Studies on Agents with Mixed NO-Dependent Vasodilating and β-Blocking Activities

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Purpose. A series of derivatives having a propranolol-like moiety linked to NO-donor furoxan substructures were synthesized. The main objective of this investigation was to obtain agents with mixed NO-dependent vasodilating and β-blocking activities.

Methods. Most of the target compounds were synthesized from the appropriate furoxans bearing $XCH_2CH_2NH_2$ (X = O, S, SO_2) chains at the 4 position of the ring, using $Al(C_2H_5)_3$ in methylene chloride solution and (±)2,3-epoxypropyl 1-naphtyl ether. Two of the final products (X = CONH) were obtained by coupling the appropriate furoxancarboxylic acids with *N*-[2-hydroxy-3-(1-naphthoxy)propyl]-ethylenediamine. β_1- and β_2-blocking activities were examined on isolated guinea pig right atria and on guinea pig trachea respectively. Vasodilating properties were assessed on endothelium denuded strips of rat aorta.

Results. Some derivatives behave as well balanced "hybrids" displaying NO-dependent vasodilating and β-blocking properties in the same concentration range. Some others display either prevalent β-blocking or vasodilating activity. Generally speaking hybrid formation lowers the affinity for β-receptors, in particular for β_2-type, to give an increase in β_1/β_2 selectivity.

Conclusions. The furoxan system is a flexible tool in designing analogues of propranolol whose NO-donating and β-blocking properties are modulated over a wide range.

KEY WORDS: NO-donors; β-blockers; hybrid drugs; furoxans; vasodilation.

INTRODUCTION

The combination of different pharmacophoric groups in a single molecule to obtain "hybrid" drugs is a strategy frequently used to design new compounds potentially useful in the management of diseases with complex and heterogeneous pathogenesis (1–3). The necessary restrictions of this approach have been recently discussed (3). They concern principally the necessity that the combined pharmacophors exert their action *via* different mechanisms in the same concentration range. Cardiovascular diseases are a classical area in which hybrid drugs could be used. In fact, these complex pathologies are frequently treated by simultaneous administration of different drugs. The use of "hybrids" could solve a number of pharmacokinetic problems connected with these therapies and have some advantages in comparison with the use of mixture of drugs, in particular when different therapeutic effects should occur in the same body compartment and with the same onset. Combinations of pharmacophoric moieties giving antihypertensive, antiarrhytmic, antianginal and cardiotonic drugs have been reviewed (1–3). The finding that nitric oxide (NO) exerts important roles in maintaining homeostatic equilibrium, principally by dilating arterial blood vessels, inhibiting platelet adherence and aggregation, and attenuating leukocyte adhesion and activation, has opened new possibilities in the design of hybrid cardiovascular drugs (4). A number of possibilities have been recently explored (3,5). They are principally the combination of a nitrate (-O-NO_2) NO-donor moiety with Ca^{2+} blocking, with K^+ channel activating, with α_1- and β_1-adrenoceptor antagonist, and with H_2-agonist pharmacophoric groups. Also a nitrosothiol (-S-NO) NO-donor moiety has been combined with captopril, a well known ACE inhibitor. Furoxans (1,2,5-oxadiazole 2-oxide derivatives) appear to be particularly flexible tools for this approach. There is interesting evidence that furoxans could behave as NO-prodrugs under physiological conditions. In fact many of these compounds possess biological properties typical of nitric oxide. In particular they induce inhibition of platelet aggregation, vasodilation and these effects are mediated by stimulation of guanylate cyclase (6,7). In cell free system (purified rat liver or lung guanylate cyclase) (7,8) and in RFL-6 cells (9), enzyme stimulation is markedly enhanced by the presence of thiol cofactors. Using furoxancarbonamides as models, NO release was evidenced in the presence of a number of thiols. This reaction is accompanied by formation of nitrites and nitrates ions (7). In tissues enzymatic NO generation could also be involved (10). NO production by furoxans appears to be a complex matter. Some mechanisms were hypothesised (7,11). They take into account the intermediacy of nitrosothiols, direct release of NO center dot and/or release of intermediate nitroxyl anion NO^-. Indeed this subject has been treated principally under a speculative point of view and still needs an experimental mechanistic study. Using appropriate substituents at the ring, it is possible to modulate over a wide range the extent and the initial rate of thiol induced NO production, and hence the NO dependent vasodilating properties (5,12). Furthermore these substances can be easily grafted to appropriate pharmacophoric groups by suitable spacers. As far as the pharmacological properties are concerned, furoxan moieties exert the positive antianginal effects of nitrovasodilators, but could lack significant tolerance development (10,13). Differences in metabolic activation, as well as in the onset and in the potency of antianginal activity, are other properties which may render these structures useful drugs in the treatment of coronary heart disease. We recently described a series of hybrids obtained by substituting furoxan derivatives for the furan ring in Prazosin, a well known α_1-blocking drug. We obtained well-balanced hybrids potentially useful as vasodilating compounds (14).

In this paper we describe a series of models obtained according to the scheme depicted in Fig. 1, by combining a series of furoxan derivatives with NO-donor properties modulated over a wide range, with a propranol-like structure. The

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NOTATION: DMSO-d_6, dimethylsulfoxide-d_6; THF, tetrahydrofuran; EtOAc, ethyl acetate; NHS, N-hydroxysuccinimide; CMC, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluensulfonate; DCC, dicyclohexylcarbodiimide; $(BOC)_2O$, di-*tert*-butyl dicarbonate.

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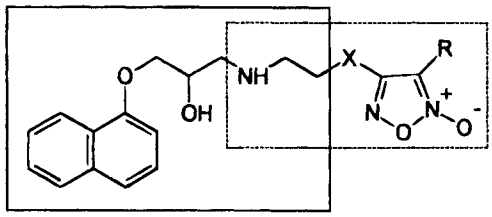


A

Fig. 1. General structure of studied hybrid models.

resulting structure **A** (Fig. 1) can be considered as a naphthyloxypropanol-like β-antagonist bearing R-furoxanyl-X(O,S,SO_2,CONH,)- substituents at the lateral alkylamino substructure. Introduction of aryl-X(O,S,SO_2,CONH)-substituents into lateral alkylamino moieties of aryloxy β-blockers, is a known structural modification which can afford selective and in some cases potent β_1-antagonists (15). A number of furazan analogues have been also prepared and investigated for control purpose, since they are unable to release NO.

MATERIALS AND METHODS

Chemistry

Melting points were measured on a Büchi 530 capillary apparatus and are uncorrected. Melting points with decomposition were determined after introducing the sample into the bath at a temperature 10°C lower than the melting point. A heating rate of 3°C min^{-1} was used. The compounds were routinely checked by infrared spectrometry (Shimadzu FT-IR 8101M), ^1H and ^{13}C nuclear magnetic resonance at 200 and 50 MHz respectively (Bruker AC-200 spectrometer), and mass spectrometry (Finnigan-Mat TSQ-700 spectrometer). All the spectra were in keeping with the proposed structures. Complete ^1H and ^{13}C NMR characterization is available from the authors as Supplementary Material. Only the ^{13}C NMR spectra of the final compounds (**18–24, 27, 28**) are reported in Table I. The assignments of these spectra were confirmed by DEPT pulse sequence. Column chromatography was performed on silica gel (Merck Kieselgel 60, 230–400 mesh ASTM) with the indicated solvent system. Thin layer chromatography (TLC) was carried out on 5 × 20 cm plates precoated with Merck silica gel 60 F_{254}, with a layer thickness of 0.25 mm. Anhydrous magnesium sulphate was used as the drying agent of the organic extracts. Solvent removal was achieved under reduced pressure at room temperature. Elemental analyses of the new compounds were performed by REDOX (Cologno M.) and the results are within ± 0.4% of theoretical values.

Intermediates **1** (16), **3** (17), **6** (12), **10** (16), **13** (18), **15** (12), **17** (19), **25** (20), **26** (21), **29** (22) were synthesised according to literature methods.

4-(2-Aminoethyloxy)-3-phenylfuroxan (2) Oxalate

We first added 2-aminoethanol (0.39 mL, 6.6 mmol) and then 0.80 g of 50% w/w water NaOH solution (9.9 mmol) to a stirred solution of 1 (1.0 g, 3.3 mmol) in THF (20 mL). NaOH solution was added portionwise, while the temperature was maintained at 25°C. The reaction mixture was stirred at 25°C for 7 h. Solvent removal gave a residue which was treated with water and extracted with CH_2Cl_2. The combined organic layers were evaporated *in vacuo* to obtain the pure title compound (0.70 g, 96%) as white solid. The base was immediately transformed into the corresponding oxalate: mp 183–184°C dec (MeOH/H_2O); anal. ($C_{10}H_{11}N_3O_3 \cdot H_2C_2O_4 \cdot 0.5\ H_2O$) C,H,N.

3-(2-Aminoethyloxy)-4-phenylfurazan (4) Oxalate

Derivative **3** (0.57 g, 3.0 mmol) was dissolved in dry *tert*-butanol (10 mL) and then 2-aminoethanol (0.19 g, 3.0 mmol) and potassium *tert*-butoxide (0.34 g, 3.0 mmol) were added to the stirred solution at room temperature. The stirring was continued for 1 h, and then the solvent was removed *in vacuo*. The residue was treated with water saturated with NaCl and extracted with CH_2Cl_2. The dried combined organic layers were evaporated *in vacuo* and the base so obtained (0.50 g, 80%) was transformed into the corresponding oxalate and recrystallized from EtOH. After drying for 6 days at 40°C in vacuum drying pistol, the compound had a melting point of 188–189°C with decomposition. Anal. ($C_{10}H_{11}N_3O_2 \cdot H_2C_2O_4 \cdot 0.5\ H_2O$) C, H, N.

3-(2-Aminoethylthio)-4-phenylfurazan (5) Oxalate

We first added 2-aminoethanethiol hydrochloride (0.34 g, 3.0 mmol) and then 0.48 g of 50% w/w water NaOH solution (6 mmol) to a stirred solution of **3** (0.57 g, 3.0 mmol) in THF (15 mL) kept under nitrogen. NaOH solution was added portionwise maintaining the temperature at 25°C. The reaction mixture was stirred under nitrogen at room temperature for 30 min. *In vacuo* solvent removal gave a residue which was treated with water and extracted with CH_2Cl_2. The combined organic layers were extracted with 1N HCl. The acid layers were extracted once with CH_2Cl_2 and then basified with 1N NaOH and extracted with CH_2Cl_2. The dried combined organic layers were evaporated *in vacuo* to give the pure title compound as an oil (0.5 g, 75%), which solidified when kept in dessicator. The free base was transformed into the corresponding oxalate and crystallized from MeOH. After drying for 6 days at 40°C in vacuum drying pistol, the compound had a melting point of 187–188°C with decomposition. Anal. ($C_{10}H_{11}N_3OS \cdot H_2C_2O_4 \cdot 0.5\ H_2O$) C, H, N.

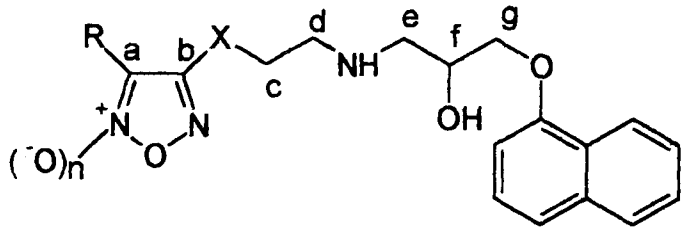
tert-Butyl N-[2-(3-phenyl-4-furoxanylthio)ethyl]carbamate (7)

Di-*tert*-butyl dicarbonate (0.48 g, 2.2 mmol) was added to a stirred solution of **6** (0.50 g, 2.1 mmol) in CH_2Cl_2 (15 mL). Stirring was continued for 4 h and then the solvent was removed *in vacuo* to give quantitative yields of the title product, pure enough to be used for further transformation. mp 98°C (EtOH/water); EI-MS: 337 (M^+). Anal. ($C_{15}H_{19}N_3O_4S$) C, H, N.

tert-Butyl N-[2-(3-phenyl-4-furoxansulfonyl)ethyl]carbamate (8)

To a stirred and ice-cooled solution of **7** (7.47 g, 22.1 mmol) in glacial acetic acid (50 mL), $KMnO_4$ (6.98 g, 44.2 mmol) was added portionwise. The water-cooled reaction mixture was stirred for 3 h and then diluted with water. Na_2SO_3 was added portionwise until the violet colour disappeared. The white precipitate formed was filtered and then the solid dis-

Table I. ^{13}C-NMR Chemical Shifts (ppm) of the Propranolol Analogues as Oxalates[a] (DMSO-d_6, TMS-int)[b]



Compound	n	R	X	PhC_4	PhC_2/C_3	PhC_1	a	b	c	d	e	f	g	$CONH_2$	CONH
18	1	Ph	O	130.7	126.6[c]/129.0	121.9[c]	107.8	162.0	66.9	45.8	50.2	65.4	70.1		
19	0	Ph	O	131.1	127.6/129.2	124.2	145.5	163.2	68.9	45.9	50.2	65.4	70.2		
20	1	Ph	S	131.1	127.7/129.3	121.9[c]	114.5	154.1	26.8	45.9	50.0	65.5	70.1		
21	0	Ph	S	131.6	128.0/129.5	124.4	152.8[d]	151.1[d]	28.5	45.8	50.0	65.5	70.1		
22	1	Ph	SO_2	131.5	129.7/128.8	120.6	113.4	156.9	51.6[d]	51.9[d]	50.2	65.7	70.2		
23	1	$PhSO_2$	O	136.2	128.6/130.0	136.9	111.0	158.7	67.3	45.4	50.2	65.3	70.1		
24	0	$PhSO_2$	O	136.2	128.6/130.3	137.0	149.0	161.0	69.9	45.5	50.1	65.4	70.1		
27	1	NH_2CO	CONH				110.1	151.1	36.0	46.3	50.0	65.5	70.1	155.2[d]	156.6[d]
28	1	Ph	CONH	130.8	128.9/128.6	121.9[c]	113.9	151.8	35.9	46.3	49.9	65.4	70.1		157.3

[a] ^{13}Cδ $H_2C_2O_4$: 163–165 ppm.
[b] Naphtalene ring carbons show the same chemical shifts in all the compounds, within a range of ± 0.1 ppm: 153.8, 134.1, 127.5, 126.6, 126.3, 125.3, 124.9, 121.9, 120.3, 105.3.
[c] Overlapping with naphtalene ring carbons.
[d] Interchangeable.

solved in CH_2Cl_2 and the solution was dried and evaporated *in vacuo* to give a residue which was recrystallized from EtOH (6.86 g, 84%): mp 134–135°C (EtOH); EI-MS: 369 (M^+). Anal. ($C_{15}H_{19}N_3O_6S$) C, H, N.

4-(2-Aminoethansulfonyl)-3-phenylfuroxan (9)

A solution of **8** (1.48 g, 4.0 mmol) in CF_3COOH (2.5 mL) was stirred for 10 min at room temperature and then was poured into 10 ml of ice-cooled water basified with 0.5 M Na_2CO_3 and extracted with CH_2Cl_2. The dried combined organic layers were evaporated *in vacuo* to give **9** (0.64 g; 60%) as an unstable oil that was immediately used for the preparation of the final compound.

Bis-(benzenesulfonyl)furazan (11)

A solution of **10** (1.82 g, 5.0 mmol) in trimethylphosphite (10 mL) was refluxed for 1h and then was poured into 30 mL of ice-cooled 5M H_2SO_4. The white precipitate formed was collected by filtration, washed with water and dried (1.57 g, 90%). After crystallization from MeOH and drying for 40 h at 65°C in vacuum drying pistol it melted at 119–120°C. EI-MS: 350 (M^+). Anal. ($C_{14}H_{10}N_2O_5S_2$) C, H, N.

3-(2-Aminoethyloxy)-4-benzenesulfonylfurazan (12)

A solution of 2-aminoethanol (0.26 mL, 4.3 mmol) and potassium *tert*-butoxide (0.48 g, 4.3 mmol) in dry *tert*-butanol (10 mL) was dropped into a refluxing solution of **11** (1.50 g, 4.3 mmol) in dry *tert*-butanol (20 ml). After 1.5 h the reaction was terminated and the solvent was evaporated *in vacuo*. The residue was treated with water and extracted with CH_2Cl_2. The combined organic layers were washed with a small amount of water and dried. *In vacuo* solvent removal gave the pure title compound (0.58 g, 50%) which was immediately transformed into the corresponding oxalate. The product decomposes during crystallization. After drying for 72 h at 40°C in vacuum drying pistol it melted at 171–172°C with decomposition. Anal. ($C_{10}H_{11}N_3O_4S \cdot H_2C_2O_4 \cdot 0.5\ H_2O$) C, H, N.

3-Carbamoyl-4-furoxancarboxylic Acid (14)

A stirred and ice-cooled solution of **13** (7.0 g) in EtOH (70 mL) was saturated with ammonia. The reaction mixture was stirred at room temperature for 1 h. *In vacuo* solvent removal afforded a residue which was treated with water, acidified with 6N HCl and extracted with EtOAc. The dried combined organic layers were evaporated *in vacuo* to give the pure title compound (4.20 g, 65%): mp 150–151°C dec. (EtOAc/petroleum ether); CI-MS : 174 $(M + 1)^+$. Anal. ($C_4H_3N_3O_5$) C, H, N.

4-(2-Aminoethylaminocarbonyl)-3-furoxancarboxamide (31) Hydrochloride and 4-(2-Aminoethylaminocarbonyl)-3-phenylfuroxan (32) Hydrochloride.

NHS (1.01 g, 8.8 mmol) and CMC (3.73 g, 8.8 mmol) were added under dry atmosphere to a stirred mixture of the appropriate furoxancarboxylic acid **14** or **15** (8.0 mmol) in dry CH_2Cl_2 (70 mL). The stirring was continued for 1 h and then a solution of **29** (1.28 g, 8.0 mmol) in dry CH_2Cl_2 (15 mL) was added. After 1.5 h the reaction mixture was washed first with water and then with phosphate buffer (0.5 M, pH 5.4). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford **14a** or **25a** as a white solid. The BOC group was removed dissolving the compound in ice-cooled EtOAc (**14a**) or in THF (**25a**) and bubbling gaseous HCl until saturation. After 1 h the solution was removed in vacuo and the residue was triturated with diethyl ether, filtered and dried.

31: 0.71g, yield 35%. Darkening at 202° C, decomposed 213° C (MeOH/diethyl ether). Anal. ($C_6H_9N_5O_4 \cdot HCl \cdot 0.25\ H_2O$), C, H, N.

32: 1.49 g, yield 65%. M.p. 220–221° C dec. (MeOH/diethyl ether). Anal. ($C_{11}H_{12}N_4O_3 \cdot HCl$) C, H, N.

General Procedure for the Synthesis of 1-(1-naphthyloxy)-3-[2-(3-R-4-furoxanyl-X-)ethylamino]propan-2-ol ***18*** *(R=Ph, X=O),* ***20*** *(R=Ph, X=S),* ***22*** *(R=Ph, X=SO_2),* ***23*** *(2 = PhSO_2, X=O) Oxalates and of the Related Furazans* ***19, 21, 24*** *Oxalates*

A solution of Et_3Al (5.5 mmol) in dry toluene (3 mL) was added dropwise over 5 min to a stirred solution of the appropriate intermediate amino derivative (5.0 mmol) in dry CH_2Cl_2 (15 mL), kept under nitrogen. The reaction mixture was stirred for 30 min and then a solution of (±)2,3-epoxypropyl 1-naphtyl ether (1.1 g, 5.5 mmol) in dry CH_2Cl_2 was added. The stirring was continued for 6–18 h at 45°C and then the mixture was treated with a saturated solution of Na_2SO_4 (15 mL) and celite. After 4 h of vigorous stirring at room temperature the mixture was filtered and the dried organic layer was evaporated *in vacuo*. The residue was purified by flash chromatography (CH_2Cl_2:MeOH 95:5 for **18, 19, 20, 22, 23, 24,** and CH_2Cl_2:EtOAc 90:10 for **21**). Reaction times, yields of free bases, recrystallisation solvents, melting points of the corresponding oxalates, formula and elemental analyses are reported below.

18: 18 h reaction time; yield 49%; m.p. 184–187° C dec. (MeOH); anal. ($C_{23}H_{23}N_3O_5 \cdot H_2C_2O_4$) C, H, N.

19: 18 h reaction time; yield 62%; m.p. 189–190° C dec. (EtOH); anal. ($C_{23}H_{23}N_3O_4 \cdot H_2C_2O_4$) C, H, N.

20: 18 h reaction time; yield 46%; m.p. 175–178° C dec. (MeOH); anal. ($C_{23}H_{23}N_3O_4S \cdot H_2C_2O_4$) C, H, N.

21: 4 h reaction time; yield 67%; m.p. 197–198° C dec. (EtOH); anal. ($C_{23}H_{23}N_3O_3S \cdot H_2C_2O_4$) C, H, N.

22: 15 h reaction time; yield 40%; m.p. 185–187° C dec. (decomposition during crystallisation); anal. ($C_{23}H_{23}N_3O_6S \cdot H_2C_2O_4$) C, H, N.

23: 18 h reaction time; yield of the oxalate 20%; m.p. 168–171° C dec. (MeOH/H_2O); anal. ($C_{23}H_{23}N_3O_7S \cdot H_2C_2O_4$) C, H, N.

24: 6 h reaction time; yield 56%; m.p. 174–175° C dec. (EtOH); anal. ($C_{23}H_{23}N_3O_6S \cdot H_2C_2O_4$) C, H, N.

1-(1-Naphthyloxy)-3-[2-(3-carbamoyl-4-furoxancarboxamido)ethylamino]propan-2-ol ***(27)*** *Oxalate and 1-(1-naphthyloxy)-3-[2-(3-phenyl-4-furoxancarboxamido)ethylamino]propan-2-ol* ***(28)*** *Oxalate*

NHS (0.25 g, 2.2 mmol) and DCC (0.45 g, 2,2 mmol) were added under dry atmosphere to a stirred solution of the appropriate furoxancarboxylic acid **14** or **25** (2.0 mmol) in dry THF (20 mL). The stirring was continued for 1 h and then the reaction mixture was filtered into a solution of **26** (0.52 g, 2.0 mmol) in dry THF (20 mL). Solvent was removed *in vacuo* to give a residue which was treated with EtOAc. The organic solution was filtered and washed twice with water, dried and treated with oxalic acid (0.36 g, 4.0 mmol), to give the title products as oxalates. Yields, melting points, recrystallisation solvents, formula and analytical data are reported below.

27: Yield 35%; after crystallization from EtOH and drying for 5 days at 50°C in vacuum drying pistol it melted at 145–150°C with decomposition. Anal. ($C_{19}H_{21}N_5O_6 \cdot H_2C_2O_4 \cdot H_2O$) C, H, N;

28: Yield 47%; m.p. 194–196° C dec. (MeOH); anal. ($C_{24}H_{24}N_4O_5 \cdot H_2C_2O_4$) C, H, N.

Pharmacology

Thoracic aorta were isolated from male Wistar rats (180–200 g) while right atria and trachea were isolated from male guinea pigs (200–250 g). All the animals were anaesthetised with CO_2, then sacrified by decapitation. Responses were recorded by means of isotonic transducers connected to two-channel Gemini recorders (Ugo Basile, Comerio, (VA) Italy).

Rat Aortic Strips Preparation and Vasoactivity Determination

The vessels were helically cut, the endothelium removed and two strips were obtained from each aorta. The tissues were mounted under 1 g tension 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, $NaHCO_3$ 12.0, glucose 11.1, maintained at 37 °C and gassed with 95% O_2–5% CO_2 (pH = 7.4)

The aortic strips were allowed to equilibrate for 1 h and then were contracted with 1 μM noradrenaline, which causes a submaximal response. During this first contraction the absence of intact endothelium was verified by adding 1 μM acetylcholine, which was found not to induce relaxation. The preparations were then extensively washed with Krebs bicarbonate buffer and after equilibration for 2.5 h from the beginning, a second contraction was evoked by 1 μM noradrenaline. When the response reached its plateau, cumulative concentrations of the vasodilating agent were added. The relaxing effect observed cannot be ascribed to competitive antagonism to noradrenaline on adrenoceptors, since a similar vasodilator profile was also evident in aortic strips depolarized by K^+ (data not shown). Effects of oxyhemoglobin (and of methylene blue) on relaxation were evaluated in separate series of experiments, in which 10 μM oxyhemoglobin (or 10 μM methylene blue) were added to precontracted aortic strips (or 5 min before contraction with 1 μM noradrenaline, respectively).

Right Atria Preparation and Functional Antagonism on β_1-Adrenoceptor

The hearts were rapidly removed and the atria were suspended in organ baths containing 30 ml of Ringer-Locke modified solution of the following composition (mM): NaCl 154, KCl 5.9, $CaCl_2$ 1.5, $NaHPO_4$ 0.29, $NaHCO_3$ 4.2, glucose 8.3, maintained at 31 °C and gassed with O_2 (pH = 7.6).

Tissues were allowed to equilibrate for 2 h and then a cumulative concentration-response curve to (−)-isoprenaline was constructed recording right atria beating frequency after each agonist addition (subsequent additions were made only after the response to the previous (−)-isoprenaline concentration had attained a maximal level and a steady beating frequency had been recorded). Following 1 h washing, tissues were incubated with the antagonist for 20 min and a second concentration-response curve to the agonist was obtained.

Trachea Preparation and Functional Antagonism on β_2-Adrenoceptor

The tracheal tube was quickly excised and cleaned free of excess tissues, then was cut into single rings. Five to six rings were joined together by thread to form a chain and mounted in organ baths containing 30 ml of the same Krebs bicarbonate buffer used for aortic strips, at 37° and gassed with 95% O_2 – 5% CO_2. After 1 h equilibration carbachol 1 μM was added to the organ baths to induce a spasm of the tracheal rings. Tissues were then washed thoroughly for 1 h. After a 2.5 h equilibration period, carbachol 1 μM was added again and, when a constant level was reached, a cumulative dose-response curve to (−)-isoprenaline was determined. Each concentration of (−)-isoprenaline was allowed to act until the response had stabilized before the next cumulative concentration was added. At the end of this curve tracheal chains were washed again extensively for 1 h and then incubated with the antagonist for 20 min. Immediately after the dose of the antagonist was added, tracheas were contracted with carbachol 1 μM, as they required about 20 min to reach the plateau. Then the cumulative concentration-response curve to (−)-isoprenaline was determined again.

A number of right atria and tracheal chains did not receive any antagonist in order to determine if any changes in sensitivity occurred during the course of the experiment.

RESULTS AND DISCUSSION

Synthesis of the intermediates **2, 4, 5, 9, 12, 14,** used for the preparation of the final compounds **18, 19, 21, 22, 24, 27** is reported in Figure 2. To prepare the derivatives **18–24** we synthesised diethylaluminium amides **16** of the appropriate intermediates **2, 4, 6, 5, 9, 15, 12,** using $Al(C_2H_5)_3$ in CH_2Cl_2 solution. Reaction *in situ* of diethylaluminium amides **16** with (±)2,3-epoxypropyl 1-naphtyl ether (**17**) afforded the expected derivatives in poor—fairly good yields (Figure 3). Compounds **27, 28** were synthesised in THF solution from **26** and the appropriate activated esters, obtained in situ treating the acids **14, 25** with NHS and DCC. Derivatives **31, 32** are two furoxan models we synthesised (Figure 3) in order to have a whole view of the NO dependent vasodilating activity of all furoxan moieties we introduced in structure A. NMR spectroscopy shows that the compounds, isolated in the reaction of the appropriate intermediates with **17,** are those deriving from the attack of diethylaluminium amides **16** at the terminal position of the oxirane ring (23). In fact in these derivatives the methine groups show ^1H and ^{13}C chemical shifts in the range 4.3 ± 0.1 and 65.5 ± 0.2 ppm respectively.

For all the furoxan derivatives the extent of thiol-induced NO-release was evaluated by detecting nitrites, the oxidative products of nitric oxide, by the Griess reaction according to the method previously described (12). The results expressed as % (mol/mol) NO_2^- are summarized in Table II. For selected compounds (derivatives **15, 27** and **31**) the production of nitric oxide was confirmed by a spectrophotometric technique based on the induced NO-oxidation of oxyhemoglobin (HbO_2^{2+}) to methemoglobin ($MetHb^{3+}$) according to the equation $HbO_2^{2+} + NO \rightarrow MetHb^{3+} + NO_3^-$ (see footnotes in Table II).

All the compounds displayed β_1 and β_2 competitive reversible antagonism. The pA_2 values and the β_1/β_2 selectivity, evaluated as ratio between the association constants $K\beta_1/K\beta_2$, are reported in Table II. The affinity of propranolol for β_1 receptors was not modified by the presence of typical NO-donors, like sodium nitroprusside (SNP, 0.1 μM) and glyceryl trinitrate (GTN, 0.1 μM). Vasodilating properties were assessed on endo-

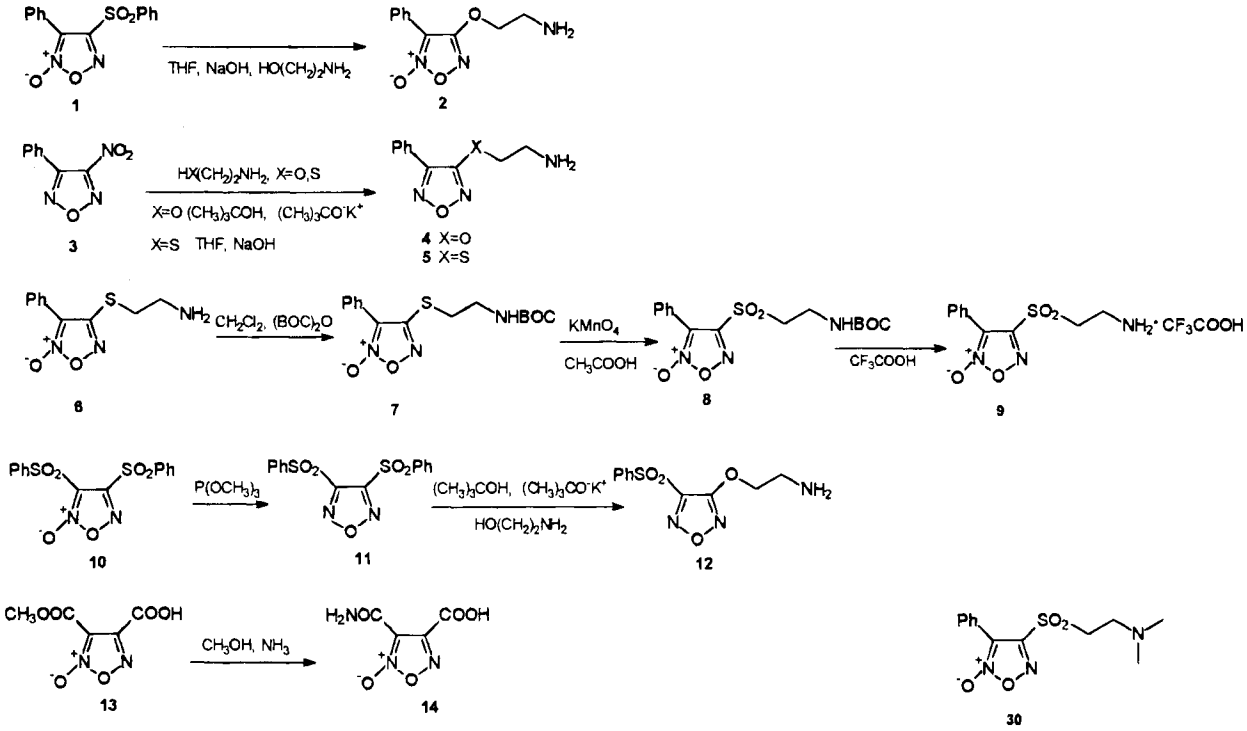


Fig. 2. Scheme illustrating the synthesis of the intermediates

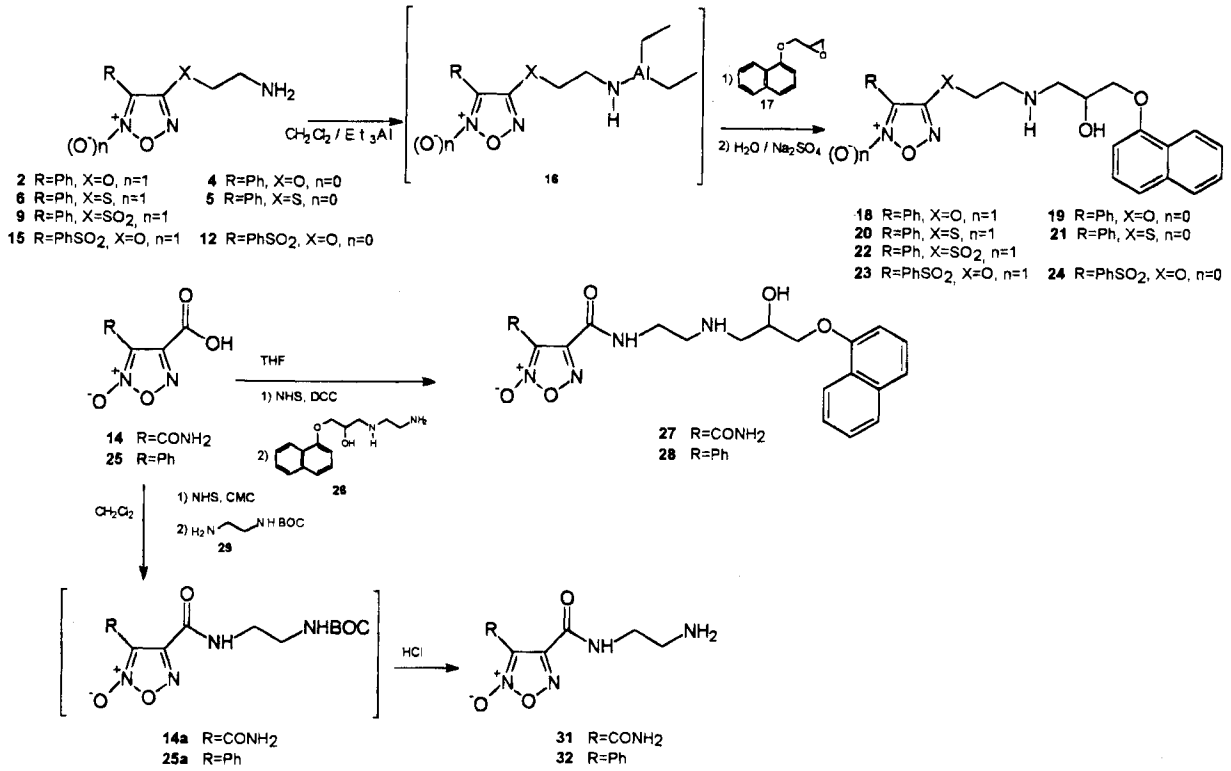


Fig. 3. Scheme illustrating the synthesis of the final compounds.

Table II. Pharmacological Activity of Compounds under Evaluation

Comp.	Guinea pig right atrium (β_1)[a]	Guinea pig trachea (β_2)[a]	β_1/β_2 selectivity	Rat thoracic aorta				$\%NO_2^-$ (mol/mol)±E.S. +L-Cys
				$-HbO_2$		$+HbO_2$	+MB	
	pA_2±C.L. 95%	pA_2±C.L. 95%		$-\log(EC_5)$±S.E.	EC_{50}±S.E.(μM)	EC_{50}±S.E.(μM)	EC_{50}±S.E. (μM)	
18	7.02±0.11	5.67±0.10	22	6.42±0.06	2.5±0.2	6.0±0.5	9.6±0.8	1.3±0.2
2	—	—	—	—	24±4	93±11	—	3±0.3
19	6.85±0.31	5.86±0.12	10	—	11±2	12±1	—	—
20	6.18±0.10	5.08±0.03[b]	12	6.31±0.05	4.0±0.2	13±3	15±4	0
6	—	—	—	—	15±1	78±9	—	3±0.1
21	6.17±0.18	5.24±0.03[b]	8.5	—	—	—	—	—
22	6.82±0.16	5.24±0.07[b]	38	8.09±0.06	0.066±0.009	0.15±0.04	0.55±0.05	4.8±0.1
30	—	—	—	—	0.10±0.01	0.43±0.02	—	8.8±0.3
23	8.56±0.18	7.23±0.10	21	8.17±0.10	0.11±0.02	0.84±0.09	0.75±0.09	33.7±1.3
15[c]	—	—	—	—	0.013±0.003	0.046±0.009	—	19.7±0.6
24	8.23±0.14	6.97±0.15	18	—	14±2	16±1	—	—
27[c]	7.91±0.19	7.09±0.12	7	7.49±0.07	0.26±0.03	0.37±0.04	3.4±0.8	48.7±0.6
31[c]	—	—	—	—	1.1±0.2	1.7±0.2	21±6	51.6±1.4
28	7.88±0.20	[d]	—	6.77±0.11	1.3±0.2	5.4±0.6	5.3±0.2	2.1±0.2
32	—	—	—	—	29±4	53±2	—	3.5±0.1
propranolol	8.69±0.14	8.49±0.14	1.7	—	—	—	—	—

[a] pA_2 values were calculated according to Arunlakshana and Schild and the experimental slopes of the plots did not differ significantly from unity.
[b] The pA_2 value (±S.E.) was calculated from the formula: $-\log K_B$-log(B/DR−1) at one concentration only of antagonist.
[c] The concentrations of derivatives **15**, **27** and **31** able to release 2.6 $\mu molL^{-1}$ min^{-1} of NO were $1.64 \cdot 10^{-5}$M (12), $3.06 \cdot 10^{-4}$M and $2.51 \cdot 10^{-5}$M respectively. In the case of derivatives **27** and **31** these figures were evaluated according to the method previously described (12). For derivative **23** it was not possible to obtain this figure because of its low solubility. At concentration 10^{-5}M this compound developed 0.77 $\mu molL^{-1}min^{-1}$ NO.
[d] It was impossible to evaluate the β_2-antagonistic activity of compound **28** because a constant and non dose-dependent reduction of the maximum response was observed from 1×10^{-6}M.

thelium denuded strips of rat aorta precontracted with noradrenaline, in the presence and in the absence of oxyhemoglobin (HbO_2, 10 μM), a well known NO scavenger. Since NO is able to diffuse at distances relatively far from its site of production, HbO_2 should be able to trap endogenously produced nitric oxide even without crossing cell membranes (24). The experiments were also performed in the presence of methylene blue (MB, 10 μM) an inhibitor of the cytosolic guanylate cyclase. The potencies, expressed as EC_{50}, are reported in Table II. Decrease in potency, when the experiments were performed either in the presence of HbO_2 or of MB, were taken as an evidence of NO involvement in the vasorelaxant action. In Table II, -log EC_5 values are also reported in order to demonstrate the lowest concentration of each drug able to trigger vasodilating action. These values are useful in evaluating the degree of balance between β-blocking and NO-dependent vasodilation activity in each drug.

Analysis of the data collected in Table II shows that insertion of the furoxan moieties into propranolol-like structure affords derivatives which display β-blocking and vasodilating activities spread over a wide range and balanced in different degree, as well as tendency towards β_1-selectivity. Derivative **20** has an affinity for β_1 and β_2 receptors about three hundred and two thousand five hundred-fold lower than propranolol respectively. Its β_1/β_2 selectivity index is 12. It displays vasodilating activity in a concentration range similar to the one in which it behaves as β_1-antagonist. Its fairly good potency (EC_{50}) as vasodilator is four fold greater than that of furoxan **6** present in it as a substructure. The potency decrease which occurs when vasodilating experiments were repeated in the presence of HbO_2, is in keeping with NO involvement in the vasorelaxant action. In summary, derivative **20** is a moderately active and well-balanced hybrid. Derivative **22** is the sulfone analogue of **20.** It displays greater affinity for β_1-receptors (about four-fold) than the parent thio compound, similar β_2-antagonist activity and therefore greater β_1/β_2 selectivity. The presence in **22** of a furoxan structure similar to that of derivative **30,** which is a compound with good NO-depending vasodilator properties, makes this derivative able to trigger vasodilating action in a concentration range lower than that in which it displays β_1-blocker activity. Therefore **22** is a hybrid biased towards NO-dependent behaviour. Substitution in **20** of the ethyloxy bridge for the ethylthio one, affords **18.** This molecular modification enhances the affinity for β_1-receptor of one order of magnitude and of four fold the affinity for β_2-receptor, consequently β_1/β_2 selectivity is improved. NO-dependent vasodilating activity of this derivative is fairly good; it is about ten fold greater than that shown by the reference furoxan **2**, and similar to that of **20.** So this compound is a hybrid less well balanced than its thio analogue, owing to greater β_1-affinity. Substitution in **20** of benzene sulfonyl group for 3-phenyl one affords **23,** which has the same potency at β_1-receptors as propranolol, but better (twenty one fold) β_1/β_2 selectivity. This compound shows a high level of NO-dependent vasodilating action too, according to the high vasodilating properties and the characteristics of NO production of the furoxan substructure **15** inserted in it. Interestingly, the use of **15** to build the hybrid affords a derivative with lower vasodilating activity. Both the mixed pharmacological properties are triggered in the same concentration range, and therefore this compound is a potent, well-constructed hybrid drug. The behaviour of the amide derivatives **27** and **28** is interesting. Derivative **28** is a hybrid biased towards β_1-blocking properties showing fairly good vasodilating action, while **27** is a quite potent well-balanced hybrid. Interestingly, the potency as vasodilator of the latter compound, and in part that of the parent furoxan **31,** does not change appreciably when evaluated in the presence of HbO_2, but it is dependent on the presence of methylene blue. This behaviour could indicate that, in spite of the extent of the thiol induced NO-production, the vasodilating action, in the concentration range analysed, is not NO-dependent. This behaviour suggests that, when the furoxan derivatives are appropriately substituted, other vasodilating mechanisms can become paramount. Work is in progress to have more insight into this hypothesis. Furazan analogues **21, 19, 24** showed very feeble vasodilating properties, not NO-dependent, as expected.

Analysis of Table II shows also that, compared with propranolol, hybrid formation lowers the affinity for β-receptors, in particular for the β_2-type, to give an increase in β_1/β_2 selectivity. This is true also for the furazan analogues, which display similar β-blocking potency. The pA_2 values at β_1 receptors for the furoxan series rank as follows: propranolol ≅ **23** > **27** ≅ **28** > **18** ≅ **22** > **20**. The hybridisation also leads to variation in NO-dependent vasodilating activity compared with the related furoxans alone. In all of the hybrid drugs this activity is, in a variable extent, greater than that of the corresponding simple furoxans; the only exception is the pair **15/23**. These results are in keeping with the knowledge that the process of chemical hybridisation can be accompanied by mutual interactions of the combined pharmacophoric groups. Thus the final compounds can display levels of activities different from those which could be predicted by simple additivity. In conclusion, this paper confirms that the furoxan system is a useful tool in designing, by an iterative approach, potent and well-balanced hybrids displaying NO-dependent activities.

SUPPLEMENTARY MATERIAL

^1H NMR spectra of the final compounds (**18–24, 27, 28**) and ^1H and ^{13}C NMR of all the intermediates.

4-(2-Aminoethyloxy)-3-phenylfuroxan (2) oxalate: ^1H NMR (DMSO-d_6) δ 3.4 (t, 2H, CH_2N), 4.7 (t, 2H, OCH_2), 6.1 (s, br, exchangeable protons), 8.2-7.6 (m, 5H, C_6H_5); ^{13}C NMR (DMSO-d_6) δ 37.8 (NCH_2), 67.8 (OCH_2), 107.6 (*C*3), 121.9 (Ph*C*1), 126.7/129.1 (Ph*C*2/*C*3), 131.7 (Ph*C*4), 162.1 (*C*4), 164.9 (oxalate).

3-(2-Aminoethyloxy)-4-phenylfurazan (4) oxalate: ^1H NMR (DMSO-d_6) δ 3.4 (t, 2H CH_2N), 4.7 (t, 2H, OCH_2), 6.2 (s, br, exchangeable protons), 8.1-7.6 (m, 5H, C_6H_5); ^{13}C NMR (DMSO-d_6) δ 37.8 (NCH_2), 69.8 (OCH_2), 124.3 (Ph*C*1), 127.8/129.3 (Ph*C*2/*C*3), 131.2 (Ph*C*4), 145.5 (*C*4), 163.2 (*C*3), 164.9 (oxalate).

3-(2-Aminoethylthio)-4-phenylfurazan (5) oxalate: ^1H NMR (DMSO-d_6) δ 3.1 (t, 2H, SCH_2, 3.4 (t, 2H, CH_2N), 4.1 (S, br, exchangeable protons), 7.9-7.6 (m, 5H, C_6H_5; ^{13}C NMR (DMSO-d_6) δ 33.2 (SCH_2), 39.1 (NCH), 124.5 Ph*C*1), 128.1 (SCH_2), 129.5 (Ph*C*2/*C*3), 131.3 (Ph*C*4), 151.6/152.8 (*C*3/*C*4), 164.6 (oxalate).

tert-Butyl N-[2-(3-phenyl-4-furoxanythio)ethyl]carbate (7): ^1H NMR ($CDCl_3$) δ 1.4 (s, 9H, CH_3), 3.4 (m, 2H, SCH_2), 3.6 (m, 2H CH_2N), 4.9 (s, br, 1H, N*H*CO), 7.9-7.5 (m, 5H, C_6H_5) ^{13}C NMR ($CDCl_3$) δ 28.2 (CH_3), 31.2 (SCH_2), 39.3

(NCH_2), 79.7 ($C(CH_3)_3$), 114.0 (*C*3), 122.2 (Ph*C*1), 127.2/128.9 (Ph*C*2/*C*3), 130.6 (Ph*C*4), 153.7 (*C*4), 155.8 (*C*ONH).

tert-Butyl N-[2-(3-phenyl-4-furoxansulfonyl)ethyl]carbamate (8): ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9H, CH_3), 3.7-3.8 (m, 4H, SCH_2, CH_2N), 4.9 (s, br, 1H, N*H*CO), 7.9-7.5 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$) δ 28.1 (CH_3), 34.5 (NCH_2), 54.1 (SO_2CH_2), 80.3 ($C(CH_3)_3$), 112.0 (*C*3), 119.9 (Ph*C*1), 128.7/129.0 (Ph*C*2/*C*3), 131.6 (Ph*C*4), 155.8 (*C*ONH), 157.2 (*C*4).

Bis-(benzenesulfonyl)furazan (11): ^{1}H NMR ($CDCl_3$) δ 8.2-7.6 (m, 10H, C_6H_5); ^{13}C NMR ($CDCl_3$) δ 129.6 (Ph*C*2/*C*3 coincident signals), 135.8 (Ph*C*4), 137.2 (Ph*C*1), 155.4 (*C*=N).

3-(2-Aminoethyloxy)-4-benzenesulfonylfurazan (12) oxalate: ^{1}H NMR (DMSO-d_6) δ 3.3 (t, 2H, CH_2N), 4.6 (t, 2H, OCH_2), 7.1 (s, br, exchangeable protons), 8.2-7.7 (m, 5H, C_6H_5); ^{13}C NMR (DMSO-d_6) δ 37.5 (NCH_2), 70.7 (OCH_2), 129.0/130.3 (Ph*C*2/*C*3), 136.3 (Ph*C*4), 137.1 (Ph*C*1), 149.0 (*C*4), 161.5 (*C*3) 164.7 (oxalate).

3-Carbamoyl-4-furoxancarboxylic acid (14): ^{1}H NMR (DMSO-d_6) δ 8.43, 8.36 (2s,2H,NH_2), 11.00 (s,br,1H, COO*H*); ^{13}C NMR (DMSO-d_6) δ 110.7 (*C*3), 149.7 (*C*4), 155.4, 158.2 (*C*OOH, *C*ONH_2).

4-(2-Aminoethylaminocarbonyl)-3-furoxancarboxamide (31) hydrochloride: ^{1}H NMR (DMSO-d_6) δ 3.0 (m, br, 2H, $CH_2NH_3^+$), 3.6 (q, 2H, CONHCH_2), 8.2 (s, br, 3H $CH_2NH_3^+$), 8.45, 8.54, (2s, 2H, NH_2CO), 9.5 (t, 1H, CON*H*); ^{13}C NMR (DMSO-d_6) δ 36.9/38.1 (NCH_2CH_2N), 110.1 (*C*3), 151.2 (*C*4), 155.2/156.9 (*C*ONH_2/*C*ONH).

4-(2-Aminoethylaminocarbonyl)-3-phenylfuroxan (32) hydrochloride: ^{1}H NMR (DMSO-d_6) δ 3.0 (m, br, 2H, $CH_2NH_3^+$), 3.6 (q, 2H, CONHCH_2), 7.8-7.5 (m, 5H, C_6H_5), 8.3 (s, br, 3H, $CH_2NH_3^+$), 9.5 (t, 1H, CON*H*); ^{13}C NMR (DMSO-d_6) δ 36.9/38.0 ($NHCH_2$/CH_2NH_2), 113.9 (*C*3), 121.9 (Ph*C*1), 128.6/129.0 (Ph*C*2/*C*3), 130.8 (Ph*C*4), 151.9 (*C*4), 157.3 (*C*ONH).

1-(1-Naphthyloxy)-3-[2-(3-phenyl-4-furoxanyloxy)-ethylamino]propan-2-ol (18) oxalate: ^{1}H NMR (DMSO-d_6) δ 3.2–3.5 (m, 2H, NCH_2CHOH), 3.6 (t, br, 2H, CH_2CH_2N), 4.2 (m, 2H, NaphOCH_2), 4.4 (m, 1H, C*H*OH), 4.8 (t, br, 2H, OCH_2CH_2), 6.5 (s, br, exchangeable protons), 7.0–8.3 (m, 12H, aromatic protons). ^{13}C NMR δ are reported Table I.

1-(1-Naphthyloxy)-3-[2-(3-phenyl-4-furazanyloxy)-ethylamino]propan-2-ol(19) oxalate: ^{1}H NMR (DMSO-d_6) δ 3.2–3.5 (m, 2H, NCH_2CHOH), 3.6 (t, br, 2H, CH_2CH_2N), 4.2 (m, 2H, NaphOCH_2), 4.4 (m, 1H, C*H*OH), 4.8 (t, br, 2H, OCH_2CH_2), 6.5 (s, br, exchangeable protons), 7.0–8.3 (m, 12H, aromatic protons). ^{13}C NMR δ are reported in Table I.

1-(1-Naphthyloxy)-3-[2-(3-phenyl-4-furoxanylthio)-ethylamino]propan-2-ol (20) oxalate: ^{1}H NMR (DMSO-d_6 δ 3.2–3.4 (m, 2H, NCH_2CHOH), 3.4 (m, 2H, SCH_2CH_2), 3.6 (m 2H, CH_2CH_2N), 4.2 (m, 2H, NaphOCH_2), 4.3 (m, 1H, C*H*OH), 6.7 (s, br, exchangeable protons), 7.0–8.3 (m, 12H, aromatic protons). ^{13}C NMR δ are reported in Table I.

1-(1-Naphthyloxy)-3-[2-(3-phenyl-4-furazanylthio)-ethylamino]propan-2-ol (21) oxalate: ^{1}H NMR (DMSO-d_6) δ 3.1–3.4 (m, 2H, NCH_2CHOH), 3.4 (m, 2H, SCH_2CH_2), 3.7 (m, 2H, CH_2CH_2N), 4.2 (m, 2H, NaphOCH_2), 4.3 (m, 1H, C*H*OH) 6.7 (s, br, exchangeable protons), 7.0–8.3 (m, 12H, aromatic protons). ^{13}C NMR δ are reported in Table I.

1-(1-Naphthyloxy)-3-[2-(3-phenyl-4-furoxansulfonyl)ethylamino]propan-2-ol (22) oxalate: ^{1}H NMR (DMSO-d_6) δ 3.2–3.4 (m, 2H, NCH_2CHOH), 3.4 (t, br, 2H, CH_2CH_2N), 4.1 (m, 4H, NaphOCH_2, SO_2CH_2), 4.3 (m, 1H, C*H*OH), 7.2, 6.9–8.3 (m, aromatic protons and exchangeable protons overlapped). ^{13}C NMR δ are reported in Table I.

1-(1-Naphthyloxy)-3-[2-(3-benzenesulfonyl-4-furoxanyloxy)ethylamino]propan-2-ol (23) oxalate: ^{1}H NMR (DMSO-d_6) δ 3.1–3.4 (m, 2H, NCH_2CHOH), 3.5 (t, br, 2H, CH_2CH_2N), 4.2 (m, 2H, NaphOCH_2), 4.4 (m, 1H, C*H*OH), 4.8 (t, br, 2H, OCH_2CH_2), 6.0–8.3 (m, aromatic protons and exchangeable protons overlapped). ^{13}C NMR δ are reported in Table I.

1-(1-Naphthyloxy)-3-[2-(3-benzensulfonyl-4-furazanyloxy)ethylamino]propan-2-ol (24) oxalate: ^{1}H NMR (DMSO-d_6) δ 3.2–3.5 (m, 2H, NCH_2CHOH), 3.4 (t, br, 2H, CH_2CH_2N), 4.2 (m, 2H, NaphOCH_2), 4.4 (m, 1H, C*H*OH), 4.7 (t, br, 2H, OCH_2CH_2), 5.4 (s, br, exchangeable protons), 7.0–8.3 (m, 12H, aromatic protons). ^{13}C NMR δ are reported in Table I.

1-(1-Naphthyloxy)-3-[2-(3-carbamoyl-4-furoxancarboxamido)ethylamino]propan-2-ol (27) oxalate: ^{1}H NMR (DMSO-d_6) δ 3.2–3.4 (m, 2H, NHCH_2CHOH), 3.2 (m, br, 2H, CONHCH_2), 3.7 (m, br, 2H, CH_2CH_2N), 4.2 (m, 2H, CH_2O-Naph), 4.3 (m, br, 1H, C*H*OH), 4.8 (s, br, exchangeable protons), 7.0–8.3 (m, 7H, aromatic protons), 8.49, 8.43 (2s, 2H, CONH_2), 9.6 (br,1H,CO*NH*). ^{13}C NMR δ are reported in Table I.

1-(1-Naphthyloxy)-3-[2-(3-phenyl-4-furoxancarboxamido)ethylamino]propan-2-ol (28) oxalate: ^{1}H NMR (DMSO-d_6) δ 3.2–3.4 (m, 4H, NHCH_2CHOH, CONHCH_2), 3.6 (m, br, 2H, CH_2CH_2N), 4.2 (m, 2H, CH_2ONaph), 4.3 (br, 1H, C*H*OH), 4.8 (s, br, exchangeable protons), 7.0–8.3 (m, 12H, aromatic protons), 9.4 (t, br, 1H, CO*NH*). ^{13}C NMR δ are reported in Table I.

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