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Hantzsch 1,4-dihydropyridines containing a diazen-1-ium-1,2-diolate nitric oxide donor moiety to study calcium channel antagonist structure–activity relationships and nitric oxide release

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Abstract—A group of racemic 3-isopropyl 5-[(2-piperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**12a**–**c**), 3-isopropyl 5-{2-[4-nitrosopiperazinyl]ethyl} 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**14a**–**c**) and 3-isopropyl 5-{2-[(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)(*N*,*N*-dialkylamino or 4-piperazin-1-yl)]ethyl} 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**22**–**30**) were prepared using modified Hantzsch reactions. This group of compounds (**12a**–**c**, **14a**–**c**, and **22**–**30**) exhibited less potent calcium channel antagonist activity (IC_{50} = 0.11 to 3.35 μM range) than the reference drug nifedipine (IC_{50} = 0.01 μM). The point of attachment of the isomeric C-4 substituent was a determinant of calcium channel antagonist activity providing the potency profile 2-pyridyl ⩾ 3-pyridyl ⩾ 4-pyridyl. The *N*-nitrosopiperazinyl compounds (**14a**–**c**) did not release nitric oxide. The prodrugs **22**–**30** that have a C-5 2-[(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)(*N*,*N*-dialkylamino or 4-piperazin-1-yl)]ethyl ester substituent, upon incubation with guinea pig serum, undergo consecutive cleavage of the O^2-acetoxymethyl moiety to give a nitric oxide donor diazenium-1-ium-1,2-diolate species that subsequently releases nitric oxide. The extent of nitric oxide released from the diazen-1-ium-1,2-diolate group is dependent upon the nature of the amino functionality attached directly to the diazen-1-ium N-1 position where the nitric oxide release profile is 1,4-piperazinyl > *N*-Et > *N*-(*n*-Bu) ≫ *N*-Me upon exposure to guinea pig serum esterase(s). The results from this study suggest this class of hybrid calcium channel antagonist/nitric oxide donor prodrugs should release the vasodilator nitric oxide in vivo, preferentially in the vascular endothelium, to enhance the smooth muscle calcium channel antagonist effect to produce a combined synergistic antihypertensive effect.
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1. Introduction

1,4-Dihydropyridine (DHP) calcium channel (CC) antagonists inhibit calcium (Ca^{2+}) currents through voltage-dependent L-type CCs.[1] In this regard, DHP CC antagonists that produce vascular smooth muscle relaxation, especially in arterial beds, are useful for the treatment of peripheral vascular diseases such as angina pectoris and hypertension.[2] Alternatively, nitric oxide (·NO), that is constitutively produced by endothelial nitric oxide synthase and acts upon the guanylate cyclase-*c* GMP pathway, is required for the maintenance of blood-vessel homeostasis, blood pressure, and organ perfusion. There is strong evidence suggesting that endothelium-derived ·NO is deficient in atherosclerosis and other related cardiovascular disorders. Thus, ·NO donors that exhibit vasodilator activity, in conjunction with the ability to inhibit platelet aggregation, are useful for either acute or preventative management of myocardial ischemia or heart failure.[3] From a clinical perspective, ·NO donor drugs reduce cardiac preload, whereas DHPs reduce cardiac afterload.

Hybrid cardiovascular drugs that incorporate more than one pharmacological action into a single drug provide an attractive alternative to multiple drug therapy.[4] In an earlier study, a group of *organic nitrates* (**1**), were described that were designed to act as hybrid CC antagonist/·NO donor drugs (see Fig. 1). Although, these hybrid compounds (**1**), exhibited good CC antagonist activity on smooth muscle, the extent of ·NO release

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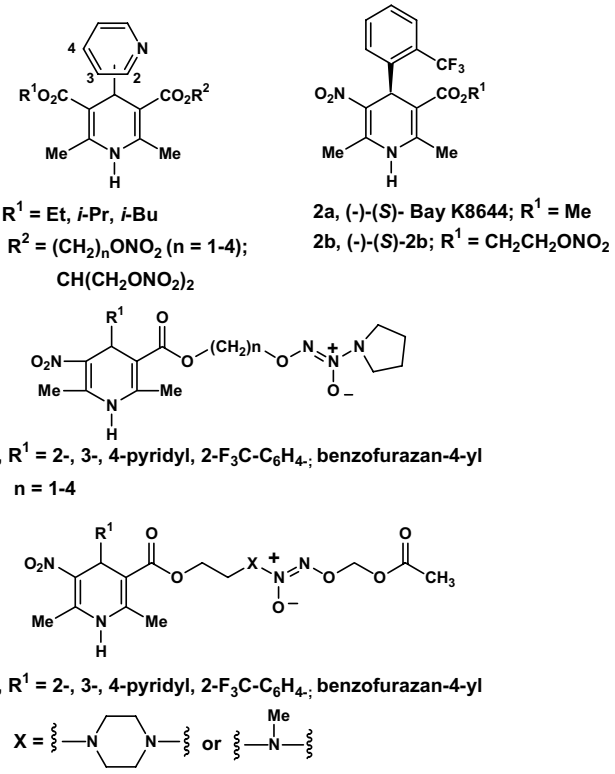


Figure 1. Structures of 3-(nitrooxyalkyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**1**), (−)-(*S*)-Bay K8644 (**2a**), (−)-(*S*)-3-nitrooxyethyl 1,4-dihydro-2,6-diemthyl-3-nitro-4-(2-trifluoromethylphenyl)-5-pyridinecarboxylate (**2b**), O^2-alkyl-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate 1,4-dihydro-2,6-dimethyl-3-nitro-4-aryl(heteroaryl)pyridine-5-carboxylates (**3**), and 4-aryl(heteroaryl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylates possessing an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate, or O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate, C-5 ester substituent (**4**).

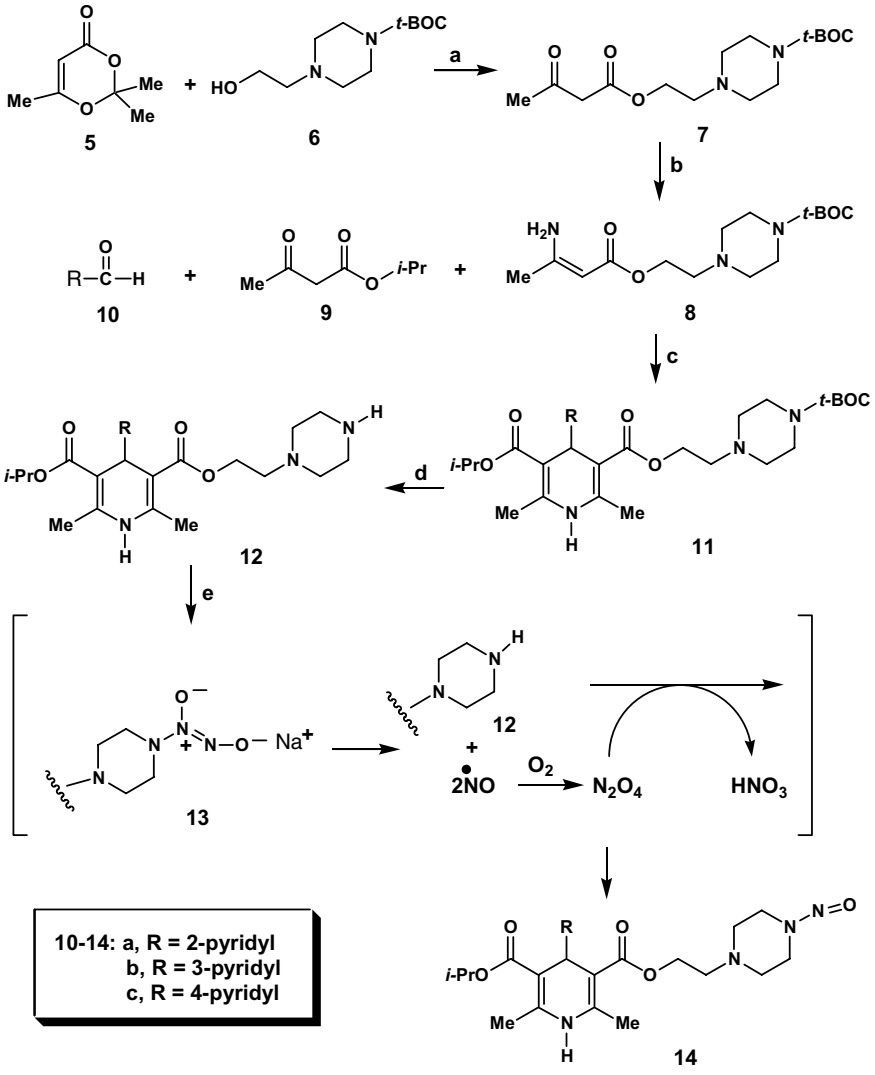
(0.03–0.43%/ONO_2 group) was very low relative to the reference drug glyceryl trinitrate (2.81%/ONO_2 group).[5] In contrast, this hybrid design concept was used successfully to abolish the undesired CC agonist effect of (−)-(*S*)-Bay K8644 (**2a**) on smooth muscle. Thus, replacement of the methyl group of (−)-(***S***)-**2a** by a ·NO donor 2-nitrooxyethyl group at the C-3 ester substituent furnished the (−)-(*S*)-2-nitrooxyethyl analog [(−)-(***S***)-**2b**] that *acted as a dual cardioselective CC agonist (positive inotrope)/smooth muscle selective CC antagonist*.[6] On the other hand, incubation of the structurally related prodrug hybrid compounds (**3**), having an O^2-alkyl-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate ester moiety, with pig liver esterase or rat serum released less than 1% of the theoretical amount (two molecules of nitric oxide/diazen-1-ium-1,2-diolate moiety) of ·NO. This inefficient release of ·NO indicates that the ester moiety present in prodrugs **3** is highly resistant to cleavage by esterases.[7] A subsequent study showed that the prodrug hybrids **4**, having an O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate or O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate C-5 ester substituent, effectively released ·NO (relative to a theoretical maximum release of 2 mol of ·NO/mol of test compound) in phosphate buffered saline (PBS) at pH 7.4 (6.5–9.4% range), in the presence of pig liver esterase (21.8–34.9% range), or guinea pig serum (60.7–75.8% range).[8]

It has been shown that the CC antagonist nitrendipine enhances the release of ·NO from vascular endothelium, which may contribute to the vascular relaxant effect,[9] that the combined effects of basal ·NO release produce an inhibition greater than additive, which increased the observed CC antagonist potency by 3-fold,[10] and that organic nitrates and ·NO donor agents induce a small but constant positive inotropic effect in vivo that is not due to coronary dilation.[11] In our ongoing program to develop structure–activity relationships for hybrid 1,4-DHP CC antagonists containing a ·NO donor moiety, and to study the structure–function relationship of CCs, we now report the smooth muscle CC antagonist activities and ·NO data for dialkyl 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**22**–**30**) having O^2-acetoxymethyldiazen-1-ium-1,2-diolate (*N*,*N*-dialkylamino or 4-piperazin-1-yl)]ethyl ·NO donor moieties in the C-3 ester substituent.

2. Chemistry

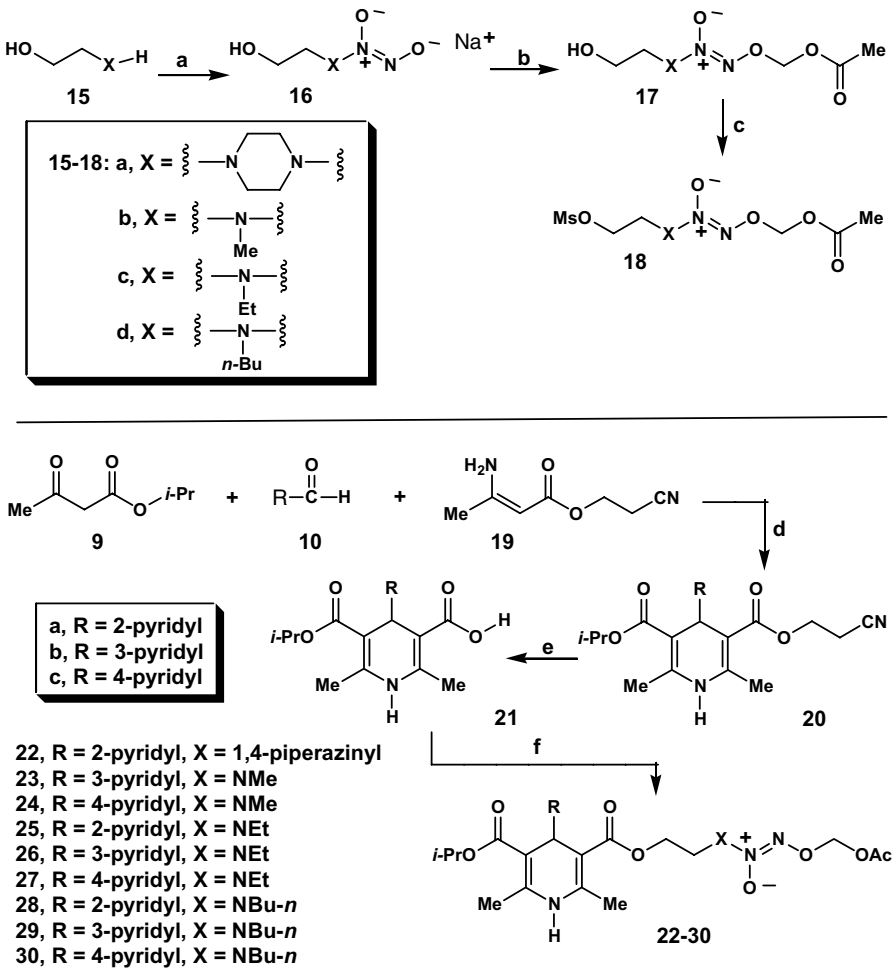
A group of racemic 3-isopropyl 5-[2-(4-nitrosopiperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**14a**–**c**) were prepared using the sequence of reactions illustrated in Scheme 1. 2-[4-(*tert*-Butoxycarbonyl)piperazin-1-yl]ethyl acetoacetate (**7**), prepared by reaction of diketene (**5**) with the alcohol (**6**), was elaborated to the 3-aminocrotonate derivative (**8**) by reaction with ammonia gas. The subsequent Hantzsch condensation of **8** with a pyridinecarboxaldehyde (**10a**–**c**) and isopropyl acetoacetate (**9**) afforded the respective 3-isopropyl 5-[2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylate (**11a**–**c**) in 31–59% chemical yield. Deprotection of the *N*-(*tert*-butoxycarbonylpiperazinyl) compound (**11a**–**c**) using trifluoroacetic acid afforded the corresponding piperazinyl derivative (**12a**–**c**). Reaction of the piperazinyl compounds **12a**–**c**, containing a 2°-amino moiety, with nitric oxide gas at 40 psi yielded the 3-isopropyl 5-[2-(4-nitrosopiperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**14a**–**c**) having an unexpected *N*-nitroso substituent in 19–69% isolated yield. A plausible mechanism for the formation of the *N*-nitroso product (**14**) involves the initial formation of the desired, but unstable, O^2-sodium N^1-substituted-diazen-1-ium-1,2-diolate salt (**13**) that then eliminates two molecules of nitric oxide, which undergo transformation to N_2O_4. Reaction of the released piperazinyl compound (**12**) with N_2O_4 could then afford the respective *N*-nitroso product (**14a**–**c**) as illustrated in Scheme 1.[12]

A group of 3-isopropyl 5-{2-[(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)(*N*,*N*-dialkylamino or 4-piperazin-1-yl)]ethyl} 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**22**–**30**) were synthesized using the sequence of reactions illustrated in Scheme 2. In this group of compounds **22**–**30**, the O^2 anion of the diazen-



Scheme 1. Reagents and conditions: (a) xylene, reflux, 72 h; (b) NH_3 gas, Et_2O, 22 °C, 9 h; (c) *i*-PrOH, 55 °C, 20 h; (d) CF_3CO_2H, CH_2Cl_2, 22 °C, 20 h; (e) $CHCl_3$, NaOMe in MeOH (25% w/w), nitric oxide gas at 40 psi, 22 °C, 20 h.

1-ium-1,2-diolate moiety was protected as a biolabile O^2-acetoxymethyl derivative that is expected to be stable in neutral aqueous media, but which will release ·NO after enzymatic cleavage of the terminal acetoxymethyl group by esterases.[8,13] The O^2-acetoxymethyl derivatives (**17c**,**d**) were prepared initially using procedures similar to those reported previously for the synthesis of the structurally related compounds **17a**,**b**.[8] Accordingly, treatment of the secondary aminoalcohols **15c**,**d** with nitric oxide gas at 40 psi in the presence of NaOMe afforded the respective O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-alkylamino]diazen-1-ium-1,2,-diolate (**16c**,**d**). The subsequent elaboration of **16c**,**d** to the corresponding O^2-acetoxymethyl derivatives upon reaction with $AcOCH_2I$ in the absence of a base following the reported procedure[8] gave low chemical yields of **17c** (9%) and **17d** (8%). It was anticipated that HI produced during the coupling reaction decomposes the sodium 1,2-diolate precursors (**16c**,**d**) since it has been reported that ·NO release from diazen-1-ium-1,2-diolates is enhanced under acidic conditions.[14] Addition of Na_2CO_3 to the reaction mixture to neutralize the HI improved the yields of **17c** (30%) and **17d** (40%). Reaction of the alcohols **17c**,**d** with $MeSO_2Cl$ in the presence of 4-dimethylaminopyridine afforded the respective methansulfonyloxy derivatives **18c**,**d**. The Hantzsch condensation of isopropyl acetoacetate (**9**) with 2-, 3-, or 4-pyridinecarboxaldehyde (**10a**–**c**) and 2-cyanoethyl 3-aminocrotonate (**19**) afforded the respective 3-isopropyl 5-(2-cyanoethyl) 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylate isomer (**20a**–**c**). Treatment of the 2-cyanoethyl esters (**20a**–**c**) with NaOMe induces a base-catalyzed β-elimination of acrylonitrile ($H_2C{=}CH{-}CN$) to afford the respective carboxylic acid product (**21a**–**c**). Condensation of the methanesulfonyloxy compounds **18a**–**d** with the carboxylic acid compounds **21a**–**c** afforded the respective 3-isopropyl 5-{2-[(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)(*N*,*N*-dialkylamino or 4-piperazin-1-yl)]ethyl} 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylate (**22**–**30**) target compounds that were designed to act as prodrugs to hybrid CC antagonist/·NO donor agents. These hybrid compounds (**22**–**30**) do not have a high chemical stability, especially those compounds having a piperazinyl (**22**), or



Scheme 2. Reagents and conditions: (a) NaOMe in MeOH (25% w/w), Et_2O, nitric oxide gas at 40 psi, 22 °C, 48–72 h; (b) Na_2CO_3, acetone, $AcOCH_2I$, 22 °C under argon, dark; (c) $MeSO_2Cl$, 4-dimethylaminopyridine (DMAP), MeCN, 22 °C under argon, dark; (d) *i*-PrOH, 55 °C, 20 h; (e) (1) NaOMe in MeOH (25% w/w), $CHCl_3$ and (2) then adjust pH to 5.5 using 2 N H_2SO_4; (f) Na_2CO_3, MeCN, **18a**, 70 °C for 48 h (compound **22**), and Na_2CO_3, MeCN, **18b**, **18c**, or **18d**, at 50 °C for 5 days (compounds **23**–**30**).

methylamino (**23**–**24**), X-moiety, because decomposition was observed during column chromatography purification and drying under vacuum at temperatures in excess of 60 °C.

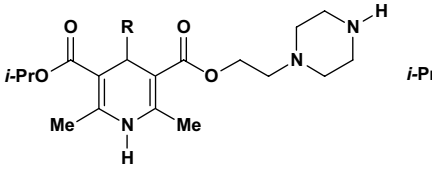
3. Results and discussion

A group of 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-alkylamino]ethyl} 3-isopropyl 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**22**–**30**) were synthesized for evaluation as hybrid calcium channel (CC) antagonist/nitric oxide (·NO) donor agents. The 3-isopropyl 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-dicarboxylate moiety was selected as the CC antagonist component since previously acquired structure–activity relationships showed that (i) a larger branched substituent such as a C-3 isopropyl ester substituent provided more potent CC antagonist activity and (ii) a C-4 pyridyl substituent is bioisosteric with the classical CC antagonist nifidepine's C-4 nitrophenyl substituent where *ortho*-, *meta*-, and *para*-nitrophenyl are bioisosteric with 2-pyridyl, 3-pyridyl, and 4-pyridyl, respectively.[15] A C-5 2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-alkylamino]ethyl ester substituent was selected as the ·NO donor prodrug component of this potential hybrid class of compounds based on the following rationale: (i) the O^2-acetoxymethyl moiety is expected to undergo facile cleavage by esterases[8] to liberate the diazen-1-ium-1,2-dilate that can theoretically release two molecules of ·NO, (ii) the rate of ·NO release from the diazen-1-ium-1,2-diolate moiety can be controlled by the nature of the R-substituent in R–$N^+(O^-)$=N–O^- compounds,[14,16] *and* compounds of general structure R^1 (R^2)N–$N^+(O^-)$=N–O(CH_2)*n*NH_2 with $t_{1/2}$ values of 1.3–3400 min at 22 °C and pH 7.4 in phosphate buffer have been reported,[17] and (iii) after release of ·NO from the diazen-1-ium-1,2-diolate moiety at pH 7.4 (no enzyme required), the DHP released with a C-5 aminoalkyl ester substituent is expected to have a longer duration of action that would be amenable to once-a-day dosing that may circumvent the peak and trough plasma levels observed with short acting CC antagonist drugs such as nifedipine. This latter expectation is consistent with a report that 1,4-dihydropyridine CC antagonists possessing an alkylpiperazinyl ester sub-

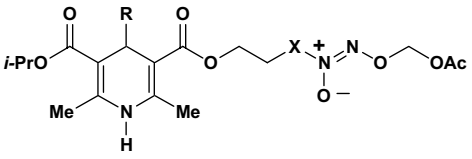
stituent exhibit a potent and long-lasting antihypertensive effect.[18]

Smooth muscle CC antagonist activity was determined as the molar concentration of the test compound required to produce 50% inhibition of the muscarinic receptor-mediated (carbachol, 0.167 μM) calcium-dependent contraction (tonic response) of guinea pig ileum longitudinal smooth muscle (GPILSM). The 1,4-dihydropyridine compounds investigated in this study (**12**, **14**, and **22**–**30**, see data in Table 1) exhibited less potent CC antagonist activity (IC_{50} = 0.11 to 3.35 μM range) than the reference drug nifedipine (IC_{50} = 0.01 μM). A comparison of the CC antagonist activities of the piperazinyl compounds **12a**,**b** (IC_{50} = 0.26–0.47 μM range) with the respective *N*-nitrosopiperazinyl derivatives **14a**,**b** (IC_{50} = 0.22–0.29 μM range) indicates that the *N*-nitroso substituent is not a major determinant of CC antagonist activity. On the other hand, the O^2-acetoxymethyldiazen-1-ium-1,2-diolate)piperazin-1-yl derivative **22** is a significantly weaker CC antagonist (IC_{50} = 1.19 μM) than the piperazinyl compound **12a** or the *N*-nitrosopiperazinyl compound **14a** that could be due to the larger steric size and/or electronic properties of this moiety that alters its binding to the CC receptor. The group of O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-alkylamino compounds **23**–**30** in which the X-moiety attached to N-1 of the diazen-1-ium-1,2-diolate group was varied [X = NMe, NEt, N-(*n*-Bu)] exhibited CC antagonist activity (IC_{50} values) over a wider

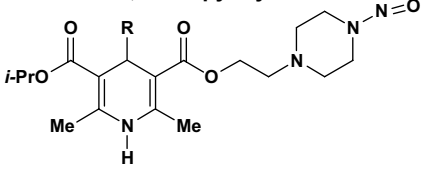
Table 1. In vitro calcium channel antagonist activities and nitric oxide release data for 5-[2-(piperazin-1-yl)ethyl]- (**12a**,**b**), 5-[2-(4-nitrosopiperazin-1-yl)ethyl]-, and 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-alkylamino]ethyl}- (**22**–**30**) derivatives of 3-isopropyl 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylate



12a, R = 2-pyridyl
12b, R = 3-pyridyl



22, R = 2-pyridyl, X = 1,4-piperazinyl
23, R = 3-pyridyl, X = NMe
24, R = 4-pyridyl, X = NMe
25, R = 2-pyridyl, X = NEt
26, R = 3-pyridyl, X = NEt
27, R = 4-pyridyl, X = NEt
28, R = 2-pyridyl, X = NBu-*n*
29, R = 3-pyridyl, X = NBu-*n*
30, R = 4-pyridyl, X = NBu-*n*



14a, R = 2-pyridyl
14b, R = 3-pyridyl
14c, R = 4-pyridyl

Compound no.	Antagonism	Percent nitric oxide released (mol/mol)	
	IC_{50} (μM)[a]	L-Cysteine[b]	Guinea Pig serum[c]
12a	0.26 ± 0.04	—	—
12b	0.47 ± 0.09	—	—
14a	0.29 ± 0.02	2 ± 0	0 ± 0
14b	0.22 ± 0.01	1 ± 0	0 ± 0
14c	1.16 ± 0.16	1 ± 0	0 ± 0
22	1.19 ± 0.08	2 ± 0	141 ± 6
23	0.11 ± 0.02	1 ± 0	24 ± 4
24	0.37 ± 0.11	3 ± 0	70 ± 8
25	1.28 ± 0.15	3 ± 0	132 ± 3
26	3.35 ± 0.14	3 ± 0	146 ± 5
27	0.82 ± 0.13	1 ± 0	101 ± 4
28	0.27 ± 0.03	3 ± 0	109 ± 3
29	0.73 ± 0.22	2 ± 0	109 ± 2
30	0.85 ± 0.29	3 ± 0	103 ± 9
Nifedipine	0.01 ± 0.00	—	—
Glyceryl trinitrate	—	20 ± 1	1 ± 0

[a] The micromolar 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 induced by the muscarinic agonist carbachol (0.167 μM) was determined graphically from the dose–response curves.

[b] The percent nitric oxide released (±SEM, n = 3) was estimated as the percent nitrite produced per mole of the test compound (0.1 mM) in the presence of L-cysteine (3.2 mM) in DMSO/phosphate buffer solution (5:95, v/v) for 1 h at 37 °C using the Griess reaction. Numerical values were obtained graphically from a standard nitrite concentration curve.

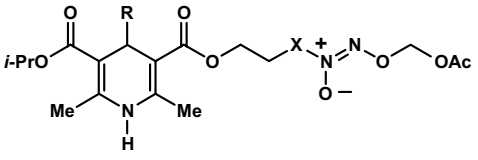
[c] The percent nitric oxide released (±SEM, n = 3) was estimated as the percent nitrite produced per mole of the test compound (10 mM) in acetonitrile and guinea pig serum (5:95 v/v) for 1 h at 37 °C using the Griess reaction. Numerical values were obtained graphically from a standard nitrite concentration curve.

range of 0.11–3.35 μM. The effect of the X-moiety on CC antagonist activity is difficult to interpret since the relative activity profiles were variable (NMe = 0.11–0.37 μM range; NEt = 0.82–3.35 μM range; N-Bu = 0.27–0.85 μM range). A comparison of the overall effect which the point of attachment of the isomeric C-4 pyridyl substituent has upon CC antagonist activity shows a potency order where 2-pyridyl ⩾ 3-pyridyl ⩾ 4-pyridyl. This activity profile is consistent with results from a previous study[15] employing related 3,5-dialkyl analogs, and well-documented structure–activity relationship that the relative potency order of a C-4 phenyl ring substituent is generally *ortho* > *meta* > *para*.[19]

The O^2-acetoxymethyl protecting group in compounds **22**–**30** must be removed by esterase cleavage to afford the terminal diazen-1-ium-1,2-diolate moiety, which is then able to release ·NO. In this regard, it is not likely that there is a sufficient in vitro concentration of esterase present in GPILSM tissue to cleave the O^2-acetoxymethyl moiety, which would preclude the release of ·NO from this hybrid class of compounds (**22**–**30**) under the experimental conditions employed in this in vitro CC antagonist assay.

The ability of the hybrid compounds **14a**–**c** and **22**–**30**, dissolved in a DMSO/phosphate buffer solution (5:95, v/v) in the presence of the L-cysteine, to release ·NO was determined (see data in Table 1). Under these experimental conditions, the release of nitric oxide [quantitated as nitrite anion (NO_2^-)] was in the 1–3% range due to the fact that the O^2-acetoxymethyldiazen-1-ium-1,2-diolate group is stable under these conditions. It is likely that the acidic reaction condition used to convert nitrite anion to nitrous acid (HNO_2) in the Griess reaction induces a small amount of hydrolysis of the O^2-acetoxymethyl group to afford the diazen-1-ium-1,2-diolate, which can then release NO, on reaction with H_2O and O_2, is rapidly converted to nitrite anion. In comparison, the reference drug glyceryl trinitrate released 20% ·NO. It was therefore of interest to carry out ·NO release studies where the test compounds were incubated with guinea pig serum that contains esterases. Although it is known that some *N*-nitroso compounds can act as ·NO donors,[3] compounds **14a**–**c** having a *N*-nitrosopiperazinyl moiety did not release ·NO (Table 1). In contrast, incubation of the hybrid compounds **22**–**30**, dissolved in acetonitrile/guinea pig serum (5:95, v/v) resulted in a 24–146% release of ·NO, relative to the reference drug glyceryl trinitrate (1% ·NO release). The release of ·NO in excess of 100% is consistent with the fact that two molecules of ·NO can be theoretically released by one molecule of a diazen-1-ium-1,2-diolate moiety.[12] In the case of those compounds for which the extent of ·NO release exceeds 100%, the rate of conversion of ·NO to NO_2^- must be faster than the reaction of released ·NO with the secondary amine to give a *N*-nitrosoamine product.

According to Hrabie and Keefer,[14] the rate of ·NO release from compounds **22**–**30**, after cleavage of the O^2-acetoxymethyl from the diazen-1-ium-1,2-diolate moiety, should be dependent upon the nature of the X-group. The extent of ·NO released from compounds **22**–**30** generally followed a release profile with respect to the X-moiety of 1,4-piperazinyl > *N*-Et > *N*-Bu-



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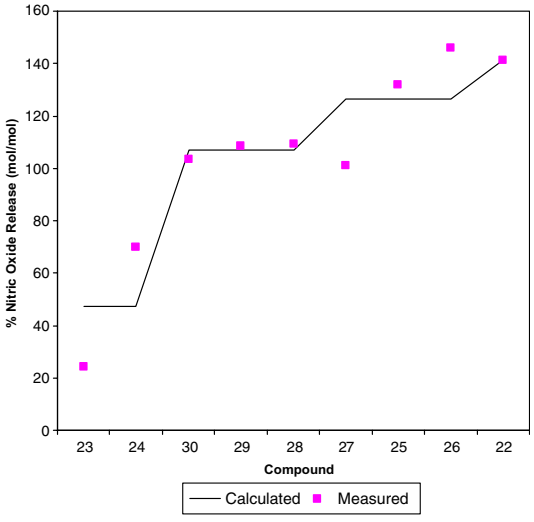
24, R = 4-pyridyl, X = NMe
25, R = 2-pyridyl, X = NEt
26, R = 3-pyridyl, X = NEt
27, R = 4-pyridyl, X = NEt
28, R = 2-pyridyl, X = NBu-*n*
29, R = 3-pyridyl, X = NBu-*n*
30, R = 4-pyridyl, X = NBu-*n*



% NO Release = X + 47.160

n = 9; R^2 = 0.81; p = 0.001

X-moiety	
1,4-piperazinyl	94.070
N-ethyl	79.223
N-(*n*-butyl)	59.833
N-methyl	0.000

Figure 2. A quantitative structure–activity relationship equation for the extent of nitric oxide released from 5-{2-[(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)(*N*,*N*-dialkylamino or 4-piperazin-1-yl)]ethyl} 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**22**–**30**).

$n \gg N$-Me. The % ·NO release data listed in Table 1 was used to generate a linear regression equation. Inclusion of the C-4 pyridyl substituents as a parameter of a multiple collinear regression equation decreased the validity of the equation as determined by the coefficient of determination (R^2). This observation suggests that there is no definitive relationship between the C-4 2-, 3-, and 4-pyridyl substituents with respect to the extent of ·NO release. Accordingly, the following quantitative structure–activity relationship equation was generated for the % ·NO release with respect to the nature of the X-moiety present in compounds **22**–**30** (see Fig. 2).

% ·NO release = X+47.160 ($n = 9$; $R^2 = 0.81$; $p = 0.001$).

4. Conclusions

The results of this study provide credence for the drug design concepts that (i) hybrid CC antagonist/·NO donor prodrugs such as 3-isopropyl 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-alkylamino]ethyl} 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**22**–**30**) may have useful in vivo antihypertensive properties, (ii) the O^2-acetoxymethyl prodrug moiety is readily cleaved by serum esterase(s), (iii) the 1,2-diazen-1-ium-1,2-diolate group is an effective ·NO donor, and (iv) the extent of ·NO release is dependent upon the nature of the amino (1,4-piperazinyl, NMe, NEt, N-Bu) moiety attached to the N-1 position of the diazen-1-ium-1,2-diolate group.

5. Experimental

Melting points were determined using a Thomas–Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Nicolet 550 Groups II Magna FT-IR spectrometer. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on a Bruker AM-300 spectrometer and chemical shifts are expressed in parts per million (ppm, δ) relative to tetramethylsilane as internal standard. Spin multiplets are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet). Coupling constants (J) are given in Hertz (Hz). ^{13}C NMR spectra were acquired using the J modulated spin echo technique where methyl and methane carbons appear as positive deflections, and methylene and quarternary carbon resonances appear as negative deflections. The assignment of exchangeable protons (N*H*, O*H*) was confirmed by the addition of D_2O. Ultraviolet spectra and quantitative analyses were measured using a Thermo Labsystem Multiscan Ascent spectrometer. Quantitative structure–activity correlations were determined using Microsoft Excel (Ver. 2003). Microanalyses were performed for C, H, and N (Micro Analytical Service Laboratory, Department of Chemistry, University of Alberta). Nominal mass, positive polarity, electrospray spectra were acquired using a Kratos MS-50 (for compounds **14a**–**c**, and **21b**) and a Waters Micromass ZQ mass spectrometer (for compounds **11a** and **22**–**30**). Preparative thin layer chromatography (PTLC) was performed using Macherey Nagel P/UV254 plates, 2.0 mm in thickness. Silica gel column chromatography was performed using Silicycle silica gel (70–230 mesh). Glyceryl trinitrate,[20] 1-(*tert*-butoxycarbonyl)-4-(2-hydroxyethyl)piperazine (**6**),[21] chloromethyl acetate,[22] 2-cyanoethyl 3-aminocrotonate (**19**),[23] O^2-acetoxymethyl-1-[4-(2-hydroxyethyl)piperazin-1-yl]diazen-1-ium-1,2,-diolate (**18a**), and O^2-acetoxymethyl-1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2,-diolate (**18b**)[8] were prepared according to literature procedures. All other reagents were purchased from Aldrich Chemical (Milawukee, WI).

5.1. 2-[4-(*tert*-Butoxycarbonyl)piperazin-1-yl]ethyl acetoacetate (7)

A solution of 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (**5**, 9.82 g, 69 mmol) and 1-(*tert*-butoxycarbonyl)-4-(2-hydroxyethyl)piperazine (**6**, 15.90 g, 69 mmol) in xylene (50 mL) was refluxed for 3 days prior to removal of the solvent in vacuo. The residue was dissolved in Et_2O (100 mL), washed with water (3 × 75 mL), dried (Na_2SO_4), and the solvent was removed in vacuo to afford **7** as an oil (61%); ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H, C*Me*$_3$), 2.29 (s, 3H, CO*Me*), 2.44 (t, $J = 5.0$ Hz, 4H, piperazinyl H-2, H-6), 2.65 (t, $J = 5.8$ Hz, 2H, C*H*$_2$N), 3.42 (t, $J = 5.0$ Hz; 4H, piperazinyl H-3, H-5), 3.48 (s, 2H, COC*H*$_2$CO), 4.28 (t, $J = 5.8$ Hz, 2H, C*H*$_2$O).

5.2. 2-[4-(*tert*-Butoxycarbonyl)piperazin-1-yl]ethyl 3-aminocrotonate (8)

2-[4-(*tert*-Butoxycarbonyl)piperazin-1-yl]ethyl 3-aminocrotonate (**8**) was prepared according to a general method described by Koo.[24] Optimal yield and ease of purification were attained when the reaction was allowed to proceed for 9 h at 22 °C to yield **8** as an oil (97%); ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H, C*Me*$_3$), 1.91 (s, 3H, aminocrotonate *Me*), 2.46 (t, $J = 5.0$ Hz, 4H, piperazinyl H-2, H-6), 2.65 (t, $J = 5.9$ Hz, 2H, C*H*$_2$N), 3.43 (t, $J = 5.0$ Hz, 4H, piperazinyl H-3, H-5), 4.19 (t, $J = 5.9$ Hz, 2H, C*H*$_2$O), 4.54 (s, 1H, aminocrotonate C*H*).

5.3. General procedure for the synthesis of 3-isopropyl 5-{2-[4-(*tert*-butoxycarbonyl)piperazin-1-yl]ethyl} 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (11a–c)

A solution of the pyridinecarboxaldehyde (**10a**–**c**, 2.36 g, 22 mmol) and isopropyl acetoacetate (**9**, 3.17 g, 22 mmol) in isopropanol (10 mL) was stirred for 10 min at 55 °C. 2-[4-(*tert*-Butoxycarbonyl)piperazin-1-yl]ethyl 3-aminocrotonate (**8**, 6.27 g, 20 mmol) was added and the reaction was allowed to proceed for 20 h, after which the solvent was removed in vacuo. The residue was dissolved in diethyl ether (100 mL), washed with water (3 × 75 mL), dried (Na_2SO_4), and the solvent was removed in vacuo. The powder obtained was recrystallized consecutively from diethyl ether/petroleum ether at 5 °C and then from diethyl ether. The percent yield, melting point, and spectral data for products **11a**–**c** are listed below.

5.4. 3-Isopropyl 5-{2-[4-(*tert*-butoxycarbonyl)piperazin-1-yl]ethyl} 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylate (11a)

Yield: 31% (solid); mp: 145 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 and 1.22 (two d, J = 6.2 Hz, 3H each, CH*Me*$_2$), 1.47 (s, 9H, C*Me*$_3$), 2.26 and 2.36 (two s, 3H each, C-2 *Me*, C-6 *Me*), 2.38 (t, J = 5.0 Hz, 4H, piperazinyl H-2, H-6), 2.58 (t, J = 5.8 Hz, 2H, C*H*$_2$N), 3.38 (t, J = 5.0 Hz, 4H, piperazinyl H-3, H-5), 4.16 (t, J = 5.8 Hz, 2H, C*H*$_2$O), 4.95 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 5.17 (s, 1H, H-4), 7.13 (ddd, $J_{4,5}$ = 8.1, $J_{5,6}$ = 4.8, $J_{3,5}$ = 0.5 Hz, 1H, pyridyl H-5), 7.41 (d, $J_{3,4}$ = 8.3 Hz, 1H, pyridyl H-3), 7.56 (ddd, $J_{3,4}$ = 8.3, $J_{4,5}$ = 8.1, $J_{4,6}$ = 0.9 Hz, 1H, pyridyl H-4), 8.17 (broad s, 1H, N*H*), 8.49 (d, $J_{5,6}$ = 4.8 Hz, 1H, pyridyl H-6). Anal. Calcd for $C_{28}H_{40}N_4O_6$: C, 63.62; H, 7.63; N, 10.60. Found: C, 63.19; H, 7.81; N, 10.47; MS: *m*/*z* (ES+) Calcd for $C_{28}H_{40}N_4O_6$ (MH^+): 529.2. Found 529.3.

5.5. 3-Isopropyl 5-{2-[4-(*tert*-butoxycarbonyl)piperazin-1-yl]ethyl} 1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)-3,5-pyridinedicarboxylate (11b)

Yield: 59% (solid); mp: 132–134 °C; ^{1}H NMR ($CDCl_3$) δ 1.11 and 1.25 (two d, J = 6.4 Hz, 3H each, CH*Me*$_2$), 1.46 (s, 9H, C*Me*$_3$), 2.35 and 2.36 (two s, 3H each, C-2 *Me*, C-6 *Me*), 2.33–2.43 (m, 4H, piperazinyl H-2, H-6), 2.58 and 2.59 (two dd, J_{gem} = 11.6, J_{vic} = 4.9 Hz, 1H each, C*H*$_2$N), 3.39 (t, J = 5.0 Hz, 4H, piperazinyl H-3, H-5), 4.16 and 4.17 (two dd, J_{gem} = 12.1, J_{vic} = 4.9 Hz, 1H each, C*H*$_a$C*H*$_b$O), 4.96 (septet, J = 6.4 Hz, 1H, C*H*Me$_2$), 4.97 (s, 1H, H-4), 5.9 (sharp s, 1H, N*H*), 7.15 (dd, $J_{4,5}$ = 8.7, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 7.61 (dd, $J_{4,5}$ = 8.7, $J_{4,6}$ = 2.6 Hz, 1H, pyridyl H-4), 8.37 (d, $J_{4,6}$ = 2.6, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.54 (d, $J_{2,4}$ = 1.9 Hz, 1H, pyridyl H-2). Anal. Calcd for $C_{28}H_{40}N_4O_6 \cdot 1/10H_2O$: C, 63.40; H, 7.64; N, 10.56. Found: C, 63.19; H, 7.96; N, 10.38.

5.6. 3-Isopropyl 5-{2-[4-(*tert*-butoxycarbonyl)piperazin-1-yl]ethyl} 1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)-3,5-pyridinedicarboxylate (11c)

Yield: 58% (solid); mp: 62–63 °C; ^{1}H NMR ($CDCl_3$) δ 1.12 and 1.24 (two d, J = 6.1 Hz, 3H each, CH*Me*$_2$), 1.46 (s, 9H, CH*Me*$_3$), 2.34 and 2.35 (two s, 3H each, C-2 *Me*, C-6 *Me*), 2.33–2.43 (m, 4H, piperazinyl H-2, H-6), 2.58 and 2.59 (two dd, J_{gem} = 11.6, J_{vic} = 4.9 Hz, 1H each, C*H*$_2$N), 3.37 (t, J = 4.6 Hz, 4H, piperazinyl H-3, H-5), 4.16 and 4.17 (two dd, J_{gem} = 12.1, J_{vic} = 4.9 Hz, 1H each, C*H*$_a$C*H*$_b$O), 4.97 (septet, J = 6.1 Hz, 1H, C*H*Me$_2$), 4.99 (s, 1H, H-4), 5.90 (sharp s, 1H, N*H*), 7.21 (d, $J_{2,3}$ = $J_{5,6}$ = 6.0 Hz, 2H, pyridyl H-3 and H-5), 8.43 (d, $J_{2,3}$ = $J_{5,6}$ = 6.0 Hz, 2H, pyridyl H-2 and H-6). Anal. Calcd for $C_{28}H_{40}N_4O_6 \cdot 1/10H_2O$: C, 63.40; H, 7.64; N, 10.56. Found: C, 63.01; H, 7.78; N, 10.22.

5.7. General procedure for the synthesis of 3-isopropyl 5-[2-(piperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (12a–c)

Trifluoroacetic acid (19.3 mL, 250 mmol) was added dropwise to a solution of **11a**, **11b**, or **11c** (2.65 g, 5 mmol) in dichloromethane (20 mL), and the reaction mixture was stirred at 22 °C for 20 h. The mixture was adjusted to pH 8 using a saturated solution of aqueous $NaHCO_3$, extracted with dichloromethane (4 × 100 mL), washed with water (4 × 75 mL), the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was dissolved in a minimal amount of EtOAc to which Et_2O was added dropwise until oiling-out occurred. The oiled-out portion was discarded. Removal of the solvent in vacuo gave a waxy residue that was further dried in vacuo while using Et_2O as a co-solvating agent. The percent yield, melting point, spectral and analytical data for products **12a–c** are listed below.

5.8. 3-Isopropyl 5-[2-(piperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylate (12a)

Yield: 34% (solid); mp: 65–66 °C; IR ($CHCl_3$): 3436 (NH), 1690 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.09 and 1.21 (two d, J = 6.3 Hz, 3H each, CH*Me*$_2$), 2.24 and 2.25 (two s, 3H each, C-2 *Me*, C-6 *Me*), 2.37–2.47 (m, 4H, piperazinyl H-2, H-6), 2.56 (t, J = 6.0 Hz, 2H, C*H*$_2$N), 2.85 (t, J = 4.7 Hz, 4H, piperazinyl H-3, H-5), 4.15 (t, J = 6.0 Hz, 2H, C*H*$_2$O), 4.95 (septet, J = 6.3 Hz, 1H, C*H*Me$_2$), 5.17 (s, 1H, H-4), 7.14 (ddd, $J_{4,5}$ = 8.1, $J_{5,6}$ = 4.8, $J_{3,5}$ = 0.5 Hz, 1H, pyridyl H-5), 7.44 (d, $J_{3,4}$ = 8.3 Hz, 1H, pyridyl H-3), 7.57 (ddd, $J_{3,4}$ = 8.3, $J_{4,5}$ = 8.1, $J_{4,6}$ = 0.9 Hz, 1H, pyridyl H-4), 8.41 (sharp s, 1H, DHP N*H*), 8.48 (d, $J_{5,6}$ = 4.8 Hz, 1H, pyridyl H-6), the piperazinyl N*H* was not observed. Anal. Calcd for $C_{23}H_{32}N_4O_4 \cdot 1/2H_2O$: C, 63.14; H, 7.60; N, 12.81. Found: C, 63.51; H, 7.46; N, 12.37.

5.9. 3-Isopropyl 5-[2-(piperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)-3,5-pyridinedicarboxylate (12b)

Yield: 45% (solid); mp: 64–66 °C; IR ($CHCl_3$): 3429 (NH), 1697 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.15 and 1.21 (two d, J = 6.1 Hz, 3H each, CH*Me*$_2$), 2.28 and 2.29 (two s, 3H each, C-2 *Me*, C-6 *Me*), 2.27–2.37 (m, 4H, piperazinyl H-2, H-6), 2.53 and 2.54 (two dd, J_{gem} = 12.0, J_{vic} = 4.9 Hz, 1H each, C*H*$_2$N), 2.81 (t, J = 4.8 Hz, 4H, piperazinyl H-3, H-5), 4.11 and 4.12 (two dd, J_{gem} = 12.1, J_{vic} = 4.9 Hz, 1H each, C*H*$_a$C*H*$_b$O), 4.91 (septet, J = 6.1 Hz, 1H, C*H*Me$_2$), 4.94 (s, 1H, H-4), 7.12 (dd, $J_{4,5}$ = 8.7, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 7.14 (broad s, 1H, DHP N*H*), 7.60 (dd, $J_{4,5}$ = 8.7, $J_{4,6}$ = 2.6 Hz, 1H, pyridyl H-4), 8.31 (d, $J_{4,6}$ = 2.6, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.49 (d, $J_{2,4}$ = 1.9 Hz, 1H, pyridyl H-2), the piperazinyl N*H* was not observed. ^{13}C NMR ($CDCl_3$) δ 19.04 and 19.13 (C-2 *Me* and C-6 *Me*), 21.78 and 22.07 (C*Me*$_2$), 37.65 (C-4), 46.00 (piperazinyl C-3 and C-5), 54.58 (piperazinyl C-2 and C-6), 57.19 (*C*H$_2$N), 61.06 (*C*H$_2$O), 67.00 (*C*HMe$_2$), 102.52 and 103.31 (C-3 and C-5), 122.92 (pyridyl C-5), 135.63 (pyridyl C-4), 143.42 (pyridyl C-3), 144.77 and 145.46 (C-2 and C-6), 146.84 (pyridyl C-6), 149.35 (pyridyl C-2), 166.53 and 166.99 (C=O). Anal. Calcd for $C_{23}H_{32}N_4O_4$: C, 64.46; H, 7.53; N, 13.07. Found: C, 64.38; H, 7.67; N, 11.54.

5.10. 3-Isopropyl 5-[2-(piperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)-3,5-pyridinedicarboxylate (12c)

Yield: 47% (solid); mp: 67–68 °C; IR ($CHCl_3$): 3436 (NH), 1690 (C═O); ^{1}H NMR ($CDCl_3$) δ ^{1}H NMR ($CDCl_3$): δ 1.12 and 1.24 (two d, J = 6.1 Hz, 3H each, CH*Me*$_2$), 2.35 and 2.36 (two s, 3H each, C-2 *Me*, C-6 *Me*), 2.35–2.45 (m, 4H, piperazinyl H-2, H-6), 2.57 (two dd, J_{gem} = 12.0, J_{vic} = 4.9 Hz, 1H each, C*H*$_2$N), 2.85 (t, J = 4.7 Hz, 4H, piperazinyl H-3, H-5), 4.16–4.17 (two dd, J_{gem} = 12.1, J_{vic} = 4.9 Hz, 1H each, C*H*$_a$C*H*$_b$O), 4.97 (septet, J = 6.1 Hz, 1H, C*H*Me$_2$), 5.00 (s, 1H, H-4), 5.75 (broad s, 1H, DHP N*H*), 7.23 (d, $J_{2,3} = J_{5,6}$ = 5.34 Hz, 2H, pyridyl H-3 and H–5), 8.43 (d, $J_{2,3} = J_{5,6}$ = 5.34 Hz, 2H, pyridyl H-2 and H-6), the piperazinyl N*H* was not observed. Anal. Calcd for $C_{23}H_{32}N_4O_4$: C, 64.46; H, 7.53; N, 13.07. Found: C, 64.65; H, 7.77; N, 11.50.

5.11. General procedure for the synthesis of 3-isopropyl 5-[2-(4-nitrosopiperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (14a–c)

A solution of NaOMe (81 mg, 1.5 mmol) in MeOH (25% w/w solution) was added dropwise to a solution of **12a**, **12b**, or **12c** (0.64 g, 1.5 mmol) in chloroform (5 mL). The mixture was stirred at 22 °C under nitric oxide gas at 40 psi for 20 h, after which the solvent was removed in vacuo. Dichloromethane (50 mL) was added, the insoluble solids were discarded, and the solvent was removed in vacuo to give an oily residue. This residue was dissolved in a minimal amount of EtOAc to which Et_2O was added dropwise until oiling out occurred. The oiled-out material was discarded, and the solvent was removed in vacuo to give a waxy residue that was further dried in vacuo while using Et_2O as a co-solvating agent. The residue was recrystallized from EtOAc/Et_2O to give a powder. The percent yield, melting point, spectral and analytical data for products **14a**–**c** are listed below.

5.12. 3-Isopropyl 5-[2-(4-nitrosopiperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylate (14a)

Yield: 19% (solid); mp: 143–145 °C; IR ($CHCl_3$): 3436 (NH), 1690 (C═O), 1484 (N═O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.11 and 1.22 (two d, J = 6.1 Hz, 3H each, CH*Me*$_2$), 2.26 and 2.28 (two s, 3H each, C-2 *Me*, C-6 *Me*), 2.39 and 2.40 (two dd, J_{gem} = 10.0, J_{vic} = 4.6 Hz, 1H each, C*H*$_2$N), 2.63 and 2.64 (two dd, J_{gem} = unknown, J_{vic} = 5.2 Hz, 2H each, piperazinyl H-2, H-6), 3.75 and 3.76 (two dd, J_{gem} = 11.9, J_{vic} = 4.6 Hz, 1H each, C*H*$_a$C*H*$_b$O), 4.15–4.20 (four dd, J_{gem} = unknown, J_{vic} = 5.2 Hz, 1H each, piperazinyl H-3, H-5), 4.96 (septet, J = 6.1 Hz, 1H, C*H*Me$_2$), 5.17 (s, 1H, H-4), 7.13 (ddd, $J_{4,5}$ = 8.1, $J_{5,6}$ = 4.8, $J_{3,5}$ = 0.5 Hz, 1H, pyridyl H-5), 7.39 (d, $J_{3,4}$ = 8.3 Hz, 1H, pyridyl H-3), 7.57 (ddd, $J_{3,4}$ = 8.3, $J_{4,5}$ = 8.1, $J_{4,6}$ = 0.9 Hz, 1H, pyridyl H-4), 8.01 (sharp s, 1H, N*H*), 8.50 (d, $J_{5,6}$ = 4.8 Hz, 1H, pyridyl H-6); MS: *m*/*z* (ES+) Calcd for $C_{23}H_{31}N_5O_5$ (MH^+): 458.2. Found 458.2.

5.13. 3-Isopropyl 5-[2-(4-nitrosopiperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)-3,5-pyridinedicarboxylate (14b)

Yield: 41% (solid); mp: 193–195 °C; IR ($CHCl_3$): 3436 (NH), 1696 (C═O), 1486 (N═O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.13 and 1.25 (two d, J = 6.2 Hz, 3H each, CH*Me*$_2$), 2.35 and 2.37 (two s, 3H each, C-2 *Me*, C-6 *Me*), 2.40 (t, J = 5.5 Hz, 2H, C*H*$_2$N), 2.62 and 2.64 (two dd [data acquired from a decoupling study by irradiating δ 4.1–4.3 (piperazinyl H-3, H-5)], J_{gem} = 10.7, J_{vic} = 5.3 Hz, 2H each, piperazinyl H-2, H-6), 3.77 (t, J = 5.5 Hz, 2H, C*H*$_2$O), 4.13, 4.16, 4.21, and 4.26 (four dd [data acquired from a decoupling study by irradiating δ 2.6–2.7 (piperazinyl H-2, H-6)], J_{gem} = 12.7, J_{vic} = 5.3 Hz, 1H each, piperazinyl H-3, H-5), 4.97 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.98 (s, 1H, H-4), 5.98 (sharp s, 1H, N*H*), 7.16 (dd, $J_{4,5}$ = 8.7, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 7.61 (dd, $J_{4,5}$ = 8.7, $J_{4,6}$ = 2.6 Hz, 1H, pyridyl H-4), 8.37 (d, $J_{4,6}$ = 2.6, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.56 (d, $J_{2,4}$ = 1.9 Hz, 1H, pyridyl H-2); ^{13}C NMR ($CDCl_3$) δ 19.19 and 19.22 (C-2 *Me* and C-6 *Me*), 21.22 and 22.11 (CH*Me*$_2$), 37.65 (C-4), 39.54 and 49.67 (piperazinyl C-3, C-5), 51.65 and 53.09 (piperazinyl C-2, C-6), 56.18 (*C*H$_2$N), 60.61 (*C*H$_2$O), 67.04 (*C*HMe$_2$), 102.22 and 103.43 (C-3, C-5), 123.07 (pyridyl C-5), 135.62 (pyridyl C-4), 143.38 (pyridyl C-3), 144.67 and 145.85 (C-2, C-6), 146.91 (pyridyl C-6), 149.33 (pyridyl C-2), 166.51 and 166.88 (C═O); MS: *m*/*z* (ES+) Calcd for $C_{23}H_{31}N_5O_5$ (MH^+): 458.2. Found 458.2.

5.14. 3-Isopropyl 5-[2-(4-nitrosopiperazin-1-yl)ethyl] 1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)-3,5-pyridinedicarboxylate (14c)

Yield: 69% (solid); mp: 140–142 °C; IR ($CHCl_3$): 3443 (NH), 1696 (C═O), 1488 (N═O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.15 and 1.25 (two d, J = 6.1 Hz, 3H each, CH*Me*$_2$), 2.35 and 2.38 (two s, 3H each, C-2 *Me*, C-6 *Me*), 2.40 and 2.41 (two dd, J_{gem} = 11.0, J_{vic} = 4.6 Hz, 1H each, C*H*$_a$H$_b$N), 2.37 (two dd, J_{gem} = unknown, J_{vic} = 5.6 Hz, 2H each, piperazinyl H-2, H-6), 3.76 and 3.77 (two dd, J_{gem} = 11.9, J_{vic} = 4.6 Hz, 1H each, CH$_a$-C*H*$_b$O), 4.12–4.29 (m, 4H, piperazinyl H-3, H-5), 4.97 (septet, J = 6.1 Hz, 1H, C*H*Me$_2$), 5.00 (s, 1H, H-4), 5.84 (sharp s, 1H, N*H*), 7.23 (d, $J_{2,3} = J_{5,6}$ = 6.0 Hz, 2H, pyridyl H-3, H–5), 8.45 (d, $J_{2,3} = J_{5,6}$ = 6.0 Hz, 2H, pyridyl H-2, H-6). ^{13}C NMR ($CDCl_3$) δ 19.24 (C-2 *Me* and C-6 *Me*), 21.82 and 22.10 (CH*Me*$_2$), 39.31 (C-4), 39.52 and 49.66 (piperazinyl C-3, C-5), 51.67 and 53.11 (piperazinyl C-2, C-6), 56.17 (*C*H$_2$N), 60.74 (*C*H$_2$O), 67.26 (*C*HMe$_2$), 101.50 and 102.79 (C-3, C-5), 123.20 (pyridyl C-3 and C-5), 144.92 and 146.16 (C-2, C-6), 149.00 (pyridyl C-2, C-6), 156.15 (pyridyl C-4), 166.51 and 166.81 (C═O); MS: *m*/*z* (ES+) Calcd for $C_{23}H_{31}N_5O_5$ (MH^+): 458.2. Found 458.2.

5.15. General procedure for the synthesis of O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-alkylamino]diazen-1-ium-1,2,-diolates (16c,d)

O^2-Sodium 1-[*N*-(2-hydroxyethyl)-*N*-ethylamino]-diazen-1-ium-1,2,-diolate (**16c**) and O^2-sodium

1-[*N*-(2-hydroxyethyl)-*N*-(*n*-butylamino)]diazen-1-ium-1,2,-diolate (**16d**) were prepared from the corresponding *N*-(2-hydroxyethyl)-*N*-ethylamine (**15c**) and *N*-(2-hydroxyethyl)-*N*-butylamine (**15d**) according to the method previously described[8] for the syntheses of O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2,-diolate and O^2-sodium 1-[4-(2-hydroxyethyl)piperazin-1-yl]diazen-1-ium-1,2,-diolate. Upon isolation, these unstable products **16c** and **16d** were used immediately for the corresponding synthesis of O^2-acetoxymethyl-1-[*N*-2-hydroxyethyl)-*N*-ethylamino]diazen-1-ium-1,2,-diolate (**17c**) and O^2-acetoxymethyl-1-[*N*-(2-hydroxyethyl)-*N*-(*n*-butylamino)]diazen-1-ium-1,2,-diolate (**17d**). Yield (oil): **16c**, 82%; **16d**, 52%.

5.16. General method for the synthesis of O^2-acetoxymethyl-1-[*N*-(2-hydroxyethyl)-*N*-alkylamino]diazen-1-ium-1,2,-diolates (17c,d)

Sodium carbonate (1.47 g, 14 mmol) and then the O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-alkylamino]diazen-1-ium-1,2,-diolate (12 mmol, 2.04 g of **16c**, or 2.37 g of **16d**) was added to a solution of iodomethyl acetate (1.99 g, 10 mmol) in acetone (40 mL) at 22 °C in the dark under an argon atmosphere. The reaction was allowed to proceed for 48 h at 22 °C, the reaction mixture was exposed to air for 5 min, and the solvent was removed in vacuo. Dichloromethane (30 mL) was added, the mixture was maintained at 5 °C for 1 h, the insoluble inorganic salts were removed by filtration, and the solvent was removed in vacuo. The oily residue obtained was purified by silica gel column chromatography using MeOH/$CHCl_3$ (5:95, v/v) as eluant. The percent yield and spectral data for products **17c**,**d** are listed below.

5.17. O^2-Acetoxymethyl-1-[*N*-(2-hydroxyethyl)-*N*-ethylamino]diazen-1-ium-1,2,-diolate (17c)

Yield: 30% (oil); ^{1}H NMR ($CDCl_3$): δ 1.14 (t, J = 7.0 Hz, 3H, NCH_2CH_3), 2.12 (s, 3H, CO*Me*), 3.30 (q, J = 7.0 Hz, 2H, NCH_2CH_3), 3.34 (t, J = 4.9 Hz, 2H, OCH_2CH_2N), 3.69 (t, J = 4.9 Hz, 2H, OCH_2CH_2N), 5.81 (s, 2H, OCH_2O).

5.18. O^2-Acetoxymethyl-1-[*N*-(2-hydroxyethyl)-*N*-*n*-butylamino]diazen-1-ium-1,2,-diolate (17d)

Yield: 40% (oil); ^{1}H NMR ($CDCl_3$) δ 0.93 (t, J = 7.2 Hz, 3H, $NCH_2CH_2CH_2CH_3$), 1.38 (sextet, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 1.49 (quintet, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 2.12 (s, 3H, CO*Me*), 3.23 (t, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 3.34 (t, J = 5.2 Hz, 2H, OCH_2CH_2N), 3.70 (t, J = 5.2 Hz, 2H, OCH_2CH_2N), 5.81 (s, 2H, OCH_2O).

5.19. General method for the synthesis of O^2-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-alkylamino]-diazen-1-ium-1,2,-diolates (18c,d)

Methanesulfonyl chloride (1.20 g, 10 mmol), and then 4-dimethylaminopyridine (1.28 g, 10 mmol), was added slowly to a solution of the O^2-acetoxymethyl-1-[*N*-(2-hydroxyethyl)-*N*-alkylamino]diazen-1-ium-1,2,-diolate (2.6 mmol: 0.58 g of **17c**, 0.654 g of **17d**) in MeCN (10 mL) at 22 °C under an argon atmosphere. The reaction was allowed to proceed for 20 h at 22 °C with stirring, the solvent was removed in vacuo, dichloromethane (50 mL) was added, and the insoluble material was removed by filtration. The solvent was removed in vacuo, and the oil-like residue obtained was purified by silica gel column chromatography using 10% (**18c**) and 20% (**18d**) EtOAc in $CHCl_3$ as eluant. Compounds **17c**–**d** are relatively unstable so they were used immediately for the subsequent preparation of **25**–**30**. The percent yield and spectral data for products **18c**–**d** are listed below.

5.20. O^2-Acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-ethylamino]diazen-1-ium-1,2,-diolate (18c)

Yield: 56% (oil); ^{1}H NMR ($CDCl_3$) δ 1.16 (t, J = 7.2 Hz, 3H, NCH_2CH_3), 2.13 (s, 3H, CO*Me*), 3.07 (s, 3H, $MeSO_2$), 3.30 (q, J = 7.2 Hz, 2H, NCH_2CH_3), 3.57 (t, J = 5.2 Hz, 2H, OCH_2CH_2N), 4.38 (t, J = 5.2 Hz, 2H, OCH_2CH_2N), 5.81 (s, 2H, OCH_2O).

5.21. O^2-Acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-*n*-butylamino]diazen-1-ium-1,2,-diolate (18d)

Yield: 43% (oil); ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J = 7.3 Hz, 3H, $NCH_2CH_2CH_2CH_3$), 1.38 (sextet, J = 7.3 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 1.51 (quintet, J = 7.3 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 2.12 (s, 3H, CO*Me*), 3.07 (s, 3H, $MeSO_2$), 3.31 (t, J = 7.3 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 3.58 (t, J = 5.2 Hz, 2H, OCH_2CH_2N), 4.37 (t, J = 5.2 Hz, 2H, OCH_2CH_2N), 5.81 (s, 2H, OCH_2O).

5.22. General method for the synthesis of 3-isopropyl 5-(2-cyanoethyl) 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (20a–c)

3-Isopropyl 5-(2-cyanoethyl) 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (**20a**–**c)** were prepared according to the general method described previously for the synthesis of **11a**–**c** except that 2-cyanoethyl 3-aminocrotonate (**19**) was used in place of 2-[4-(*tert*-butoxycarbonyl)piperazin-1-yl]ethyl 3-aminocrotonate (**8**). At completion of the reaction, the solvent was removed in vacuo prior to washing with Et_2O (3 × 30mL). The percent yield, melting point and spectral data for products **20a**–**c** are listed below.

5.23. 3-Isopropyl 5-(2-cyanoethyl) 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylate (20a)

Yield: 28% (oil); mp: 146–147 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 and 1.23 (two d, J = 6.3 Hz, 3H each, $CHMe_2$), 2.26 and 2.27 (two s, 3H each, C-2 Me, C-6 *Me*), 2.61 and 2.63 (two dd, J_{gem} = 9.9, J_{vic} = 5.3 Hz, 1H each, CH_aH_bN), 4.22 and 4.26 (two dd, J_{gem} = 9.9, J_{vic} = 5.3 Hz, 1H each, CH_aCH_bO), 4.95 (septet, J = 6.3 Hz, 1H, $CHMe_2$), 5.18 (s, 1H, H-4), 7.15 (ddd, $J_{4,5}$ = 8.1, $J_{5,6}$ = 4.8, $J_{3,5}$ = 0.5 Hz, 1H, pyridyl H-5), 7.45 (d, $J_{3,4}$ = 8.3 Hz, 1H, pyridyl H-3), 7.62 (ddd, $J_{3,4}$ = 8.3, $J_{4,5}$ = 8.1, $J_{4,6}$ = 0.9 Hz, 1H, pyridyl H-4),

8.49 (d, $J_{5,6}$ = 4.8 Hz, 1H, pyridyl H-6), 8.59 (sharp s, 1H, N*H*).

5.24. 3-Isopropyl 5-(2-cyanoethyl) 1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)-3,5-pyridinedicarboxylate (20b)

Yield: 46% (oil); mp: 180–181 °C (lit.[25]) mp: 192 °C; ^{1}H NMR (DMSO-d_6) δ 1.03 and 1.18 (two d, J = 6.2 Hz, 3H each, CHMe_2), 2.28 and 2.29 (two s, 3H each, C-2 Me, C-6 *Me*), 2.82 and 2.84 (two dd, J_{gem} = 11.5, J_{vic} = 5.8 Hz, 1H each, CH_aCH_bN), 4.13 and 4.14 (two dd, J_{gem} = 11.5, J_{vic} = 5.8 Hz, 1H each, CH_aCH_bO), 4.81 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.85 (s, 1H, H-4), 7.24 (dd, $J_{4,5}$ = 8.7, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 7.53 (dd, $J_{4,5}$ = 8.7, $J_{4,6}$ = 2.6 Hz, 1H, pyridyl H-4), 8.30 (d, $J_{4,6}$ = 2.6, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.41 (d, $J_{2,4}$ = 1.9 Hz, 1H, pyridyl H-2), 8.98 (sharp s, 1H, N*H*).

5.25. 3-Isopropyl 5-(2-cyanoethyl) 1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)-3,5-pyridinedicarboxylate (20c)

Yield: 39% (oil); mp: 151–152 °C; ^{1}H NMR ($CDCl_3$) δ 1.13 and 1.26 (two d, J = 6.2 Hz, 3H each, CHMe_2), 2.36 and 2.38 (two s, 3H each, C-2 Me, C-6 *Me*), 2.63 and 2.64 (two dd, J_{gem} = 10.5, J_{vic} = 6.1 Hz, 1H each, CH_aH$_b$N), 4.26 and 4.28 (two dd, J_{gem} = 12.3, J_{vic} = 6.1 Hz, 1H each, CH_aCH_bO), 4.96 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 5.00 (s, 1H, H-4), 6.06 (sharp s, 1H, N*H*), 7.24 (d, $J_{2,3}$ = $J_{5,6}$ = 6.9 Hz, 2H, pyridyl H-3, H-5), 8.46 (d, $J_{2,3}$ = $J_{5,6}$ = 6.9 Hz, 2H, pyridyl H-2 and H-6).

5.26. General method for the synthesis of 3-isopropyl 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (21a–c)

A solution of NaOMe in MeOH (1.32 g, 24 mmol, 25% w/w solution) chilled to 5 °C was added dropwise to a suspension of a 3-isopropyl 5-(2-cyanoethyl) 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylate (**20a**, **20b**, or **20c**, 3.01 g, 8 mmol) in chloroform (10 mL) at 5 °C with stirring. The mixture was stirred at 5 °C for 5 min, and then at 22 °C for 3 days. Removal of the solvent in vacuo gave a residue that was dissolved in ice water (10 mL) prior to acidification with sulfuric acid (2 N) to pH 5.5. The precipitate was filtered and washed with Et$_2$O. The percent yield, melting point and spectral data for products **21a**–**c** are listed below.

5.27. 3-Isopropyl 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylate (21a)

Yield: 98% (solid); mp: 185–186 °C; ^{1}H NMR (DMSO-d_6) δ 0.93 and 1.12 (two d, J = 6.1 Hz, 3H each, CHMe_2), 2.18 and 2.19 (two s, 3H each, C-2 *Me*, C-6 *Me*), 4.74 (septet, J = 6.1 Hz, 1H, C*H*Me$_2$), 5.17 (s, 1H, H-4), 6.99 (ddd, $J_{4,5}$ = 8.1, $J_{5,6}$ = 4.8, $J_{3,5}$ = 0.5 Hz, 1H, pyridyl H-5), 7.21 (d, $J_{3,4}$ = 8.3 Hz, 1H, pyridyl H-3), 7.48 (ddd, $J_{3,4}$ = 8.3, $J_{4,5}$ = 8.1, $J_{4,6}$ = 0.9 Hz, 1H, pyridyl H-4), 7.82 (sharp s, 1H, N*H*), 8.30 (d, $J_{5,6}$ = 4.8 Hz, 1H, pyridyl H-6).

5.28. 3-Isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)-3,5-pyridinedicarboxylate (21b)

Yield: 88% (solid); mp: 191–193 °C (lit.[26]) mp: 208 °C; ^{1}H NMR ($CDCl_3$) δ 0.99 and 1.15 (two d, J = 6.4 Hz, 3H each, CHMe_2), 2.26 and 2.27 (two s, 3H each, C-2 Me, C-6 *Me*), 4.84 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.91 (s, 1H, H-4), 7.07 (dd, $J_{4,5}$ = 8.7, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 7.22 (sharp s, 1H, N*H*), 7.57 (dd, $J_{4,5}$ = 8.7, $J_{4,6}$ = 2.6 Hz, 1H, pyridyl H-4), 8.25 (d, $J_{4,6}$ = 2.6, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.47 (d, $J_{2,4}$ = 1.9 Hz, 1H, pyridyl H-2). Anal. Calcd for $C_{17}H_{20}N_2O_4 \cdot 1/4H_2O$: C, 63.63; H, 6.29; N, 8.73. Found: C, 63.94; H, 6.34; N, 8.90; MS: *m*/*z* (ES+) Calcd for $C_{17}H_{20}N_2O_4$ (MH^+): 317.1. Found 317.1.

5.29. 3-Isopropyl 1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)-3,5-pyridinedicarboxylate (21c)

Yield: 50% (solid); mp: 198–199 °C; ^{1}H NMR ($CDCl_3$) δ 1.03 and 1.17 (two d, J = 6.1 Hz, 3H each, CHMe_2), 2.27 and 2.28 (two s, 3H each, C-2 *Me*, C-6 *Me*), 4.88 (septet, J = 6.1 Hz, 1H, C*H*Me$_2$), 4.94 (s, 1H, H-4), 7.03 (broad s, 1H, N*H*), 7.18 (d, $J_{2,3}$ = $J_{5,6}$ = 6.1 Hz, 2H, pyridyl H-3, H–5), 8.33 (d, $J_{2,3}$ = $J_{5,6}$ = 6.1 Hz, 2H, pyridyl H-2, H-6).

5.30. 3-Isopropyl 5-{2-[4-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)piperazin-1-yl]ethyl} 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylate (22)

Sodium carbonate (32 mg, 0.30 mmol) was added to a solution of **21a** (79 mg, 0.25 mmol) in MeCN (5 mL) at 22 °C, and the mixture was stirred for 30 min. O^2-acetoxymethyl-1-[4-(2-methanesulfonyloxyethyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (**18a**) was added and the mixture was stirred at 70 °C for 2 days. After removal of the solvent in vacuo, EtOAc (70 mL) was added to the residue, the suspension was washed with water (3 × 30 mL), the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was partially purified by PTLC using MeOH/$CHCl_3$ (5:95, v/v) as eluant. The residue was further purified by silica gel column chromatography using MeOH/$CHCl_3$ (5:95, v/v) as eluant to afford **22** as an oil (16%); IR ($CHCl_3$): 3436 (NH), 1703 (C=O), 1427 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.09 and 1.21 (two d, J = 6.1 Hz, 3H each, CHMe_2), 2.12 (s, 3H, CO*Me*), 2.24 and 2.25 (two s, 3H each, C-2 *Me*, C-6 *Me*), 2.58–2.63 (m, J_{gem} = unknown, $J_{vic(ethyl\ CH2N)}$ = 5.5, $J_{vic(piperazinyl\ H\text{-}2\ and\ H\text{-}6)}$ = 4.6 Hz, 3 × 2H, ethyl CH_2N and piperazinyl H-2, H-6), 3.40–3.41 (m, J_{gem} = unknown, J_{vic} = 4.6 Hz, 2 × 2H, piperazinyl H-3, H-5), 4.13–4.17 (m, J_{gem} = unknown, J_{vic} = 5.5 Hz, 2H, ethyl CH_2O), 4.94 (septet, J = 6.1 Hz, 1H, C*H*Me$_2$), 5.16 (s, 1H, H-4), 5.79 (s, 2H, acetoxymethyl OCH_2O), 7.13 (ddd, $J_{4,5}$ = 8.1, $J_{5,6}$ = 4.8, $J_{3,5}$ = 0.5 Hz, 1H, pyridyl H-5), 7.42 (d, $J_{3,4}$ = 8.3 Hz, 1H, pyridyl H-3), 7.58 (ddd, $J_{3,4}$ = 8.3, $J_{4,5}$ = 8.1, $J_{4,6}$ = 0.9 Hz, 1H, pyridyl H-4), 8.50 (d, $J_{5,6}$ = 4.8 Hz, 1H, pyridyl H-6), 8.52 (sharp s, 1H, N*H*); MS: *m*/*z* (ES+) Calcd for $C_{26}H_{36}N_6O_8$ (MH^+): 561.3. Found 561.3.

5.31. General method for the synthesis of 3-isopropyl 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-alkylamino]ethyl} 1,4-dihydro-2,6-dimethyl-4-(pyridyl)-3,5-pyridinedicarboxylates (23–30)

Sodium carbonate (42 mg, 0.40 mmol) was added to a solution of either **21a**, **21b**, or **21c** (105 mg, 0.33 mmol) in MeCN (5 mL) at 22 °C, and the mixture was stirred for 30 min. An O^2-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-alkylamino]diazen-1-ium-1,2,-diolate (**18b**–**d**) was added, and the mixture was stirred at 50 °C for 5 days. After removing the solvent in vacuo, dichloromethane (25 mL) was added to the residue, the insoluble solid was removed by filtration, washed with dichloromethane (2 × 25 mL), and the solid was discarded. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using MeOH/$CHCl_3$ (3:97, v/v) as eluant. The percent yield and spectral data for products **23**–**30** are listed below.

5.32. 3-Isopropyl 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-methylamino]ethyl} 1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)-3,5-pyridinedicarboxylate (23)

Yield: 40% (oil); IR ($CHCl_3$): 3429 (NH), 1696 (C═O), 1484 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.12 and 1.25 (two d, J = 6.4 Hz, 3H each, CH*Me*$_2$), 2.11 (s, 3H, CO*Me*), 2.33 and 2.34 (two s, 3H each, C-2 Me, C-6 *Me*), 3.00 (s, 3H, N*Me*), 3.62 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.23 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.94 (overlap of septet, J = 6.4 Hz, 1H, C*H*Me$_2$; and s, 1H, H-4), 5.76 (s, 2H, OCH_2O), 6.34 (sharp s, 1H, N*H*), 7.17 (dd, $J_{4,5}$ = 8.7, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 7.60 (dd, $J_{4,5}$ = 8.7, $J_{4,6}$ = 2.6 Hz, 1H, pyridyl H-4), 8.36 (d, $J_{4,6}$ = 2.6, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.51 (d, $J_{2,4}$ = 1.9 Hz, 1H, pyridyl H-2); MS: *m*/*z* (ES+) Calcd for $C_{23}H_{31}N_5O_8$ (MH^+): 506.2. Found 506.2.

5.33. 3-Isopropyl 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-methylamino]ethyl} 1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)-3,5-pyridinedicarboxylate (24)

Yield: 33% (oil); IR ($CHCl_3$): 3436 (NH), 1683 (C═O), 1427 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 and 1.26 (two d, J = 6.3 Hz, 3H each, CH*Me*$_2$), 2.12 (s, 3H, CO*Me*), 2.34 and 2.36 (two s, 3H each, C-2 *Me*, C-6 *Me*), 3.00 (s, 3H, N*Me*), 3.62 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.25 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.98 (overlap of septet, J = 6.3 Hz, 1H, C*H*Me$_2$; and s, 1H, H-4), 5.76 (s, 2H, OCH_2O), 6.0 (sharp s, 1H, N*H*), 7.23 (d, $J_{2,3}$ = $J_{5,6}$ = 5.6 Hz, 2H, pyridyl H-3, H-5), 8.43 (d, $J_{2,3}$ = $J_{5,6}$ = 5.6 Hz, 2H, pyridyl H-2, H-6); MS: *m*/*z* (ES+) Calcd for $C_{23}H_{31}N_5O_8$ (MH^+): 506.2. Found 506.2.

5.34. 3-Isopropyl 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-ethylamino]ethyl} 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylate (25)

Yield: 52% (oil); IR ($CHCl_3$): 3449 (NH), 1703 (C═O), 1501 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.07 and 1.20 (two d, J = 6.2 Hz, 3H each, CH*Me*$_2$), 1.07 (t, J = 7.0 Hz, NCH_2CH_3), 2.08 (s, 3H, CO*Me*), 2.21 (s, 2 × 3H, C-2 *Me*, C-6 *Me*), 3.44 (q, 2H, J = 7.0 Hz, 2H, NCH_2CH_3), 3.44 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.16 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.92 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 5.15 (s, 1H, H-4), 5.76 (s, 2H, OCH_2O), 7.14 (ddd, $J_{4,5}$ = 8.1, $J_{5,6}$ = 4.8, $J_{3,5}$ = 0.5 Hz, 1H, pyridyl H-5), 7.42 (d, $J_{3,4}$ = 8.3 Hz, 1H, pyridyl H-3), 7.59 (ddd, $J_{3,4}$ = 8.3, $J_{4,5}$ = 8.1, $J_{4,6}$ = 0.9 Hz, 1H, pyridyl H-4), 8.46 (d, $J_{5,6}$ = 4.8 Hz, 1H, pyridyl H-6), 9.12 (sharp s, 1H, N*H*); MS: *m*/*z* (ES+) Calcd for $C_{24}H_{33}N_5O_8$ (MH^+): 520.2. Found 520.3.

5.35. 3-Isopropyl 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-ethylamino]ethyl} 1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)-3,5-pyridinedicarboxylate (26)

Yield: quantitative (oil); IR ($CHCl_3$): 3435 (NH), 1691 (C═O), 1494 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.09 and 1.24 (two d, J = 6.3 Hz, 3H each, CH*Me*$_2$), 1.10 (t, J = 7.0 Hz, NCH_2CH_3), 2.09 (s, 3H, CO*Me*), 2.32 (s, 6H, C-2 *Me*, C-6 *Me*), 3.27 (q, 2H, J = 7.0 Hz, 2H, NCH_2CH_3), 3.44 (t, J = 5.6 Hz, 2H, OCH_2CH_2N), 4.18 (t, J = 5.6 Hz, 2H, OCH_2CH_2N), 4.94 (overlap of septet, J = 6.3 Hz, 1H, C*H*Me$_2$; and s, 1H, H-4), 5.76 (s, 2H, OCH_2O), 6.68 (broad s, 1H, N*H*), 7.16 (dd, $J_{4,5}$ = 8.7, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 7.63 (dd, $J_{4,5}$ = 8.7, $J_{4,6}$ = 2.6 Hz, 1H, pyridyl H-4), 8.35 (d, $J_{4,6}$ = 2.6, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.51 (d, $J_{2,4}$ = 1.9 Hz, 1H, pyridyl H-2); MS: *m*/*z* (ES+) Calcd for $C_{24}H_{33}N_5O_8$ (MH^+): 520.2. Found 520.2.

5.36. 3-Isopropyl 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-ethylamino]ethyl} 1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)-3,5-pyridinedicarboxylate (27)

Yield: quantitative (oil); IR ($CHCl_3$): 3436 (NH), 1696 (C═O), 1494 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.06 (t, J = 7.0 Hz, 3H, NCH_2CH_3), 1.10 and 1.23 (two d, J = 6.3 Hz, 3H each, CH*Me*$_2$), 2.09 (s, 3H, CO*Me*), 2.32 (s, 6H, C-2 Me, C-6 *Me*), 3.26 (q, 2H, J = 7.0 Hz, 2H, NCH_2CH_3), 3.45 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.19 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.96 (septet, J = 6.3 Hz, 1H, C*H*Me$_2$), 4.97 (s, 1H, H-4), 5.75 (s, 2H, OCH_2O), 6.67 (broad s, 1H, N*H*), 7.23 (d, $J_{2,3}$ = $J_{5,6}$ = 5.5 Hz, 2H, pyridyl H-3, H-5), 8.41 (d, $J_{2,3}$ = $J_{5,6}$ = 5.5 Hz, 2H, pyridyl H-2, H-6); MS: *m*/*z* (ES+) Calcd for $C_{24}H_{33}N_5O_8$ (MH^+): 520.2. Found 520.2.

5.37. 3-Isopropyl 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-*n*-butylamino]ethyl} 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylate (28)

Yield: 71% (oil); IR ($CHCl_3$): 3436 (NH), 1690 (C═O), 1497 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.07 (t, J = 7.2 Hz, 3H, $NCH_2CH_2CH_2CH_3$), 1.08 and 1.21 (two d, J = 6.1 Hz, 3H each, CH*Me*$_2$), 1.31 (sextet, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 1.42 (quintet, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 2.09 (s, 3H, CO*Me*), 2.23 (s, 6H, C-2 Me, C-6 *Me*), 3.20 (t, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 3.45 (t,

J = 5.3 Hz, 2H, OCH_2CH_2N), 4.16 (t, J = 5.3 Hz, 2H, OCH_2CH_2N), 4.93 (septet, J = 6.1 Hz, 1H, $CHMe_2$), 5.15 (s, 1H, H-4), 5.77 (s, 1H, OCH_2O), 7.14 (ddd, $J_{4,5}$ = 8.1, $J_{5,6}$ = 4.8, $J_{3,5}$ = 0.5 Hz, 1H, pyridyl H-5), 7.44 (d, $J_{3,4}$ = 8.3 Hz, 1H, pyridyl H-3), 7.59 (ddd, $J_{3,4}$ = 8.3, $J_{4,5}$ = 8.1, $J_{4,6}$ = 0.9 Hz, 1H, pyridyl H-4), 8.48 (d, $J_{5,6}$ = 4.8 Hz, 1H, pyridyl H-6), 8.75 (broad s, 1H, NH); MS: *m*/*z* (ES+) Calcd for $C_{26}H_{37}N_5O_8$ (MH^+): 548.3. Found 548.3.

5.38. 3-Isopropyl 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-*n*-butylamino]ethyl} 1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)-3,5-pyridinedicarboxylate (29)

Yield: 46% (oil); IR ($CHCl_3$): 3432 (NH), 1690 (C=O), 1492 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, J = 7.0 Hz, 3H, $NCH_2CH_2CH_2CH_3$), 1.10 and 1.26 (two d, J = 6.1 Hz, 3H each, $CHMe_2$), 1.31 (sextet, J = 7.0 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 1.42 (quintet, J = 7.0 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 2.10 (s, 3H, COMe), 2.33 (s, 6H, C-2 *Me*, C-6 *Me*), 3.18 (t, J = 7.0 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 3.45 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.18 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.95 (overlap of septet, J = 6.1 Hz, 1H, $CHMe_2$; and s, 1H, H-4), 5.77 (s, 2H, OCH_2O), 6.22 (broad s, 1H, NH), 7.18 (dd, $J_{4,5}$ = 8.7, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 7.62 (dd, $J_{4,5}$ = 8.7, $J_{4,6}$ = 2.6 Hz, 1H, pyridyl H-4), 8.40 (d, $J_{4,6}$ = 2.6, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.52 (d, $J_{2,4}$ = 1.9 Hz, 1H, pyridyl H-2); MS: *m*/*z* (ES+) Calcd for $C_{26}H_{37}N_5O_8$ (MH^+): 548.3. Found 548.2.

5.39. 3-Isopropyl 5-{2-[*N*-(O^2-acetoxymethyldiazen-1-ium-1,2-diolate)-*N*-*n*-butylamino]ethyl} 1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)-3,5-pyridinedicarboxylate (30)

Yield: 47% (oil); IR ($CHCl_3$): 3442 (NH), 1691 (C=O), 1494 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, J = 7.2 Hz, 3H, $NCH_2CH_2CH_2CH_3$), 1.12 and 1.25 (two d, J = 6.1 Hz, 3H each, $CHMe_2$), 1.32 (sextet, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 1.43 (quintet, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 2.10 (s, 3H, COMe), 2.35 (s, 6H, C-2 *Me*, C-6 *Me*), 3.18 (t, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 3.45 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.19 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.97 (overlap of septet, J = 6.1 Hz, 1H, $CHMe_2$; and s, 1H, H-4), 5.76 (s, 2H, OCH_2O), 5.95 (broad s, 1H, NH), 7.23 (d, $J_{2,3}$ = $J_{5,6}$ = 5.5 Hz, 2H, pyridyl H-3 and H-5), 8.41 (d, $J_{2,3}$ = $J_{5,6}$ = 5.5 Hz, 2H, pyridyl H-2 and H-6); MS: *m*/*z* (ES+) Calcd for $C_{26}H_{37}N_5O_8$ (MH^+): 548.3. Found 548.2.

5.40. In vitro calcium channel antagonist assay

The in vitro calcium channel antagonist assay was performed using a protocol approved by the Health Sciences Animal Welfare Committee at the University of Alberta according to the previously reported procedures.[27] In this regard, smooth muscle calcium channel antagonist activity was determined as the molar concentration of the test compound required to produce 50% inhibition of the muscarinic receptor-mediated (carbachol, 0.167 μM) calcium-dependent contraction (tonic response) of guinea pig ileum longitudinal smooth muscle (GPILSM). The IC_{50} value (±SEM) was determined graphically from the dose–response curve.

5.41. In vitro nitric oxide release assay in the presence of L-cysteine

In vitro nitric oxide release in the presence of L-cysteine, determined by the quantification of nitrite, produced by the reaction of nitric oxide with oxygen and water using the Griess reaction,[28] was measured for the test compounds **11a**–**c**, **22**–**30** and the reference drug glyceryl trinitrate using our previously reported procedure.[29]

5.42. Nitric oxide release assay in the presence of guinea pig serum

Nitric oxide release in guinea pig serum, determined by quantification of nitrite produced by the reaction of nitric oxide with oxygen and water using the Griess reaction, was measured for the test compounds **22**–**30** and the reference drug glyceryl trinitrate using the following procedure.

After sacrificing guinea pigs, whole blood was immediately collected from their hearts. The blood was allowed to stand at 5 °C for 20 h so as to permit the serum to separate. The blood was centrifuged at 2000 rpm for 15 min and the clear serum was removed. The Griess reagent was prepared by dissolving sulfanilamide (4.0 g) and *N*-(1-naphthyl)ethylenediamine·2HCl (0.2 g) in a mixture of H_3PO_4 (85% grade, 10 mL) and distilled water (90 mL). A standard nitrite-absorbance concentration plot was prepared as follows. A solution of $NaNO_2$ (25 mM) in acetonitrile and serum (5:95 v/v, 400 μL) was prepared by serial dilution. Sodium nitrite (3.5 mg, 0.05 mmol) was dissolved in serum (200 μL). An aliquot of this solution (40 μL) was diluted with serum (320 μL) and acetonitrile (20 μL) was mixed in. Eleven dilutions with acetonitrile and serum (5:95 v/v) were prepared from this solution (25 mM). An undiluted nitrite sample (25 mM), the eleven dilutions, and a nitrite-free serum sample (0 mM) (100 μL each) were incubated in a multiple-well cuvette at 37 °C with gentle shaking for 1 h. Griess reagent (33 μL) was added to each sample, and the mixtures were incubated at 37 °C with gentle shaking for 30 min. The solution ultraviolet absorbances were measured at 540 nm. The absorbances were used to prepare the calibration curve from which nitrite concentrations were calculated (±SEM, n = 3). The percentage nitric oxide released from the test compounds was determined as followed. A solution of the test compound dissolved initially in acetonitrile (20 μL) was added to serum (380 μL) to provide a solution of the test compound (400 μL of 10 mM). Three aliquots of this solution (100 μL each) were incubated with the test compound in a multiple-well cuvette at 37 °C with gentle shaking for 1 h. Griess reagent (33 μL) was added to each part, and the mixtures were incubated at 37 °C with gentle shaking for 30 min. The solution ultraviolet absorbance was measured at 540 nm. The nitrite concentration was determined from the standard nitrite

concentration–absorbance curve to calculate the percent nitric oxide released from the test compound (mol/mol).

5.43. Nitric oxide release structure–activity relationship equation

The rank of the X-moieties and linear regression equation describing the percentage of nitric oxide released from compounds **22**–**30** were derived using the following method. The X-moieties were each given an initial score: 1,4-piperazinyl was assigned 141% which is the percent nitric oxide released from **22**; *N*-ethyl was assigned 47%, which is the average percent nitric oxide release for **23** and **24**; *N*-(*n*-butyl) was assigned 126% (average of **25**–**27**); and *N*-methyl was assigned 107% (average of **28**–**30**). The scores were adjusted by subtracting the smallest score (47%), resulting in the final scores shown in Figure 2. A linear regression equation was derived using the least square method (Microsoft-Excel's 'linest' function) from which the coefficient of determination (R^2), Fischer statistic (F) and degrees of freedom (v_2) were obtained. The probability statistic (p) was converted, using Microsoft-Excel's 'fdist' function, from the Fischer statistic and v_2, with v_1 being '1' because the regression equation only uses one parameter.

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