New 1,4-Dihydropyridines Conjugated to Furoxanyl Moieties, Endowed with Both Nitric Oxide-like and Calcium Channel Antagonist Vasodilator Activities

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A series of 4-phenyl-1,4-dihydropyridines substituted at the *ortho* and *meta* positions of the phenyl ring with NO-donating furoxan moieties and their non-NO-releasing furazan analogues were synthesized and pharmacologically characterized. The vasodilator activities of these compounds were evaluated on rat aorta and expressed as EC_{50} values or as EC_{50}^{iGC} values when obtained in the presence of inhibitors of guanylate cyclase methylene blue (MB) and 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ). Affinities to 1,4-DHP receptors on Ca^{2+} channels, expressed as IC_{50} values, were determined through displacement experiments of [^{3}H]-nitrendipine on rat cortex homogenates. A linear correlation between IC_{50} and EC_{50} values was found for compounds unable to release NO. EC_{50}^{calcd} values for derivatives containing NO-donor moieties, expression of the Ca^{2+}-blocking component of their vasodilator activity, were interpolated on this linear regression. They showed a good correspondence with EC_{50}^{iGC} values determined in the presence of soluble guanylate cyclase inhibitors. Analysis of EC_{50}^{iGC}/EC_{50} ratios provided a useful tool to distinguish well-balanced hybrids from derivatives biased toward Ca^{2+}-blocking or NO-dependent vasodilator activity. A detrimental effect on affinity to the 1,4-DHP receptor, due to substitution at the *ortho* and *meta* positions of the 4-phenyl ring, was observed. SAR to explain this effect is proposed.

Introduction

1,4-Dihydropyridines (DHPs) are an important class of drugs which are potent blockers of calcium (Ca^{2+}) currents through voltage-dependent L class Ca^{2+} channels.[1] They induce relaxation of vascular smooth muscle, preferentially in arterial beds, and display a negative inotropic effect on isolated cardiac muscle. In therapy, this class of drugs is principally used in the treatment of cardiac arrhythmias, peripheral vascular disorders, and hypertension.[2] Organic nitrates are also able to relax vascular smooth muscle, but their effect is more pronounced in venous beds than on arterial ones.[2] This action is a consequence of their ability to generate nitric oxide (NO), a physiological messenger which displays a variety of biological actions.[3] In particular NO plays important roles in maintaining microvascular and macrovascular homeostasis by dilating blood vessels, inhibiting platelet adhesion and aggregation, and attenuating leukocyte adhesion and activation. Combining of 1,4-dihydropyridine with an organic nitrate could be interesting for the treatment of a number of cardiovascular diseases. Several attempts aimed at achieving these results have been published recently.[4−7] They deal with the introduction of the nitroxy group ($-O-NO_2$) in the alkyl ester chain at the 3- and/or 5-position of the 4-phenyl-1,4-DHP system, as well as of nitroxyalkyloxy moieties at the 4-phenyl ring. The limit of this approach is that it is difficult to modulate the NO-donor properties of the $-O-NO_2$ substructure and consequently to balance the activity of the hybrid. This aspect is paramount in the design of a hybrid because the combined pharmacophoric groups must display their activity in the same concentration range.[5] There is interesting evidence that furoxans (1,2,5-oxadiazole 2-oxides) can behave as NO prodrugs, in the presence of thiol cofactors.[8,9] The overall reaction mechanism appears to be very complex, and direct NO radical release or intermediate formation of nitroxyl anion (NO^-), or both, could be involved. Using appropriate substituents at the heteroring, it is possible to modulate the properties of the NO production as well as the NO-dependent vasodilator activity of these compounds over a wide range. The pharmacological profile of furoxans as vasodilators is similar to that of nitrates, but they could lack significant tolerance development.[10,11] This behavior renders furoxan derivatives interesting tools in the design of hybrid cardiovascular drugs.

In previous works we reported the use of furoxan substructures to obtain well-balanced hybrids with mixed α_1-antagonist[12] or β_1-antagonist[13] and NO-dependent vasodilator properties. In this paper we discuss the preparation and the pharmacological characterization of a series of 4-phenyl-1,4-dihydropyridines, having furoxan moieties at the *ortho* or *meta* position of the phenyl ring (Chart 1, derivatives **24**−**29a**,**b**). The furoxan substituents were appropriately chosen in order to modulate the NO release of the final products. The related furazan derivatives (Chart 1, derivatives **24**−**29**) were also considered for control purpose, since they are unable to release NO. Derivatives **3** and **4**, in which the nitrate NO-donor function ($-O-NO_2$) is present, were considered for a comparison. Nifedipine (**1**) and its 3-NO_2 isomer **2** were taken as references.

Results and Discussion

Chemistry. Synthesis of a number of 1,4-DHPs containing methyl- or carbamoylfuroxan moieties re-

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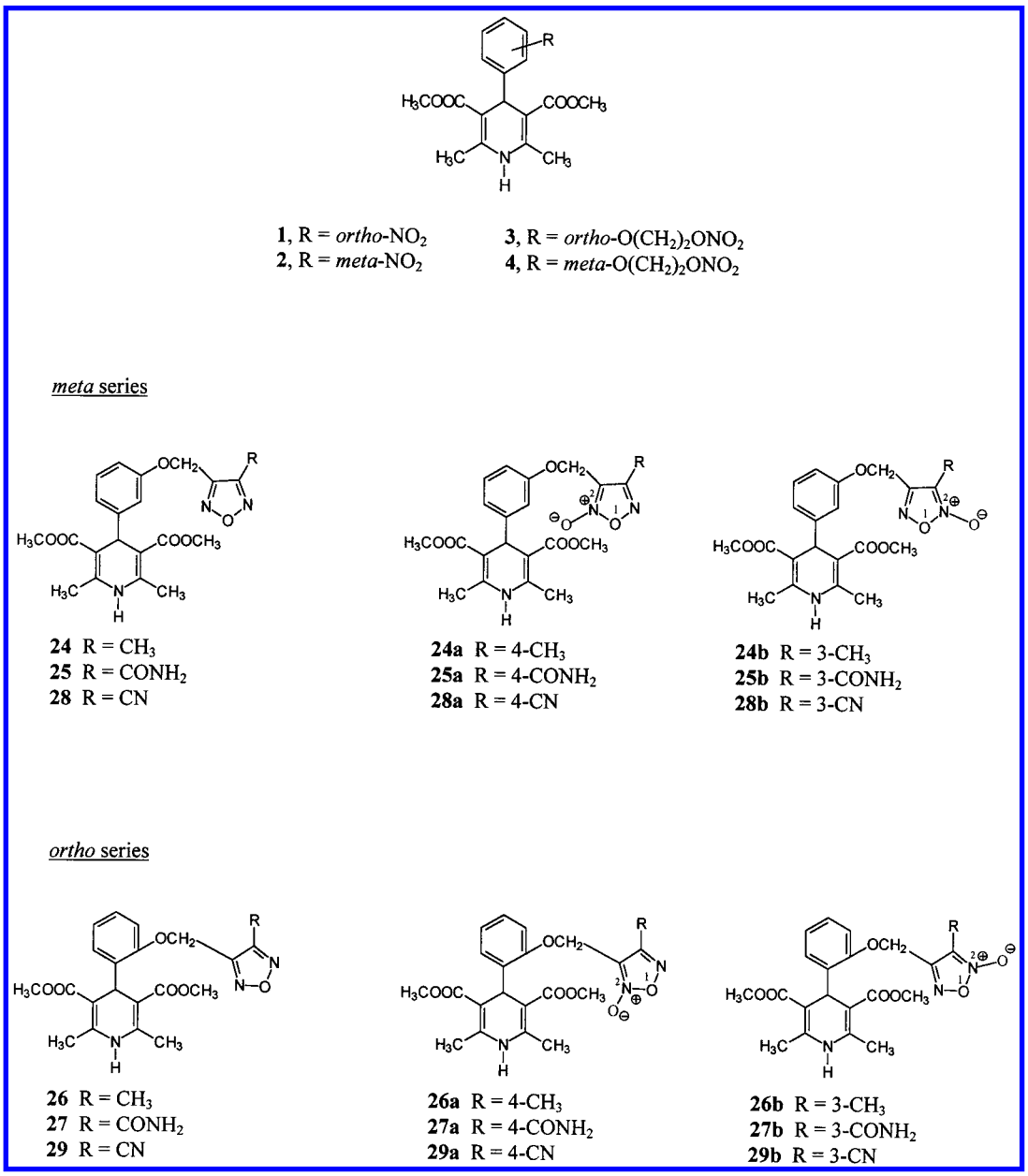
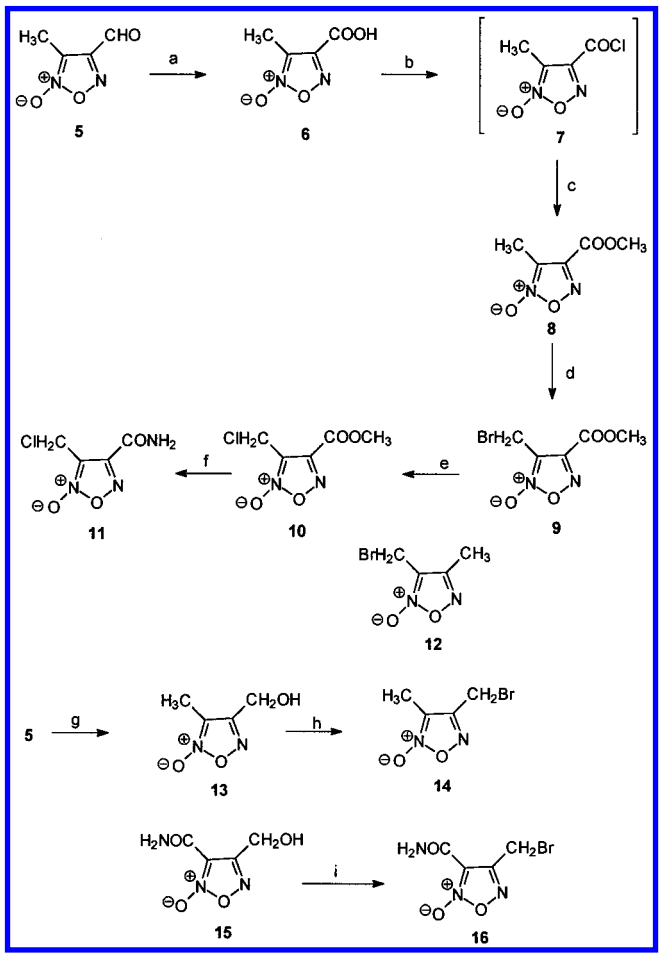
Chart 1



quired preliminary preparation of a series of (halomethyl)furoxans (Scheme 1). Selective bromination of the methyl group linked at the 3-position of the ester **8**, obtained by the classical sequence of reactions **5** → **6** → **7** → **8**, afforded **9**. In this reaction *N*-bromosuccinimide (NBS) and benzoyl peroxide in boiling carbon tetrachloride were used, according to a procedure we previously described for synthesis of **12**.[22] Derivative **9** was transformed into the chlorinated analogue **10** by the action of sodium chloride, under phase-transfer catalysis conditions. Derivative **10** was converted to **11** in the presence of 32% ammonia. Treatment of **9** in the same conditions gave 3-(aminomethyl)-4-furoxancarbonamide. Reduction of **5** with sodium borohydride afforded **13**, which was transformed into the expected bromomethyl derivative **14** by the action of thionyl bromide. Isomer **16** was obtained from **15** using NBS.

The aldehydes **19a**−**22a** and **19b**−**22b**, en route to the final 1,4-DHPs, were synthesized starting from the related (halomethyl)furoxans through nucleophilic substitution of the halogen by *m*-hydroxybenzaldehyde (**17**) or *o*-hydroxybenzaldehyde (**18**) in DMF, in the presence of sodium hydroxide (Scheme 2A). The final 1,4-DHPs (**24a**−**27a**, **24b**−**27b**) were obtained using the modified Hantzsch approach outlined in Scheme 2A. The appropriate aldehyde dissolved in absolute ethanol was treated with methyl 3-aminocrotonate (**23**) in the presence of trifluoroacetic acid to give the expected products. The furazan 1,4-DHPs **24**−**27** were obtained by reduction of the furoxan analogues in boiling trimethyl phosphite (Scheme 2B). In this reaction either series **a** or **b** furoxans can be used. Generally we started from isomers **a**, owing to the better yields in their preparation. Finally the cyano-substituted 1,4-DHPs (**28**, **29**, **28a**,**b**, **29a**,**b**) were prepared by dehydration of the amide analogues dissolved in dry pyridine in the presence of trifluoroacetic anhydride (Scheme 2C). All of the final furoxan DHPs were assessed for their ability to

Scheme 1[a]

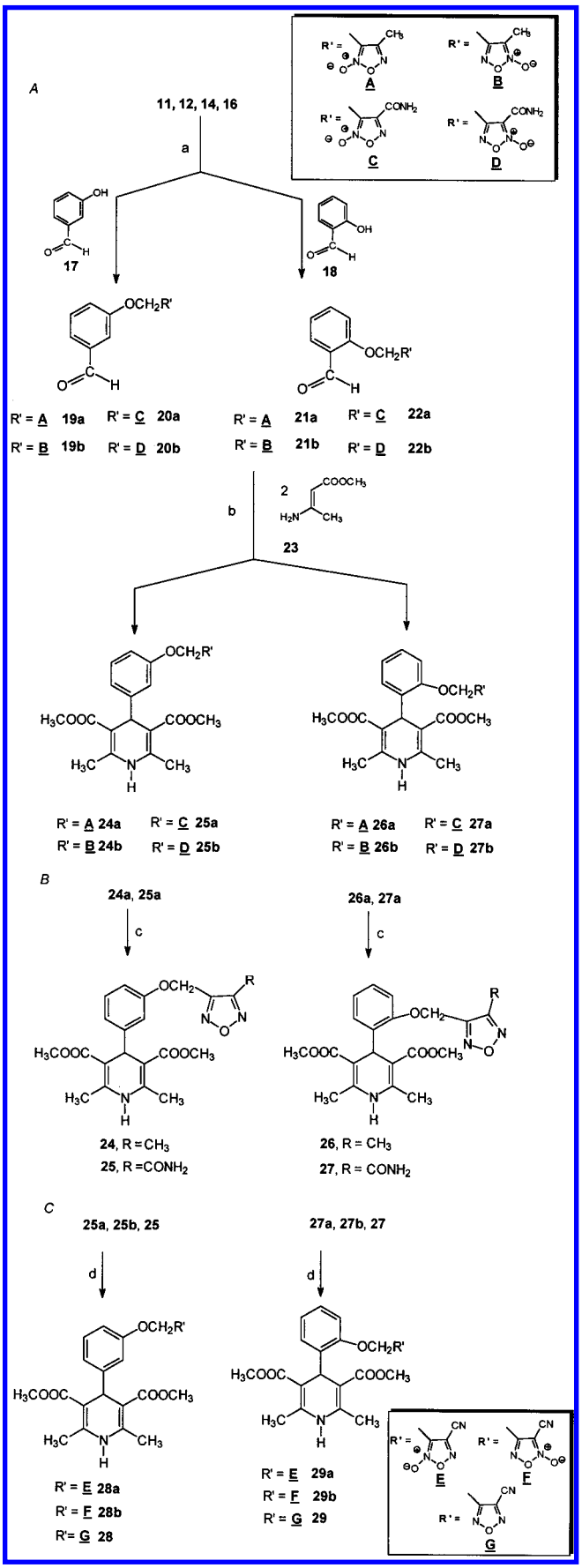


[a] (a) $KMnO_4$, H_2SO_4, acetone; (b) $SOCl_2$, DMF, reflux; (c) MeOH, 0 °C; (d) NBS, CCl_4, benzoyl peroxide, reflux; (e) NaCl, BTEAC, $CHCl_3$; (f) NH_3 (32%), MeOH; (g) $NaBH_4$, dioxane, 25 °C; (h) $SOBr_2$, DMF, CH_2Cl_2, 25 °C; (i) NBS, $(CH_3)_2S$, dry CH_2Cl_2.

generate NO in the presence of a large excess of cysteine (1:50) in buffer solution (pH = 7.4), at 37 °C. The extent of NO production after 1 h was determined by detection of nitrites, which are the oxidative metabolites of nitric oxide (Griess reaction).[9] The results expressed as percentages of NO_2^- (mol/mol) are reported in Table 1.

Pharmacology and Structure−Activity Relationship (SAR). Vasodilator activities of all 1,4-DHP derivatives were evaluated on K^+-depolarized rat thoracic aorta strips. Concentration−response curves were determined for each compound, and the potency, expressed as EC_{50} value, was calculated (Table 1). The experiments were repeated in the presence of methylene blue (MB) and, in some cases, in the presence of 1*H*-[1,2,4]-oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), well-known inhibitors of the soluble guanylate cyclase (sGC). A rightward shift of the concentration−response curves was taken as evidence of nitric oxide involvement in the vasodilator action. Potencies determined in these experiments, expressed as EC_{50}^{iGC} values, are reported in Table 1. A problem which arises in the study of hybrid DHPs containing NO-donor moieties is individual evaluation of the NO-dependent vasodilator component and of the component due to Ca^{2+} channel block. We addressed this aspect by subjecting all the compounds to

Scheme 2[a]



[a] (a) DMF, 50% NaOH, 50 °C; (b) absolute EtOH, CF_3COOH, 0 °C; (c) $P(OCH_3)_3$, reflux; (d) dry Pyr, $(CF_3CO)_2O$, 0 °C.

Table 1. Vasodilator Potencies, Radioligand Binding Affinities, and NO Generation Properties of 1,4-DHP Derivatives

compd	$EC_{50} \pm SE$ (nM)	$EC_{50}^{iGC} \pm SE$ (nM)	$IC_{50} \pm SE$ (nM)	$K_i \pm SE$ (nM)	$\%NO_2^- \pm SE$ (mol/mol)
2	3.4 ± 0.6	3.7 ± 0.3	2.6 ± 0.7	1.2 ± 0.3	
4	750 ± 90	750 ± 100	970 ± 350	440 ± 160	<0.5
24	640 ± 40	620 ± 60	720 ± 190	320 ± 90	
24a	400 ± 20	400 ± 50	610 ± 110	280 ± 50	<0.5
24b	500 ± 60	540 ± 60	820 ± 290	370 ± 130	<0.5
25	3500 ± 400	3300 ± 400	5100 ± 2400	2300 ± 1100	
25a	1200 ± 100	3700 ± 400	3600 ± 200	1600 ± 100	7.6 ± 0.5
25b	710 ± 70	1500 ± 100	2000 ± 400	920 ± 200	6.0 ± 0.2
28	310 ± 50	300 ± 30	590 ± 170	270 ± 80	
28a	11 ± 2	140 ± 20 520 ± 100[a]	500 ± 60	220 ± 30	31.5 ± 0.3
28b	3.3 ± 0.6	63 ± 17 560 ± 90[a]	500 ± 220	230 ± 100	43.8 ± 0.6
1	3.2 ± 0.4	3.4 ± 0.8	2.7 ± 0.4	1.2 ± 0.2	
3	56 ± 7	54 ± 7	85 ± 24	38 ± 11	<0.5
26	41 ± 2	46 ± 10	59 ± 14	27 ± 6	
26a	78 ± 10	76 ± 8	72 ± 2	32 ± 1	1.8 ± 0.1
26b	43 ± 4	46 ± 6	40 ± 6	18 ± 3	0.9 ± 0.2
27	970 ± 80	910 ± 60	880 ± 300	400 ± 140	
27a	560 ± 40	640 ± 40	780 ± 120	350 ± 60	12.3 ± 0.2
27b	550 ± 50	940 ± 120	1500 ± 500	670 ± 220	13.0 ± 0.1
29	6.6 ± 0.8	6.6 ± 0.7	10 ± 4	4.7 ± 2	
29a	9.4 ± 1.4	50 ± 6	67 ± 22	30 ± 10	24.0 ± 0.2
29b	4.4 ± 0.7	22 ± 6	27 ± 14	21 ± 6	40.8 ± 0.2

[a] Determined in the presence of ODQ. For all the other furoxan derivatives EC_{50}^{iGC} values determined in the presence of ODQ were the same as those determined in the presence of MB within the experimental error.

a competition study of inhibition of [^{3}H]nitrendipine binding to cerebral cortices, following the procedure reported in the Experimental Section. The concentration of each compound able to display 50% of [^{3}H]nitrendipine binding was determined from the competition curves (IC_{50} values, Table 1), and then the corresponding K_i constant was calculated (Table 1). These figures represent pure DHP receptor affinities. When log $1/EC_{50}$ values for the compounds not containing NO-donor moieties, namely, the furazan derivatives **24**−**29** and the models **1** and **2**, were plotted against the corresponding log $1/IC_{50}$ values, the following very satisfactory correlation (eq 1) was obtained:

$$\log 1/EC_{50} = 0.947(\pm 0.042) \log 1/IC_{50} + 0.443(\pm 0.299) \quad (1)$$

$$n = 8,\ r^2 = 0.988,\ s = 0.14$$

The equation indicates a very good relationship between DHP receptor occupancy and functional effect. By using eq 1 and the tabulated log $1/IC_{50}$ values, it is possible to calculate log $1/EC_{50}^{calcd}$ values (Table 2) for all the DHPs containing NO-donor moieties, namely, for the derivatives **3**, **4**, **24a**−**29a**, and **24b**−**29b**. A recent paper reports interesting evidence that NO released by *N*-acetyl-*S*-nitrosopenicillamine (SNAP; 100−800 μM) could inhibit expressed cardiovascular L-type Ca^{2+} channels through a non-cGMP-dependent pathway.[14] EC_{50}^{calcd} values, obtained for NO-donor models from eq 1, could then be partially influenced by this mechanism, if it were operating. However, our findings suggest that this mechanism is not involved, because nifedipine binding is not modified by increasing amounts (10^{-6}−10^{-9} M) of 4-phenyl-3-furoxancarbonitrile,[15] a typical NO-donor furoxan (unpublished data). Analysis of data in Table 2 shows that there is a good correspondence between log $1/EC_{50}^{calcd}$ values interpolated on the line (eq 1) and log $1/EC_{50}^{iGC}$ values determined in the presence of MB. The sole exceptions are the values relating to the cyano derivatives **28a**,**b**, which are potent NO donors belonging to the *meta* series. A good correspondence is again restored when EC_{50}^{iGC} values for these compounds, determined in the presence of ODQ, a more potent inhibitor of sGC, are taken into account. When log $1/EC_{50}^{iGC}$ values (in the presence of ODQ for derivatives **28a**,**b**) are plotted against log $1/EC_{50}^{calcd}$ values, a very satisfactory regression is obtained, in which the slope and the intercept are 1 and 0, respectively, in the range of the confidence limits (Figure 1). The above-reported picture indicates that EC_{50}^{iGC} values can be considered a measure of the vasodilator potencies of this set of structures, largely dependent on their Ca^{2+}-blocker properties. In the following discussion we assume that when $EC_{50}^{iGC}/EC_{50} \cong 1$, the compounds behave as practically pure Ca^{2+} antagonists (e.g., **26b** in Figure 2a). This applies to all the furazan derivatives (Chart 1, **24**−**29**) and to the references **1** and **2**, since they are unable to release NO. Analysis of the rightward

Table 2. Interpolated Values (EC_{50}^{calcd}, M) from Regression Eq 1 and Vasodilator Potencies Obtained in the Presence of sGC Inhibitors (EC_{50}^{iGC}, M), Expressed as Cologarithms, for NO-Donor 1,4-DHPs

compd	log $1/EC_{50}^{calcd} \pm CL$ (95%)	log $1/EC_{50}^{iGC} \pm CL$ (95%)	
4	6.14 ± 0.16	6.12 ± 0.15	
24a	6.33 ± 0.15	6.40 ± 0.16	
24b	6.21 ± 0.15	6.27 ± 0.14	
25a	5.61 ± 0.20	5.43 ± 0.12	
25b	5.84 ± 0.18	5.82 ± 0.08	
28a	6.41 ± 0.14	6.85 ± 0.21	6.28 ± 0.34[a]
28b	6.41 ± 0.14	7.20 ± 0.38	6.25 ± 0.26[a]
3	7.24 ± 0.12	7.27 ± 0.13	
26a	7.21 ± 0.14	7.12 ± 0.12	
26b	7.46 ± 0.13	7.34 ± 0.10	
27a	6.23 ± 0.15	6.19 ± 0.07	
27b	5.97 ± 0.17	6.03 ± 0.12	
29a	7.24 ± 0.12	7.30 ± 0.12	
29b	7.63 ± 0.14	7.66 ± 0.18	

[a] Determined in the presence of ODQ.

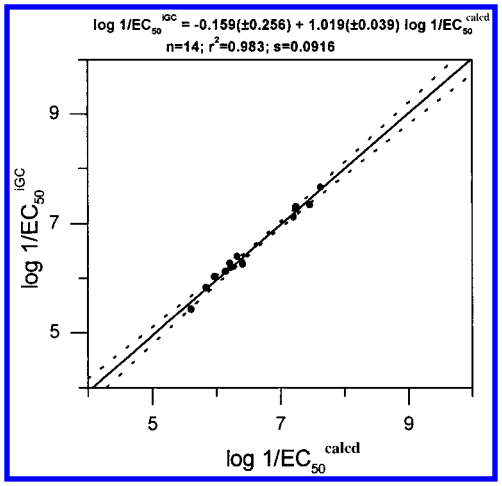


Figure 1. Correlation between log $1/EC_{50}^{iGC}$ and log $1/EC_{50}^{calcd}$ values for NO-donor 1,4-DHPs. Dashed lines represent 95% confidence limits for the regression line.

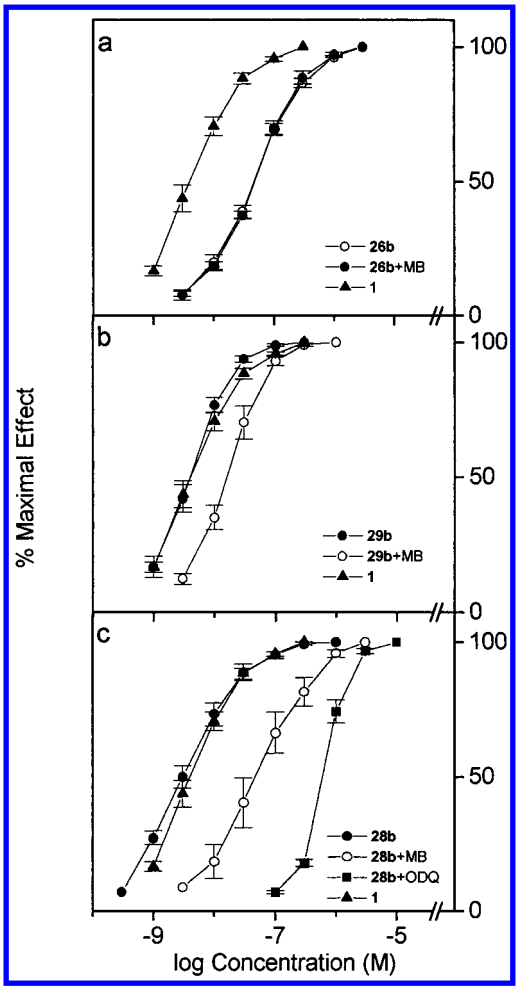


Figure 2. Concentration−response curves for vasodilator activity of compounds **26b** (a), **29b** (b), and **28b** (c) in the presence and absence of MB or ODQ. All points are mean values ± SE from independent experiments.

shift of the concentration curves was used to determine the relative contributions of Ca^{2+}-blocker and NO-mediated properties whereby values for EC_{50}^{iGC}/EC_{50} of 2−5 indicate well-balanced hybrids (e.g., **29b** in Figure 2b) and values greater than these indicate a predominantly NO-mediated vasodilator action (e.g., **28b** in Figure 2c).

Table 1 shows that substitution of methylfurazanylmethyloxy and methylfuroxanylmethyloxy moieties for the *m*-nitro group in **2** affords derivatives with similar potency, about 2 logarithm units lower than that of the reference. Since the methylfuroxan analogues **24a**,**b** have $EC_{50} \cong EC_{50}^{iGC}$, we conclude that they behave as pure Ca^{2+} channel blockers. Correspondingly these compounds produced relatively small amounts of NO_2^- in the presence of cysteine. Carbamoyl congeners of this series (**25**, **25a**,**b**) again display low potency. Furoxan isomers produce similar amounts of NO_2^- and behave as low-potency, well-balanced hybrids, since EC_{50}^{iGC}/EC_{50} is equal to 3 and 2, respectively. Cyano-substituted analogues **28** and **28a**,**b** are the most active members of the series; in particular **28b** is equipotent with the reference. Both the furoxan isomers display vasodilator activity that is principally dependent on their NO-donor properties (EC_{50}^{iGC}/EC_{50} for **28a** ≅ 47, for **28b** ≅ 170). This behavior parallels production of nitrite by the two derivatives. Study of derivative **3**, a recently described 1,4-dihydropyridine with a nitrate ester moiety,[7] shows that this compound is practically a pure Ca^{2+} channel blocker ($EC_{50}^{iGC}/EC_{50} \cong 1$) in the tested concentration range.

Substitution of methylfurazanylmethyloxy and methylfuroxanylmethyloxy moieties for the *o*-nitro group in **1** affords derivatives **26** and **26a**,**b**, which are 1 logarithm unit less potent than the lead. In this series, too, methylfuroxan congeners are very feeble NO donors and display $EC_{50} \cong EC_{50}^{iGC}$. Therefore their vasodilator activity is principally due to Ca^{2+} channel block. Carbamoyl analogues **27** and **27a**,**b** are the least active members of the group. Unlike the isomer **27a**, which has $EC_{50}^{iGC}/EC_{50} \cong 1$, **27b** shows $EC_{50}^{iGC}/EC_{50} \cong 2$, and so it can be considered a well-constructed hybrid of low potency. Also in the *ortho* series, cyano derivatives **29** and **29a**,**b** are the most active compounds. Their potency is comparable to that of **1**. Analysis of EC_{50}^{iGC}/EC_{50} ratios (**29a**,**b** $EC_{50}^{iGC}/EC_{50} \cong 5$) indicates that both the furoxans are potent, well-balanced hybrid structures. Model **4**[7] behaves as a pure Ca^{2+} channel antagonist in the range of the considered concentrations.

Analysis of the Ca^{2+} channel-blocking activities (Table 1, EC_{50}^{iGC}, IC_{50}) shows some qualitative structure−activity relationships. The compounds belonging to the *ortho* series display higher Ca^{2+} channel-blocker potency than the corresponding compounds of the *meta* series. In both series, NH_2CO derivatives are markedly less potent than all the other compounds. In addition, the furoxan isomers together with the furazan analogue display similar potency, with the exception of derivative **29**. Finally, the substitution of R-furazanyl-CH_2O− and R-furoxanyl-CH_2O− moieties for the NO_2 group in **1** and **2**, respectively, is detrimental to Ca^{2+}-blocker activity. The results of a QSAR study by Coburn et al.[16] on a series of 4-phenyl-1,4-dihydropyridine derivatives with *ortho* and *meta* monosubstitutions at the phenyl ring can help to explain these findings. In Coburn's paper the effect of a number of substituents on the tonic contractile response of longitudinal muscle strips of

guinea pig ileum was correlated satisfactorily by eq 2:

$$\log 1/IC_{50} = 0.69(\pm 0.10)\pi + 2.32(\pm 0.49)\sigma_m - 0.49(\pm 0.10)L_m + 8.01(\pm 0.32) \quad (2)$$

$$n = 29,\ r = 0.87,\ s = 0.67,\ F = 25.35$$

in which the appropriate σ_m is entered for the substituent regardless of whether it is in the *ortho* or *meta* position. Use of eq 2 in analyzing structure−activity relationships in the two series of derivatives described here is questionable, because the correlation was obtained using pharmacological data from a different tissue. In addition, the substituents present at the phenyl ring in our compounds have different shapes and are significantly larger than any other substituent used to derive the equation, so they could behave differently with respect to the original group, particularly in the *ortho* series. Nevertheless eq 2 seems to explain some trends. In a recent paper we showed that methylfurazanyl and methylfuroxanyl substructures display σ_I values close to that of the chlorine group.[17] Therefore, since σ_I of the $-OCH_2Cl$ moiety is $+0.41$,[18] we expect values near this for all our substituents. Calculation of their L constants, using the CCPK program,[19] shows that they range between 7.21 and 7.96. Finally, π constants were calculated by the CLogP algorithm[20] ($\pi_{OCH_2-Fx-R} = 0.045, -0.442, -1.010$; $\pi_{OCH_2-Fz-R} = 0.203, -0.072, -1.018$; R = CN, CH_3, $CONH_2$; Fx = furoxanyl; Fz = furazanyl). In this approximation, π values for isomer furoxan substituents are identical. On the basis of these molecular descriptors, we could infer that the extensive length of the substituents is responsible for the decrease in potency of the *meta* series with respect to the *ortho* one. Since σ_m and L have similar values for all the substituents, the low potency of NH_2CO derivatives should principally highlight the low lipophilicity of the carbamoyl group. In each series, the similar potency displayed by furoxan isomers and the furazan analogue should reflect the general resemblance between their molecular descriptors. Electronic and steric properties, combined with lipophilicity in the case of NH_2CO derivatives, should be chiefly responsible for the decrease in the activity of all the compounds of the *meta* series with respect to NO_2 derivative **2** (NO_2, $\sigma_m = 0.71$, $\pi = -0.337$, $L = 3.44$). Finally, electronic properties, again combined with lipophilicity in the carbamoyl derivatives, could be paramount in determining the lower potency of the derivatives of the *ortho* series compared to the nitro lead **1**. However this analysis is made more difficult for the possible presence of *ortho* effects not scaled in eq 2.

In conclusion this work demonstrates the possibility of obtaining "well-balanced" hybrids with mixed Ca^{2+} channel-blocker and NO-dependent vasodilator activities by using appropriate furoxan substructures. In addition, a procedure for the individual evaluation of the two kinds of activities is proposed.

Experimental Section

Chemistry. Melting points (uncorrected) were obtained on a Büchi 530 apparatus after introducing the sample into the bath at a temperature 10 °C lower than the melting point. A heating rate of 1 °C min^{-1} was used, 3 °C min^{-1} in the case of decomposition. All of the compounds were routinely checked by infrared spectrophotometry (Shimadzu FT-IR 8101M). ^1H and ^{13}C nuclear magnetic resonance spectra were recorded at 200 and 50 MHz, respectively, on a Brucker AC-200 spectrometer and are reported in δ (ppm) units (§, tentatively assigned; ∗, not assigned). Column chromatography was performed on silica gel (Merck Kieselgel 60, 230−400 mesh ASTM) with the indicated solvent system. Anhydrous $MgSO_4$ was used as drying agent. 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 the theoretical values. Intermediates **5**[21] and **12**[22] and derivatives **2**,[23] **3**, and **4**[7] were synthesized according to the literature. Derivative **15** was a gift from Dr. K. Schönafinger, H.M.R., Frankfurt, and **1** was purchased from Sigma. Nitrite production by furoxans in the presence of L-cysteine was evaluated according to the procedure described in ref 9.

3-Methyl-4-furoxancarboxylic Acid (6). $KMnO_4$ (7.9 g, 50 mmol) was added portionwise to a stirred, iced water-cooled mixture of **5** (7.7 g, 60 mmol) dissolved in acetone (5 mL) and 12 N H_2SO_4 (50 mL). The temperature was not permitted to rise above 15 °C. Stirring was continued for 12 h at room temperature, and the excess of $KMnO_4$ was destroyed with Na_2SO_3. The mixture was extracted with EtOAc, and the combined organic phases were treated with 2 N NaOH (3 × 15 mL). The aqueous phase was strongly acidified by adding 12 N H_2SO_4 and then extracted with EtOAc and dried. Solvent removal afforded a residue which was filtered on a short silica gel column (petroleum ether/EtOAc, 7:3) (4.3 g, 50%): mp 92 °C (benzene) (lit.[24] mp 92 °C).

Methyl 3-Methyl-4-furoxancarboxylate (8). A solution of **6** (18 g, 125 mmol) in thionyl chloride (60 mL) containing a catalytic amount of dry DMF was refluxed for 1 h. Solvent removal gave **7** which, without any further purification, was treated under stirring and external cooling with dry methanol (30 mL). The resulting precipitate was filtered off, dried, and purified by flash chromatography (petroleum ether/EtOAc, 7:3) to give the title product (11.8 g, 60%): mp 80−81 °C (EtOAc/petroleum ether). ^1H NMR (DMSO-d_6): 2.36 (s, 3H, CH_3); 4.03 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6): 156.3 (COO); 148.9 (C4); 112.1 (C3); 53.3 (OCH_3); 8.37 (CH_3). Anal. ($C_5H_6N_2O_4$) C, H, N.

Methyl 3-(Bromomethyl)-4-furoxancarboxylate (9). NBS and a catalytic amount of benzoyl peroxide were added to a solution of **8** (4.7 g, 30 mmol) in CCl_4 (70 mL). The mixture was refluxed for 48 h under stirring. Solvent removal gave a residue which was purified by flash chromatography (petroleum ether/CH_2Cl_2, 1:1). The title product was obtained as a pure pale-yellow oil (4.4 g, 63%). ^1H NMR ($CDCl_3$): 4.50 (s, 2H, CH_2); 4.02 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$): 157.0 (COO); 146.8 (C4); 112.7 (C3); 53.2 (s, 3H, OCH_3); 15.8 (CH_2). Anal. ($C_5H_5N_2O_4Br$) C, H, N.

3-(Chloromethyl)-4-furoxancarbonamide (11). Benzyltriethylammonium chloride (BTEAC) (6.6 g, 29 mmol) and NaCl (16.9 g, 290 mmol) were added to a well-stirred solution of **9** (7.0 g, 29 mmol) in $CHCl_3$ (30 mL). The mixture was kept under stirring at room temperature for 3 days. The formed precipitate was filtered and washed with $CHCl_3$, and the organic phase was dried and evaporated. The residue obtained was extracted with EtOAc. Solvent removal gave **10** which was filtered on a short column (petroleum ether/EtOAc, 9:1). The oil obtained after evaporation of the solvent, without any additional purification, was dissolved in methanol (20 mL), and 32% ammonia (15 mL) was added dropwise to the iced water-cooled and well stirred solution. The resulting white precipitate was filtered off, washed with water, and dried (4.1 g, 70%): mp 149 °C ($CHCl_3$). ^1H NMR (DMSO-d_6): 4.84 (s, 2H, CH_2); 8.32, 8.86 (2s, 2H, $CONH_2$). ^{13}C NMR (DMSO-d_6): 158.1 ($CONH_2$); 150.4 (C4); 113.7 (C3); 32.7 (CH_2). Anal. ($C_4H_4N_3O_3Cl$) C, H, N.

3-Methyl-4-furoxanmethanol (13). $NaBH_4$ (3.0 g, 80 mmol) was added portionwise to a solution of **5** (5.1 g, 40 mmol) in dioxane (40 mL), keeping the temperature at 20−25 °C. The reaction mixture was stirred for 12 h at room temperature. The residue obtained by concentration of the solution was treated with water and extracted with EtOAc. The organic

phase was evaporated, and the crude oil obtained was purified by flash chromatography (petroleum ether/EtOAc, 7:1). The title product was obtained as a pale-yellow oil (4.6 g, 50%) (lit.[25] oil).

4-(Bromomethyl)-3-methylfuroxan (14). Thionyl bromide (1.55 mL, 20 mmol) was added dropwise to a stirred and ice−salt-cooled solution (−10 °C) of **13** (1.3 g, 10 mmol) in CH_2Cl_2 (50 mL) containing a few drops of DMF. The reaction mixture was kept under stirring at room temperature for 12 h. After this time, water (50 mL) was added, and the mixture was extracted with CH_2Cl_2. By evaporation of the dried combined organic layers, an oily residue was obtained which was purified by flash chromatography (petroleum ether/EtOAc, 7:3) to give the title product (1.0 g, 50%) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): 2.20 (s, 3H, CH_3); 4.38 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$): 154.4 (C4); 111.8 (C3); 18.5 (CH_2); 7.4 (CH_3). Anal. ($C_4H_5N_2O_2Br$) C, H, N.

4-(Bromomethyl)-3-furoxancarbonamide (16). NBS (5.0 g, 20 mmol) and methyl sulfide (2.6 mL, 35 mmol) were mixed under dry atmosphere in CH_2Cl_2 (90 mL) at 0 °C. The reaction mixture was cooled at −15 °C, and then **15** (3.0 g, 19 mmol) was added under stirring. The mixture was allowed to reach room temperature and was stirred for 24 h. After this time, equivalent amounts of NBS (3.3 g, 19 mmol) and methyl sulfide (1.40 mL, 19 mmol) were added, and the stirring was continued for 4 h. Then the reaction mixture was extracted with water, and the dried organic phases were evaporated to give the title product as a pale-yellow oil which was purified by flash chromatography (CH_2Cl_2) (3.0 g, 72%): mp 91−92 °C ($CHCl_3$, petroleum ether). ^{1}H NMR ($CDCl_3$): 5.0 (s, 2H, CH_2); 7.65, 8.52 (2s, 2H, $CONH_2$). ^{13}C NMR ($CDCl_3$): 155.4[§] ($CONH_2$); 155.8[§] (C4); 109.8 (C3); 20.4 (CH_2). Anal. ($C_4H_4N_3O_3Br$) C, H, N.

General Method of Preparation of Substituted Benzaldehydes 19a−22a and 19b−22b. NaOH (50%) (6 mmol) was added to a stirred solution of the appropriate (halomethyl)-furoxans (5 mmol) and hydroxybenzaldehyde **17** or **18** (6 mmol) in DMF (20 mL) kept at 50 °C. The stirring at 50 °C was continued for 30 min, and then the reaction mixture was poured into water. The resulting precipitate was filtered off and dissolved in EtOAc. The organic solution, washed with 2 N NaOH and then with water, was dried and evaporated to give the expected compounds.

3-[(4-Methylfuroxan-3-yl)methoxy]benzaldehyde (19a): mp 81−82 °C ($CHCl_3$/petroleum ether, 50%). ^{1}H NMR (DMSO-d_6): 10.0 (s, 1H, CHO); 7.4−7.8 (m, 4H, Ph); 5.24 (s, 2H, OCH_2); 2.42 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): 192.8 (CHO); 157.8, 137.8, 130.8, 123.9, 121.7, 114.2 (Ph); 58.2 (OCH_2); 113.4 (C3); 155.8 (C4); 10.8 (CH_3). Anal. ($C_{11}H_{10}N_2O_4$) C, H, N.

3-[(3-Methylfuroxan-4-yl)methoxy]benzaldehyde (19b): mp 103−104 °C ($CHCl_3$/petroleum ether, 50%). ^{1}H NMR (DMSO-d_6): 10.0 (s, 1H, CHO); 7.4−7.6 (m, 4H, Ph); 5.43 (s, 2H, OCH_2); 2.27 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): 192.8 (CHO); 158.0, 137.8, 130.7, 123.8, 121.8, 114.3 (Ph); 61.0 (OCH_2); 113.1 (C3); 155.4 (C4); 7.59 (CH_3). Anal. ($C_{11}H_{10}N_2O_4$) C, H, N.

3-[(4-Carbamoylfuroxan-3-yl)methoxy]benzaldehyde (20a): mp 155−156 °C (EtOAc/petroleum ether). ^{1}H NMR (DMSO-d_6): 10.0 (s, 1H, CHO); 7.4−7.6 (m, 4H, Ph); 5.33 (s, 2H, OCH_2); 8.29, 8.62 (2s, 2H, $CONH_2$). ^{13}C NMR (DMSO-d_6): 197.2 (CHO); 158.0*, 137.8, 130.7, 123.5, 121.6, 114.4 (Ph); 58.3 (OCH_2); 112.0 (C3); 151.4 (C4); 158.2* ($CONH_2$). Anal. ($C_{11}H_{10}N_2O_4$) C, H, N.

3-[(3-Carbamoylfuroxan-4-yl)methoxy]benzaldehyde (20b): mp 150−151 °C (EtOAc/petroleum ether). ^{1}H NMR (DMSO-d_6): 10.0 (s, 1H, CHO); 5.53 (s, 2H, OCH_2); 7.4−7.6 (m, 4H, Ph); 7.86, 8.52 (2s, 2H, $CONH_2$). ^{13}C NMR (DMSO-d_6): 192.9 (CHO); 158.2, 137.8, 130.6, 123.7, 121.7, 114.4 (Ph); 61.5 (OCH_2); 110.6 (C3); 155.8* (C4); 155.2* ($CONH_2$). Anal. ($C_{11}H_{10}N_2O_4$) C, H, N.

2-[(4-Methylfuroxan-3-yl)methoxy]benzaldehyde (21a): mp 103−104 °C ($CHCl_3$/petroleum ether, 70%). ^{1}H NMR (DMSO-d_6): 10.3 (s, 1H, CHO); 7.2−7.8 (m, 4H, Ph); 5.31 (s, 2H, OCH_2); 2.44 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): 189.1 (CHO); 159.4, 136.4, 128.3, 125.0, 122.2, 114.2 (Ph); 58.7 (OCH_2); 113.4 (C3); 155.8 (C4); 10.8 (CH_3). Anal. ($C_{11}H_{10}N_2O_4$) C, H, N.

2-[(3-Methylfuroxan-4-yl)methoxy]benzaldehyde (21b): mp 97−98 °C ($CHCl_3$/petroleum ether, 65%). ^{1}H NMR (DMSO-d_6): 10.4 (s, 1H, CHO); 7.1−7.8 (m, 4H, Ph); 5.49 (s, 2H, OCH_2); 2.22 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): 189.2 (CHO); 159.6, 136.4, 128.3, 124.8, 121.9, 114.1 (Ph); 61.3 (OCH_2); 113.4 (C3); 155.8 (C4); 7.6 (CH_3). Anal. ($C_{11}H_{10}N_2O_4$) C, H, N.

2-[(4-Carbamoylfuroxan-3-yl)methoxy]benzaldehyde (22a): mp 149−150 °C ($CHCl_3$/petroleum ether, 75%). ^{1}H NMR (DMSO-d_6): 10.3 (s, 1H, CHO); 7.1−7.8 (m, 4H, Ph); 5.42 (s, 2H, OCH_2); 8.28, 8.61 (2s, 2H, $CONH_2$). ^{13}C NMR (DMSO-d_6): 189.2 (CHO); 158.2[§], 136.4, 127.8, 125.1, 122.2, 114.6 (Ph); 59.1 (OCH_2); 112.0 (C3); 151.4 (C4); 159.8[§] ($CONH_2$). Anal. ($C_{11}H_{10}N_2O_4$) C, H, N.

2-[(3-Carbamoylfuroxan-4-yl)methoxy]benzaldehyde (22b): mp 150−151 °C (EtOAc/petroleum ether, 178−179 °C). ^{1}H NMR (DMSO-d_6): 10.4 (s, 1H, CHO); 5.59 (s, 2H, OCH_2); 7.13−7.76 (m, 4H, Ph); 7.85, 8.50 (2s, 2H, $CONH_2$). ^{13}C NMR (DMSO-d_6): 189.2 (CHO); 160.1, 136.4, 127.7, 124.7, 121.8, 114.5 (Ph); 62.1 (OCH_2); 110.7 (C3); 155.8* (C4); 155.7* ($CONH_2$). Anal. ($C_{11}H_{10}N_2O_4$) C, H, N.

General Method of Preparation of 1,4-DHPs 24a−27a and 24b−27b. A solution of methyl 3-aminocrotonate (**23**) (1.0 g, 9 mmol) in absolute ethanol (10 mL) was added dropwise to a stirred and ice-cooled (0 °C) solution of the appropriate benzaldehyde (3 mmol) in absolute ethanol (10 mL) and trifluoroacetic acid (0.7 g, 6 mmol). Stirring was continued for 3 h at 0 °C. Then the resulting precipitate was filtered off, washed with a small amount of absolute ethanol, and dried.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[3-[(4-methylfuroxan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (24a): mp 159−160 °C ($CHCl_3$/petroleum ether, 60%). ^{1}H NMR (DMSO-d_6): 8.90 (s, 1H, −NH); 3.56 (s, 3H, $-OCH_3$); 4.88 (s, 1H, C(4)−H); 2.27 (s, 3H, 2,6-CH_3); 6.7−7.2 (m, 4H, Ph); 5.04 (s, 2H, $-OCH_2$); 2.34 (s, 3H, $-CH_3$). ^{13}C NMR (DMSO-d_6): 18.3 (2,6-CH_3); 38.6 (4-CH); 50.8 (OCH_3); 101.3 (3,5-C); 146.0 (2,6-C); 167.4 (COO); 157.0, 149.7, 129.5, 120.9, 114.0, 113.5[§] (Ph); 58.0 (OCH_2); 112.2[§] (C3); 155.7 (C4); 8.0 (CH_3). Anal. ($C_{21}H_{23}N_3O_7$) C, N, H.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[3-[(3-methylfuroxan-4-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (24b): mp 163−164 °C ($CHCl_3$/petroleum ether, 60%). ^{1}H NMR (DMSO-d_6): 8.92 (s, 1H, −NH); 3.57 (s, 3H, $-OCH_3$); 4.89 (s, 1H, C(4)−H); 2.28 (s, 3H, 2,6-CH_3); 6.8−7.2 (m, 4H, Ph); 5.24 (s, 2H, $-OCH_2$); 2.17 (s, 3H, $-CH_3$). ^{13}C NMR (DMSO-d_6): 18.3 (2,6-CH_3); 38.6 (4-CH); 50.8 (OCH_3); 101.3 (3,5-C); 146.0 (2,6-C); 167.4 (COO); 157.3, 149.7, 129.4, 120.9, 114.1, 113.1[§] (Ph); 60.7 (OCH_2); 112.2[§] (C3); 155.8 (C4); 7.67 (CH_3). Anal. ($C_{21}H_{23}N_3O_7$) C, N, H.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[3-[(4-carbamoylfuroxan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (25a): mp 215−216 °C dec (EtOAc/petroleum ether, 85%). ^{1}H NMR (DMSO-d_6): 8.87 (s, 1H, −NH); 3.57 (s, 3H, $-OCH_3$,); 4.88 (s, 1H, C(4)−H); 2.27 (s, 3H, 2,6-CH_3); 6.7−7.2 (m, 4H, Ph); 5.13 (s, 2H, $-OCH_2$); 8.59, 8.26 (2s, 2H, $CONH_2$). ^{13}C NMR (DMSO-d_6): 18.7 (2,6-CH_3); 38.6 (4-CH); 50.7 (OCH_3); 101.3 (3,5-C); 145.9 (2,6-C); 167.4 (COO); 157.2, 149.7, 129.2, 120.6, 113.6*, 111.7* (Ph); 57.7 (OCH_2); 112.2* (C3); 151.4 (C4); 158.2 ($CONH_2$). Anal. ($C_{21}H_{22}N_4O_8$) C, N, H.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[3-[(3-carbamoylfuroxan-4-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (25b): mp 209−210 °C dec (EtOAc/petroleum ether, 85%). ^{1}H NMR (DMSO-d_6): 8.93 (s, 1H, −NH); 3.58 (s, 3H, $-OCH_3$), 4.90 (s, 1H, C(4)−H); 2.28 (s, 3H, 2,6-CH_3); 6.8−7.2 (m, 4H, Ph); 5.36 (s, 2H, $-OCH_2$); 8.52, 7.86 (2s, 2H, $CONH_2$). ^{13}C NMR (DMSO-d_6): 18.3 (2,6-CH_3); 38.5 (4-CH); 50.8 (OCH_3); 101.4 (3,5-C); 146.0 (2,6-C); 167.5 (COO); 157.5, 149.6, 129.4, 120.4, 113.8, 111.9[§] (Ph); 61.0 (OCH_2); 110.5[§] (C3); 155.4* (C4); 155.9* ($CONH_2$). Anal. ($C_{21}H_{22}N_4O_8$) C, N, H.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[2-[(4-methylfuroxan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (26a): mp 184−185 °C ($CHCl_3$/petroleum ether, 70%). ^{1}H NMR

(DMSO-d_6): 8.64 (s, 1H, −NH); 3.45 (s, 3H, $-OCH_3$); 5.14 (s, 1H, C(4)−H); 2.16 (s, 3H, 2,6-CH_3); 6.9−7.1 (m, 4H, Ph); 5.02 (s, 2H, $-OCH_2$); 2.45 (s, 3H, $-CH_3$). ^{13}C NMR (DMSO-d_6): 18.0 (2,6-CH_3); 35.1 (4-CH); 50.4 (OCH_3); 101.6 (3,5-C); 145.2 (2,6-C); 167.7 (COO); 155.9*, 135.3, 127.5, 130.1, 112.2§ (Ph); 58.1 (OCH_2); 113.7§ (C3); 154.9* (C4); 10.7 (CH_3). Anal. ($C_{21}H_{23}N_3O_7$) C, N, H.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[2-[(3-methylfuroxan-4-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (26b): mp 168−169 °C ($CHCl_3$/petroleum ether, 60%). ^{1}H NMR (DMSO-d_6): 8.71 (s, 1H, −NH); 3.44 (s, 3H, $-OCH_3$), 5.18 (s, 1H, C(4)−H); 2.16 (s, 3H, 2,6-CH_3); 6.9−7.2 (m, 4H, Ph); 5.21 (s, 2H, $-OCH_2$); 2.25 (s, 3H, $-CH_3$). ^{13}C NMR (DMSO-d_6): 18.1 (2,6-CH_3); 34.5 (4-CH); 61.0 (OCH_3); 100.9 (3,5-C); 145.2 (2,6-C); 167.7 (COO); 156.0, 135.8, 129.8, 127.5, 121.5, 112.6§ (Ph); 61.0 (OCH_2); 113.5 (C3); 154.7 (C4); 7.5 (CH_3). Anal. ($C_{21}H_{23}N_3O_7$) C, N, H.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[2-[(4-carbamoylfuroxan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (27a): mp 167−168 °C (EtOAc/petroleum ether, 85%). ^{1}H NMR (DMSO-d_6): 8.48 (s, 1H, −NH); 3.44 (s, 3H, $-OCH_3$), 5.04 (s, 1H, C(4)−H); 2.11 (s, 3H, 2,6-CH_3); 6.9−7.1 (m, 4H, Ph); 5.10 (s, 2H, $-OCH_2$); 8.59, 8.30 (2s, 2H, $CONH_2$). ^{13}C NMR (DMSO-d_6): 18.0 (2,6-CH_3); 36.0 (4-CH); 50.3 (OCH_3); 101.2 (3,5-C); 145.6 (2,6-C); 167.6 (COO); 155.8, 135.1, 130.7, 127.4, 120.4, 112.4* (Ph); 57.9 (OCH_2); 112.1* (C3); 151.8 (C4); 158.2 ($CONH_2$). Anal. ($C_{21}H_{22}N_4O_8$) C, N, H.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[2-[(3-carbamoylfuroxan-4-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (27b): mp 161−163 °C (EtOAc/petroleum ether, 85%). ^{1}H NMR (DMSO-d_6): 8.64 (s, 1H, −NH); 3.46 (s, 3H, $-OCH_3$), 5.15 (s, 1H, C(4)−H); 2.14 (s, 3H, 2,6-CH_3); 6.8−7.1 (m, 4H, Ph); 5.31 (s, 2H, $-OCH_2$); 8.51, 7.82 (2s, 2H, $CONH_2$). ^{13}C NMR (DMSO-d_6): 18.0 (2,6-CH_3); 35.1 (4-CH); 50.4 (OCH_3); 101.7 (3,5-C); 145.5 (2,6-C); 167.7 (COO); 155.8*, 136.2, 130.3, 127.4, 121.2, 113.0§ (Ph); 61.7 (OCH_2); 110.5§ (C3); 155.5* (C4); 155.9* (CH_3). Anal. ($C_{21}H_{22}N_4O_8$) C, N, H.

General Method of Preparation of 1,4-DHPs 24−27. A solution of the appropriate furoxan 1,4-DHPs **24a**−**27a** (2 mmol) in trimethyl phosphite (15 mL) was refluxed for 9 h. Then the reaction mixture, cooled at room temperature, was poured into 2 N HCl (150 mL). The resulting precipitate was filtered off, washed several times with water, and dried.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[3-[(4-methylfurazan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (24): mp 155−156 °C ($CHCl_3$/petroleum ether, 58%). ^{1}H NMR (DMSO-d_6): 8.93 (s, 1H, −NH); 3.56 (s, 3H, $-OCH_3$); 4.88 (s, 1H, C(4)−H); 2.27 (s, 3H, 2,6-CH_3); 6.7−7.2 (m, 4H, Ph); 5.34 (s, 2H, $-OCH_2$); 2.42 (s, 3H, $-CH_3$). ^{13}C NMR (DMSO-d_6): 18.3 (2,6-CH_3); 38.6 (4-CH); 50.8 (OCH_3); 101.3 (3,5-C); 146.0 (2,6-C); 167.4 (COO); 157.4, 149.7, 129.4, 120.6, 114.0, 112.2 (Ph); 58. 9 (OCH_2); 152.0* (C3); 151.9* (C4); 8.0 (CH_3). Anal. ($C_{21}H_{23}N_3O_6$) C, H, N.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[3-[(4-carbamoylfurazan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (25): mp 202−203 °C (EtOAc/petroleum ether, 90%). ^{1}H NMR (DMSO-d_6): 8.91 (s, 1H, −NH); 3.57 (s, 3H, $-OCH_3$), 4.88 (s, 1H, C(4)−H); 2.27 (s, 3H, 2,6-CH_3); 6.7−7.2 (m, 4H, Ph); 5.46 (s, 2H, $-OCH_2$); 8.58, 8.22 (2s, 2H, $CONH_2$). ^{13}C NMR (DMSO-d_6): 18.4 (2,6-CH_3); 38.6 (4-CH); 50.8 (OCH_3); 101.4 (3,5-C); 146.1 (2,6-C); 167.5 (COO); 157.5, 149.6, 129.4, 120.4, 113.7, 112.0 (Ph); 59.5 (OCH_2); 148.9 (C3); 152.5 (C4); 158.4 ($CONH_2$). Anal. ($C_{21}H_{22}N_4O_7$) C, N, H.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[2-[(4-methylfurazan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (26): mp 153−154 °C ($CHCl_3$/petroleum ether, 70%). ^{1}H NMR (DMSO-d_6): 8.64 (s, 1H, −NH); 3.40 (s, 3H, $-OCH_3$); 5.20 (s, 1H, C(4)−H); 2.16 (s, 3H, 2,6-CH_3); 6.9−7.1 (m, 4H, Ph); 5.32 (s, 2H, $-OCH_2$); 2.51 (s, 3H, $-CH_3$). ^{13}C NMR (DMSO-d_6): 18.0 (2,6-CH_3); 34.0 (4-CH); 50.4 (OCH_3); 101.1 (3,5-C); 145.1 (2,6-C); 167.7 (COO); 154.6, 135.9, 129.6, 127.4, 121.4, 112.4 (Ph); 59. 0 (OCH_2); 152.1* (C3); 155.7 (C4); 8.0 (CH_3). Anal. ($C_{21}H_{23}N_3O_6$) C, N, H.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[2-[(4-carbamoylfurazan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (27): mp 182−184 °C (EtOAc/petroleum ether, 85%). ^{1}H NMR (DMSO-d_6): 8.60* (s, 1H, −NH); 3.45 (s, 3H, $-OCH_3$), 5.18 (s, 1H, C(4)−H); 2.16 (s, 3H, 2,6-CH_3); 6.8−7.1 (m, 4H, Ph); 5.45 (s, 2H, $-OCH_2$); 8.58*, 8.26 (2s, 2H, $CONH_2$). ^{13}C NMR (DMSO-d_6): 18.0 (2,6-CH_3); 34.3 (4-CH); 50.4 (OCH_3); 101.1 (3,5-C); 145.3 (2,6-C); 167.7 (COO); 155.0, 136.6, 130.1, 127.4, 121.4, 112.9 (Ph); 60.0 (OCH_2); 148.8 (C3); 152.5 (C4); 158.3 ($CONH_2$). Anal. ($C_{21}H_{22}N_4O_7$) C, N, H.

General Method of Preparation of 1,4-DHPs 28, 29, 28a,b, and 29a,b. Trifluoroacetic anhydride (3 mmol) was added dropwise to a stirred and ice−salt-cooled solution of the appropriate DHPs **25**, **25a**,**b**, **27**, and **27a**,**b** (1 mmol) in dry pyridine (5 mL). The cooling bath was removed, and the stirring was continued for 30 min at room temperature. The reaction mixture was poured into water, and the solution, acidified with 2 N HCl, was extracted with EtOAc. The combined organic layers were washed with 2 N HCl, dried, and evaporated to afford a residue which was purified by flash chromatography (petroleum ether/EtOAc, 7:3).

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[3-[(4-cyanofurazan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (28): 157−158 °C (EtOAc/petroleum ether, 60%). ^{1}H NMR (DMSO-d_6): 8.95 (s, 1H, −NH); 3.57 (s, 3H, $-OCH_3$); 4.90 (s, 1H, C(4)−H); 2.28 (s, 3H, 2,6-CH_3); 6.8−7.3 (m, 4H, Ph); 5.55 (s, 2H, $-OCH_2$). ^{13}C NMR (DMSO-d_6): 18.2 (2,6-CH_3); 38.6 (4-CH); 50.8 (OCH_3); 101.3 (3,5-C); 146.0 (2,6-C); 167.4 (COO); 157.0, 149.8, 129.4, 120.9, 114.0, 111.9 (Ph); 59.4 (OCH_2); 134.0 (C3); 154.6 (C4); 107.0 (CN). Anal. ($C_{21}H_{20}N_4O_6$) C, H, N.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[3-[(4-cyanofuroxan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (28a): 145−146 °C (EtOAc/petroleum ether, 70%). ^{1}H NMR (DMSO-d_6): 8.88 (s, 1H, −NH); 3.57 (s, 3H, $-OCH_3$); 4.90 (s, 1H, C(4)−H); 2.28 (s, 3H, 2,6-CH_3); 6.8−7.3 (m, 4H, Ph); 5.17 (s, 2H, $-OCH_2$). ^{13}C NMR (DMSO-d_6): 18.1 (2,6-CH_3); 38.6 (4-CH); 50.6 (OCH_3); 101.2 (3,5-C); 145.9 (2,6-C); 167.3 (COO); 156.7, 149.8, 129.4, 121.2, 114.2§, 111.7 (Ph); 58. 9 (OCH_2); 113.1§ (C3); 135.0 (C4); 107.8 (CN). Anal. ($C_{21}H_{20}N_4O_7$) C, H, N.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[3-[(3-cyanofuroxan-4-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (28b): 142−143 °C (EtOAc/petroleum ether, 80%). ^{1}H NMR (DMSO-d_6): 8.94 (s, 1H, −NH); 3.58 (s, 3H, $-OCH_3$), 4.91 (s, 1H, C(4)−H); 2.28 (s, 3H, 2,6-CH_3); 6.8−7.3 (m, 4H, Ph); 5.36 (s, 2H, $-OCH_2$). ^{13}C NMR (DMSO-d_6): 18.2 (2,6-CH_3); 38.5 (4-CH); 50.8 (OCH_3); 101.3 (3,5-C); 146.0 (2,6-C); 167.4 (COO); 157.0, 149.8, 129.4, 121.0, 114.3, 111.7 (Ph); 60.7 (OCH_2); 98.4 (C3); 155.3 (C4); 106.4 (CN). Anal. ($C_{21}H_{20}N_4O_7$) C, H, N.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[2-[(4-cyanofurazan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (29): mp 153−156 °C (EtOAc/petroleum ether, 50%). ^{1}H NMR (DMSO-d_6): 8.60 (s, 1H, −NH); 3.42 (s, 3H, $-OCH_3$), 5.22 (s, 1H, C(4)−H); 2.15 (s, 3H, 2,6-CH_3); 6.9−7.1 (m, 4H, Ph); 5.49 (s, 2H, $-OCH_2$). ^{13}C NMR (DMSO-d_6): 18.1 (2,6-CH_3); 34.6 (4-CH); 50.8 (OCH_3); 101.0 (3,5-C); 145.2 (2,6-C); 167.7 (COO); 155.4, 136.5, 130.1, 129.8, 121.9, 112.8 (Ph); 59.3 (OCH_2); 154.2* (C3); 153.7* (C4); 107.7 (CN). Anal. ($C_{21}H_{20}N_4O_6$) C, H, N.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[2-[(4-cyanofuroxan-3-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (29a): mp 130−133 °C (EtOAc/petroleum ether, 70%). ^{1}H NMR (DMSO-d_6): 8.65 (s, 1H, −NH); 3.46 (s, 3H, $-OCH_3$), 5.14* (s, 1H, C(4)−H); 2.15 (s, 3H, 2,6-CH_3); 6.9−7.2 (m, 4H, Ph); 5.13 (s, 2H, $-OCH_2$). ^{13}C NMR (DMSO-d_6): 18.1 (2,6-CH_3); 35.4 (4-CH); 50.5 (OCH_3); 101.7 (3,5-C); 145.3 (2,6-C); 167.6 (COO); 155.0, 136.1*, 130.4, 127.5, 122.0, 113.5* (Ph); 58. 9 (OCH_2); 113.0* (C3); 135.3* (C4); 107.7 (CN). Anal. ($C_{21}H_{20}N_4O_7$) C, H, N.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-[2-[(3-cyanofuroxan-4-yl)methoxy]phenyl]-3,5-pyridinedicarboxylate (29b): mp 149−151 °C (EtOAc/petroleum ether, 50%). ^{1}H NMR (DMSO-d_6): 8.74 (s, 1H, −NH); 3.47 (s, 3H, $-OCH_3$), 5.18 (s, 1H, C(4)−H); 2.17 (s, 3H, 2,6-CH_3); 6.9−7.2 (m, 4H, Ph); 5.31 (s, 2H, $-OCH_2$). ^{13}C NMR (DMSO-d_6): 18.1 (2,6-CH_3); 35.2 (4-

CH); 50.5 (OCH_3); 101.8 (3,5-C); 146.3 (2,6-C); 167.6 (COO); 155.1*, 136.4, 130.4, 127.4, 122.0, 113.8 (Ph); 61.2 (OCH_2); 98.4 (C3); 154.6* (C4); 106.1 (CN). Anal. ($C_{21}H_{20}N_4O_7$) C, H, N.

Pharmacology. Vasoactivity Determination. Thoracic aortae were isolated from male Wistar rats weighing 180−200 g. The vessels were helically cut, and three strips were obtained from each aorta. The tissues were mounted under 0.7 g tension in organ baths containing 30 mL of Krebs bicarbonate buffer (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 mM) 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 depolarized by addition of a solution of KCl to a final K^+ concentration of 50 mM. The preparations were then extensively washed with Krebs bicarbonate buffer, and a second contraction was evoked by K^+ depolarization (50 mM). When the contraction plateaued, cumulative concentrations of the vasodilator agents were added every 45 min, according to Christiaans.[26] Development of antagonism occurred so slowly that increasing doses had to be given at established times, without waiting for a complete equilibrium to be reached.

The effects of 10 μM MB and 1 μM ODQ on relaxation were evaluated in a separate series of experiments by adding the sGC inhibitors 5 min before K^+ depolarization.

Receptor Binding Assay. Cerebral cortices, isolated from male Wistar rats (180−200 g), were homogenized in 20 volumes (w/v) of 50 mM Tris-HCl buffer (pH = 7.4 at 4 °C) in an Ultra Turrax homogenizer. The homogenate was centrifugated at 43000*g* for 10 min, and the pellet was resuspended in the buffer. This process was repeated a further two times. The final pellet was stored at −80 °C until required. Binding experiments were performed avoiding exposure to light, because of the photolability of [^{3}H]nitrendipine.

All binding assays were carried out according to Christiaans[26] by adding 200 μL of 50 mM Tris buffer (pH = 7.4), 100 μL of rat brain membrane suspension (170 μg of protein/mL), 100 μL of [^{3}H]nitrendipine solution (1 nM), and 100 μL of the drug concentration to each incubation tube for a final volume of 0.5 mL. Triplicate tubes were used for each condition. Specific binding was defined as the difference between total binding (measured in the absence of any added ligand) and nonspecific binding (determined in the presence of 1 μM nifedipine). Reaction tubes were incubated for 60 min at 37 °C, then diluted with 4 mL of ice-cold Tris buffer (50 mM, pH = 7.4), and filtered under reduced pressure through Whatman GF/C glass fiber filters, treated with a 0.1% poly(ethylenimine) solution. Tubes and filters were washed two additional times with ice-cold buffer. The amount of radioactivity retained on the filters was quantitated by liquid scintillation counting, using a Beckman liquid scintillation spectrophotometer.

Saturation experiments were performed by incubating increasing concentrations of [^{3}H]nitrendipine from 0.03 up to 2 nM with 50 μL of rat cortical membranes (170 mg of protein/mL) and with 50 mM Tris-HCl buffer (pH = 7.4) at 37 °C for a total volume of 0.250 mL. Nonspecific binding was determined in the presence of 1 μM nifedipine. Equilibrium dissociation constant (K_D) of 0.83 ± 0.16 nM and the maximal binding (B_{max}) of 245 ± 70 fmol/mg of protein of [^{3}H]nitrendipine and IC_{50} values and K_i values of all the compounds tested were calculated with the nonlinear fitting program INPLOT 4.0.

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