and evaporated to yield a crude solid. The latter was dissolved in DMF (40 mL) and cooled to 0 °C, then $SOCl_2$ (8.35 mL, 114 mmol) was added. After 30 min, the reaction mixture was poured into ice/water (400 mL) and extracted with Et_2O (4 × 50 mL). The combined ethereal layers were washed with 10% $NaHCO_3$ (6 × 25 mL), brine (25 mL), dried and evaporated to yield a crude oil, which was purified by flash chromatography (eluent: petroleum ether with 20% EtOAc) obtaining **9a** (5.03 g, 47%) as a white solid. CIMS: m/z 188 (MH^+); mp 116–117 °C (n-hexane/$CHCl_3$). ^{1}H NMR (DMSO-d_6): δ, 10.44 (s, 1H, OH), 7.84 (d, 2H, J = 8.7 Hz, Ph-2,6-H), 7.04 (d, 2H, J = 8.7 Hz, Ph-3,5-H); ^{13}C NMR (DMSO-d_6): δ, 161.0 (Ph-4-C), 154.4 (Fz-4-C), 131.8 (Fz-3-C), 129.6 (Ph-2,6-C), 116.4 (Ph-3,5-C), 112.9 (Ph-1-C), 109.1 (CN). Anal. Calcd for $C_9H_5N_3O_2 \cdot 0.1H_2O$ (188.96): C, 57.21; H, 2.77; N, 22.24. Found: C, 57.48; H, 2.73; N, 21.85.

4.15. 4-(4-Hydroxyphenyl)furoxan-3-carboxamide (10)

To a solution of **9** (3.12 g, 15.4 mmol) in acetone (20 mL), 0.5 M K_2CO_3 (120 mL) was added. After 5 days under stirring, the reaction mixture was diluted with H_2O (100 mL), adjusted to pH 5 with acetic acid and then extracted with EtOAc (4 × 25 mL). The combined organic layers were washed with $NaHCO_3$ (10 × 10 mL), brine (10 mL), dried and evaporated to yield a crude product, which was purified by FC (eluent: petroleum ether/EtOAc 1:1) obtaining **10** (1.60 g, 47%) as a pale yellow solid. CIMS: m/z 222 (MH^+); mp 184 °C (dec) (iPr$_2$O). ^{1}H NMR (DMSO-d_6): δ, 10.19 (s, 1H, OH), 8.46 (s, NH_2), 8.38 (s, NH_2), 7.62 (d, 2H, J = 8.2 Hz, Ph-2,6-H), 6.93 (d, 2H, J = 8.2 Hz, Ph-3,5-H); ^{13}C NMR (DMSO-d_6): δ, 161.1 (Ph-4-C), 157.2* (Fx-4-C), 156.0* (C=O), 130.2 (Ph-2,6-C), 116.9 (Ph-1-C), 116.6 (Ph-3,5-C), 112.1 (Fx-3-C). Anal. Calcd for $C_9H_7N_3O_4$ (221.17): C, 48.88; H, 3.19; N, 19.00. Found: C, 48.72; H, 3.37; N, 18.79.

4.16. 4-(4-Hydroxyphenyl)furazan-3-carboxamide (10a)

To a solution of **9a** (3.56 g, 19.0 mmol) in acetone (20 mL), 0.5 M K_2CO_3 (120 mL) was added. After 5 days under stirring, the reaction mixture was worked up as for **10** obtaining **10a** (3.43 g, 88%) as a white solid. CIMS: *m*/*z* 206 (MH^+); mp 191–192 °C (dec) (*n*-hexane/*i*Pr_2O). ^{1}H NMR (DMSO-d_6): δ, 10.16 (s, 1H, OH), 8.65 (s, NH_2), 8.32 (s, NH_2), 7.72 (d, 2H, J = 8.4 Hz, Ph-2,6-H), 6.94 (d, 2H, J = 8.4 Hz, Ph-3,5-H); ^{13}C NMR (DMSO-d_6): δ, 160.0 (Ph-4-C), 159.6 (C=O), 152.4 (Fz-4-C), 148.9 (Fz-3-C), 130.0 (Ph-2,6-C), 115.9 (Ph-3,5-C), 114.7 (Ph-1-C). Anal. Calcd for $C_9H_7N_3O_3$ (205.17): C, 52.69; H, 3.44; N, 20.48. Found: C, 52.84; H, 3.66; N, 20.08.

4.17. General procedure for the synthesis of derivatives 13–15 and 13a–16a

To a solution of **12** (1.00 g, 2.71 mmol) in dry THF (30 mL) kept under N_2, the appropriate oxadiazole (Scheme 2, 3.25 mmol) and Ph_3P (1.42 g, 5.42 mmol) were added. The reaction mixture was cooled to 0 °C and DIAD (1.07 mL, 5.42 mmol) was added dropwise over 10 min; after 12 h under stirring at rt, the reaction mixture was evaporated to yield an orange oil. The latter was dissolved in 10% TFA in CH_2Cl_2 (40 mL) and stirred for 12 h; the reaction mixture was evaporated and the residue was dissolved in 1 N HCl (50 mL), filtering the insoluble matter on a celite pad. The filtrate was washed with Et_2O (3 × 20 mL), then the pH was adjusted to 8 with $NaHCO_3$, precipitating a white solid, which was collected on a Buchner funnel and washed with H_2O.

4.18. 4-(4-(3-(1*H*-Imidazol-4-yl)propoxy)phenyl)-3-methylfuroxan hydrochloride (13)

The crude precipitate was purified by FC (eluent: CH_2Cl_2 in gradient from 2.5% to 3.5% of MeOH) obtaining a white solid. A stream of $HCl_{(g)}$ was bubbled in a methanolic solution of the latter for 10 min; after 1 h, the solvent was evaporated and the residue was recrystallised from cold MeOH/Et_2O obtaining **13** (0.55 g, 60%) as a white solid. Mp 185–186 °C (MeOH/Et_2O). ^{1}H NMR (DMSO-d_6): δ, 9.08 (s, 1H, Im-2-H), 7.72 (d, 2H, J = 8.8 Hz, Ph-2,6-H), 7.49 (s, Im-5-H), 7.15 (d, 2H, J = 8.8 Hz, Ph-3,5-H), 4.12 (t, 2H, J = 6.1 Hz, CH_2–O), 2.86 (t, 2H, J = 7.5 Hz, Im-CH_2), 2.30 (s, 3H, CH_3), 2.16 (qn, 2H, J = 7.2 Hz, Im-CH_2–CH_2–CH_2–O); ^{13}C NMR (DMSO-d_6): δ, 160.7 (Ph-4-C), 157.1 (Fx-4-C), 133.6 (Im-2-C), 132.7 (Im-4-C), 129.3 (Ph-2,6-C), 118.7 (Ph-1-C), 115.6 (Im-5-C), 115.3 (Ph-3,5-C), 113.0 (Fx-3-C), 66.9 (CH_2–O), 27.5 (Im-CH_2), 20.8 (Im-CH_2–CH_2–CH_2–O), 9.1 (CH_3). Anal. Calcd for $C_{15}H_{17}ClN_4O_3$ (336.78): C, 53.50; H, 5.09; N, 16.64. Found: C, 53.60; H, 5.23; N, 16.54.

4.19. 3-(4-(3-(1*H*-Imidazol-4-yl)propoxy)phenyl)-4-methylfurazan hydrochloride (13a)

Operating as for **13**, **13a** was obtained (0.51 g, 59%) as a white solid. Mp 162–163 °C (MeOH/Et_2O). ^{1}H NMR (DMSO-d_6): δ, 9.07 (s, 1H, Im-2-H), 7.76 (d, 2H, J = 8.8 Hz, Ph-2,6-H), 7.50 (s, Im-5-H), 7.14 (d, 2H, J = 8.8 Hz, Ph-3,5-H), 4.11 (t, 2H, J = 6.1 Hz, CH_2–O), 2.86 (t, 2H, J = 7.5 Hz, Im-CH_2), 2.56 (s, 3H, CH_3), 2.15 (qn, 2H, J = 7.0 Hz, Im-CH_2–CH_2–CH_2–O); ^{13}C NMR (DMSO-d_6): δ, 160.3 (Ph-4-C), 153.7 (Fz-4-C), 150.5 (Fz-3-C), 133.6 (Im-2-C), 132.8 (Im-4-C), 129.8 (Ph-2,6-C), 117.7 (Ph-1-C), 115.6 (Im-5-C), 115.3 (Ph-3,5-C), 66.8 (CH_2–O), 27.6 (Im-CH_2), 20.8 (Im-CH_2–CH_2–CH_2–O), 9.4 (CH_3). Anal. Calcd for $C_{15}H_{17}ClN_4O_2 \cdot 0.2H_2O$ (324.38): C, 55.54; H, 5.41; N, 17.27. Found: C, 55.65; H, 5.37; N, 17.19.

4.20. (4-(4-(3-(1*H*-Imidazol-4-yl)propoxy)phenyl)furoxan-3-yl)methanol hydrochloride (14)

The crude precipitate was dissolved in warm MeOH (20 mL) and then Boc_2O (0.50 g, 2.29 mmol) was added. After 1 h, the solvent was evaporated and the residue was purified by FC (eluent: CH_2Cl_2 in gradient from 1 to 5% of MeOH) obtaining a white solid. A stream of $HCl_{(g)}$ was bubbled in a methanolic solution of the latter for 10 min; after 1 h, the solvent was evaporated and the residue was recrystallised from cold MeOH/Et_2O obtaining **14** (0.36 g, 38%) as a white solid. Mp 166–167 °C (dec) (MeOH/Et_2O). ^{1}H NMR (D_2O + CD_3OD): δ, 8.46 (s, 1H, Im-2-H), 7.53 (d, 2H, J = 8.7 Hz, Ph-2,6-H), 7.09 (s, Im-5-H), 6.90 (d, 2H, J = 8.7 Hz, Ph-3,5-H), 4.49 (s, 2H, CH_2–OH), 3.97 (t, 2H, J = 5.8 Hz, –CH_2–CH_2–O), 2.77 (t, 2H, J = 7.4 Hz, Im-CH_2), 2.01 (qn, 2H, J = 6.6 Hz, Im-CH_2–CH_2–CH_2–O); ^{13}C NMR (D_2O + CD_3OD): δ, 161.5 (Ph-4-C), 157.3 (Fx-4-C), 134.0 (Im-4-C), 133.5 (Im-2-C), 129.5 (Ph-2,6-C), 119.2 (Ph-1-C), 115.9 (Im-5-C), 115.3 (Ph-3,5-C), 115.3 (Fx-3-C), 67.1 (–CH_2–CH_2–O), 52.5 (CH_2–OH), 28.1 (Im-CH_2), 21.3 (Im-CH_2–CH_2–CH_2–O). Anal. Calcd for $C_{15}H_{16}N_4O_4 \cdot 1.5HCl \cdot 0.3H_2O$ (376.41): C, 47.86; H, 4.85; N, 14.88. Found: C, 47.51; H, 4.68; N, 15.22.

4.21. (4-(4-(3-(1*H*-Imidazol-4-yl)propoxy)phenyl)furazan-3-yl)methanol hydrochloride (14a)

Operating as for **14**, **14a** was obtained (0.29 g, 32%) as a white solid. Mp 159–160.5 °C (abs EtOH/acetone). ^{1}H NMR (DMSO-d_6): δ, 8.99 (s, 1H, Im-2-H), 7.89 (d, 2H, J = 8.8 Hz, Ph-2,6-H), 7.46 (s, Im-5-H), 7.12 (d, 2H, J = 8.8 Hz, Ph-3,5-H), 5.97 (br s, 1H, OH), 4.80 (s, 2H, CH_2–OH), 4.11 (t, 2H, J = 6.1 Hz, –CH_2–CH_2–O), 2.84 (t, 2H, J = 7.5 Hz, Im-CH_2), 2.13 (qn, 2H, J = 6.8 Hz, Im-CH_2–CH_2–CH_2–O); ^{13}C NMR (DMSO-d_6): δ, 160.4 (Ph-4-C), 153.6* (Fz-4-C), 153.4* (Fz-3-C), 133.6 (Im-2-C), 132.7 (Im-4-C), 130.0 (Ph-2,6-C), 117.5 (Ph-1-C), 115.6 (Im-5-C), 115.2 (Ph-3,5-C), 66.8 (–CH_2–CH_2–O), 52.5 (CH_2–OH), 27.5 (Im-CH_2), 20.8 (Im-CH_2–CH_2–CH_2–O). Anal. Calcd for $C_{15}H_{16}N_4O_3 \cdot HCl \cdot 0.4H_2O$ (343.98): C, 52.38; H, 5.22; N, 16.29. Found: C, 52.65; H, 5.24; N, 16.00.

4.22. 4-(4-(3-(1*H*-Imidazol-4-yl)propoxy)phenyl)furoxan-3-carboxamide hydrochloride (15)

The crude precipitate was dissolved in warm MeOH (20 mL) and then Boc_2O (0.30 g, 1.37 mmol) was added. After 1 h, the solvent was evaporated and the residue

was purified by FC (eluent: petroleum ether/EtOAc 1:1) obtaining a white solid. To a solution of the latter in acetone (20 mL), Et_2O saturated with $HCl_{(g)}$ (10 mL) was added. After refluxing for 1 h, the solvent was evaporated and the residue was recrystallised from abs EtOH/acetone obtaining **15** (0.28 g, 28%) as a white solid. Mp 179–180 °C (abs EtOH/acetone). ^{1}H NMR (CD_3OD): δ, 8.83 (s, 1H, Im-2-H), 7.75 (d, 2H, J = 8.8 Hz, Ph-2,6-H), 7.37 (s, Im-5-H), 7.04 (d, 2H, J = 8.8 Hz, Ph-3,5-H), 4.15 (t, 2H, J = 5.9 Hz, CH_2–O), 2.98 (t, 2H, J = 7.5 Hz, Im-CH_2), 2.22 (qn, 2H, J = 7.0 Hz, Im-CH_2–CH_2–CH_2–O); ^{13}C NMR (CD_3OD): δ, 161.5 (Ph-4-C), 157.6 (Fx-4-C), 156.8 (C═O), 134.0 (Im-4-C), 133.7 (Im-2-C), 130.6 (Ph-2,6-C), 118.8 (Ph-1-C), 115.9 (Im-5-C), 114.5 (Ph-3,5-C), 111.3 (Fx-3-C), 66.9 (CH_2–O), 28.1 (Im-CH_2), 21.2 (Im-CH_2–CH_2–CH_2–O). Anal. Calcd for $C_{15}H_{15}N_5O_4$·HCl (365.78): C, 49.26; H, 4.41; N, 19.15. Found: C, 49.19; H, 4.49; N, 18.78.

4.23. 4-(4-(3-(1*H*-Imidazol-4-yl)propoxy)phenyl)furazan-3-carboxamide hydrochloride (15a)

Operating as for **15**, **15a** was obtained (0.32 g, 34%) as a white solid. Mp 178.5–179.5 °C (abs EtOH/acetone). ^{1}H NMR (D_2O): δ, 8.46 (s, 1H, Im-2-H), 7.63 (d, 2H, J = 8.8 Hz, Ph-2,6-H), 7.12 (s, Im-5-H), 6.95 (d, 2H, J = 8.8 Hz, Ph-3,5-H), 4.06 (t, 2H, J = 6.0 Hz, CH_2–O), 2.83 (t, 2H, J = 7.3 Hz, Im-CH_2), 2.08 (qn, 2H, J = 6.6 Hz, Im-CH_2–CH_2–CH_2–O); ^{13}C NMR (D_2O): δ, 161.4 (C═O), 160.6 (Ph-4-C), 153.5 (Fz-4-C), 148.1 (Fz-3-C), 133.3 (Im-4-C), 133.1 (Im-2-C), 130.8 (Ph-2,6-C), 116.5 (Ph-1-C), 115.6 (Im-5-C), 115.1 (Ph-3,5-C), 67.3 (CH_2–O), 27.4 (Im-CH_2), 20.9 (Im-CH_2–CH_2–CH_2–O). Anal. Calcd for $C_{15}H_{15}N_5O_3$·HCl (349.78): C, 51.51; H, 4.61; N, 20.02. Found: C, 51.34; H, 4.61; N, 19.72.

4.24. 4-(4-(3-(1*H*-Imidazol-4-yl)propoxy)phenyl)furazan-3-carbonitrile hydrochloride (16a)

The crude precipitate was dissolved in acetone (20 mL) and then Boc_2O (0.50 g, 2.29 mmol) was added. After 1 h, the solvent was evaporated and the residue was purified by FC (eluent: petroleum ether/EtOAc 7:3) obtaining a white solid. To a solution of the latter in acetone (20 mL), Et_2O saturated with $HCl_{(g)}$ (10 mL) was added. After refluxing for 1 h, the solvent was evaporated and the residue was recrystallised from acetone/iPr$_2$O obtaining **16a** (0.24 g, 26%) as a white solid. Mp 190–191 °C (dec) (acetone/iPr$_2$O). ^{1}H NMR (DMSO-d_6): δ, 9.06 (s, 1H, Im-2-H), 7.92 (d, 2H, J = 8.7 Hz, Ph-2,6-H), 7.48 (s, Im-5-H), 7.23 (d, 2H, J = 8.7 Hz, Ph-3,5-H), 4.14 (t, 2H, J = 6.1 Hz, CH_2–O), 2.85 (t, 2H, J = 7.5 Hz, Im-CH_2), 2.16 (qn, 2H, J = 6.8 Hz, Im-CH_2–CH_2–CH_2–O); ^{13}C NMR (DMSO-d_6): δ, 162.3 (Ph-4-C), 155.2 (Fz-4-C), 134.3 (Im-2-C), 133.4* (Fz-3-C), 133.0* (Im-4-C), 130.6 (Ph-2,6-C), 116.5 (Ph-3,5-C), 116.4 (Im-5-C), 115.6 (Ph-1-C), 110.0 (CN), 67.8 (CH_2–O), 28.2 (Im-CH_2), 21.5 (Im-CH_2–CH_2–CH_2–O). Anal. Calcd for $C_{15}H_{13}N_5O_2$·HCl (331.76): C, 54.31; H, 4.25; N, 21.11. Found: C, 54.14; H, 4.30; N, 20.78.

4.25. 4-(4-(3-(1*H*-Imidazol-4-yl)propoxy)phenyl)furoxan-3-carbonitrile hydrochloride (16)

To a solution of **12** (1.48 g, 4.02 mmol) in dry THF (40 mL) kept under N_2, **11** (1.47 g, 4.82 mmol) and Ph_3P (2.11 g, 8.04 mmol) were added. The reaction mixture was cooled to 0 °C and DIAD (1.58 mL, 8.04 mmol) was added dropwise over 10 min; after 12 h under stirring at rt, the reaction mixture was evaporated to yield an orange oil, which was dissolved in 1 N p-toluenesulfonic acid in MeOH (50 mL). After 4 h, the reaction mixture was cooled to 0 °C and Et_3N (7 mL) was added, then it was poured into water (500 mL) and extracted with Et_2O (4 × 50 mL). The combined organic layers were washed with 5% citric acid (50 mL), brine (25 mL), dried and evaporated to yield a yellow oil. To a solution of the latter in DMF (20 mL) kept under N_2 at −40 °C $SOCl_2$ (4 mL, 54.8 mmol) was added dropwise over 30 min and then the cooling bath was removed. When the reaction mixture reached 18 °C, it was poured into water (500 mL) and extracted with Et_2O (4 × 50 mL). The combined organic layers were washed with 10% $NaHCO_3$ (3 × 50 mL), brine (25 mL), dried and evaporated to yield a crude oil, which was purified by FC (eluent: petroleum ether/EtOAc 6:4). The product was dissolved in 10% TFA in CH_2Cl_2 (40 mL) and stirred for 12 h; the reaction mixture was evaporated and the residue was dissolved in 1 N HCl (50 mL), filtering the insoluble matter on a celite pad. The filtrate was washed with Et_2O (3 × 20 mL), then the pH was adjusted to 8 with $NaHCO_3$, precipitating a white solid, which was collected on a Buchner funnel and washed with H_2O. The crude precipitate was dissolved in warm CH_3CN (20 mL) and then Boc_2O (0.50 g, 2.29 mmol) was added. After 1 h, the solvent was evaporated and the residue was purified by FC (eluent: petroleum ether/EtOAc 7:3) obtaining a white solid. To a solution of the latter in acetone (20 mL), Et_2O saturated with $HCl_{(g)}$ (10 mL) was added. After refluxing for 1 h, the solvent was evaporated and the residue was recrystallised from CH_3CN/acetone obtaining **16** (0.31 g, 22%) as a white solid. Mp 174 °C (dec) (CH_3CN/acetone). ^{1}H NMR (DMSO-d_6): δ, 9.08 (s, 1H, Im-2-H), 7.82 (d, 2H, J = 7.7 Hz, Ph-2,6-H), 7.48 (s, Im-5-H), 7.23 (d, 2H, J = 7.7 Hz, Ph-3,5-H), 4.14 (s, 2H, CH_2–O), 2.86 (s, 2H, Im-CH_2), 2.17 (s, 2H, Im-CH_2–CH_2–CH_2–O); ^{13}C NMR (DMSO-d_6): δ, 161.6 (Ph-4-C), 154.6 (Fx-4-C), 133.3 (Im-2-C), 132.5 (Im-4-C), 128.6 (Ph-2,6-C), 115.9 (Ph-1-C), 115.6 (Ph-3,5-C), 115.4 (Im-5-C), 107.5 (CN), 98.1 (Fx-3-C), 66.9 (CH_2–O), 27.3 (Im-CH_2), 20.6 (Im-CH_2–CH_2–CH_2–O). Anal. Calcd for $C_{15}H_{13}N_5O_3$·HCl (347.76): C, 51.81; H, 4.06; N, 20.14. Found: C, 51.76; H, 4.05; N, 19.96.

4.26. Lipophilicity studies

Potentiometric titrations were performed with the GLpK_a apparatus (Sirius Analytical Instruments Ltd, Forrest Row, East Sussex, UK).[9] Ionisation constants were determined by four separate titrations for each compound (ca. 0.5 mM).[17] The low aqueous solubility of the compounds required titrations in the presence of variable amounts of methanol as cosolvent (20–50 wt%); pK_a values were determined by extrapolation to

zero content of cosolvent according to the Yasuda–Shedlovsky procedure.[18] To obtain lipophilicity data, at least four separate titrations for each compound (ca. 0.5 mM), containing various volumes of octan-1-ol (from 1 mL of organic solvent/15 mL of H_2O to 10 mL of organic solvent/5 mL of H_2O), were performed in the pH range 2–11.5. Log *P* data were obtained by the Multiset approach.[17,19] All titrations were carried out under Ar at 25.0 ± 0.1 °C.[19]

4.27. Functional studies

Ileum segments were isolated from male albino guinea-pigs weighing 250–350 g, which had been anaesthetised with CO_2 and killed by decapitation. All animals were treated humanely in accordance with recognised guidelines on experimentation. As few animals as possible were used. The purposes and the protocols of our studies have been approved by the Ministero della Salute, Rome, Italy. The tissues were mounted in organ baths containing 30 mL of Krebs-bicarbonate buffer of the following composition (mM): NaCl 111.2, KCl 5.0, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.0, $NaHCO_3$ 12 and glucose 11.1. The solution was maintained at 37 °C and continuously gassed with 95% O_2–5% CO_2 (pH 7.4). After equilibration for 2 h, with washings every 20 min, the muscle segments were stimulated with square wave pulses (10 V, 24 PPM, 0.5 ms) for 30 min and then cumulative concentration–response curves (half-log increments) of the histamine H_3 agonist MHA were recorded until no change in response was found. To avoid desensitisation phenomena, a single 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 (1 μM pyrilamine and 1 μM ranitidine) to prevent the activation of these receptors by the highest concentration of MHA. In the case of derivative **16**, we determined the pA_2 value in the presence of 1 μM ODQ, which was added to the bath at least 15 min before the addition of the antagonist. pA_2 values are means of 6–12 determinations and were estimated at two concentrations for **13** and **13a** (0.1 and 0.3 μM) and at one concentration for the other compounds (1 μM). Responses were recorded by an isometric transducer connected to the MacLab System PowerLab.

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