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Nicorandil Analogues Containing NO-Donor Furoxans and Related Furazans

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Abstract—The synthesis and in vitro vasodilating properties of hybrid compounds in which furoxan (1,2,5-oxadiazole 2-oxide) moieties, endowed with different NO-donor properties, were substituted for the nitroxy function of Nicorandil are reported. The corresponding cyanoguanidine analogues are also considered. This approach has led to a series of vasorelaxing compounds devoid of affinity for K_{ATP} channels, whose activity is prevalently due to their ability to activate sGC, at the concentrations of the experiments. Related furazan (1,2,5-oxadiazole) derivatives, unable to release nitric oxide were also prepared and studied for control. The amide analogues of Nicorandil display feeble vasorelaxing action not involving the activation of K^+ channels, while in the guanidine analogues, this mechanism seems to underlie this action.

Introduction

Potassium channel activators (KCAs) represent an important class of compounds with a great therapeutic potential in the management of disorders caused by smooth muscle contraction.[1,2] Several chemically diverse types of compounds, endowed with K^+channel opening properties, have been identified. Among these, pyridine derivatives, such as Nicorandil (**1**, Fig. 1), have been the object of several structural modifications.[3,4] This compound displays strong vasodilating activity through hyperpolarisation of the cell membrane in vascular smooth muscle due to its ability to open principally ATP-dependent K^+ channels (K_{ATP}).[1] This action is antagonised by Glibenclamide, an inhibitor of this type of channel,[1,5] and it is associated to the pyridyl moiety.[3] The vasodilating properties of Nicorandil are also partly due to soluble guanylate cyclase (sGC) activation generated by the NO-donor nitroxy side chain.[1,3] This latter group is important not only for the activation of sGC, but also for its degree of potency as KCA.[6] The dual action of this compound can produce a more beneficial coronary vasodilation than a pure K^+ channel activator.

Recently, attention has been devoted to furoxan derivatives as NO-donors.[7,8] These compounds are able, normally under the action of thiol cofactors, to produce NO through a complex mechanism, not yet completely understood. Different redox forms of nitric oxide could be involved in the process. The possibility of modulating the NO release by changing the substitution pattern at the ring,[9] renders these structures flexible tools in designing compounds with a dual activity, one of which is dependent on their NO donor capacity.[10] In this paper we describe the synthesis and in vitro vasodilating properties of hybrid compounds in which furoxan moieties, endowed with different NO-donor properties, were substituted for the nitroxy function of Nicorandil (derivatives **7** and **9**) and of their cyanoguanidine analogues (derivatives **18** and **20**). Related furazan derivatives **8**, **10**, **19**, **21**, unable to release nitric oxide, were also prepared and studied for control.

Results

Chemistry

Synthesis. Synthesis of the models **7** and **8** was realised according to the pathway reported in Scheme 1a. Nucleophilic substitution of the nitro group in derivatives **3** and **4** by the *N*-(2-hydroxyethyl)nicotinamide (**2**), was run in THF at room temperature, under basic

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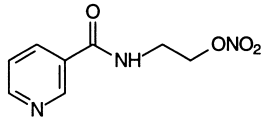

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Figure 1.

conditions. Benzenesulfonyl analogues **9** and **10** were prepared in a similar manner by nucleophilic substitution of one of the two benzenesulfonyl groups present in **5** and **6**. It is known that **5**, dissolved in THF, reacts with ethanol under basic conditions giving 3-benzenesulfonyl-4-ethoxyfuroxan (**5a**).[11] For this reason we assigned a 3-benzenesulfonylfuroxan-4-yl structure to **9**. This assignment was confirmed by the analysis of ^{13}C NMR spectra (Table 1). In particular, the signals at 110.7 and at 159.0 ppm are indicative for C(3) and C(4) carbons respectively of a 4-alkoxy-3-benzenesulphonyl substituted furoxan ring.[12] Cyanoguanidine analogues **18–21** were synthesised according to the sequential pathway reported in Scheme 1b. *O,O*-Diphenyl-*N*-cyanocarbonimidate (**11**) was used as starting material. This compound shows a very high reactivity towards nucleophiles.[13] Displacement of the first phenoxy group occurs under mild conditions, even with poor nucleophiles. Thus, intermediate **13** was obtained simply on stirring **11** in *iso*-propanol (*i*-PrOH) at room temperature with 3-aminopyridine (**12**). Longer reaction times were necessary for the displacement of the second phenoxy group by amines **14–17** in order to obtain the final derivatives **18–21**. ^{13}C and ^{1}H NMR data for these compounds are in keeping with the proposed structures (Table 1 and experimental session).

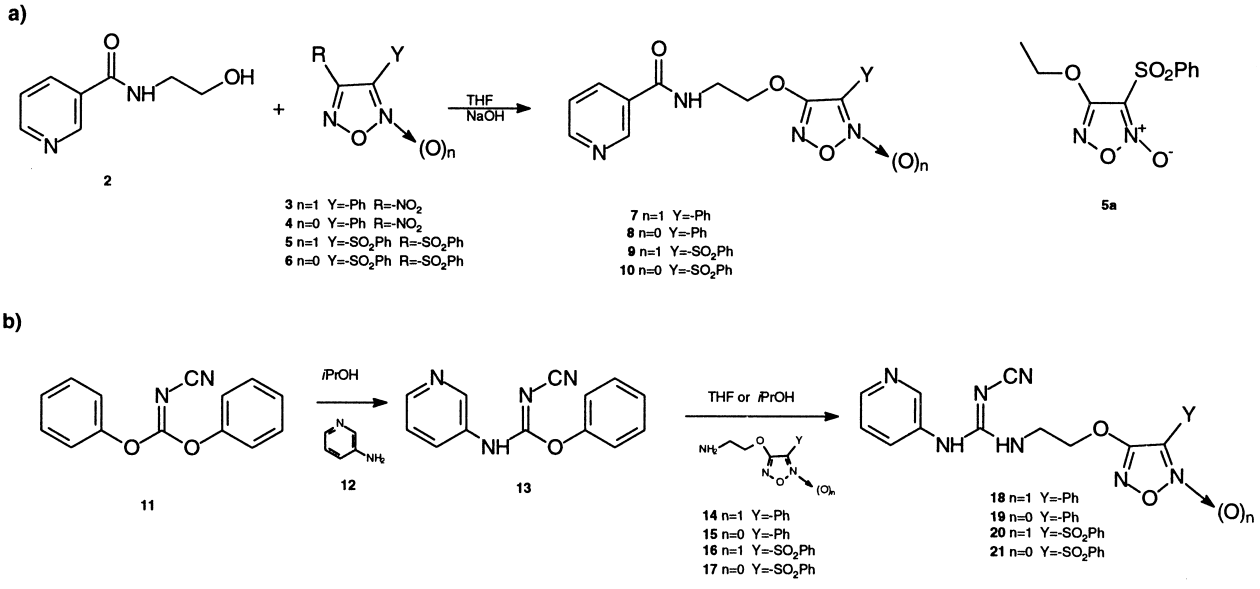
Nitrite detection. Nitrites are the major oxidation products of NO in an aqueous system and their detection serves as a basis for inferring its previous presence. In the presence of thiols, besides this oxidation, a variety of interrelated reactions with distinct reaction rates can occur.[7] In order to evaluate thiol-induced NO generation by furoxan models, the appropriate compound was incubated in pH 7.4 buffered water at 37 °C for 1 h, in the presence of a large excess of cysteine (1:50). Nitrites were determined by Griess reaction.[9] The results expressed as percentage NO_2^- (mol/mol) are entered in Table 2.

Pharmacology

The vasodilating activity of all the final furoxan and furazan derivatives was examined by measuring their inhibitory effects on the 25 mM KCl-induced contractions of endothelium denuded rat thoracic aorta strips. Nicorandil and LY211808, an analogue of Pinacidil,[3] were considered as reference compounds. All the derivatives triggered a concentration-dependent response. EC_{50} values were calculated from the cumulative concentration-response curves. In order to prove the involvement of nitric oxide in the relaxation process, the experiments were repeated in the presence of methylene blue (MB), an inhibitor of sGC. Similarly, in order to evaluate the involvement of K^+ channel opening in the process, the experiments were also run in the presence either of Glibenclamide or of a co-administration of MB-Glibenclamide. The results, expressed as mean EC_{50} (μM) ± S.E. are reported in Table 2.

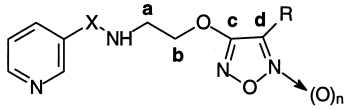
Discussion

Analysis of the data collected in Table 2 shows that the strict analogues of Nicorandil, compounds **7** and **9**, display good vasodilating activity. EC_{50} of benzenesulfonyl derivative **9** is about one hundred times less than EC_{50} of the reference **1**, while the potency of the phenyl analogue **7** is about the same. When vasodilating



Scheme 1.

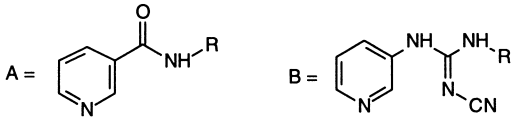
Table 1. ^{13}C NMR chemical shifts (ppm) of the final compounds (DMSO-d_6, TMS int.)

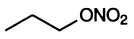
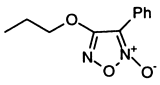
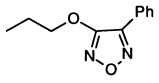
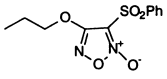
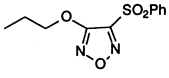
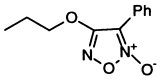
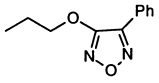
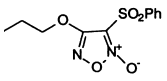
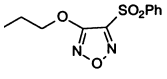


Compound	n	R	X	C2(Pyr)	C6(Pyr)	C3(Pyr)	C4(Pyr)	C5(Pyr)	C=O	c	d	C1(Ph)	C2(Ph)/ C3(Ph)	C4(Ph)	C=N	C≡N	a	b
7	1	Ph	C=O	152.1	148.4	129.8	135.0	123.6	165.4	162.3	107.6	122.1	128.9/126.3	130.7			38.2	69.3
8	0	Ph	C=O	152.0	148.4	129.8	135.0	123.6	165.4	163.4	145.3	124.5	129.2/127.4	131.1			38.3	71.3
9	1	$PhSO_2$	C=O	152.1	148.5	129.8	135.1	123.6	165.4	159.0	110.7	137.3	129.9/128.4	136.1			38.0	69.6
10	0	$PhSO_2$	C=O	152.1	148.5	129.7	135.1	123.6	165.3	161.1	148.9	137.0	130.1/128.8	136.1			38.1	72.1
18	1	Ph	-NHC=N-C≡N	146.0/145.4		134.3	131.5[a]	123.8	123.8	162.2	107.7	122.0	129.1/126.3	130.7[a]	158.5	116.9	40.4	69.1
19	0	Ph	-NHC=N-C≡N	145.9/145.3[b]		134.4	131.8[a]	123.8	123.8	163.4	145.3[b]	124.4	129.4/127.4	131.4[a]	158.5	116.9	40.5	71.2
20	1	$PhSO_2$	-NHC=N-C≡N	146.0/145.4		134.4	131.5	123.8	123.8	159.0	110.7	137.2	130.1/128.5	136.2	158.4	116.9	40.5	69.4
21	0	$PhSO_2$	-NHC=N-C≡N	146.0/145.4		134.3	131.6	123.9	123.9	161.1	148.9	137.0	130.3/128.8	136.3	158.4	116.9	40.5	71.9

[a]Interchangeable.
[b]Overlapped.

Table 2. Pharmacological activity of compounds under evaluation



Compound	Structure	R	EC_{50} (μM)				NO_2^-% (+L-cys)
				+ MB	+ GLIB	+ MB + GLIB	
1	A		2.9±0.4	24±2	6.3±1.2	107±23	1.0±0.1
7	A		3.1±0.5	20±3	3.8±0.6	22±1	1.3±0.1
8	A		28±4	26±2	26±3	—	—
9	A		0.024±0.005	0.38±0.05	0.023±0.003	0.40±0.04	39±4
10	A		26±6	25±4	22±4	—	—
LY211808	B		7.6±1.1	7.8±1.0	415±32	438±23	—
18	B		1.5±0.2	6.9± 0.4	1.6±0.6	7.8±0.7	1.4± 0.2
19	B		86±8	82±14	166±30	—	—
20	B		0.021±0.003	0.44±0.03	0.023±0.003	0.47±0.09	26±1
21	B		29±4	28±4	47±5	—	—

experiments were performed in the presence of Glibenclamide, no rightward shift of the dose–response curve was observed for either compound and consequently no changes in their EC_{50} values. On the other hand, both the models displayed a marked increase in EC_{50} values when the experiments were done in the presence of MB. Co-administration of MB-Glibenclamide resulted in no further modification of the potencies. This means that in these hybrids nitric oxide has a predominant role in the vasodilating action. Their order of potency is in keeping with their ability to produce nitrite ions. Interestingly the most potent compound **9** displays a potency similar to that of the related simple furoxan **5a** (EC_{50} = 0.0070±0.0004 μM), under the same experimental conditions. Furazan analogues **8** and **10** show similar potency, about ten times lower than that of Nicorandil. No shift to the right of concentration–response curves was observed either in the presence of MB or of Glibenclamide. This strongly suggests that neither NO, as anticipated, nor the activation of K^+ channels underlies this feeble action. Other mechanisms must be involved. Phosphodiesterase inhibition could be one of them. In fact it is known that some nicotinamide derivatives display this kind of activity.[14] Furoxan cyanoguanidine hybrids **18** and **20** trigger vasodilating responses very close to the ones of the amide analogues **7** and **9**. At the concentrations of the experiments, their activity is prevalently NO-like, as the decrease in the potency in the presence of MB and the constancy of the potency in the presence of Glibenclamide demonstrate. Derivative **20** displays a higher activity than **18**, in keeping with its higher capacity to produce nitrite ions. Its potency is similar to that of the related simple furoxan **5a**, as for **9**. The furazan analogues of this series (derivatives **19** and **21**) behave as feeble vasodilating products, similarly to the related amide derivatives, but their activity is in part due to the activation of K^+ channels. In fact the response is partially inhibited in the presence of Glibenclamide. The potencies of the two models are ten and four times less than that of LY211808, respectively. The lower affinity of the two compounds for K_{ATP} channels with respect to the reference compound is probably due to the lack of the lateral short branched alkyl moiety. In fact it is known that in LY211808 and related compounds this substructure confers optimal activity and tissue selectivity.[3]

Conclusions

The attempt to use two NO-donor furoxan substructures, endowed with different NO-donor properties, to prepare hybrid drugs related to Nicorandil, has led to a class of vasorelaxing compounds devoid of affinity for K_{ATP} channels, whose activity is prevalently due to their ability to activate sGC. Related furazan structures, unable to release NO, behave in different manners. Strict analogues of Nicorandil display vasorelaxing action not involving the activation of K^+ channels, while in guanidine analogues this mechanism seems to underlie the action.

Experimental

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 NMR spectra were recorded in DMSO-d_6 at 200 and 50 MHz, respectively, with a Bruker AC-200 spectrometer. The ^{13}C-chemical shifts of the final compounds are reported in Table 1. 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. 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. Compounds **2**,[15] **3**,[16] **4**,[17] **5**,[11] **6**,[18] **11**,[13] **14**,[18] **15**,[18] **16**,[9] **17**[18] were synthesised according to literature methods.

General procedure for the preparation of compounds 7–10. 0.48 g of 50% w/w water NaOH solution (6.0 mmol) were added portionwise to a stirred solution of **2** (0.50 g, 3.0 mmol) and of the appropriate oxadiazoles **3**–**6** (3.0 mmol) in THF (20 mL), while the temperature was maintained at 25 °C. The reaction mixture was stirred at 25 °C for 2–5 h. Solvent removal gave a residue which was treated with water and filtered (for **7**, **8** and **10**) or extracted with ethyl acetate (for **9**) to obtain the expected product.

***N*-(2-(3-Phenylfuroxan-4-yloxy)ethyl)nicotinamide (7).** Reaction time 3 h; yield 52%; mp 166–167 °C dec. (EtOH). ^1H NMR (DMSO-d_6) δ 9.02 (d, 2H, 2-pyr and N*H*), 8.71 (dd, H, 6-pyr), 8.19 (m, H, 4-pyr), 8.07 (m, 2H, Ph), 7.53 (m, 4H, 5-pyr and Ph), 4.65 (t, 2H, CH_2O), 3.85 (q, 2H, NCH_2). Anal. for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N, 17.17. Found: C, 59.14; H, 4.33; N, 17.15.

***N*-(2-(4-Phenylfurazan-3-yloxy)ethyl)nicotinamide (8).** Reaction time 4 h; yield 87%; mp 149–150 °C (MeOH). ^1H NMR (DMSO-d_6) δ 9.01 (m, 2H, 2-pyr and N*H*), 8.71 (m, H, 6-pyr), 8.18 (m, H, 4-pyr), 7.97 (m, 2H, Ph), 7.55 (m, 4H, 5-pyr and Ph), 4.63 (t, 2H, CH_2O), 3.84–3.87 (q, 2H, NCH_2). Anal. for $C_{16}H_{14}N_4O_3$: C, 61.93; H, 4.55; N, 18.05. Found: C, 62.05; H, 4.53; N: 17.95.

***N*-(2-(3-Benzensulfonylfuroxan-4-yloxy)ethyl)nicotinamide (9).** Reaction time 5 h; yield 81%; mp 171–172 °C dec. (MeOH). ^1H NMR (DMSO-d_6) δ 9.04 (m, H, 2-pyr), 8.91 (t, H, N*H*), 8.76 (m, H, 6-pyr), 8.23 (m, H, 4-pyr), 8.03–7.60 (m, 5H, Ph), 7.55 (m, H, 5-pyr), 4.60 (t, 2H, CH_2O), 3.77 (q, 2H, NCH_2). Anal. for $C_{16}H_{14}N_4O_6S$: C, 49.23; H, 3.61; N, 14.35. Found: C, 49.69; H, 3.56; N, 14.21.

***N*-(2-(4-Benzensulfonylfurazan-3-yloxy)ethyl)nicotinamide (10).** Reaction time 2 h; yield 60%; mp 162–163 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 9.01 (m, H, 2-pyr), 8.87 (t, H, N*H*), 8.76 (m, H, 6-pyr), 8.19 (m, H, 4-pyr), 8.11–7.53 (m, 6H, Ph and 5-pyr), 4.57 (t, 2H, CH_2O), 3.75 (q, 2H, NCH_2). Anal. for $C_{16}H_{14}N_4O_5S$: C, 51.33; H, 3.77; N, 14.97. Found: C, 51.30; H, 3.72; N, 14.86.

3-Cyano-1-(3-pyridinyl)-2-phenylisourea (13). The product was prepared according to a similar scheme described in literature:[19] 3-aminopyridine (1.88 g; 20.0 mmol) was added to a mixture of **11** (4.76 g; 20.0 mmol) in isopropanol (25 mL) and the mixture was stirred at room temperature for 24 h. Then the mixture was cooled in an ice bath, filtered, and the residue washed with cooled isopropanol and dried. Yield 74%; mp 147–148 °C dec. (*i*-PrOH). ^{1}H NMR (DMSO-d_6) δ 11.02 (br, H, N*H*), 8.71 (s, H, 2-pyr), 8.46 (d, H, 6-pyr), 7.95 (m, H, 4-pyr), 7.51–7.29 (m, 6H, Ph and 5-pyr); ^{13}C NMR (DMSO-d_6) δ 160.5, 151.4, 146.8, 145.0, 133.2, 131.6, 129.9, 126.6, 123.6, 121.3, 113.3. Anal. for $C_{13}H_{10}N_4O$: C, 65.54; H, 4.23; N, 23.52. Found: C, 65.48; H, 4.26; N, 23.51.

General procedure for the preparation of compounds 18–21. Compound **13** (0.95 g, 4.0 mmol) was added to a solution of the appropriate amine **14**–**17** (4.0 mmol) in isopropanol (50 mL) or THF (30 mL). The mixture was stirred at room temperature over the reported time. The formed precipitated was obtained by filtration for the compounds **18**, **19** and **21**. In the case of **20** no precipitation occurred and so the reaction mixture was evaporated and the residue was purified by flash chromatography.

2-Cyano-1-(2-(3-phenylfuroxan-4-yloxy)ethyl)-3-(3-pyridinyl)guanidine (18). Isopropanol; reaction time: 10 h; yield 54%; mp 186–187 °C dec. (EtOH). ^{1}H NMR (DMSO-d_6) δ 9.36 (s, H, N*H*-pyr), 8.48 (d, H, 2-pyr), 8.37 (m, H, 6-pyr), 8.12–8.07 (m, 2H, Ph), 7.69–7.56 (m, 5H, Ph and 4-pyr and N*H*CH$_2$), 7.34 (m, 1H, 5-pyr), 4.60 (t, 2H, CH_2O), 3.79 (q, 2H, NCH_2). Anal. for $C_{17}H_{15}N_7O_3$: C, 55.89; H, 4.14; N, 26.84. Found: C, 55.67; H, 4.10; N, 26.66.

2-Cyano-1-(2-(4-phenylfurazan-3-yloxy)ethyl)-3-(3-pyridinyl)guanidine (19). THF; reaction time: 24 h; yield 65%; mp 200–201 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 9.34 (s, H, pyr-N*H*), 8.46 (d, H, 2-pyr), 8.36 (m, H, 6-pyr), 8.06–7.97 (m, 2 H, Ph), 7.67–7.51 (m, 5H, Ph and 4-pyr and N*H*CH$_2$), 7.30 (m, 1H, 5-pyr), 4.58 (t, 2H, CH_2O), 3.76 (q, 2H, NCH_2). Anal. $C_{17}H_{15}N_7O_2$: C, 58.45; H, 4.33; N, 28.07. Found: C, 58.37; H, 4.27; N, 28.10.

1-(2-(3-Benzensulfonylfuroxan-4-yloxy)ethyl)-2-cyano-3-(3-pyridinyl)guanidine (20). Reagent ratio 1:1.2; THF; reaction time: 96 h; eluent: CH_2Cl_2:CH_3OH 9.5:0.5; yield 52%; mp 145–147 °C dec. (EtOAc). ^{1}H NMR (DMSO-d_6) δ 9.35 (s, H, pyr-N*H*), 8.52 (d, H, 2-pyr), 8.39 (m, H, 6-pyr), 8.06–7.71 (m, 6H, Ph and 4-pyr), 7.60 (t, H, N*H*CH$_2$), 7.41 (m, H, 5-pyr), 4.59 (t, 2H,CH_2O), 3.72 (q, 2H, NCH_2). Anal. for $C_{17}H_{15}N_7O_5S$: C, 47.55; H, 3.52; N, 22.83. Found: C, 47.34; H, 3.51; N, 22.70.

1-(2-(4-Benzensulfonylfurazan-3-yloxy)ethyl)-2-cyano-3-(3-pyridinyl)guanidine (21). Isopropanol; reaction time: 6 days; yield 44%; mp 141–144 °C dec. ($CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 9.34 (s, H, pyr-N*H*), 8.49 (d, H, 2-pyr), 8.39 (m, H, 6-pyr), 8.14–7.68 (m, 6H, Ph and 4-pyr), 7.58 (t, H, N*H*CH$_2$), 7.44–7.36 (m, H, *5*-pyr), 4.53 (t, 2H, CH_2O), 3.70 (q, 2H, NCH_2). Anal. for $C_{17}H_{15}N_7O_4S0.25H_2O$: C, 48.86; H, 3.74; N, 23.46. Found: C, 48.87; H, 3.71; N, 23.41.

Pharmacology

Vasoactivity determination. Thoracic aortas were isolated from male Wistar rats weighing 180–200 g, which had been anaesthetised with CO_2 and killed by decapitation. All animals were dealt with in a humane way in accordance with recognised guidelines on experimentation. As few rats as possible were used and generally three strips per animal were obtained. The purposes and the protocols of our studies have been approved by the Ministero della Sanità, Rome, Italy. The vessels were helically cut and the endothelium removed. The tissues were mounted under 0.7 g tension in organ baths containing 30 mL of Krebs-bicarbonate solution (NaCl 112 mM, KCl 5.0 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.0 mM, $NaHCO_3$ 12 mM, glucose 11 mM) maintained at 37 °C and gassed with 95% O_2–5% CO_2 (pH 7.4). Responses were recorded on a 7070 Gemini Recorder through an Isotonic Transducer (Ugo Basile, Varese, Italy). The aortic strips were allowed to equilibrate for 1 h and then were depolarized by addition of a solution of KCl to a final K^+ concentration 25 mM. During this first contraction the absence of intact endothelium was verified by adding 1 μM acetylcholine, which failed to induce relaxation. The preparations were then extensively washed with Krebs-bicarbonate buffer and after equilibration for 2.5 h, a second contraction was evoked by K^+-depolarization (25 mM). When the response reached its plateau, cumulative concentrations of the vasodilating agent were added. The effect of MB 10 μM, Glibenclamide 10 μM and of MB-Glibenclamide co-administration on relaxation were evaluated in a separate series of experiments by adding the inhibitors 5 min before K^+-depolarization.

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