Syntheses, Calcium Channel Agonist−Antagonist Modulation Activities, and Nitric Oxide Release Studies of Nitrooxyalkyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate Racemates, Enantiomers, and Diastereomers

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A novel group of hybrid calcium channel (CC) modulators was prepared where the isopropyl ester moiety of isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate (PN 202-791) was replaced by a variety of nitric oxide (•NO) donor nitrooxyalkyl ester substituents. Enantiomers, or diastereomers, having the (*R*)-configuration at the C-4 position of the 1,4-dihydropyridine ring (1,4-DHP) exhibited more potent in vitro CC antagonist activity on guinea pig ileum longitudinal smooth muscle (GPILSM) than compounds having the (*4S*)-configuration. None of the nitrooxyalkyl compounds exhibited a contraindicated CC agonist effect on GPILSM that would cause smooth muscle contraction. Structure−activity studies showed the enantiomers having the (*S*)-configuration at the C-4 position of the 1,4-DHP ring or diastereomers having the a (*4S*)-configuration at the C-4 position of the 1,4-DHP ring in conjunction with a (*1R*-)-1-methyl-2-nitrooxyethyl ester substituent exhibited the most potent cardiac CC agonist (positive inotropic) activity on guinea pig left atrium (GPLA). This class of compounds releases •NO in vitro that is enhanced by the presence of a thiol such as *N*-acetylcysteamine. The novel •NO donor (−)-(*S,R*)-1-methyl-2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate [(−)-(*S,R*)-**38**], which acts as a *dual cardioselective calcium channel agonist (GPLA)/smooth muscle selective calcium channel antagonist (GPILSM),* is a useful lead compound for drug discovery targeted to the treatment of congestive heart failure, and it provides a useful research probe to study the structure−function relationship of calcium channels.

Introduction

The design of tissue selective 1,4-dihydropyridine (1,4-DHP) calcium channel (CC) agonists to treat congestive heart failure (CHF) necessitates removal of their contraindicated smooth muscle vasoconstrictor effect while the target cardiac positive inotropic action is maintained.[1] In this regard, racemic methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)pyridine-5-carboxylate (Bay K8644) produces a CC agonist effect on both smooth and cardiac muscle since the agonist (−)-(*S*)-enantiomer (**1**) is about 10-fold more potent relative to the antagonist (+)-(*R*)-enantiomer (**3**).[2] In a previous study, hybrid 1,4-DHP CC modulators were prepared where the methyl ester substituent of Bay K8644 was replaced by a 2-nitrooxyethyl ester moiety, which also have the potential to simultaneously release nitric oxide (•NO).[3] This hybrid design concept was based on observations that •NO is an endogenous activator of guanylate cyclase that is responsible for vascular relaxation and that organic nitrovasodilators elicit their effect in vivo by bypassing the •NO-production system in the endothelium to deliver •NO directly to muscle cells in the artery. Accordingly, the (−)-(*S*)-**2** 2-nitrooxyethyl enantiomer, like (−)-(*S*)-Bay K8644 (**1**), acts as a cardiac CC agonist, but unlike (−)-(*S*)-Bay K8644, (−)-(*S*)-**2** is a smooth muscle CC antagonist that also acts as a •NO donor in vitro (see structures in Figure 1). In contrast, the 2-nitrooxyethyl (+)-(*R*)-enantiomer (**4**) exhibited a CC antagonist effect on both cardiac and smooth muscle. The *dual cardioselective CC agonist/smooth muscle selective CC antagonist actions* of the 2-nitrooxyethyl (−)-(*S*)-**2** enantiomer fulfill the clinical requirements for the treatment of CHF. The two enantiomers of PN 202-791 (**5**) also show differences in CC modulation effects where the (−)-(*R*)-enantiomer is 100-fold more potent as an antagonist compared to the agonist (+)-(*S*)-enantiomer.[4] In our ongoing program to acquire CC modulation structure−activity relationships and to design compounds to study structure−function relationships with respect to CC modulation, we now report the syntheses of nitrooxyalkyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate racemates, enantiomers, and/or diastereo-

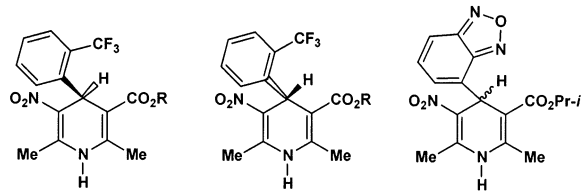

Figure 1. Structures for the (−)-(*S*)-**1** and (+)-(*R*)-**3** enantiomers of Bay K8644, the (−)-(*S*)-**2** and (+)-(*R*)-**4** enantiomers of 2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)pyridine-5-carboxylate, and PN 202-791 (**5**).

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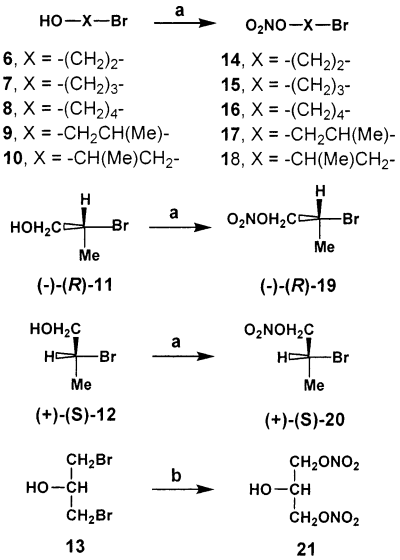
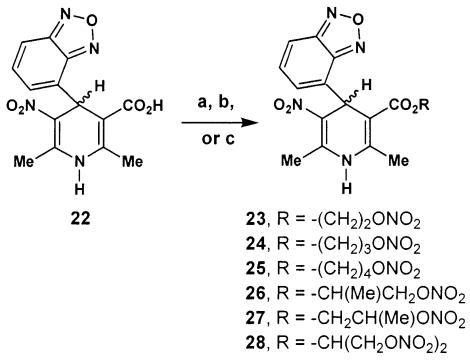
Scheme 1[a]



[a] Reagents and conditions: (a) 70% HNO_3, 95% H_2SO_4, 25 °C, 1 h; (b) $AgNO_3$, MeCN, 80 °C, 3 h.

Scheme 2[a]



[a] Reagents and conditions: (a) O_2NO-X-Br (**14**, **15**, **16**, **18**), DMF, K_2CO_3, 25 °C, 24 h; (b) O_2NO-X-Br (**17**), DMF, K_2CO_3, 25 °C, 7 days; (c) $SOCl_2$, DMF−CH_2Cl_2, $HOCH(CH_2ONO_2)$ (**21**), 0 °C, 6 h.

mers (**23**−**28**, **35**−**39**), their in vitro CC modulating effects on smooth and cardiac muscle, and •NO release data.

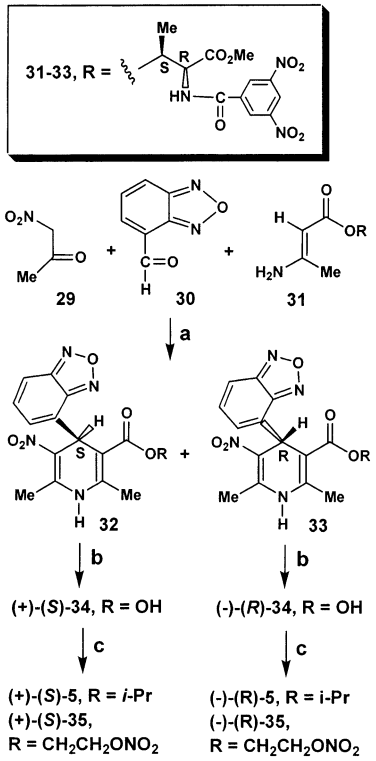
Chemistry

Nitration of the bromoalkanols (**6**−**12**) using a mixture of 70% HNO_3 and 95% H_2SO_4 afforded the respective nitrooxyalkyl bromides (**14**−**20**) in 83−99% yield. In contrast, 1,3-dinitrooxy-2-propanol (**21**) was prepared in 96% yield by reaction of 1,3-dibromo-2-propanol (**13**) with $AgNO_3$ in MeCN at 80 °C, as illustrated in Scheme 1.

Condensation of the nitrooxyalkyl bromides (**14**−**18**) with racemic 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate (**22**) in the presence of K_2CO_3 afforded the respective nitrooxyalkyl ester product as a racemate (**23**−**25**) or as a mixture of diastereomers (**26**, **27**). The racemic 1,3-dinitrooxy-2-propyl ester (**28**) was synthesized by conversion of the racemic acid (**22**) to the acid chloride using $SOCl_2$ and then condensation with 1,3-dinitrooxy-2-propanol (**21**) as illustrated in Scheme 2.

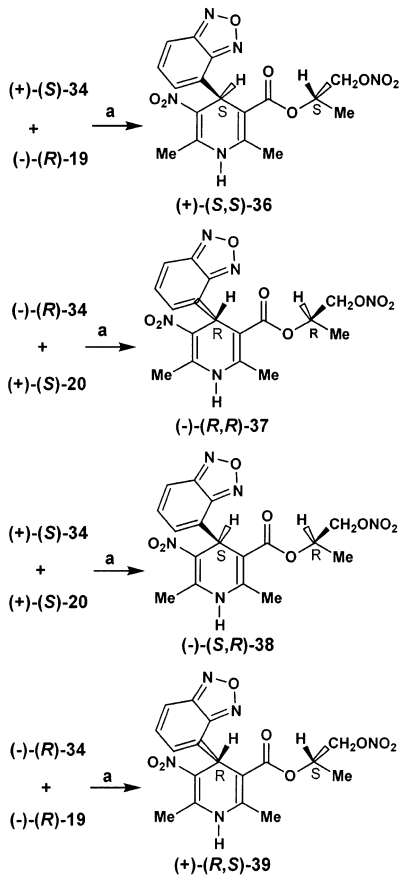
Attempts to resolve the racemic acid **22**, to acquire the individual acid enantiomers (+)-(*S*)-**34** and (−)-(*R*)-**34**, by fractional crystallization of the (−)-cinchonidine or (+)-cinchonine salts using a procedure similar to that reported by Shibanuma et al.[5] for the synthesis of nicardipine enantiomers was not successful, since the racemic acid **22** was extremely insoluble in solvents such as EtOAc, EtOH, or MeOH. An alternate synthetic strategy was therefore investigated, since it was expected that use of a chiral esterification group such as D-threonine, which could be efficiently removed by a β-elimination mechanism using a nonnucleophilic base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), would be a desirable derivative for separation of the diastereomeric esters (**32**, **33**) that could be converted to the respective target chiral acids (+)-(*S*)-**34** and (−)-(*R*)-**34**.[6] Accordingly, the modified Hantzsch condensation of nitroacetone (**29**), 2,1,3-benzoxadiazol-4-carboxaldehyde (**30**), and (1*S*,2*R*)-2-(3,5-dinitrophenylcarbonylamino)-2-methoxycarbonyl-1-methylethyl 3-aminocrotonate (**31**) afforded a mixture of the two diastereomers **32** and **33** that differ in configuration (*S* or *R*) at the C-4 position of the 1,4-DHP ring (see Scheme 3). Silica gel column separation of diastereomers **32** and **33** and then cleavage of the individual esters using DBU at 25 °C yielded the individual (+)-(*S*)-**34** and (−)-(*R*)-**34** carboxylate enantiomers. The absolute configuration for (+)-(*S*)-**34** and (−)-(*R*)-**34** was determined by their conversion to the respective (+)-(*S*)-**5** and (−)-(*R*)-**5** isopropyl esters for which the absolute configuration and optical rotation are known.[7] Reaction of (+)-(*S*)-**34** or (−)-(*R*)-**34** with $BrCH_2CH_2ONO_2$ in the presence of K_2CO_3 in DMF afforded the respective (+)-(*S*)-**35** or (−)-(*R*)-**35** enantiomer of 2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-

Scheme 3[a]



[a] Reagents and conditions: (a) EtOH, 80 °C, 17 h; (b) 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), MeOH, 25 °C, 4 h; (c) $BrCHMe_2$ for (+)-(*S*)-**5** and (−)-(*R*)-**5**, or $BrCH_2CH_2ONO_2$ (**14**) for (+)-(*S*)-**35** and (−)-(*R*)-**35**, DMF, K_2CO_3, 25 °C, 36 h for (+)-(*S*)-**5** and (−)-(*R*)-**5** and 24 h for (+)-(*S*)-**35** and (−)-(*R*)-**35**.

Scheme 4[a]



[a] Reagents and conditions: (a) dry DMF, K_2CO_3, 25 °C, 7 days.

nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate. Each enantiomer [(+)-(*S*)-**5**, (−)-(*R*)-**5**, (+)-(*S*)-**35**, (−)-(*R*)-**35**] exhibited a single resonance for the dihydropyridine C-6 methyl resonance upon addition of the ^{1}H NMR chiral shift reagent (+)-Eu(hfc)$_3$, indicating a very high optical purity (≥96% ee).

1-Methyl-2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate (**26**), prepared by reaction of racemic **22** with racemic 2-bromo-1-nitrooxypropane (**17**) as illustrated in Scheme 2, possesses two asymmetric centers, which resulted in the formation of four diastereomeric products (*SS*, *RR*, *SR*, *RS*). The four diastereomeric compounds (+)-(*S*,*S*)-**36**, (−)-(*R*,*R*)-**37**, (−)-(*S*,*R*)-**38**, and (+)-(*R*,*S*)-**39** have been prepared by reaction of the optically pure acid (+)-(*S*)-**34** or (−)-(*R*)-**34** with a chiral 2-bromo-1-nitrooxypropane [(−)-(*R*)-**19** or (+)-(*S*)-**20**] in DMF at 25 °C as illustrated in Scheme 4. This S_N2 reaction, which occurs with inversion of configuration at the asymmetric center in the 2-bromo-1-nitrooxypropane [(−)-(*R*)-**19** or (+)-(*S*)-**20**], is favored when a relatively unhindered alkyl halide, strong nucleophile, polar solvent, and high concentration of nucleophile are employed. The ^{1}H NMR spectra of diastereomers **36**−**39** exhibited a single set of resonances [diastereomer excess (de) >96%] relative to the diastereomeric mixture (**26**), which exhibited dual resonances.

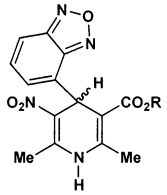
Results and Discussion

A group of Hantzsch 1,4-dihydropyridines having a variety of nitrooxyalkyl ester nitric oxide (•NO) donor moieties in place of the isopropyl ester substituent present in isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate (**5**, PN 202-791) were synthesized. Hybrid agents of this type that release the smooth muscle vasorelaxant •NO constitute a useful drug design strategy to eliminate the contraindicated smooth muscle vasoconstrictor effect exhibited by the (+)-(*S*)-enantiomer of PN 202-791 while its useful cardiac positive inotropic (calcium agonist) effect is maintained. Additional support for this concept is based on reports that •NO modulates the activity of Ca^{2+} release channels by preventing oxidation of regulatory sulfhydryls,[8] and replacement of the methyl group of the ester substituent of (−)-(*S*)-Bay K8644 by a 2-nitrooxyethyl •NO donor substituent abolished the undesirable calcium channel agonist effect of (−)-(*S*)-Bay K8644 on vascular smooth muscle.[3]

The in vitro calcium channel modulating activities of the nitrooxyalkyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylates (**23**−**28**, **35**−**39**) were determined using guinea pig ileum longitudinal smooth muscle (GPILSM) and guinea pig left atrium (GPLA) assays.[6] The molar concentration of the antagonist compound required to produce 50% inhibition of GPILSM Ca^{2+}-dependent contractility (IC_{50}) induced by the muscarinc agonist carbachol (1.6×10^{-7} M) are presented in Table 1. Structure−activity data for CC antagonist activity showed that the racemic (**23**−**25**, **28**) and diastereomeric (**26**, **27**) nitrooxyalkyl derivatives were weaker CC antagonists (IC_{50} values in the 6.38×10^{-6} to 2.41×10^{-7} M range) than the reference drugs nifedipine ($IC_{50} = 1.40 \times 10^{-8}$ M) or racemic PN 202-791 ($IC_{50} = 4.0 \times 10^{-8}$ M). Nitrooxyalkyl substituent chain length [$(CH_2)_nONO_2$] had a variable effect on CC antagonist activity, where the relative potency order was **24** ($n = 3$) > **25** ($n = 4$) > **23** ($n = 2$). The number of nitrooxy moieties present in the ester substituent was not a determinant of activity, since **23** [$(CH_2)_2ONO_2$] and **28** [$CH(CH_2ONO_2)_2$] were equipotent CC antagonists. The point of attachment of a methyl group in a nitrooxyethyl ester substituent had a modest effect, where the relative potency order was $CH_2CH(Me)ONO_2$ (**27**) > $CH(Me)CH_2ONO_2$ (**26**). The configuration at the C-4 position of the 1,4-DHP ring was an important determinant of CC antagonist activity, since compounds having the (*4R*)-configuration were always more potent (63−194-fold) than the corresponding (*4S*)-enantiomer [(−)-(*R*)-**35** > (+)-(*S*)-**35**] or diastereomer [(−)-(*R*,*R*)-**37** > (+)-(*S*,*S*)-**36**; (+)-(*R*,*S*)-**39** > (−)-(*S*,*R*)-**38**]. It is also highly relevant that none of these nitrooxyalkyl compounds exhibited a contraindicated CC agonist effect on GPILSM that would produce smooth muscle vasoconstriction.

The structure−activity data acquired (see Table 1) for cardiac CC agonist (positive inotropic) activity on GPLA showed that some members of this group of nitrooxyalkyl compounds, with the exception of the inactive agents (−)-(*R*)-**35** and (+)-(*R*,*S*)-**39**, exhibit appreciable cardiac CC agonist activity (EC_{50} values in the 9.15×10^{-5} to 1.56×10^{-7} M range) relative to the reference drug racemic PN 202-791 ($EC_{50} = 9.4 \times 10^{-6}$ M). The relative cardiac CC agonist potency order with respect to nitrooxyalkyl substituent chain length [$(CH_2)_nONO_2$] was $n = 4$ (**25**) > $n = 3$ (**24**) and $n = 2$ (**23**). The point

Table 1. In Vitro Calcium Channel Modulation Activities for Nitrooxyalkyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylates (**23**−**28**, **35**−**39**)



compound	R	calcium channel antagonist activity GPILSM: IC_{50} (M)[a]	calcium channel agonist activity (cardiac inotropy) GPLA: EC_{50} (M)[b]
rac-**23**	$(CH_2)_2ONO_2$	$3.89 \pm 0.25 \times 10^{-6}$	$3.11 \pm 2.31 \times 10^{-5}$
rac-**24**	$(CH_2)_3ONO_2$	$2.41 \pm 0.30 \times 10^{-7}$	$5.23 \pm 4.92 \times 10^{-5}$
rac-**25**	$(CH_2)_4ONO_2$	$8.78 \pm 1.02 \times 10^{-7}$	$4.03 \pm 0.55 \times 10^{-6}$
26[c]	$CH(Me)CH_2ONO_2$	$6.38 \pm 0.52 \times 10^{-6}$	$1.86 \pm 0.59 \times 10^{-6}$
27[c]	$CH_2CH(Me)ONO_2$	$1.70 \pm 2.30 \times 10^{-6}$	$9.72 \pm 4.19 \times 10^{-7}$
rac-**28**	$CH(CH_2ONO_2)_2$	$3.01 \pm 0.51 \times 10^{-6}$	$9.15 \pm 9.07 \times 10^{-5}$
(+)-(*S*)-**35**	$(CH_2)_2ONO_2$	$2.24 \pm 0.17 \times 10^{-5}$	$3.13 \pm 2.30 \times 10^{-6}$
(-)-(*R*)-**35**	$(CH_2)_2ONO_2$	$1.15 \pm 0.09 \times 10^{-7}$	inactive
(+)-(*S*,*S*)-**36**	(*S*)-$CH(Me)CH_2ONO_2$	$3.65 \pm 0.05 \times 10^{-5}$	$1.92 \pm 0.29 \times 10^{-6}$
(−)-(*R*,*R*)-**37**	(*R*)-$CH(Me)CH_2ONO_2$	$5.77 \pm 0.82 \times 10^{-7}$	$3.37 \pm 3.37 \times 10^{-5}$
(−)-(*S*,*R*)-**38**	(*R*)-$CH(Me)CH_2ONO_2$	$9.43 \pm 0.80 \times 10^{-6}$	$1.56 \pm 0.19 \times 10^{-7}$
(+)-(*R*,*S*)-**39**	(*S*)-$CH(Me)CH_2ONO_2$	$5.96 \pm 0.50 \times 10^{-8}$	inactive
nifedipine		$1.40 \pm 0.19 \times 10^{-8}$	−
rac-PN 202-791	$CH(Me)_2$	$4.0 \pm 0.07 \times 10^{-8}$	$9.4 \pm 2.6 \times 10^{-6}$

[a] The molar concentration of the test compound causing a 50% decrease in the slow component or tonic contractile response (IC_{50} ± SEM, $n = 3$) in guinea pig ileum longitudinal smooth muscle (GPILSM) induced by the muscarinic agonist carbachol (1.6×10^{-7} M) was determined graphically from the dose−response curves. [b] The molar concentration of the test compound causing a 50% increase in the cardiac contractile force (EC_{50} ± SEM, $n = 3$) in guinea pig left atrium (GPLA) was determined graphically from the dose−response curves. [c] Mixture of diastereomers.

of attachment of a Me group to the nitrooxyethyl chain in diastereomers **26** [R = $CH(Me)CH_2ONO_2$] and **27** [R = $CH_2CH(Me)ONO_2$] was not a determinant of activity, since their <2-fold difference in cardiac CC agonist potency is small. All four possible diastereomers **36**−**39** (*SS*, *RR*, *SR*, *RS*) of the compound having a 1-methyl-2-nitrooxyethyl substituent were prepared to determine the effect of configuration at the C-4 position of the 1,4-DHP ring and the C-1 position of the 1-methyl-2-nitrooxyethyl moiety on cardiac CC agonist activity. The configuration at the C-4 position of the 1,4-DHP is a major determinant of activity, since compounds having the (*4S*)-configuration are much more potent than the corresponding (*4R*)-enantiomer [(+)-(*S*)-**35** ≫ inactive (−)-(*R*)-**35**] or diastereomer [(+)-(*S*,*S*)-**36** ≫ inactive (+)-(*R*,*S*)-**39**; (−)-(*S*,*R*)-**38** > (−)-(*R*,*R*)-**37**]. In contrast, diastereomers having a (*1R*)-1-methyl-2-nitrooxyethyl configuration were more potent than the corresponding diastereomers having a (*1S*)-1-methyl-2-nitrooxyethyl configuration [(−)-(*S*,*R*)-**38** > (+)-(*S*,*S*)-**36**; (−)-(*R*,*R*)-**37** ≫ inactive (+)-(*R*,*S*)-**39**]. These latter structure−activity correlations are consistent with the observation that (−)-(*S*,*R*)-1-methyl-2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate [(−)-(*S*,*R*)-**38**] is the most potent cardiac CC agonist, being about 60-fold more active than racemic PN 202-791 ($EC_{50} = 9.4 \times 10^{-6}$ M).

In vitro •NO release was determined by quantitation of nitrite using the Griess reaction,[9] and the results are listed in Table 2. The percentage •NO released was generally greater in the presence of *N*-acetylcysteamine than in the absence of *N*-acetylcysteamine. This •NO release data is consistent with reports that •NO release from organic nitrates is facilitated by thiols.[10] A plausible explanation for the observation that the nitrooxyalkyl compounds described do not induce a CC agonist effect on GPILSM is attributed to their ability to release •NO. In contrast, (+)-(*S*)-PN 202-791 acts as a CC agonist on vascular smooth muscle.[4]

Table 2. Nitric Oxide Release Data for Nitrooxyalkyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)-pyridine-5-carboxylates (**23**−**28**)

		% nitric oxide released[a]	
compound	R	absence of *N*-acetyl-cysteamine[b]	presence of *N*-acetyl-cysteamine[c]
23	$(CH_2)_2ONO_2$	1.08	2.71
24	$(CH_2)_3ONO_2$	0.00	1.31
25	$(CH_2)_4ONO_2$	0.00	1.10
26	$CH(Me)CH_2ONO_2$	1.84	1.53
27	$H_2CH(Me)ONO_2$	0.58	1.74
28	$CH(CH_2ONO_2)_2$	0.19	1.02
glycerol trinitrate[d]		0.18	5.30

[a] Percent nitric oxide released (mean value, $n = 3$) from one nitrooxy (ONO_2) group present in the test compound. Variation from the mean % value was ≤0.02%. [b] Incubated in the absence of *N*-acetylcysteamine in phosphate buffer (pH 7.4) at 37 °C for 1 h. [c] Incubated in the presence of *N*-acetylcyteamine (1 equiv per ONO_2 moiety) in phosphate buffer (pH 7.4) at 37 °C for 1 h. [d] Glycerol trinitrate = $O_2NOCH_2CH(ONO_2)CH_2ONO_2$.

Conclusions

The nitrooxyalkyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate racemates, enantiomers, and diastereomers invesitigated constitute a novel group of nitric oxide donor compounds with desirable calcium channel modulation activities. In particular, the 2-nitrooxyethyl ester enantiomer (+)-(*S*)-**35**, having the (*S*)-configuration at the C-4 position of the 1,4-dihydropyridine ring, or the (−)-(*S*,*R*)-**38** diastereomer, also having the (*S*)-configuration at the 1,4-DHP C-4 position in conjunction with a 1-methyl-

2-nitrooxyethyl ester substituent having the (*R*)-configuration at its C-1 position, exhibit distinctive profiles in vitro that encompass a *dual cardioselective agonist/smooth muscle selective antagonist activity* that could provide a potentially new approach to drug design directed toward the treatment of congestive heart failure. The (+)-(*S*)-**35** enantiomer and the (−)-(*S*,*R*)-**38** diastereomer are potentially valuable probes to investigate the structure−function relationship of calcium channel modulation.

Experimental Section

General. Melting points were recorded with a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer (300 MHz). The assignment of exchangeable protons (N*H*, N*H*$_2$, O*H*) was confirmed by the addition of D_2O. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Infrared spectra were acquired using a Nicolet IR-500 Series II spectrometer. Silica gel column chromatography was carried out using Merck 7734 (60−200 mesh) silica gel. Microanalyses were within ±0.4% of theoretical values for all elements listed, unless otherwise stated. 2-Bromoethanol, 3-bromo-1-propanol, isopropyl bromide, 1,3-dibromo-2-propanol, 1-bromo-2-propanol, *N*-acetylcysteamine, and tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III) [Eu(hfc)$_3$] were purchased from the Aldrich Chemical Co. Nitroacetone,[11] 2-amino-1-nitroprop-1-ene,[12] nitroglycerin (glycerol trinitrate),[13] 2,1,3-benzoxadiazol-4-carboxaldehyde,[14] 4-bromo-1-butanol,[15] and (*S*)- and (*R*)-2-bromo-1-propanol[16] were prepared according to the reported procedures.

General Method for the Preparation of Nitrooxyalkyl Bromides (14−20). A bromo alcohol (**6**−**12**, 10 mmol) was added dropwise to a solution of 70% HNO_3 (1.1 mL) and 95% H_2SO_4 (2.4 mL) at 0 °C, and the reaction was allowed to proceed at the same temperature for 1 h with stirring. The resulting suspension was poured into water (50 mL), extracted with CH_2Cl_2 (3 × 200 mL), and dried ($MgSO_4$), and the solvent was removed to give the respective nitrooxyalkyl bromide (**14**−**20**). Some physical and ^{1}H NMR spectral data for compounds **14**−**20**, which were used immediately for the subsequent syntheses of the nitrooxyalkyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylates (**23**−**27**, **35**−**39**), are listed below.

2-Nitrooxyethyl bromide (14): 94% yield; oil; ^{1}H NMR ($CDCl_3$) δ 3.56 (t, J = 6.4 Hz, 2H, H-1), 4.76 (t, J = 6.4, 2H, H-2). Compound **14** was used immediately for the syntheses of compounds **23**, **(+)-(*S*)-35**, and **(−)-(*R*)-35**.

3-Nitrooxypropyl bromide (15): 99% yield; oil; ^{1}H NMR ($CDCl_3$) δ 2.28 (p, J = 6.1 Hz, 2H, H-2), 3.49 (t, J = 6.1, 2H, BrC*H*$_2$), 4.62 (t, J = 6.1, 2H, C*H*$_2$ONO$_2$). Compound **15** was used immediately for the syntheses of compound **24**.

4-Nitrooxybutyl bromide (16): 84% yield; oil; ^{1}H NMR ($CDCl_3$) δ 1.87−2.04 (m, 4H, BrCH$_2$C*H*$_2$C*H*$_2$), 3.45 (t, J = 6.0, 2H, BrC*H*$_2$), 4.50 (t, J = 6.0, 2H, C*H*$_2$ONO$_2$). Compound **16** was used immediately for the syntheses of compound **25**.

2-Bromo-1-nitrooxypropane (17): 96% yield; oil; ^{1}H NMR ($CDCl_3$) δ 1.76 (d, J = 6.7 Hz, 3H, C*H*$_3$), 4.23 (ddq, J = 6.4, 6.7, 6.7 Hz, 1H, BrC*H*), 4.54 (dd, J = 11.9, 6.7, 1H, C*H*H′ONO$_2$), 4.70 (dd, J = 11.9, 6.4, 1H, CH*H*′ONO$_2$). Compound **17** was used immediately for the syntheses of compound **26**.

1-Bromo-2-nitrooxypropane (18): 98% yield; oil; ^{1}H NMR ($CDCl_3$) δ 1.48 (d, J = 6.0 Hz, 3H, C*H*$_3$), 3.49 (d, J = 6.0 Hz, 2H, BrC*H*$_2$), 5.25 (m, 1H, CH$_3$C*H*). Compound **18** was used immediately for the syntheses of compound **27**.

(−)-(*R*)-2-Bromo-1-nitrooxypropane (19): 83% yield; oil; $[\alpha]^{23}_D$ = −17.5° (*c* 0.4 in CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.76 (d, J = 6.7 Hz, 3H, C*H*$_3$), 4.23 (ddq, J = 6.4, 6.7, 6.7 Hz, 1H, BrC*H*), 4.54 (dd, J = 11.9, 6.7, 1H, C*H*H′ONO$_2$), 4.70 (dd, J = 11.9, 6.4, 1H, CH*H*′ONO$_2$). Compound **19** was used immediately for the syntheses of compounds (+)-(*S*,*S*)-**36** and (+)-(*R*,*S*)-**39**.

(+)-(*S*)-2-Bromo-1-nitrooxypropane (20): 83% yield; oil; $[\alpha]^{23}_D$ = +17.5° (*c* 0.4 in CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.76 (d, J = 6.7 Hz, 3H, C*H*$_3$), 4.23 (ddq, J = 6.4, 6.7, 6.7 Hz, 1H, BrC*H*), 4.54 (dd, J = 11.9, 6.7, 1H, C*H*H′ONO$_2$), 4.70 (dd, J = 11.9, 6.4, 1H, CH*H*′ONO$_2$). Compound **20** was used immediately for the syntheses of compounds (−)-(*R*,*R*)-**37** and (−)-(*S*,*R*)-**38**.

1,3-Dinitrooxy-2-propanol (21). A solution of 1,3-dibromo-2-propanol (**13**, 9.13 g, 41.89 mmol), $AgNO_3$ (25.48 g, 150 mmol), and acetonitrile (150 mL) was heated at 80 °C for 3 h with stirring. The solution was cooled to 25 °C, the solvent was removed in vacuo, and water (200 mL) was added. The precipitated AgBr was removed by filtration, the filtrate was extracted with ether (3 × 500 mL), the extract was dried ($NaSO_4$), and the solvent was removed in vacuo to give **21** (7.32 g, 96% yield) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.78 (br s 1H, O*H*), 4.31 (m, 1H, H-2), 4.54 (dd, J = 6.1, 11.7 Hz, 2H, C*H*$_2$ONO$_2$), 4.61 (dd, J = 4.5, 11.7 Hz, 2H, C*H*$_2$ONO$_2$). Compound **21** was used immediately for the syntheses of compound **28**.

General Method for the Preparation of Nitrooxyalkyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylates (23−27). A solution of the respective nitrooxyalkyl bromide (14−18, 0.22 mmol), **22** (0.20 mmol), and K_2CO_3 (0.24 mmol) in dry DMF (10 mL) was stirred at 25 °C for 24 h (7 days for preparation of **27**). Water (40 mL) was added, the mixture was extracted with EtOAc (3 × 300 mL), the extract was washed with water (2 × 40 mL) and then brine (40 mL), the organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography using EtOAc−hexane (1:1, v/v) as eluent. Physical and spectral data for **23**−**27** are listed below.

2-Nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate (23): 91% yield; yellow oil; IR ($CHCl_3$) 1313, 1379, and 1488 (NO_2), 1637 (C=N), 1710 (CO_2), 3324 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, C-6 *Me*), 2.56 (s, 3H, C-2 *Me*), 4.40−4.25 (m, 2H, CO$_2$C*H*$_2$), 4.61 (t, J = 4.5 Hz, 2H, C*H*$_2$ONO$_2$), 5.79 (s, 1H, H-4), 6.47 (br s, 1H, *NH*), 7.36 (dd, J = 9.0, 6.5 Hz, 1H, 4-benzofurazanyl H-6), 7.46 (d, J = 6.5 Hz, 1H, 4-benzofurazanyl H-5), 7.69 (d, J = 9.0 Hz, 1H, 4-benzofurazanyl H-7). Anal. ($C_{16}H_{15}N_5O_8$) C, H, N.

3-Nitrooxypropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate (24): 93% yield; yellow oil; IR ($CHCl_3$) 1313, 1382, and 1489 (NO_2), 1631 (C=N), 1706 (CO_2), 3320 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.02 (p, J = 6.1 Hz, 2H, *CH*$_2$CH$_2$ONO$_2$), 2.38 (s, 3H, C-6 *Me*), 2.56 (s, 3H, C-2 *Me*), 4.16 (t, J = 6.1 Hz, 2H, CO$_2$C*H*$_2$), 4.40 (t, J = 6.1 Hz, 2H, C*H*$_2$ONO$_2$), 5.81 (s, 1H, H-4), 6.54 (br s, 1H, *NH*), 7.35 (dd, J = 9.0, 6.5 Hz, 1H, 4-benzofurazanyl H-6), 7.44 (d, J = 6.5 Hz, 1H, 4-benzofurazanyl H-5), 7.70 (d, J = 9.0 Hz, 1H, 4-benzofurazanyl H-7). Anal. ($C_{17}H_{17}N_5O_8$) C, H, N.

4-Nitrooxybutyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate (25): 82% yield; yellow oil; IR ($CHCl_3$) 1312, 1380, and 1489 (NO_2), 1628 (C=N), 1702 (CO_2), 3318 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.69 (m, 4H, C*H*$_2$C*H*$_2$CH$_2$ONO$_2$), 2.37 (s, 3H, C-6 *Me*), 2.55 (s, 3H, C-2 *Me*), 4.08 (m, 2H, CO$_2$C*H*$_2$), 4.42 (t, J = 5.50 Hz, 2H, C*H*$_2$-ONO$_2$), 5.80 (s, 1H, H-4), 6.39 (br s, 1H, *NH*), 7.35 (dd, J = 8.8, 6.1 Hz, 1H, 4-benzofurazanyl H-6), 7.43 (d, J = 6.1 Hz, 1H, 4-benzofurazanyl H-5), 7.70 (d, J = 8.8 Hz, 1H, 4-benzofurazanyl H-7). Anal. ($C_{18}H_{19}N_5O_8$) C, H, N.

1-Methyl-2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate (26): 45% yield; yellow oil (mixture of diastereomers); IR ($CHCl_3$) 1312, 1381, and 1489 (NO_2), 1637 (C=N), 1708 (CO_2), 3321 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.10 and 1.33 (two d, J = 6.7 Hz, 3H total, CO$_2$CHC*H*$_3$), 2.36 and 2.38 (two s, 3H total, C-6 *Me*), 2.55 and 2.56 (two s, 3H total, C-2 *Me*), 4.21−4.62 (m, 2H, C*H*$_2$ONO$_2$), 5.13−5.22 (m, 1H, CO$_2$C*H*), 5.78 and 5.80 (two s, 1H, H-4), 6.53 and 6.54 (two br s, 1H total, *NH*), 7.32−7.38 (m, 1H, 4-benzofurazanyl H-6), 7.44 (d, J = 6.4 Hz, 1H, 4-benzofurazanyl H-5), 7.69 and 7.70 (two d, J = 8.8 Hz, 1H total, 4-benzofurazanyl H-7). Anal. ($C_{17}H_{17}N_5O_8$) C, H, N.

2-Methyl-2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate (27): 65% yield; yellow oil (mixture of diastereomers); IR ($CHCl_3$) 1219, 1279, and 1535 (NO_2), 1642 (C═N), 1736 (C═O), 3314 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 and 1.37 (two d, $J = 6.4$ Hz, 3H total, *Me*CHONO$_2$), 2.38 (s, 3H, C-6 *Me*), 2.56 (s, 3H, C-2 *Me*), 4.05−4.34 (m, 2H, CO_2CH_2), 5.19−5.31 (m, 1H, MeC*H*ONO$_2$), 5.77 (s, 1H, H-4), 6.46 (br s, 1H, *NH*), 7.34 (two overlapping dd, $J = 8.8$, 8.2 Hz, 1H total, 4-benzofurazanyl H-6), 7.45 and 7.47 (two d, $J = 8.2$ Hz, 1H total, 4-benzofurazanyl H-5), 7.70 (d, $J = 8.8$ Hz, 1H, 4-benzofurazanyl H-7). Anal. ($C_{17}H_{17}N_5O_8$) C, H, N.

1,3-Dinitrooxy-2-propyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate (28): A mixture of **22** (94.8 mg, 0.30 mmol), $SOCl_2$ (89.2 mg, 0.75 mmol), DMF (1 mL), and CH_2Cl_2 (5 mL) was stirred at 0 °C for 2 h, and to this suspension a mixture of **21** (60.1 mg, 0.33 mmol) in CH_2Cl_2 (1 mL) was added dropwise with stirring at the same temperature. After stirring at 0 °C for 4 h, the reaction mixture was poured into water (10 mL), basified with 1 N NaOH to pH ∼8, extracted with EtOAc (3 × 50 mL), washed with water (50 mL), and dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography using EtOAc−hexane (1:1, v/v) as eluent to afford **28** as a yellow oil (80.7 mg, 56.0%): IR ($CHCl_3$) 1314, 1381 and 1487 (NO_2), 1645 (C═N), 1714 (CO_2), 3325 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.39 (s, 3H, C-6 *Me*), 2.56 (s, 3H, C-2 *Me*), 4.41 (dd, $J = 12.3$, 5.8 Hz, 1H, C*H*H′ONO$_2$), 4.52 (dd, $J = 12.3$, 4.0 Hz, 1H, CH*H*′ONO$_2$), 4.61 (dd, $J = 12.3$, 5.8 Hz, 1H, C*H*H′ONO$_2$), 4.74 (dd, $J = 12.3$, 4.0 Hz, 1H, CH*H*′ONO$_2$), 5.36 (m, 1H, CO_2CH), 5.76 (s, 1H, H-4), 6.37 (br s, 1H, *NH*), 7.35 (dd, $J = 8.8$, 6.1 Hz, 1H, 4-benzofurazanyl H-6), 7.43 (d, $J = 6.1$ Hz, 1H, 4-benzofurazanyl H-5), 7.71 (d, $J = 8.8$ Hz, 1H, 4-benzofurazanyl H-7). Anal. ($C_{17}H_{16}N_6O_{11}$) C, H, N.

(1*S*,2*R*)-2-(3,5-Dinitrophenylcarbamoyl)-2-(methoxycarbonyl)ethyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate Diastereomers [(1*S*,2*R*)-32 and (1*S*,2*R*)-33. A solution of (1*S*,2*R*)-**31** (21.00 g, 51.2 mmol), 2,1,3-benzoxadiazol-4-carboxaldehyde (**30**) (7.58 g, 51.2 mmol), and nitroacetone (**29**) (7.35 g, 71.3 mmol) in EtOH (300 mL) was stirred at 25 °C for 1 h prior to heating at 80 °C for 17 h. Removal of the solvent in vacuo gave a foamlike solid which was purified by silica gel column chromatography using EtOAc−hexane (1:1, v/v) as eluent. Initial elution gave (1*S*,2*R*)-**32**. Further elution provided a mixture of the two diastereomers (1*S*,2*R*)-**32** and (1*S*,2*R*)-**33**, followed by fractions containing pure (1*S*,2*R*)-**33**. The fractions containing the mixture of (1*S*,2*R*)-**32** and (1*S*,2*R*)-**33** were rechromatographed using EtOAc−CH_2Cl_2 (1:6, v/v) as eluent. In this way, after five column purifications, similar fractions were combined, and the solvent was removed in vacuo to afford (1*S*,2*R*)-**32** and (1*S*,2*R*)-**33** as yellow crystals after recrystallization from EtOAc/hexane, respectively.

Diastereomer (1*S*,2*R*)-32: 20% yield; 6.49 g; mp 244−245 °C; $[\alpha]^{23}_D = -129.25°$ (*c* 0.4 in CH_2Cl_2); IR ($CHCl_3$): 1353 and 1541 (NO_2), 1703 (C═O), 3314 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.26 (d, $J_{Me,CH} = 6.1$ Hz, 3H, CH*Me*), 2.33 (s, 3H, C-6 *Me*), 2.44 (s, 3H, C-2 *Me*), 3.60 (s, 3H, CO_2Me), 4.61 (m, 1H, CHC*H*NH), 5.22 (p, $J_{CH,CH} = J_{CH,Me} = 6.1$ Hz, 1H, OC*H*Me), 5.67 (s, 1H, H-4), 7.29 (dd, $J = 8.8$, 6.1 Hz, 1H, 4-benzofurazanyl H-6), 7.35 (d, $J = 6.1$ Hz, 1H, 4-benzofurazanyl H-5), 7.58 (d, $J = 8.8$ Hz, 1H, 4-benzofurazanyl H-7), 8.85 (d, $J = 2.1$ Hz, 2H, dinitrophenyl H-2 and H-6), 8.97 (dd, $J = 2.1$, 2.1 Hz, 1H, dinitrophenyl H-4), 9.26 (d, $J = 7.3$ Hz, 1H, CH−N*H*), 9.91 (br s, 1H, dihydropyridyl N*H*). Anal. ($C_{26}H_{23}N_7O_{12}$) C, H, N.

Diastereomer (1*S*,2*R*)-33. 27% yield; 8.60 g; mp 222−223 °C; $[\alpha]^{23}_D = +53.75°$ (*c* 0.4 in CH_2Cl_2); IR ($CHCl_3$): 1347 and 1541 (NO_2), 1709 and 1743 (C═O), 3314 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.00 (d, $J_{Me,CH} = 6.4$ Hz, 3H, CH*Me*), 2.25 (s, 3H, C-6 *Me*), 2.45 (s, 3H, C-2 *Me*), 3.65 (s, 3H, CO_2Me), 4.85 (m, 1H, CHC*H*NH), 5.28 (p, $J_{CH,CH} = J_{CH,Me} = 6.4$ Hz, 1H, OC*H*Me), 5.72 (s, 1H, H-4), 7.42 (d, $J = 6.7$ Hz, 1H, 4-benzofurazanyl H-5), 7.50 (dd, $J = 8.8$, 6.7 Hz, 1H, 4-benzofurazanyl H-6), 7.83 (d, $J = 8.8$ Hz, 1H, 4-benzofurazanyl H-7), 9.00 (dd, $J = 2.1$, 2.1 Hz, 1H, dinitrophenyl H-4), 9.07 (d, $J = 2.1$ Hz, 2H, dinitrophenyl H-2 and H-6), 9.66 (d, $J = 7.9$ Hz, 1H, CHN*H*), 9.86 (br s, 1H, dihydropyridyl N*H*). Anal. ($C_{26}H_{23}N_7O_{12}$) C, H, N.

1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylic Acid Enantiomers [(+)-(*S*)-34 and (−)-(*R*)-34]. A solution of either **32** or **33** (5.00 g, 8.0 mmol) and DBU (3.65 g, 24.0 mmol) in MeOH (200 mL) was stirred at 25 °C for 24 h. The solvent was removed in vacuo and water (250 mL) was added. The water layer was washed with ether (3 × 500 mL), acidified to pH 2 with 1 N HCl, and extracted with EtOAc (3 × 500 mL). The combined extracts were dried Na_2SO_4), and the solvent was removed in vacuo to give the crude product as a brownish oil which was purified by silica gel column chromatography using EtOAc−hexane (2:1, v/v) as eluent. Recrystallization from EtOH−ether afforded the respective product [(+)-(*S*)-**34** or (−)-(*R*)-**34**] as yellow crystals.

Enantiomer (+)-(*S*)-34: 50% yield; 1.27 g; mp 196 °C; $[\alpha]^{23}_D = +114.0°$ (*c* 0.4 in MeOH); IR (KBr): 1219 and 1494 (NO_2), 1649 (C═O), 3308 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.27 (s, 3H, C-6 *Me*), 2.50 (s, 3H, C-2 *Me*), 5.70 (s, 1H, H-4), 7.40 (d, $J = 6.7$ Hz, 1H, 4-benzofurazanyl H-5), 7.55 (dd, $J = 9.2$, 6.7 Hz, 1H, 4-benzofurazanyl H-6), 7.88 (d, $J = 9.2$ Hz, 1H, 4-benzofurazanyl H-7), 9.82 (s, 1H, N*H*), 12.29 (br s, 1H, COO*H*). Anal. ($C_{14}H_{12}N_4O_5$) C, H, N.

Enantiomer (−)-(*R*)-34: 50% yield; 1.27 g; mp 212 °C (dec); $[\alpha]^{23}_D = -114.0°$ (*c* 0.4 in MeOH); IR (KBr) and ^1H NMR (DMSO-d_6) spectra for (−)-(*R*)-**34** were the same as those for (+)-(*S*)-**34**. Anal. ($C_{14}H_{12}N_4O_5$) C, H, N.

General Method for the Preparation of Optically Pure Isopropyl and Nitrooxyalkyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylates [(+)-(*S*)-5, (−)-(*R*)-5, (+)-(*S*)-35, (−)-(*R*)-35, (+)-(*S*,*S*)-36, (−)-(*R*,*R*)-37, (−)-(*S*,*R*)-38, and (+)-(*R*,*S*)-39]. A solution of either (+)-(*S*)-**34** or (−)-(*R*)-**34** (126.4 mg, 0.40 mmol), K_2CO_3 (66.3 mg, 0.48 mmol), and either isopropyl bromide or nitrooxyalkyl bromide [0.44 mmol, **14**, (−)-(*R*)-**19**, or (+)-(*S*)-**20**] in dry DMF (12 mL) was stirred at 25 °C for 24 h (7 days for syntheses of compounds **36**−**39**). Water (40 mL) was added, and the mixture was extracted with EtOAc (3 × 200 mL) and washed with water (2 × 40 mL) and then brine (40 mL). The organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography using EtOAc−hexane (1:1, v/v) as eluent to yield the respective optically pure enantiomer [(+)-(*S*)-**5**, (−)-(*R*)-**5**, (+)-(*S*)-**35**, or (−)-(*R*)-**35**] or diastereomer [(+)-(*S*,*S*)-**36**, (−)-(*R*,*R*)-**37**, (−)-(*S*,*R*)-**38**, or (+)-(*R*,*S*)-**39**] product. The physical and spectral data for these compounds are listed below.

(+)-(*S*)-Isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate enantiomer [(+)-(*S*)-5]: 88.8% yield; $[\alpha]^{23}_D = +63.2°$ (*c* 0.5 in $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.07 (d, $J = 6.1$ Hz, 3H, *Me*CHMe′), 1.25 (d, $J = 6.4$ Hz, 3H, MeCH*Me*′), 2.36 (s, 3H, C-6 *Me*), 2.55 (s, 3H, C-2 *Me*), 4.95 (dq, $J = 6.4$, 6.1 Hz, 1H, MeC*H*Me), 5.80 (s, 1H, H-4), 6.30 (br s, 1H, N*H*), 7.34 (dd, $J = 8.8$, 6.1 Hz, 1H, 4-benzofurazanyl H-6), 7.45 (d, $J = 6.1$ Hz, 1H, 4-benzofurazanyl H-5), 7.68 (d, $J = 8.8$ Hz, 1H, 4-benzofurazanyl H-7).

(−)-(*R*)-Isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate enantiomer [(−)-(*R*)-5]: 89% yield; $[\alpha]^{23}_D = -63.2°$ (*c* 0.5 in $CHCl_3$); ^1H NMR ($CDCl_3$) spectrum for (−)-(*R*)-**5** was the same as that for (+)-(*S*)-**5**.

(+)-(*S*)-2-Nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate enantiomer [(+)-(*S*)-35]: 91% yield; $[\alpha]^{23}_D = +60.6°$ (*c* 0.5 in $CHCl_3$); IR ($CHCl_3$): 1313, 1379, and 1488 (NO_2), 1636 (C═N), 1710 (CO_2), 3321 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.37 (s, 3H, C-6 *Me*), 2.56 (s, 3H, C-2 *Me*), 4.40−4.25 (m, 2H, CO_2CH_2), 4.61 (t, $J = 4.5$ Hz, 2H, CH_2ONO$_2$), 5.79 (s, 1H, H-4), 6.47 (br s, 1H, *NH*), 7.36 (dd, $J = 9.0$, 6.5 Hz, 1H, 4-benzofurazanyl

H-6), 7.46 (d, J = 6.5 Hz, 1H, 4-benzofurazanyl H-5), 7.69 (d, J = 9.0 Hz, 1H, 4-benzofurazanyl H-7). Anal. ($C_{16}H_{15}N_5O_8$) C, H, N.

(−)-(*R*)-2-Nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate enantiomer [(−)-(*R*)-35]: 91% yield; $[\alpha]^{23}_D = -61.0°$ (*c* 0.5 in $CHCl_3$); IR ($CHCl_3$) and ^{1}H NMR ($CDCl_3$) spectra for (−)-(*R*)-**35** were the same as those for (+)-(*S*)-**35**. Anal. ($C_{16}H_{15}N_5O_8$) C, H, N.

(+)-(*S,S*)-1-Methyl-2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate diastereomer [(+)-(*S,S*)-36]: 25% yield; $[\alpha]^{23}_D = +59.0°$ (*c* 0.4 in CH_2Cl_2); IR ($CHCl_3$) 1312, 1381, and 1489 (NO_2), 1637 (C═N), 1708 (CO_2), 3321 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.33 (d, J = 6.7 Hz, 3H, CO_2CHCH_3), 2.38 (s, 3H, C-6 *Me*), 2.56 (s, 3H, C-2 *Me*), 4.25 (dd, J = 12.0, 6.7 Hz, 1H, $CHH'ONO_2$), 4.44 (dd, J = 12.0, 3.0 Hz, 1H, $CHH'ONO_2$), 5.16 (dp, J = 6.7, 3.0 Hz, 1H, CO_2CH), 5.80 (s, 1H, H-4), 6.43 (br s, 1H, *NH*), 7.34 (dd, J = 8.8, 6.4 Hz, 1H, 4-benzofurazanyl H-6), 7.44 (d, J = 6.4 Hz, 1H, 4-benzofurazanyl H-5), 7.69 (d, J = 8.8 Hz, 1H, 4-benzofurazanyl H-7). Anal. ($C_{17}H_{17}N_5O_8$) C, H, N.

(−)-(*R,R*)-1-Methyl-2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate diastereomer [(−)-(*R,R*)-37]: 30% yield; $[\alpha]^{23}_D = -59.0°$ (*c* 0.4 in CH_2Cl_2); IR ($CHCl_3$) and ^{1}H NMR ($CDCl_3$) spectra for (−)-(*R,R*)-**37** were the same as those for (+)-(*S,S*)-**36**. Anal. ($C_{17}H_{17}N_5O_8$) C, H, N.

(−)-(*S,R*)-1-Methyl-2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate diastereomer [(−)-(*S,R*)-38]: 30% yield; $[\alpha]^{23}_D = -30.5°$ (*c* 0.4 in CH_2Cl_2); IR ($CHCl_3$) and ^{1}H NMR ($CDCl_3$) spectra for (−)-(*S,R*)-**38** were the same as those for (+)-(*R,S*)-**39** listed below. Anal. ($C_{17}H_{17}N_5O_8$) C, H, N.

(+)-(*R,S*)-1-Methyl-2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate diastereomer [(+)-(*R,S*)-39]: 30% yield; $[\alpha]^{23}_D = +30.8°$ (*c* 0.4 in CH_2Cl_2); IR ($CHCl_3$): 1312, 1381, and 1489 (NO_2), 1637 (C═N), 1708 (CO_2), 3321 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.10 (d, J = 6.7 Hz, 3H, CO_2CHCH_3), 2.36 (s, 3H, C-6 *Me*), 2.56 (s, 3H, C-2 *Me*), 4.43 (dd, J = 12.2, 6.7 Hz, 1H, $CHH'ONO_2$), 4.59 (dd, J = 12.2, 3.4 Hz, 1H, $CHH'ONO_2$), 5.19 (dp, J = 6.7, 3.4 Hz, 1H, CO_2CH), 5.78 (s, 1H, H-4), 6.41 (br s, 1H, *NH*), 7.35 (dd, J = 8.8, 6.1 Hz, 1H, 4-benzofurazanyl H-6), 7.44 (d, J = 6.1 Hz, 1H, 4-benzofurazanyl H-5), 7.70 (d, J = 8.8 Hz, 1H, 4-benzofurazanyl H-7). Anal. ($C_{17}H_{17}N_5O_8$) C, H, N.

Optical Purity of Enantiomers and Diastereomers. The optical purity of (+)-(*S*)-**5**, (−)-(*R*)-**5**, (+)-(*S*)-**35**, and (−)-(*R*)-**35** was determined by ^{1}H NMR spectrometry. When 20 μL of a solution (100 mg in 1 mL $CDCl_3$) of the chiral shift reagent (+)-Eu(hfc)$_3$ was added to a solution of racemic 2-nitrooxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)-5-pyridinecarboxylate (**23**) (5 mg in 0.5 mL $CDCl_3$), the 1,4-dihydropyridyl C-6 methyl resonance at δ 2.37 was separated into two resonances that appeared at δ 2.54 and 2.56. Addition of (+)-Eu(hfc)$_3$ (60 μL) to (+)-(*S*)-**35** or (−)-(*R*)-**35**, as described above, resulted in retention of single resonance for the C-6 methyl resonance (≥96% ee). Similarly, addition of (+)-Eu(hfc)$_3$ (10 μL) to racemic isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)-5-pyridinecarboxylate (**5**) (5 mg in 0.5 mL $CDCl_3$) resulted in the separation of the 1,4-dihydropyridyl C-6 methyl resonance at δ 2.36 into two resonances, which appeared at δ 2.37 and 2.38. A similar addition of (+)-Eu(hfc)$_3$ (40 μL) to (+)-(*S*)-**5** or (−)-(*R*)-**5** resulted in retention of a single resonance for the C-6 methyl resonance (≥96% ee). The optical purity of diastereomers (+)-(*S,S*)-**36**, (−)-(*R,R*)-**37**, (−)-(*S,R*)-**38**, and (+)-(*R,S*)-**39** was determined by ^{1}H NMR spectrometry. Similar to previous studies, the configuration at the 1,4-dihydropyridine C-4 position was retained during the esterification reaction, whereas the S_N2 reaction with the nitrooxyalkyl bromide proceeded with inversion of configuration. Accordingly, retention of a single resonance for the C-6 methyl resonance at δ 2.36 or δ 2.38, for each diastereomer, compared to two resonance at both δ 2.36 and δ 2.38, for a mixture of the diastereomers (**26**), showed that these diastereomers were optically pure (≥96% de). In addition, the opposite optical rotation for the diastereomers (+)-(*S,S*)-**36** and (−)-(*R,R*)-**37** and for the diastereomers (−)-(*S,R*)-**38** and (+)-(*R,S*)-**39** supports this assumption.

In Vitro Calcium Channel Antagonist and Agonist Assays. Calcium channel antagonist activities were determined as the molar concentration of the test compound required to produce 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.6×10^{-7} M) Ca^{2+}-dependent contraction (tonic response) of guinea pig ileum longitudinal smooth muscle (GPILSM) using the procedure reported previously.[6] The IC_{50} value (±SEM, n =3) was determined graphically from the dose−response curve.

Calcium channel modulation activity (positive inotropic effect) was calculated as the molar concentration of the test compound required to produce a 50% increase (positive inotropic, or CC agonist, effect) in contractile force of isolated guinea pig left atrium (GPLA) relative to its basal contractile force in the absence of test compound.[6]

Nitric Oxide Release Assay. In vitro nitric oxide release was assayed using a modification of the previously reported procedure.[9] The test compound (0.0075 mmol) was added to a thoroughly mixed solution of 0.1 M phosphate buffer (pH 7.40) and acetonitrile (1:1, v/v) (1.5 mL) containing *N*-acetylcysteamine (1 equiv per $-ONO_2$ moiety) or, in the absence of *N*-acetylcysteamine, with stirring under argon at 37 °C for 1 h. After exposure to air for 10 min, the reaction mixture (0.2 mL) was diluted with water (0.6 mL), and this solution was treated with 0.2 mL of Griess reagent [sulfanilamide (4 g), *N*-naphthylenediamine dihydrochloride (0.2 g), and 85% phosphoric acid (10 mL) in distilled water (final volume: 100 mL)] for 10 min at room temperature. After dilution of this solution with water to a volume of 5 mL, the absorbance was measured at 540 nm. Solutions of 3−24 μM sodium nitrite were used to prepare a nitrite versus concentration curve. A control experiment (absence of test compound) was performed for each measurement (n = 3). The percent nitric oxide release (quantitated as nitrite ion) was calculated from the nitrite versus concentration curve.

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