New 1,4-Dihydropyridines Endowed with NO-Donor and Calcium Channel Agonist Properties

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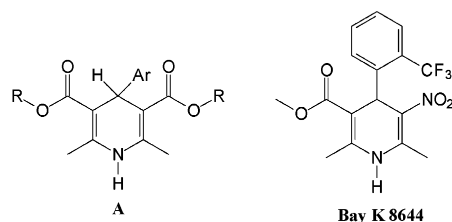
A new series of calcium channel agonists structurally related to Bay K8644, containing NO donor furoxans and the related furazans unable to release NO, is described. The racemic mixtures were studied for their action on L-type Ca^{2+} channels expressed in cultured rat insulinoma RINm5F cells. All the products proved to be potent calcium channel agonists. All the racemic mixtures, with the only exception of the carbamoyl derivatives **9**, **12** endowed with scanty solubility, were separated by chiral chromatography into the corresponding enantiomers; the (+) enantiomers were found to be potent agonists while the (−) ones were feeble antagonists. The racemic mixtures were also assessed for their positive inotropic activity on electrically stimulated rat papillary muscle and for their ability to increase Ca^{2+} entry into the vascular smooth muscle of rat aorta strips. The cyanofuroxan **8** proved to be an interesting product with dual Ca^{2+}-dependent positive inotropic and NO-dependent vasodilating activity.

Introduction

1,4-Dihydropyridines (DHPs) of general structure **A** (Chart 1) are an important class of drugs able to block the Ca^{2+} currents through voltage-dependent L-type channels.[1] It is known that when an appropriate group, such as the nitro group, is substituted in **A** for one of the two ester functions, the resulting two enantiomers display opposite pharmacological profiles.[2] Bay K 8644 is a typical example (Chart 1). The (−) *S*-antipode is a potent agonist at L-type Ca^{2+}-channels while the (+) *R*-antipode is a feeble antagonist. The Ca^{2+}-channel agonists are potentially useful in therapy as positive inotropic agents, in particular for the treatment of the congestive heart failure (CHF).[3−5] The practical use is limited by their additional capacity to increase Ca^{2+} entry into vascular smooth muscle with the consequent constriction. NO donors are a class of products which display potent vasodilating properties.[6] This is because they are able to release NO in physiological conditions. Hybrid structures in which 1,4-dihydropyridines able to activate L-type Ca^{2+} channels are connected with suitable NO-donor moieties could represent a new interesting class of positive inotropic agents, devoid of vasoconstrictor properties. Two examples of this approach were recently reported in the literature.[7,8] On these bases, we designed new 1,4-dihydropyridines formally obtained by substitution of appropriately substituted NO-donor furoxan substructures for the CF_3 group in Bay K 8644 (derivatives **8**, **9**, **10**). We herein describe the syntheses of these products and the action of their racemic mixtures on L-type Ca^{2+} channels expressed by rat insulinoma cell line (RINm5F cells). The racemic mixtures **8**, **10** were resolved by chiral chromatography, and the abilities of the single stereoisomers to modulate the calcium channels were assessed. In the case of these latter racemates, namely of products that release small and large amounts of NO respectively, their actions on rat aorta strips partially depolarized with 10 mM K^+ as well as their inotropic actions evaluated on electrically stimulated rat papillary muscle are also discussed. For control purposes parallel studies were done on the furazan analogues (derivatives **11**, **12**, **13**) devoid of the properties to release NO.

Chart 1



Results and Discussion

Chemistry. The aldehydes **3**, **4**, **5** were used as starting materials for the synthesis of the final products. Aldehydes **4**, **5** were described in a previous paper,[9] while **3** was obtained from the related (bromomethyl)-furoxan **2** through nucleophilic substitution of the bromine by *o*-hydroxybenzaldehyde (**1**) in DMF, in the presence of potassium carbonate (Scheme 1A). The furoxan 1,4-DHPs **8**, **9**, **10** were prepared by a modified Hantzsch reaction, namely by condensation of the appropriate aldehydes with nitroacetone **6** and methyl 3-aminocrotonate **7** (Scheme 1B) in 2-propanol. For compound **8** the reaction was conducted at 40 °C to avoid thermal furoxan isomerization. The furazan DHPs **11**, **12**, **13** were obtained by reduction of the furoxan analogues in boiling trimethyl phosphite.

The furoxan compounds were assessed for their ability to produce nitrite (NO_2^-) (Griess reaction) in buffer

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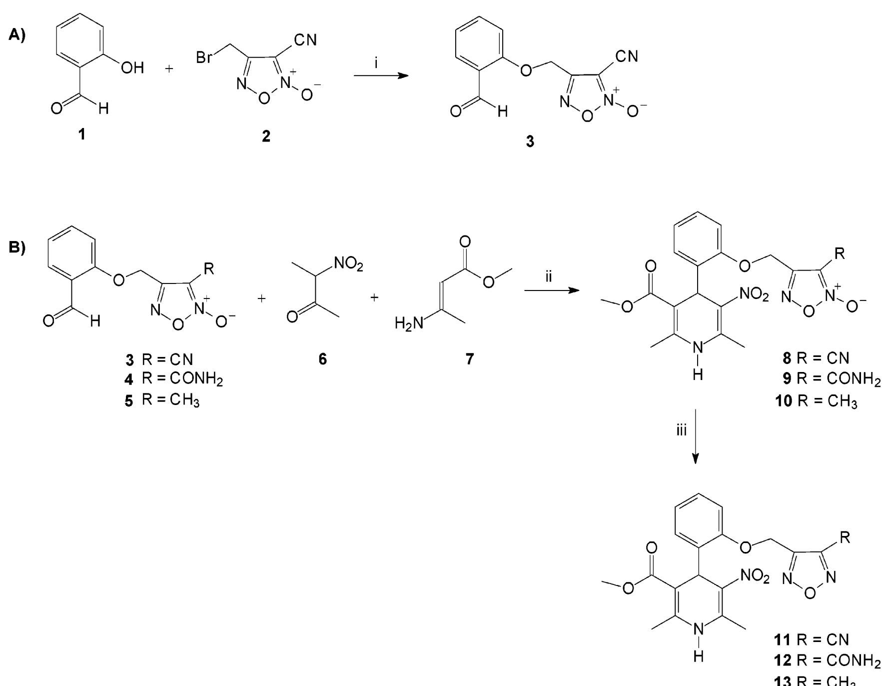
Scheme 1[a]



[a] (A): (i) DMF, K_2CO_3, 40 °C. (B): (ii) for compounds **9** and **10**: i-PrOH reflux, 8 h; for compound **8**: i-PrOH, 40 °C, 48 h; (iii) $P(OCH_3)_3$, reflux, 5 h.

Table 1. Chromatographic Conditions of Chiral Analytical Separation of Derivatives **8**, **9**, **10**, **11**, **12**, and **13**

compd	stationary phase	mobile phase	$t_R(1)$ (+)[a]	$k'(1)$ (+)	$t_R(2)$ (−)	$k'(2)$ (−)	α
8	Whelk *R,R*	hexane/chloroform 25/75	10.80	2.54	13.21	3.33	1.31
11	Whelk *R,R*	hexane/chloroform 25/75	8.30	1.72	10.16	2.33	1.35
9	Chiralpak AD	hexane/ethanol 50/50	4.86	0.61	6.13	1.04	1.69
12	Chiralcel OJ	hexane/ethanol 50/50	5.68	0.89	7.76	1.58	1.78
10	Chiralpak AD	hexane/ethanol 80/20	9.10	2.03	11.37	2.79	1.37
13	Chiralcel OJ	hexane/ethanol 80/20	15.30	3.90	18.07	4.79	1.23

[a] Sign of the rotatory power in the mobile phase given by on-line polarimeter.

solution (pH 7.4) at 37 °C, in the presence of an excess of cysteine according to a procedure previously described.[10] The results expressed as percentages of NO_2^- (mol/mol) rank the order **8** (40.3 ± 0.7) > **9** (13.6 ± 0.7) > **10** (<1). These figures can be taken as rough indexes of the ability of the products to produce NO under the chosen experimental conditions.

Racemic mixtures of the products were successfully separated by HPLC on analytical scale (Table 1). In the case of compounds **9** and **10** an amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) column and hexane/ethanol mixture as mobile phase were used. Racemic mixture of furazan derivatives **12** and **13** were resolved under similar conditions but using a cellulose tris(4-methylbenzoate) (Chiralcel OJ) column. It was not possible to use these experimental conditions to separate racemates **8** and **11** since the products, during the elution, undertook partial reaction with the alcohol present in the mobile phase. Therefore, both the mixtures were separated using a (3*S*,4*R*)-4-(3,5-dinitrobenzamido)-3-[3-(dimethylsilyloxy)propyl]-1,2,3,4-tetrahydrophenantrene ((*R,R*) Whelk-01) column and hexane/chloroform mixture as mobile phase. Chromatographic conditions reported in Table 1 were chosen for semipreparative separation. The amides **9** and **12** were not separated on the semipreparative scale because of their scanty solubility.

Voltage Clamp. Racemates **8**, **9**, **10**, **11**, **12**, **13** and (±)-enantiomers of **8**, **10**, **11**, **13** were studied for their action on the L-type Ca^{2+} channels expressed in cultured rat insulinoma RINm5F cells.[11,12] Cells were bathed in 10 mM Ba^{2+} and kept at −60 mV holding potential to increase the proportion of L-type currents. The test potential was −20 mV when either testing the agonistic and antagonistic effects of the DHP derivatives, and the return potential was set at −40 mV to better visualize the agonistic effects of the compounds through the slowing down of tail currents. Under these conditions, 3 μM nifedipine quickly blocked about 67% of the total current, and 3 μM Bay K 8644 caused an approximately 320% increase at −20 mV and a marked slowing of tail currents on return to −40 mV (Figure 1A). Figure 1B shows the variable degrees of potentiation of Ba^{2+} currents induced by the six racemates tested (**8** to **13**). To the right, the mean percentage of increased Ba^{2+} currents in reference to control currents (agonistic potency) are also reported. As noticed, all six racemates increased the size of Ba^{2+} currents at −20 mV and produced a marked prolongation of tail currents on return to −40 mV. Racemates **8**, **10**, **11**, and **13** were the most effective, with no significant difference among them (200 to 240% agonistic potency; $P < 0.1$, as established with a one-way ANOVA followed by a Student−Newman−Keuls test for multiple comparisons). In contrast to this, racemates **9** and **12** had only weak agonistic effects. Because of the weak action and due to the difficulties in separating the corresponding

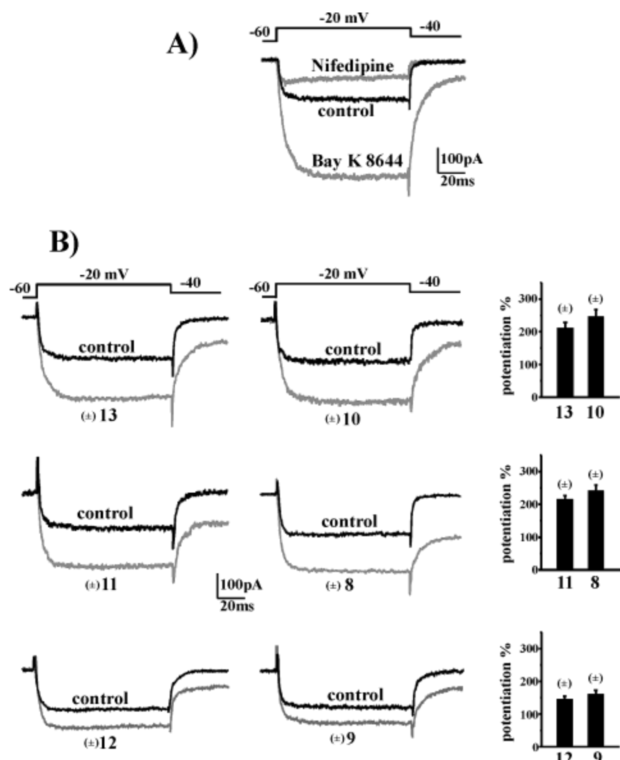


Figure 1. Action of furoxan 1,4-DHPs (racemates **8**, **9**, **10**) and furazan 1,4-DHPs (racemates **11**, **12**, **13**) on L-type Ba^{2+} currents of rat insulinoma RINm5F cells. The cells were maintained at −60 mV holding potential and depolarized to −20 mV for 100 ms. The return potential was set to −40 mV to assay the potentiating effects of 1,4-DHP agonists. This was evident through the time course of tail currents which became 10 times slower and approached nonzero steady-state values at the end of the −40 mV step. To the right are reported the mean potentiation of each racemate calculated as the percentage of L-type Ba^{2+} current increase at −20 mV from $n = 11$, 5, 5, 8, 5, and 7 cells for racemates **8**, **9**, **10**, **11**, **12**, and **13**, respectively. The potentiation was calculated after subtracting from control currents the nifedipine-insensitive component remaining after addition of 3 μM nifedipine. As shown, all racemates at 3 μM concentration produced a variable degree of Ba^{2+} current potentiation. To the top of the figure are shown the blocking potency of 3 μM nifedipine (mean 67% $n = 14$) and the potentiating effects of 3 μM (−)-Bay K 8644 (mean 340% $n = 15$) at the same testing voltage (−20 mV). Each panel is obtained from a different RINm5F cell.

enantiomers (see above), the two compounds were not further investigated.

The results of Figure 1 allow a preliminary consideration about the dual nature of furoxan derivatives being both DHP agonists and NO donors. Their agonistic action on L-type Ca^{2+} channels apparently occurs regardless of their ability to produce NO at various degrees. Notice that furoxan compounds display similar L-type channel agonists activity to their furazan analogues which are unable to release NO.

Figure 2 summarizes the agonistic and antagonistic effects of the (+/−)-enantiomers of compounds **8**, **10**, **11**, and **13**. As shown, the four (+)-enantiomers exert a similarly potent agonistic action on L-type channels, which results in an increased amplitude at −20 mV (275 to 290%, with no significant difference among them; $P < 0.2$ with ANOVA) and a marked prolongation of tail currents at −40 mV. On the contrary, the (−)-enantiomers exerted weak blocking effects, suggesting that the potent agonistic action of the four racemates derives from the predominant agonistic effects of the (+)-enantiomers. To complete this study, we also investigated the action of the cyano furoxan derivative **8** and its (±)-enantiomers on the L-type Ba^{2+} currents at various voltages, using ramp commands from −50 and +60 mV (Figure 3). The agonistic effects of the racemate and (+)-enantiomer were evident in a wide range of potentials (from −40 to +10 mV), and the same was true for the weak blocking effects of the (−)-enantiomer.

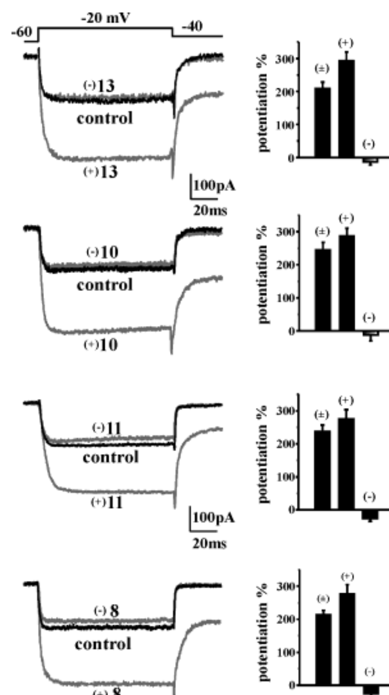


Figure 2. Agonistic and antagonistic effects of the (±)-enantiomers of compounds **8**, **10**, **11**, and **13** (3 μM). The voltage protocol to test the percentage of blockade and potentiation of the 1,4-DHPs was similar to that illustrated in Figure 1. To the right are reported the percentage of potentiation (positive ordinates) or blockade (negative ordinates) calculated at −20 mV from a number of cells varying from 15 to 22 for each compound. The effects are referred to the L-type currents estimated by subtracting the nifedipine-insensitive currents remaining after adding 3 μM nifedipine to the bath. Notice the weak degree of antagonistic effect of the (−)-enantiomers compared to the potent agonistic effects of the (+)-enantiomers.

Pharmacology. Racemic mixtures **8**, **10**, **11**, **13** were assessed for their positive inotropic activities on rat papillary muscle electrically stimulated by unipolar squarewave pulses. After an appropriate equilibration period, cumulative concentration−response curves for each Ca^{2+} agonist were constructed. The curve of the cyanofuroxan derivative **8** is reported in Figure 4A as an example. The positive inotropic potencies of each compound, expressed as EC_{50} values, are reported in Table 2. Products **13**, **10**, **11**, **8** were about 5, 7, 9, 30-fold less potent than the Bay K 8644, respectively. The furazans were more potent than the structurally related furoxans and the methyl-substituted products more potent than the analogous cyano derivatives. Since nitric oxide may behave as an inotropic agent,[13] the experiments were repeated for the NO-donor furoxan derivatives **8** and **10** in the presence of 1*H*-[1,2,4]oxadiazolo-[4,3-*a*]quinoxalin-1-one (ODQ), a well-known inhibitor of the soluble guanylate cyclase (sGC), a cytosolic en-

Table 2. Positive Inotropic Effect and Smooth Muscle Contraction of Derivatives **8**, **10**, **11**, and **13**

	rat papillary muscle	rat aortic strips	
compd	$EC_{50} \pm SE$ (μM)	$EC_{50} \pm SE$ (μM)	% max., respectively[a]
BAY K 8644	0.17 ± 0.02	0.018 ± 0.002	84 ± 4
8	5.2 ± 0.4 (5.0 ± 0.6)[b]	no effect (0.21 ±0.07)[b]	0 (29 ± 2)[b]
11	1.5 ± 0.2	0.12 ± 0.02	62 ± 5
10	1.2 ± 0.3 (1.3 ± 0.2)[b]	0.10 ± 0.02 (0.11 ± 0.01)[b]	80 ± 4 (83 ± 5)[b]
13	0.82 ± 0.07	0.057 ± 0.006	85 ± 3

[a] Relative to 50 mM KCl. [b] In the presence of 1 μM ODQ.

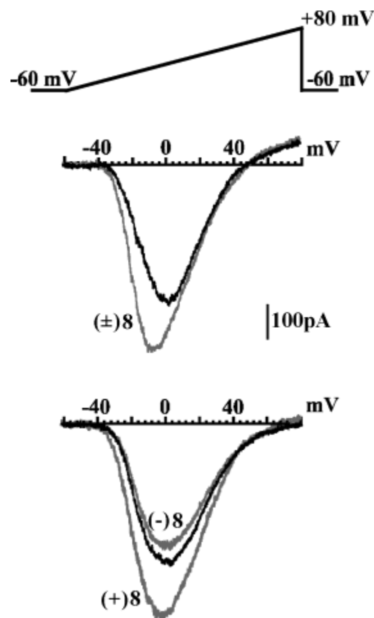


Figure 3. Current/voltage characteristics in control conditions, in the presence of racemates **8** (top) and during application of (+) and (−) enantiomers (bottom) measured with ramp commands of 1.4 V/s slope. Cells were maintained at −60 mV holding potential and depolarized linearly up to +80 mV as indicated. The two panels report experiments from two different RINm5F cells. Notice the marked potentiating effect of the (+)-**8** enantiomer (3 μM), which is evident between −35 and 0 mV where L-type channels mainly contribute to total Ba^{2+} current.

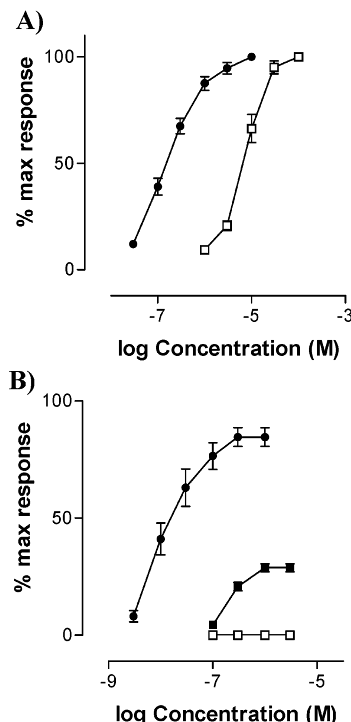


Figure 4. (A) Cumulative concentration−response curves of Bay K 8644 (solid circle) and der. **8** (open square) in rat papillary muscle. (B) Cumulative concentration−response curves of Bay K 8644 (solid circle) and der. **8** in the absence (open square) and in the presence (solid square) of ODQ in partially depolarized rat aorta. The contractile response was expressed in terms of the percentage of the 50 mM KCl-induced contractile force in each strips.

zyme involved in the nitric oxide signal transduction mechanism.[14] No shift of the concentration−response curve was observed. This suggests that no NO cGMP-dependent inotropic effects are present. Consequently the positive inotropic effects detected seem to be principally due to the Ca^{2+}-agonist properties of the products.

The capacity of the racemic mixtures to increase Ca^{2+} entry into the vascular smooth muscle was evaluated on rat aorta strips partially depolarized with 10 mM K^+. Furazan derivatives **13** and **11** were capable of contracting the tissue in a dose-dependent manner. Their potencies were less than Bay K8644's potency by about 3 and 7-fold, respectively. The behavior of the methylfuroxan derivative **10**, a feeble generator of nitrite, was similar to that of the related furazan **13**. Experiments conducted in the presence of ODQ did not induce any modification of the concentration−response curve. This suggests that the product behaves in the vasoconstrictor experiments as a pure Ca^{2+} agonist. By contrast, analogous experiments carried out with the cyanofuroxan **8**, which is a potent producer of nitrite, showed that the product was able to contract the tissue only in the presence of ODQ (Figure 4B). This indicates that the Ca^{2+}-agonist properties of the product are masked by its NO-dependent vasodilating activity. In fact this model was able to dilate rat aorta strips depolarized with 50 mM K^+ in a dose-dependent manner. The potency of the product as a vasodilator (EC_{50} = 0.0044 ± 0.0008 μM; EC_{50} in the presence of 1 μM ODQ = 1.9 ± 0.1 μM) was higher than that of glyceryl trinitrate (GTN, EC_{50} = 0.022 ± 0.004 μM).[10] In conclusion, **8** is an interesting product endowed with both Ca^{2+}-dependent positive inotropic properties and NO-dependent vasodilating activities.

Experimental Section

Melting points were determined 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. The compounds were routinely checked by infrared spectrophotometry (Shimadzu FT-IR 8101). ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-200 spectrometer and are reported in δ (ppm) units (* means either not assigned or tentatively 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 a drying agent. Solvent removal was achieved under reduced

pressure at room temperature. Elemental analyses of the new compounds were performed by REDOX (Cologno Monzese), and the results are within ±0.4% of the theoretical values.

Analytical chiral HPLC experiments on Chiralpak AD and Chiralcel OJ (250 × 4.6 mm, 10 μm) (Daicel Co., Tokyo) and (*R,R*) Whelk-01 (250 × 4.6 mm, 5 μm) (E. Merck, Darmstadt) columns were performed with a LaChrom (Merck) screening unit equipped with a L-7100 pump, a L-7200 auto sampler, a L-7360 oven which accommodates 12 columns alimented by a Valco positions valve, a L-7400 UV detector, and a Jasco OR-1590 polarimeter detector. Analysis were performed on 1 mL/min, at a controlled temperature (25 °C) with UV (λ = 254 nm) and polarimetric detection. Semipreparative separations were performed with a Merck-Hitachi LiChrograph Model L-6000 HPLC pump and a Merck-Hitachi LiChrograph L-4000 UV detector (λ = 254 nm). For semipreparative separations, a Chiralpak AD, Chiralcel OJ (250 × 10 mm, 10 μm), and (*R,R*) Whelk-01 (250 × 5 mm, 5 μm) were used. The solvents were HPLC grade from SDS (Peypin, France), filtered on a Millipore membrane 0.45 μm and degassed before use. The optical rotatory powers were measured on a 241 MC Perkin-Elmer polarimeter with a sodium lamp and a double-jacketed cell (1 dm) at 25 °C.

Cell Culture and Solutions. RINm5F cells were kindly provided by Dr. E. Sher (Eli Lilly and Co., Windlesham, Surrey, U.K.) and were cultured as previously described. The recording external solution was (mM) 125 NaCl, 10 $BaCl_2$, 1 MgCl, 10 HEPES (pH 7.3 with NaOH), and 300 nM TTX. The standard internal solution was (mM) 100 CsCl, 20 TEACl, 10 EGTA, 2 $MgCl_2$, 8 glucose, 4 ATP, 0.5 GTP, 15 phosphocreatine, 10 HEPES, (pH 7.3 with CsOH). The four racemates and their enantiomers as well as Bay K 8644 (Bayer AG, Wuppertal, Germany) were dissolved in ethanol (95%) and stored in the dark at 4 °C as 1 mM stock solutions. Dilution to the final concentration was performed daily under light protection. The external solutions were exchanged by fast superfusing using a multibarrelled glass pipet held close to the cell. Experiments were carried out at room temperature (22 °C).[15]

Whole-Cell Current Recordings. Membrane currents were measured in the whole-cell configuration using a Axopatch 200 A (Axon Instruments) patch-clamp amplifier and pipets of borosilicate glass with a resistance of 3−4 MΩ. Stimulation, acquisition, filtering, and data analysis were performed as described elsewhere.[12] Cells were clamped at −60 mV holding potential (V_h). Step depolarization of 100 ms from V_h were applied at intervals of 10 s to minimize Ca^{2+}-channel "run down". Capacitative currents were corrected with the clamp-amplifier settings. Leakage currents were compensated electronically with the P/4 pulse protocol. Data are expressed as means ± SEM for n = number of cells.

Functional Studies. Papillary muscles[16] and aortas were isolated from male Wistar rats weighing 200−250 g which had been anaesthetized with CO_2 and killed by decapitation. All animals were treated humanely in accordance with recognized guidelines on experimentation. As few rats as possible were used. The purposes and the protocols of our studies have been approved by the Ministero della Salute, Rome, Italy. The tissues were mounted in organ baths containing 30 mL of Krebs-bicarbonate buffer of the following composition (mM): NaCl 111.2, KCl 5.0, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.0, $NaHCO_3$ 12, glucose 11.1. The solution was maintained at 37 °C and continuously gassed with 95% O_2−5% CO_2 (pH = 7.4).

Papillary Muscles. Two electrodes implanted into the ventricular basis were used to drive the tissues electrically. Square wave pulses (2 Hz, 1 ms, 20% above threshold voltage) were continuously delivered, and changes in the contraction force of papillary muscles were recorded by an isometric transducer connected with a pen recorder. After a 90 min equilibration period, cumulative concentration−response curves to Ca^{2+} agonists were constructed by increasing doses by half-log units after each response had reached a plateau. To avoid indirect receptor-mediated effects of possibly released endogenous noradrenaline by the Ca^{2+}-channel activators, the muscles were incubated in the presence of the β-receptor blocker propranolol (1 μM) and the α_1-receptor blocker prazosin (0.1 μM).[16] The receptor blockers were introduced into the bath solution 15 min before addition of Ca^{2+}-channel activator. Only one concentration−response curve to a 1,4-DHP activator could be determined in a single tissue.[17] In the case of **8** and **10** we determined the inotropic effect either in the absence or in the presence of 1 μM ODQ which was added to the bath at least 15 min before the addition of the first concentration of the agonist. EC_{50} values are the mean of 5−7 determinations.

Aortic Strips. Thoracic aortas were helically cut, the endothelium was removed, and three strips were obtained from each vessel. The tissues were mounted under 0.7 g tension (isotonic transducer).

To evaluate vascular contraction, the aortic strips were allowed to equilibrate for 1 h and then depolarized twice at 60 min intervals by an addition of KCl solution of to a final concentration 50 mM. Contractile responses to Ca-channel agonists were determined in the presence of 10 mM KCl, because at that K^+ concentration the activator produced the maximum response.[18] In the case of furoxan derivatives **8** and **10**, we determined the effect either in the absence or in the presence of 1 μM ODQ which was added to the bath at least 15 min before the addition of the first concentration of the agonist. EC_{50} values are the mean of 5−7 determinations. To evaluate vasodilating activity, we followed a method previously reported.[9] All the experiments were performed avoiding exposure to light, because of the photolability of the 1,4-DHPs.

Synthesis of 3-[(3-Cyanofuroxan-4-yl)methoxy]benzaldehyde (3). K_2CO_3 (1.4 g, 10 mmol) was added to a stirred solution of compound **2** (1 g, 5 mmol) and 2-hydroxybenzaldehyde (**1**) (0.73 g, 6 mmol) in dry DMF (10 mL). The stirring was continued for 30 min at room temperature, and then the reaction mixture was poured into water. The resulting precipitate was filtered, washed with water, and purified by flash-chromatography (PE 7/EtOAc 3); mp 122 °C (EtOAc/PE); yield 87%. ^{1}H NMR (DMSO-d_6): 5.65 (s, 2H, −CH_2); 10.4 (s, 1H, CHO); 7.18−7.78 (Ph). ^{13}C NMR (DMSO-d_6): 61.3 (OCH_2); 114.1, 122.4, 124.7, 127.8, 136.5, 159.4 (Ph); 98.6 (C3-furoxan); 106.1 (CN); 154.7 (C4-furoxan); 189.1 (CHO). Anal. ($C_{11}H_7N_3O_4$) C, H, N.

General Method of Preparation of DHPs 9 and 10. A solution of the appropriate aldehyde **4** and **5** (5 mmol) and nitroacetone **6** (0.62 g, 6 mmol) in 2-propanol (70 mL) was refluxed for 4 h, and then methyl-3-aminocrotonate **7** (0.58 g, 5 mmol) was added. Reflux was continued for other 4 h; solvent removal gave a residue which was purified by flash-chromatography. Chromatographic eluents, yields, melting points, and crystallization solvents of the products were as follows.

(±)-Methyl 4-[2-[(3-Carbamoylfuroxan-4-yl]methoxy]-phenyl]-2,6-dimethyl-5-nitro 1,4-dihydropyridine-3-carboxylate (9): eluent CH_2Cl_2 8/EtOAc 2; yield 42%; mp 198−199 °C dec (EtOAc/PE). ^{1}H NMR (DMSO-d_6): 2.13, 2.33 (s, 6H, 2,6-CH_3); 3.52 (s, 3H, $COOCH_3$); 5.29 (s, 2H, OCH_2); 5.40 (s, 1H, 4-CH); 6.92−7.22 (m, 4H, Ph); 7.80−8.55 (2s, 2H, $CONH_2$); 9.38 (s br, 1H, NH). ^{13}C NMR (DMSO-d_6): 17.4 (2-CH_3); 19.9 (6-CH_3); 38.3 (C4-DHP); 50.9 (OCH_3); 61.1 (OCH_2); 104.5 (C5-DHP); 110.5 (C3-furoxan); 124.7 (C3-DHP); 143.6, 146.1 (C2,C6-DHP); 102.7, 112.8, 128.2, 131.4, 131.7, 155.7 (Ph); 155.0 (C4-furoxan); 156.6 ($CONH_2$); 166.8 ($COOCH_3$). Anal. ($C_{19}H_{19}N_5O_8$) C, H, N.

(±)-Methyl 2,6-Dimethyl-4-[2-[(3-methylfuroxan-4-yl]-methoxy]phenyl]-5-nitro-1,4-dihydropyridine-3-carboxylate (10): eluent CH_2Cl_2 9.5/EtOAc 0.5; yield 25%; mp 216 °C (EtOAc/PE). ^{1}H NMR (DMSO-d_6): 2.14, 2.36 (s, 6H, 2,6-CH_3); 2.47 (s, 3H, CH_3); 3.45 (s, 3H, $COOCH_3$); 5.33 (s, 2H, OCH_2); 5.49 (s, 1H, 4-CH); 9.29 (s br, 1H, NH). ^{13}C NMR (DMSO-d_6): 7.4 (CH_3); 17.4 (2-CH_3); 19.8 (6-CH_3); 37.1 (C4-DHP); 50.8 (OCH_3); 60.7 (OCH_2); 105.5 (C5-DHP); 113.3* (C3-furoxan); 125.3 (C3-DHP); 143.0, 147.6 (C2,C6-DHP); 112.6*, 121.3, 128.3, 130.9, 132.4, 155.6* (Ph); 155.5* (C4-furoxan); 166.7 ($COOCH_3$). Anal. ($C_{19}H_{20}N_4O_7 \times 0.5H_2O$) C, H, N.

Semipreparative separation afforded ca. 50 mg of each enantiomer with an optical purity higher than 98%: (+)**10** (c = 0.1, acetone), $[\alpha]^{25}_D$= +17; (−)**10** (c = 0.1, acetone), $[\alpha]^{25}_D$ = −16.

Synthesis of (±)-Methyl 4-[2-[(3-Cyanofuroxan-4-yl]-methoxy]phenyl]-2,6-dimethyl-5-nitro-1,4-dihydropyridine-3-carboxylate (8): A mixture of compound **3** (0.73 g, 3 mmol) and nitoacetone **6** (1.08 g, 10.5 mmol) in 2-propanol (70 mL) was heated at 40 °C for 36 h. Methyl 3-aminocrotonate **7** (0.58 g, 5 mmol) was added, and heating was continued for other 48 h; solvent removal gave a residue which was purified by flash-chromatography (eluent PE 7/EtOAc 3). Yield 30%; mp188−189 °C dec (PE/EtOAc). ^{1}H NMR (DMSO-d_6): 2.16, 2.35 (s, 6H, 2,6-CH_3); 3.49 (s, 3H, $COOCH_3$); 5.33 (s, 2H, OCH_2); 5.48 (s, 1H, 4-CH); 6.97−7.20 (Ph); 9.29 (s br, 1H, NH). ^{13}C NMR (DMSO-d_6): 17.5 (2-CH_3); 19.8 (6-CH_3); 37.7 (C4-DHP); 50.8 (OCH_3); 60.7 (OCH_2); 105.5 (C5-DHP); 106.0 (CN); 113.3* (C3-furoxan); 125.3 (C3-DHP); 143.3, 147.6 (C2,C6-DHP); 113.6*, 121.8, 128.2, 131.3, 132.6, 155.7* (Ph); 155.4* (C4-furoxan); 166.7 ($COOCH_3$). Anal. ($C_{19}H_{17}N_5O_7 \times 0.25\ H_2O$) C, H, N.

Semipreparative separation afforded ca. 10 mg of each enantiomer with an optical purity higher than 99%. Unfortunately, the rotatory power of this compound is too small to be determined on our polarimeter with this low quantity of product.

General Method of Preparation of DHPs 11, 12, and 13. The appropriate furoxan 1,4-DHP **8**−**10** (2 mmol) was refluxed in trimethyl phosphite (50 mL). Then the reaction mixture, cooled at room temperature, was poured into 2 N HCl (150 mL) and extracted with EtOAc (3 × 50 mL); the dried organic phases were evaporated to give the title product as an orange oil which was purified by flash-chromatography (eluent CH_2Cl_2 95/EtOAC 5). Reaction time, yields, melting points, and crystallization solvents of the products were as follows.

(±)-Methyl 4-[2-[(4-Cyanofurazan-3-yl]methoxy]phenyl]-2,6-dimethyl-5-nitro-1,4-dihydropyridine-3-carboxylate (11): 1 h; yield 83%; mp 178 °C (EtOAc/PE). ^{1}H NMR (DMSO-d_6): 2.20, 2.40 (s, 6H, 2,6-CH_3); 3.53 (s, 3H, $COOCH_3$); 5.34 (s, 2H, OCH_2); 5.49 (s, 1H, 4-CH); 6.96−7.25 (Ph); 9.50 (s br, 1H, NH). ^{13}C NMR (DMSO-d_6): 17.4 (2-CH_3); 19.7 (6-CH_3); 32.3 (C4-DHP); 50.9 (OCH_3); 59.1 (OCH_2); 105.3 (C5-DHP); 107.6 (CN); 133.5 (C3-furazan); 125.4 (C3-DHP); 143.1, 147.3 (C2,C6-DHP); 113.4, 121.8, 128.2, 131.6, 132.8, 155.3 (Ph); 153.9 (C4-furazan); 166.7 ($COOCH_3$). Anal. ($C_{19}H_{17}N_5O_6$) C, H, N.

Semipreparative separation afforded ca. 10 mg of each enantiomer with an optical purity higher than 98%. Unfortunately, the rotatory power of this compound is too small to be determined on our polarimeter with this low quantity of product.

(±)-Methyl 4-[2-[(4-Carbamoylfurazan-3-yl]methoxy]-phenyl]-2,6-dimethyl-5-nitro-1,4-dihydropyridine-3-carboxylate (12): 4 h; yield 55%; mp 205 °C (EtOAc/PE). ^{1}H NMR (DMSO-d_6): 2.19, 2.33 (s, 6H, 2,6-CH_3); 3.51 (s, 3H, $COOCH_3$); 5.29 (s, 2H, OCH_2); 5.40 (s, 1H, 4-CH); 6.92−7.22 (m, 4H, Ph); 8.25−8.58 (2s, 2H, $CONH_2$); 9.21 (s br, 1H, NH). ^{13}C NMR (DMSO-d_6): 17.4 (2-CH_3); 19.8 (6-CH_3); 38.0 (C4-DHP); 50.8 (OCH_3); 59.5 (OCH_2); 104.7 (C5-DHP); 143.3 (C3-furazan); 124.9 (C3-DHP); 146.7, 147.8 (C2,C6-DHP); 112.9, 120.9, 128.1, 131.4, 132.0, 156.2 (Ph); 155.0 (C4-furazan); 156.6 ($CONH_2$); 166.8 ($COOCH_3$). Anal. ($C_{19}H_{19}N_5O_7$), C, H, N.

(±)-Methyl 2,6-Dimethyl-4-[2-[(4-methylfurazan-3-yl]-methoxy]phenyl]-5-nitro-1,4-dihydropyridine-3-carboxylate (13): 16 h; yield 37%; mp 198 °C (EtOAc/PE). ^{1}H NMR (DMSO-d_6): 2.16, 2.20 (s, 6H, 2,6-CH_3); 2.38 (s, 3H, CH_3); 3.48 (s, 3H, $COOCH_3$); 5.21 (s, 2H, OCH_2); 5.47 (s, 1H, 4-CH); 6.90−7.25 (m, 4H, Ph); 9.40 (s br, 1H, NH). ^{13}C NMR (DMSO-d_6): 7.8 (CH_3); 17.3 (2-CH_3); 19.7 (6-CH_3); 36.7 (C4-DHP); 50.8 (OCH_3); 58.7 (OCH_2); 105.7 (C5-DHP); 155.4* (C3-furazan); 125.4 (C3-DHP); 147.5, 142.8 (C2,C6-DHP); 112.4, 121.2, 128.2, 130.7, 132.5, 155.4* (Ph); 151.9 (C4-furazan); 166.8 ($COOCH_3$). Anal. ($C_{19}H_{20}N_4O_6$), C, H, N.

Semipreparative separation afforded 18 mg of each enantiomer with an optical purity higher than 98%: (+)**13** (c = 0.1, acetone), $[\alpha]^{25}_D$ = +15; (−)**13** (c = 0.1, acetone), $[\alpha]^{25}_D$ = −12.

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