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Synthesis and biological evaluation of 1,4-dihydropyridine calcium channel modulators having a diazen-1-ium-1,2-diolate nitric oxide donor moiety for the potential treatment of congestive heart failure

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Abstract—A group of racemic 4-aryl(heteroaryl)-1,4-dihydro-2,6-dimethyl-3-nitropyridines possessing nitric oxide donor O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino, or 4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate, C-5 ester substituents were synthesized by coupling the respective 4-aryl(heteroaryl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylic acids with either O^2-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate, or O^2-acetoxymethyl-1-[4-(2-methylsulfonyloxyethyl)piperazin-1-yl]diazen-1-ium-1,2-diolate. Compounds having a C-4 2-pyridyl, 4-pyridyl, 2-trifluoromethylphenyl, or benzofurazan-4-yl substituent exhibited more potent smooth muscle calcium channel antagonist activity (IC_{50}'s in the 0.37–1.09 μM range) than related analogs having a C-4 3-pyridyl substituent (IC_{50}'s = 3.03–9.14 μM range) relative to the reference drug nifedipine (IC_{50} = 9.13 nM). The point of attachment of C-4 isomeric pyridyl substituents was a determinant of smooth muscle calcium channel antagonist activity where the relative potency profile was 4-pyridyl>2-pyridyl>3-pyridyl. Replacement of the C-5 methyl ester substituent of methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)pyridine-5-carboxylate (Bay K 8644) by 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 provided compounds, which exhibited a lower, yet respectable, cardiac positive inotropic effect (IC_{50}'s = 4.82 and 4.05 μM, respectively) relative to the reference drug Bay K 8644 (IC_{50} = 0.30 μM). All compounds released nitric oxide upon incubation with either phosphate buffer at pH 7, or porcine liver esterase. However, the percentage nitric oxide released was up to 3-fold higher (76%) when these O^2-acetoxymethyl-1-(alkylamino)diazen-1-ium-1,2-diolates were incubated with guinea pig serum. These results suggest that ·NO would be released in vivo, upon cleavage by nonspecific *serum* esterases, preferentially in the vascular endothelium where it may enhance smooth muscle calcium channel antagonist activity.

1. Introduction

Despite a steady decline in morbidity from coronary artery disease (CAD) during the past 25 years, cardiovascular diseases remain the leading cause of mortality in Canada accounting for 37% of total deaths.[1] Congestive heart failure (CHF), which is defined as the inability of the heart to expel sufficient blood to keep pace with the metabolic demands of the body, develops in 50–60% of patients with CAD, valvular insufficiency, and rheumatic heart disease. The design of tissue selective 1,4-dihydropyridine (1,4-DHP) calcium channel (CC) agonists to treat CHF necessitates removal of their contraindicated smooth muscle vasoconstrictor effect while the target cardiac positive inotropic action is maintained.[2] One of the classical examples in this regard is Bay K 8644 (**1**), which in spite of its significant positive inotropic activity,[3] also exhibits an undesirable vascular smooth muscle vasoconstrictor side effect that contraindicates its clinical use to treat CHF (Fig. 1).

It has been reported that increasing endothelial nitric oxide (·NO) generation may be expected to produce beneficial effects such as lowering blood pressure, prevention of atherosclerosis, and inhibition of restenosis after angioplasty, which constitute potential medical uses for nitric oxide donors.[4] In nanomolar concentrations, ·NO reversibly activates soluble guanylate cyclase[5]

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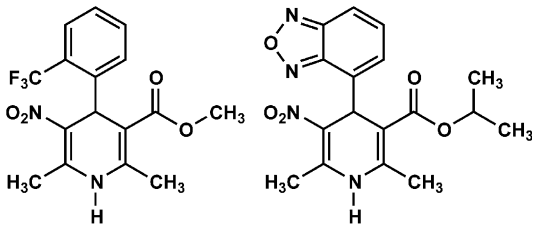


Figure 1. Structures of Bay K 8644 (**1**) and PN 202-791 (**2**).

by 400-fold, catalyzing the conversion of guanosine triphosphate to cyclic guanosine monophosphate (cGMP). Elevation of cGMP relaxes smooth muscle in blood vessels, inhibits platelet aggregation and adhesion, and blocks the adhesion of white cells to blood vessel walls.[6]

We have previously reported that it is possible to design compounds having a dual cardioselective CC agonist effect in conjunction with a smooth muscle selective CC antagonist vasorelaxant effect, by replacement of the methyl or isopropyl ester groups of Bay K 8644 (**1**) or PN 202-791 (**2**) by a ·NO donor moiety.[7–9] In this regard, we have also proposed that tissue selective 1,4-DHP CC modulators having a nitric oxide donor group, would satisfy the clinical requirements for the treatment of CHF by increasing the force of the heart contractions, while simultaneously inducing a CC vasorelaxant effect *enhanced by the release of* ·NO directly into peripheral blood vessels. However, a compound that releases ·NO spontaneously would also produce, in addition to the desired effect at the localized site, unwanted actions at other ·NO-sensitive sites throughout the organism.[10]

The chemical properties of ionic (or zwitterionic) diazen-1-ium-1,2-diolates (**3**) as ·NO-donor groups in physiological media, have shown a wide range of rates and extents of ·NO release, making them ideal agents for probing many aspects of cardiovascular functions,[11] and therefore related disorders. The recently reported O^2-acetoxymethyl-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**4**) is a novel prodrug that is stable in neutral aqueous media, but it released 1.8 equiv of ·NO per mol

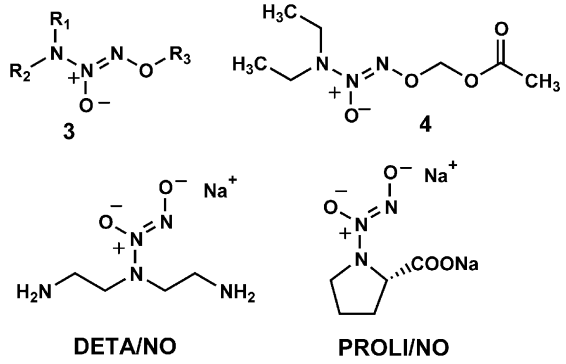


Figure 2. General structures of zwitterionic diazen-1-ium-1,2-diolates (**3**); O^2-acetoxymethyl-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**4**); O^2-sodium 1-[*N*-(2-aminoethyl)-1,2-ethanediamine-*N*-yl]diazen-1-ium-1,2-diolate (DETA/NO); 1-(prolin-1-yl-2-carboxyl)diazen-1-ium-1,2-diolate disodium salt (PROLI/NO).

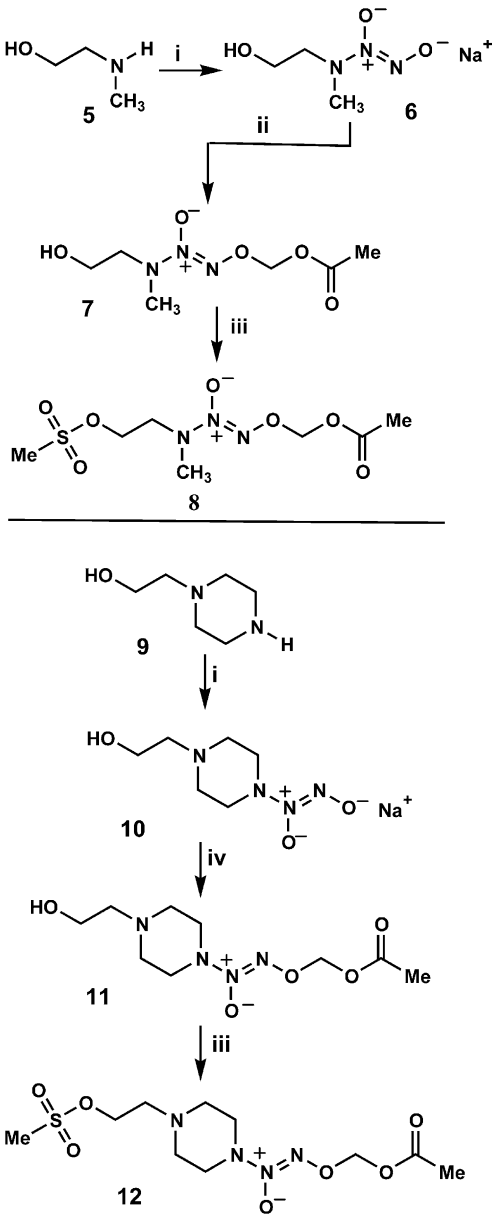
of drug upon metabolism by porcine liver esterase.[12] In an earlier attempt to combine the ·NO-donor properties of diazen-1-ium-1,2-diolates with several 1,4-DHP structures, we observed that unlike the prodrug **4**, compounds containing an O^2-alkyl-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate moiety did not release significant amounts of ·NO upon incubation with porcine liver esterase, and therefore were not suitable ·NO donors, even though they showed moderate CC modulation effects.[13]

It was therefore of interest to evaluate the efficacy of an acetoxymethyl ($CH_3CO_2CH_2$–) moiety as a protective group for the O^2-oxygen atom present in diazen-1-ium-1,2-diolates coupled to a 1,4-DHP CC modulator. Accordingly, we now report the synthesis, calcium channel modulation effects and nitric oxide release studies for a group of racemic 4-aryl(heteroaryl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylates possessing O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**18**–**22**), and O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate (**23**–**27**), C-5 ester substituents.

2. Chemistry

The synthetic reactions employed for the preparation of O^2-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**8**), and O^2-acetoxymethyl-1-[4-(2-methylsulfonyloxyethyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (**12**), are illustrated in Scheme 1. Thus, reaction of 2-(*N*-methylamino)ethanol (**5**), or 1-(2-hydroxyethyl)piperazine (**9**), with pressurized nitric oxide (at 40 psi) and sodium methoxide at room temperature, afforded the correspondent O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**6**), or O^2-sodium 1-[4-(2-hydroxyethyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (**10**), salt. Reaction of these nucleophilic 1,2-diolate sodium salts with iodomethyl acetate, previously prepared by reaction of chloromethyl acetate with sodium iodide in acetone, afforded O^2-acetoxymethyl-1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**7**), or O^2-acetoxymethyl-1-[4-(2-hydroxyethyl)piperazin-1-yl]-diazen-1-ium-1, 2-diolate (**11**), respectively. The terminal hydroxy groups of **7** and **11** were subsequently transformed into a good leaving group (mesylate) by reaction with methanesulfonyl chloride and 4-(dimethylamino)pyridine in THF (Scheme 1). It is noteworthy that compounds **7** and **11** did not react with *para*-toluenesulfonyl chloride under the same reaction conditions that worked successfully for methanesulfonyl chloride.

The final coupling reactions proceeds by nucleophilic displacement (S_N2 reaction) of the mesyloxy group present in **8** or **12** by the correspondent sodium 4-aryl-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylate (**13**–**17**) in hexamethylphosphoramide (HMPA) to afford the target products (**18**–**27**) in moderate yields (19–36%). Similar reactions using dimethylformamide, dimethylsulfoxide, tetrahydrofuran, acetonitrile or



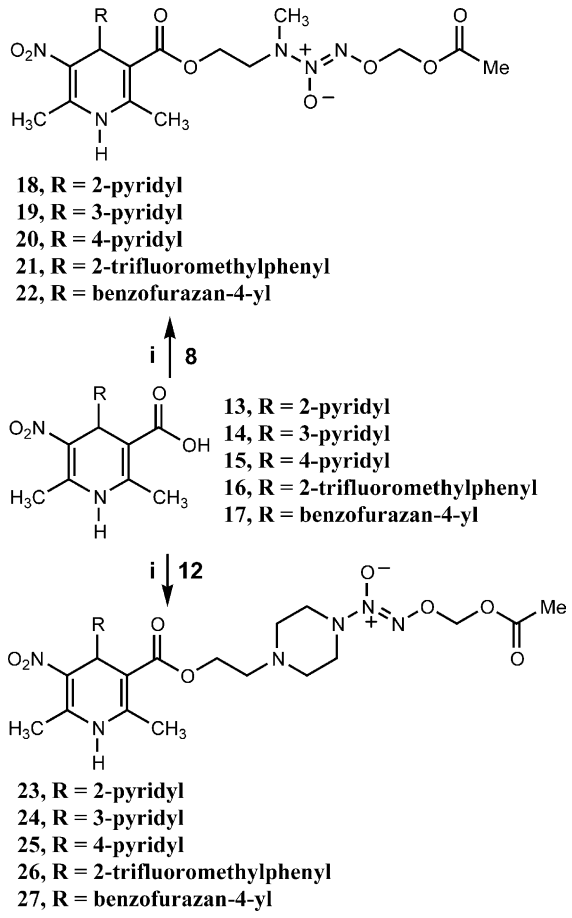
Scheme 1. Reagents and conditions: (i) Nitric oxide (at 40 psi), CH_3ONa/CH_3OH, ether, 25 °C, 72 h; (ii) $CH_3CO_2CH_2I$, CH_3CN, 25 °C, overnight; (iii) CH_3SO_2Cl, DMAP, THF, 25 °C, 15 h; (iv) $CH_3CO_2CH_2I$, THF, −10 to 25 °C, 15–18 h.

different mixtures of these solvents, in place of HMPA, gave lower product yields (Scheme 2).

3. Results and discussion

A group of racemic 4-aryl(heteroaryl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylates possessing a potential nitric oxide donor O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**18**–**22**), and O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate (**23**–**27**), C-5 ester substituent were synthesized.

The point of attachment of C-4 isomeric pyridyl substituents was a determinant of CC antagonist activity on



Scheme 2. Reagents and conditions: (i) Na_2CO_3, HMPA, 25 °C, 72 h.

guinea pig ileum longitudinal smooth muscle (GPILSM), where the relative potency profile was 4-pyridyl>2-pyridyl>3-pyridyl. Compounds having a 2-pyridyl (**18**, **23**), 4-pyridyl (**20**, **25**), 2-trifluoromethylphenyl (**21**, **26**) and benzofurazan-4-yl (**22**, **27**) substituent at the 1,4-DHP C-4 position, exhibited more potent smooth muscle calcium channel antagonist activity (IC_{50}'s in the 0.37–1.09 μM range) than related analogs having a C-4 3-pyridyl substituent (**19** and **24**, IC_{50}'s = 9.14 and 3.03 μM), relative to the reference drug nifedipine (IC_{50} = 9.13 nM). Replacement of the methyl ester moiety of Bay K 8644 by an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate group (**21**), or an O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate group (**26**), retained the desired cardiac positive inotropic effect on guinea pig left atrium (GPLA, IC_{50}'s = 4.82 and 4.05 μM) although **21** and **26** were less potent than the reference drug Bay K 8644 (IC_{50} = 0.30 μM). In contrast to Bay K 8644, which exhibited a contraindicated CC agonist effect on GPILSM (EC_{50} = 0.23 μM), none of the compounds possessing a nitric oxide donor moiety (**18**–**27**) showed a CC agonist effect on GPILSM. Unlike analogs of PN 202-791 (**2**) having nitrooxyalkyl [$O_2NO(CH_2)n$] C-5 ester substituents reported in a recent study,[14] replacement of the isopropyl ester group of PN 202-791 by an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate group (**22**), or an O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate group (**27**), decreased (**22**) or even abolished

Table 1. In vitro calcium channel modulation activities for 4-aryl(heteroaryl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylates possessing a O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**18**–**22**), or O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate (**23**–**27**), C-5 ester substituent

Compound No.	R	GPILSM IC_{50}[a] (μM)	GPILSM EC_{50}[b] (μM)	GPLA EC_{50}[c] (μM)
18	2-Pyridyl	0.89 ± 0.01	No effect[d]	No effect[e]
19	3-Pyridyl	9.14 ± 6.25	No effect[d]	No effect[e]
20	4-Pyridyl	0.37 ± 0.03	No effect[d]	32.2 ± 3.2
21	2-Trifluoromethylphenyl	0.57 ± 0.13	No effect[d]	4.82 ± 1.41
22	Benzofurazan-4-yl	0.46 ± 0.07	No effect[d]	22.7 ± 1.7
23	2-Pyridyl	1.09 ± 0.02	No effect[d]	No effect[e]
24	3-Pyridyl	3.03 ± 0.20	No effect[d]	8.18 ± 0.71
25	4-Pyridyl	0.60 ± 0.05	No effect[d]	12.7 ± 6.7
26	2-Trifluoromethylphenyl	0.84 ± 0.01	No effect[d]	4.05 ± 0.23
27	Benzofurazan-4-yl	0.97 ± 0.01	No effect[d]	No effect[e]
Nifedipine		0.0091 ± 0.0026	—	—
BAY K 8644 (**1**)		—	0.23 ± 0.01	0.30 ± 0.01
PN 202-791 (**2**)	—	—	—	9.4 ± 1.2

[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 (GPILSM) induced by the muscarinic agonist carbachol (0.167 μM) was determined graphically from the dose–response curve.

[b] The micromolar concentration of the test compound causing a 50% increase in the slow component or tonic contractile response in guinea pig ileum longitudinal smooth muscle (GPILSM), in the absence of carbachol, was determined graphically from the dose–response curves.

[c] The micromolar 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.

[d] No smooth muscle calcium channel agonist response was observed at the highest test compound concentration employed (44.66 μM).

[e] No calcium channel agonist response (positive inotropic effect) on heart was observed at the highest test compound concentration employed (44.66 μM).

(**27**) the desired cardiac positive inotropic effect on GPLA. A complete list of the CC modulation results are presented in Table 1.

It has been reported that the rate of ·NO release from O^2-sodium *N*-substituted-diazen-1-ium-1,2-diolate salts varies greatly depending on the nature of the structure of the substrate with half-lives from 1.8 s for PROLI/NO to 20 h for DETA/NO (Fig. 2).[15–19] One type of chemical modification used to control the rate of nitric oxide release from diazen-1-ium-1,2-diolates is the attachment of alkyl substituents to the O^2-position.[20] O^2-substituted diazen-1-ium-1,2-diolates are stable compounds that hydrolyze slowly even in acidic solution.[21] Consistent with these observations, when compounds **18**–**27** were incubated in PBS at pH 7.4, the percentage of ·NO released varied from 6.3% to 9.4% suggesting a slow decomposition even when the media was acidified by quenching with the Griess reagent (pH about 1–2).

As mentioned previously, the prodrug O^2-acetoxymethyl-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**4**) was reported to release up to 1.8 mol of ·NO per mol hydrolysis of the O^2-acetoxymethyl group by porcine liver esterase (PLE). The first enzymatic hydrolysis product of **4**, O^2-hydroxymethyl-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate, is reported to be unstable in water as it spontaneously eliminates formaldehyde and the zwitterion 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate, which in turn releases nitric oxide in phosphate buffer (see the mechanism in Fig. 3). ·NO release data acquired in this investigation indicated that the amount of ·NO released upon incubation of the test compounds **18**–**27** in PBS at pH 7.4 for 1.5 h at 37 °C was relatively constant (6.3–9.4% range). The O^2-acetoxymethyl moiety of O^2-acetoxymethyl-1-(alkylamino)diazen-1-ium-1,2-diolate moieties, attached to 4-aryl(heteroaryl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylic acid derivatives, upon incubation with 10 equiv of pig liver esterase (PLE) is hydrolyzed less extensively compared to when the O^2-acetoxymethyl moiety is present in smaller molecules such as the prodrug **4**. In this regard, incubation of compounds **18**–**27** in the presence of PLE produced a 21–34% release of ·NO compared with the 90% ·NO release from the prodrug **4**.

The effect of nonspecific esterases on the ·NO release properties of compounds **18**–**27** was determined by their incubation in the presence of guinea pig serum for 1.5 h at 37 °C (pH 7.4). The percentage ·NO released was substantially higher (60–75% range) than that observed upon incubation with PLE (see Table 2). These data indicate the nonspecific serum esterases present in guinea pig serum cleave these O^2-acetoxymethyl-1-(alkylamino)diazen-1-ium-1,2-diolates more effectively than pig liver esterase (PLE). Compounds **18**–**27** are prodrugs, which must be cleaved by esterases before they are able to release ·NO. In contrast, the unprotected diazen-1-ium-1,2-diolates **6** and **10**, which lack the O^2-acetoxymethyl moiety and do not require cleavage by an esterase, released about 85% (**6**) and 70% (**10**) of the theoretical amount of ·NO irrespective of whether the compound was incubated with PBS (pH 7.4), PLE, or guinea pig serum.

The hybrid CC modulation/·NO donor drugs (**18**–**27**) possess a number of potential advantages relative to using a physical mixture of a CC modulator and nitrate

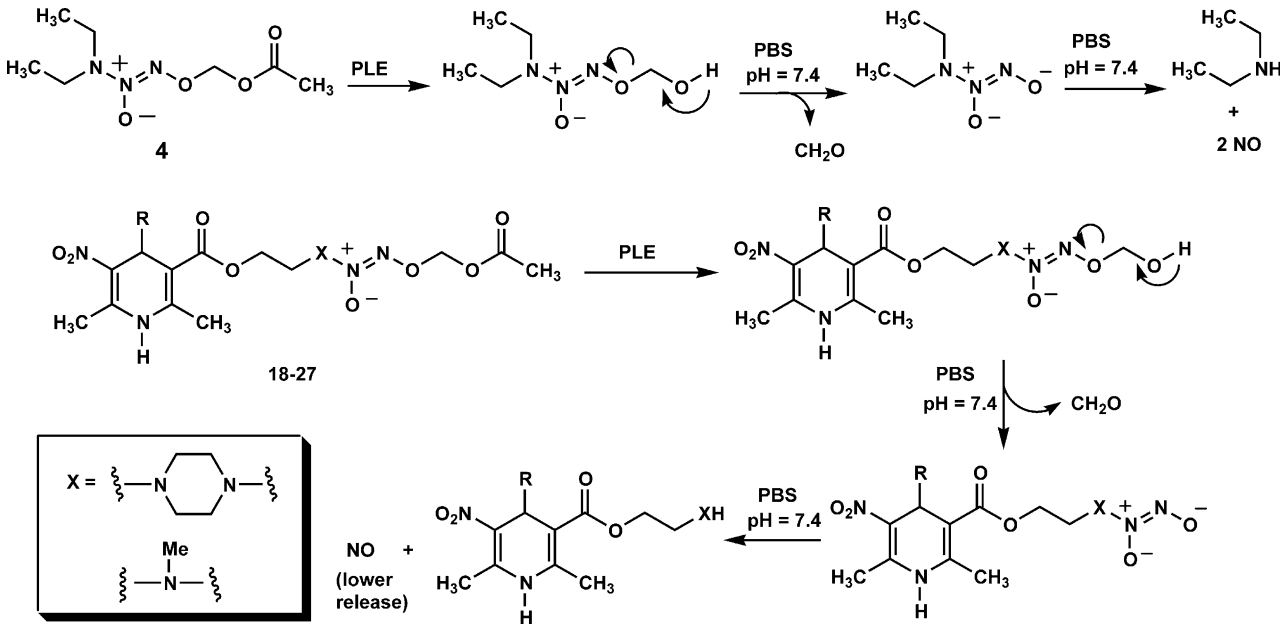


Figure 3. Theoretical ester cleavage and nitric oxide release from prodrug (**4**), and 4-aryl(heteroaryl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylates possessing a O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**18**–**22**) or O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate (**23**–**27**) C-5 ester substituent, where R = 2-, 3-, or 4-pyridyl, 2-CF_3–C_6H_4–, benzofurazan-4-yl.

Table 2. Nitric oxide release studies for 4-aryl(heteroaryl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylates possessing a O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**18**–**22**), or O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate (**23**–**27**), C-5 ester substituent

Compd	% of Nitric oxide released[a]		
	PBS (pH 7.4)[b]	PLE[c]	GP-Serum[d]
18	9.42 ± 0.01	26.43 ± 0.06	68.19 ± 0.04
19	7.71 ± 0.04	23.48 ± 0.07	63.37 ± 0.05
20	9.03 ± 0.04	26.62 ± 0.05	62.41 ± 0.06
21	7.92 ± 0.05	23.95 ± 0.03	75.80 ± 0.04
22	6.32 ± 0.09	25.12 ± 0.06	69.84 ± 0.05
23	6.92 ± 0.05	34.90 ± 0.06	61.20 ± 0.04
24	6.93 ± 0.52	31.00 ± 0.05	—
25	7.81 ± 0.04	23.20 ± 0.05	60.73 ± 0.05
26	6.54 ± 0.06	21.85 ± 0.05	69.01 ± 0.06
27	7.84 ± 0.04	32.08 ± 0.05	72.53 ± 0.09
6	84.90 ± 0.06	84.92 ± 0.06	84.94 ± 0.05
10	70.45 ± 0.04	70.41 ± 0.05	70.46 ± 0.06

[a] Percent of nitric oxide released (±SEM, $n = 3$) relative to a theoretical maximum release of 2 mol of NO/mol of test compound.
[b] Incubated in phosphate buffer solution (PBS) only (pH 7.4) at 37 °C for 1.5 h.
[c] Incubated in the presence of 10 equiv of pig liver esterase (based on a ratio of 1 mol of test compound/10 mol of esterase) in phosphate buffer solution (pH 7.4) at 37 °C for 1.5 h.
[d] Test compound (2.0×10^{-4} mmol) incubated with guinea pig serum (260 μL) in phosphate buffer solution (pH 7.4) at 37 °C for 1.5 h.

vasodilator as glycerol trinitrate: (i) the 1,4-DHP moiety can act as a carrier to simultaneously deliver the 1,4-DHP CC modulator and ·NO moiety to the target tissue that could provide a synergistic effect. CC antagonists enhance the effect of ·NO in vascular smooth muscle cells,[22] the vasodilating effect induced by CC antagonists is increased by ·NO-donor drugs,[23,24] and the combined effects of basal ·NO release and CC antagonists produce an inhibition greater than additive where the concentrations of CC antagonist drug required (IC_{50}) is 3-fold lower in the presence of basal ·NO release than in its absence.[25]; (ii) in contrast to organic nitrate vasodilators, these hybrid compounds do not require a thiol cofactor as L-cysteine or glutathione to enhance the release of ·NO from the diazen-1-ium-1,2-diolate moiety, and a redox activation is not needed for ·NO release[26]; (iii) 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,[15,27] *and* compounds of general structure $R^1(R^2)N$–$N^+(O^-)$=N–$O(CH_2)_nNH_2$ with $t_{1/2}$ values of 1.3–3400 min at 22 °C and pH 7.4 in phosphate buffer have been reported;[28] (iv) 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 amino-alkyl ester substituent is expected to have a long duration of action (like amlodipine, which has an aminoethoxymethyl substituent) that would be amenable to once-a-day dosing, which would circumvent the peak and trough plasma levels observed with short acting CC antagonist drugs such as nifedipine; and (v) hybrid compounds having an O^2-acetoxymethyldiazen-1-ium-1,2-diolate moiety may inhibit platelet aggregation like aspirin that is used chronically in low doses for prophylaxis of stroke and myocardial infarction,[29] and undergo rapid cleavage of the acetoxy group by plasma esterases prior to conversion to the diazen-1-ium-1,2-diolate ·NO donor moiety, which will subsequently release ·NO (see Fig. 3). In addition, ·NO also reduces blood clotting and inhibits platelet aggregation and adhesion, whereas a ·NO deficiency may favor thrombosis.[30]

4. Conclusions

The group of racemic 4-aryl(heteroary)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylates possessing a nitric oxide donor O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**18**–**22**) or O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate (**23**–**27**) C-5 ester substituent, constitute a novel group of nitric oxide donor compounds with desirable calcium channel modulation activities. In particular, compounds having a C-4 2-trifluoromethylphenyl substituent (**21** and **26**) exhibit *dual cardioselective agonist/smooth muscle selective antagonist activities*, in conjunction with an *enhanced nitric oxide release profile* compared with other nitric oxide functional group donors reported in the literature, such as furoxans.[31,32] The replacement of the methyl ester moiety of Bay K 8644 by an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate group (**21**), or an O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate group (**26**), retained the desired cardiac positive inotropic effect while abolishing the smooth muscle agonist effect on guinea pig ileum. Remarkably, the O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate and O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate C-5 ester substituents, released up to 3-fold higher amounts of nitric oxide in the presence of *serum esterases*, compared with the ·NO release in the presence of a liver esterase. This *selective* and *enhanced* ·NO release might provide a useful therapeutic advantage towards the treatment of cardiovascular diseases, including congestive heart failure, because it may be possible to increase endothelial ·NO with only minor effects at other sensitive sites throughout the organism.

5. Experimental

Melting points were determined using a Thomas–Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Nicolet 550 Series II Magna FT-IR spectrometer. ^{1}H NMR nuclear magnetic resonance spectra were recorded on a Bruker AM-300 spectrophotometer. The assignment of exchangeable protons (N*H*) was confirmed by the addition of D_2O. Ultraviolet (UV) spectra and quantitative analyses were measured using a Philips PU 8740 UV/vis scanning spectrophotometer. Silica gel column chromatography was performed using Silicycle® (silica gel 70–230 mesh). Elemental analyses were performed for C, H, and N (microanalytical service laboratory, Department of Chemistry, University of Alberta). Chloromethyl acetate[33] and the 4-aryl-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylic acids (**13**–**17**)[7] were prepared according to procedures reported in the literature. All other reagents (including the porcine liver esterase, 3.2 M ammonium sulfate suspension) were purchased from Aldrich Chemical (Milwaukee, WI) and used without further purification. In vitro calcium channel antagonist and agonist activities were determined using protocols approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

5.1. Synthesis of iodomethyl acetate

Chloromethyl acetate (108.5 g, 1 mol) was added slowly to a solution of sodium iodide (180 g, 1.2 mol) in dry acetone (600 mL) at 25 °C with stirring. The reaction was allowed to proceed for 3 h with stirring at 25 °C, the insoluble inorganic salts were removed by filtration, the solvent was evaporated under reduced pressure, the residue was dissolved in dichloromethane (300 mL), and this solution was washed with 2 N sodium thiosulfate solution (2 × 100 mL). The organic phase was dried (Na_2SO_4), the solvent was removed in vacuo and the residue was purified by fractional distillation (27 °C/3 mmHg) to afford iodomethyl acetate as a yellow liquid (82.4 g, 41%), which was stored under nitrogen to avoid decomposition prior to use. ^{1}H NMR ($CDCl_3$): δ 5.75 (s, 2H, ICH_2O), 2.11 (s, 3H, CH_3).

5.2. O^2-Sodium 1-[*N*-(2-hydroxyethyl)-*N*-methylamino]-diazen-1-ium-1,2-diolate (6)

2-(Methylamino)ethanol (**6**, 20 g, 0.26 mol) was added to a solution of sodium methoxide (14.6 g, 0.26 mol, 61 mL of a 25% w/v solution in MeOH) and diethyl ether (300 mL) with stirring at 25 °C. This mixture was flushed with dry nitrogen for 5 min and then the reaction was allowed to proceed under an atmosphere of nitric oxide (40 psi internal pressure) with stirring at 25 °C for 72 h. The product, which precipitated as a fine white powder, was isolated by filtration and then suspended in diethyl ether (100 mL) upon stirring for 15 min. The suspension was filtered, the solid collected was dried at 25 °C under reduced pressure until a constant weight was achieved after about 2 h to afford **6** as a fine white powder (36.1 g, 88%); mp 150–151 °C; ^{1}H NMR (D_2O) δ 4.63 (t, $J = 5.7$ Hz, 2H, CH_2CH_2OH), 3.35 (s, 3H, NCH_3), 2.86 (t, $J = 5.7$ Hz, 2H, CH_2CH_2OH). Product **6** was used immediately after drying without further purification for the synthesis of compound **7**.

5.3. O^2-Sodium 1-[4-(2-hydroxyethyl)piperazin-1-yl]-diazen-1-ium-1,2-diolate (10)

Reaction of 1-(2-hydroxyethyl)piperazine (**9**, 20 g, 0.15 mol) under an atmosphere of nitric oxide at 40 psi, using the procedure described for the synthesis of **6** above, afforded **10** (29.5 g, 91%) as a white solid; mp 137–140 °C; ^{1}H NMR (D_2O) δ 3.73 (t, $J = 6.3$ Hz, 2H, CH_2CH_2OH), 3.17 (t, $J = 4.8$ Hz, 4H, piperazin-1-yl *H*-2, *H*-6), 2.80 (t, $J = 4.8$ Hz, 4H, piperazin-1-yl *H*-3, *H*-5), 2.61 (t, $J = 6.3$ Hz, 2H, CH_2CH_2OH). Product **10** was used immediately after drying without further purification for the synthesis of compound **11**.

5.4. O^2-Acetoxymethyl-1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (7)

Freshly distilled iodomethyl acetate (27.8 g, 0.14 mol) was added drop wise to a solution of **6** (20 g, 0.12 mol) in acetonitrile (200 mL, HPLC grade) at 25 °C with

stirring. The reaction was allowed to proceed for 19 h at 25 °C with stirring, insoluble inorganic salts were removed by filtration, and the solvent was removed in vacuo. Dichloromethane was added (150 mL) to the residue, and once again insoluble inorganic salts were removed by filtration. Removal of the solvent in vacuo gave a residue that was purified by silica gel column chromatography using hexane–ethyl acetate, 1:2, v/v) as eluant to afford **7** (15.54 g, 62%) as a pale yellow liquid; ^{1}H NMR ($CDCl_3$) δ 5.78 (s, 2H, OCH_2O), 3.78 (t, $J = 5.1$ Hz, 2H, CH_2CH_2OH), 3.50 (t, $J = 5.1$ Hz, 2H, CH_2CH_2OH), 3.10 (s, 3H, NCH_3), 2.12 (s, 3H, COCH_3). Anal. Calcd for $C_6H_{13}N_3O_5$: C, 34.78; H, 6.32; N, 20.28. Found: C, 34.52; H, 6.68; N, 20.49.

5.5. O^2-Acetoxymethyl-1-[4-(2-hydroxyethyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (11)

Freshly distilled iodomethyl acetate (20.6 g, 103.7 mmol) was added dropwise with stirring to a solution of **10** (20 g, 94.3 mmol) in dry THF (200 mL) at −50 to −60 °C (acetone–dry ice bath). Once the addition was complete, the reaction mixture was allowed to warm to 25 °C, the reaction was allowed to proceed for 19 h, and ethyl acetate (200 mL) was added to quench the reaction. The organic phase was washed with a 0.2 N sodium thiosulfate solution until a pale yellow solution (organic phase) was obtained. The organic phase was dried (Na_2SO_4), the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (ethyl acetate–methanol; 9:1, v/v) to yield **11** (7.34 g, 29.6%) as a pale yellow liquid; ^{1}H NMR ($CDCl_3$) δ 5.71 (s, 2H, OCH_2O), 3.58 (t, $J = 5.4$ Hz, 2H, CH_2CH_2OH), 3.45 (t, $J = 5.1$ Hz, 4H, piperazin-1-yl *H*-2, *H*-6), 2.82 (s, 1H, O*H*), 2.63 (t, $J = 5.1$ Hz, 4H, piperazin-1-yl *H*-3, *H*-5), 2.53 (t, $J = 5.4$ Hz, 2H, CH_2CH_2OH), 2.05 (s, 3H, COCH_3). Anal. Calcd for $C_9H_{18}N_4O_5$: C, 41.22; H, 6.92; N, 21.36. Found: C, 41.02; H, 6.79; N, 21.63.

5.6. O^2-Acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (8)

Methanesulfonyl chloride (4.0 g, 35.0 mmol) in dry THF (50 mL) was added to a solution of 4-dimethylaminopyridine (4.24 g, 35.0 mmol) in dry THF (50 mL) and this mixture was stirred for 10 min at 25 °C. A solution of **7** (6.6 g, 31.9 mmol) in dry THF (90 mL) was added dropwise, and the reaction was allowed to proceed for 15 h at 25 °C with stirring. The solids were filtered off, and the solvent was removed in vacuo to give a pale yellow oil, which was purified by silica gel column chromatography (hexane–ethyl acetate; 1:2, v/v) to furnish **8** as a colorless oil (3.2 g, 35.2%); ^{1}H NMR ($CDCl_3$) δ 5.73 (s, 2H, OCH_2O), 4.37 (t, $J = 5.1$ Hz, 2H, CH_2OMs), 3.69 (t, $J = 5.1$ Hz, 2H, NCH_2), 3.11 (s, 3H, NCH_3), 3.01 (s, 3H, SO_2CH_3), 2.08 (s, 3H, COCH_3). Compound **8** was used immediately after purification for the synthesis of **18**–**22**.

5.7. O^2-Acetoxymethyl-1-[4-(2-methylsulfonyloxyethyl)-piperazin-1-yl]diazen-1-ium-1,2-diolate (12)

Methanesulfonyl chloride (1.3 g, 11.3 mmol) in dry THF (30 mL) was added to a solution of 4-dimethylaminopyridine (1.37 g, 11.3 mmol) in dry THF (30 mL) and this solution was stirred for 10 min at 25 °C. A solution of **11** (2.7 g, 10.3 mmol) in dry THF (30 mL) was added dropwise, the reaction was allowed to proceed for 16 h at 25 °C with stirring, the solids were filtered off, and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography (hexane–ethyl acetate; 1:2, v/v) to afford **12** as a pale yellow oil (2.86 g, 81%); ^{1}H NMR ($CDCl_3$) δ 5.78 (s, 2H, OCH_2O), 4.33 (t, $J = 5.1$ Hz, 2H, CH_2OMs), 3.51 (t, $J = 4.8$ Hz, 4H, piperazin-1-yl *H*-2, *H*-6), 3.06 (s, 3H, SO_2CH_3), 2.76 (t, $J = 5.1$ Hz, 2H, NCH_2), 2.72 (t, $J = 4.8$ Hz, 4H, piperazin-1-yl *H*-3, *H*-5), 2.12 (s, 3H, COCH_3). Compound **12** was used immediately after purification for the synthesis of compounds **23**–**27**.

5.8. General procedure for the synthesis of racemic 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 (18–22), or O^2-acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate (23–28), C-5 ester substituent

A mixture of a 4-aryl-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylic acid (**13**–**17**, 0.47 mmol), sodium carbonate (0.56 mmol), and hexamethylphosphoramide (HMPA, 2 mL) was stirred for 4–6 h at 25 °C to form the sodium carboxylate salt. A solution of the mesylate (either **8** or **12**, 0.56 mmol) in HMPA (1 mL) was added, the reaction was allowed to proceed for 72 h at 25 °C with stirring, and then a mixture of ice-water (1:1, v/v; 50 mL) was added to quench the reaction. Extraction with EtOAc (3 × 30 mL), washing the combined EtOAc extracts with water (5 × 30 mL), drying the EtOAc fraction (Na_2SO_4), and removal of the solvent in vacuo gave the respective product (**18**–**27**), which was purified by silica gel column chromatography (hexane–EtOAc, 1:2, v/v; or EtOAc) and then recrystallization from ether–chloroform. Physical and spectroscopic data for compounds **18**–**27** are listed below.

5.8.1. O^2-Acetoxymethyl-1-(*N*-methyl-*N*-ethylamino)diazen-1-ium-1,2-diolate 4-(2-pyridyl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylate (18). Yellow powder (32%); mp 133–135 °C; IR (KBr): 3273 (NH), 1776 (CO), 1662 (C=C), 1528 (NO_2), 1212, 829 (N–O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 10.13 (s, 1H, N*H*), 8.46 (d, $J_{5,6} = 4.5$ Hz, 1H, pyridyl *H*-6), 7.73 (dd, $J_{3,4} = 7.5$, $J_{4,5} = 6.8$ Hz, 1H, pyridyl *H*-4), 7.61 (d, $J_{3,4} = 7.5$ Hz, 1H, pyridyl *H*-3), 7.26 (dd, $J_{5,6} = 4.5$, $J_{4,5} = 6.8$ Hz, 1H, pyridyl *H*-5), 5.76 (s, 2H, OCH_2O), 5.57 (s, 1H, *H*-4), 4.14-4.26 (m, 2H, CO_2CH_2), 3.56–3.61 (m, 2H, CH_2CH_2N), 3.01 (s, 3H, NCH_3), 2.41 (s, 3H, C-2 CH_3), 2.21 (s, 3H, C-6 CH_3), 2.10 (s, 3H, COCH_3). Anal. Calcd for $C_{19}H_{24}N_6O_8$: C, 49.14; H, 5.21; N, 18.10. Found: C, 49.14; H, 5.06; N, 17.60.

5.8.2. O^2-Acetoxymethyl-1-(*N*-methyl-*N*-ethylamino)diazen-1-ium-1,2-diolate 4-(3-pyridyl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylate (19). Yellow powder (21%); mp 122–125 °C; IR (KBr): 3288 (NH), 1770

(CO), 1649 (C=C), 1508 (NO_2), 1313, 829 (N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.50 (d, $J_{2,4} = 1.8$ Hz, 1H, pyridyl *H*-2), 8.42 (dd, $J_{5,6} = 4.8$, $J_{4,6} = 1.5$ Hz, 1H, pyridyl *H*-6), 8.31 (s, 1H, N*H*), 7.77 (ddd, $J_{4,5} = 7.8$, $J_{2,4} = 1.8$, $J_{4,6} = 1.5$ Hz, 1H, pyridyl *H*-4), 7.25 (dd, $J_{4,5} = 7.8$, $J_{5,6} = 4.8$ Hz, 1H, pyridyl *H*-5), 5.76 (s, 2H, OCH_2O), 5.35 (s, 1H, *H*-4), 4.22–4.26 (m, 2H, CO_2CH_2), 3.55–3.63 (m, 2H, CH_2N), 3.01 (s, 3H, NCH_3), 2.51 (s, 3H, C-2 CH_3), 2.35 (s, 3H, C-6 CH_3), 2.11 (s, 3H, $COCH_3$). Anal. Calcd for $C_{19}H_{24}N_6O_8$: C, 49.14; H, 5.21; N, 18.10. Found: C, 49.00; H, 5.07; N, 17.87.

5.8.3. O^2-Acetoxymethyl-1-(*N*-methyl-*N*-ethylamino)diazen-1-ium-1,2-diolate 4-(4-pyridyl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylate (20). Yellow powder (19%); mp 150–152 °C; IR (KBr): 3180 (NH), 1763 (CO), 1649 (C=C), 1494 (NO_2), 1313, 829 (N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.48 (d, $J_{ortho} = 5.4$ Hz, 2H, pyridyl *H*-2, *H*-6), 7.62 (s, 1H, N*H*), 7.28 (d, $J_{ortho} = 5.4$ Hz, 2H, pyridyl *H*-3, *H*-5), 5.76 (s, 2H, OCH_2O), 5.39 (s, 1H, *H*-4), 4.23–4.29 (m, 2H, CO_2CH_2), 3.57–3.67 (m, 2H, CH_2N), 3.00 (s, 3H, NCH_3), 2.53 (s, 3H, C-2 CH_3), 2.38 (s, 3H, C-6 CH_3), 2.11 (s, 3H, $COCH_3$). Anal. Calcd for $C_{19}H_{24}N_6O_8$: C, 49.14; H, 5.21; N, 18.10. Found: C, 49.05; H, 5.08; N, 17.88.

5.8.4. O^2-Acetoxymethyl-1-(*N*-methyl-*N*-ethylamino)diazen-1-ium-1,2-diolate 4-(2-trifluoromethylphenyl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylate (21). Yellow powder (36%); mp 91–93 °C; IR (KBr): 3328 (NH), 1763 (CO), 1656 (C=C), 1494 (NO_2), 1313, 776 (N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.51 (d, $J_{3,4} = 7.8$ Hz, 1H, phenyl *H*-3), 7.40–7.44 (m, 2H, phenyl *H*-5, *H*-6), 7.27 (dd, $J_{4,5} = 6.9$, $J_{3,4} = 7.8$ Hz, 1H, phenyl *H*-4), 7.03 (s, 1H, N*H*), 5.90 (s, 1H, *H*-4), 5.75 (s, 2H, OCH_2O), 4.27–4.37 (m, 1H, CO_2CHH'), 4.07–4.15 (m, 1H, CO_2CHH'), 3.65–3.75 (m, 1H, $CHH'N$), 3.49–3.57 (m, 1H, $CHH'N$), 2.97 (s, 3H, NCH_3), 2.46 (s, 3H, C-2 CH_3), 2.29 (s, 3H, C-6 CH_3), 2.11 (s, 3H, $COCH_3$). Anal. Calcd for $C_{21}H_{24}F_3N_5O_8$: C, 47.46; H, 4.55; N, 13.18. Found: C, 47.21; H, 4.58; N, 13.06.

5.8.5. O^2-Acetoxymethyl-1-(*N*-methyl-*N*-ethylamino)diazen-1-ium-1,2-diolate 4-(benzofurazan-4-yl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylate (22). Yellow powder (28%); mp 120–122 °C; IR (KBr): 3348 (NH), 1756 (CO), 1649 (C=C), 1474 (NO_2), 1273, 803 (N–O) cm^{-1} ; ^1H NMR ($CDCl_3$) δ 7.60 (d, $J_{6,7} = 8.7$ Hz, 1H, benzofurazan-4-yl *H*-7), 7.39 (d, $J_{5,6} = 6.3$ Hz, 1H, benzofurazan-4-yl *H*-5), 7.30 (dd, $J_{6,7} = 8.7$, $J_{5,6} = 6.3$ Hz, 1H, benzofurazan-4-yl *H*-6), 6.46 (s, 1H, N*H*), 5.72 (s, 1H, *H*-4), 5.70 (s, 2H, OCH_2O), 4.12–4.17 (m, 2H, CO_2CH_2), 3.50–3.55 (m, 2H, CH_2N), 2.95 (s, 3H, NCH_3), 2.48 (s, 3H, C-2 CH_3), 2.29 (s, 3H, C-6 CH_3), 2.05 (s, 3H, $COCH_3$). Anal. Calcd for $C_{20}H_{23}N_7O_9$: C, 47.53; H, 4.59; N, 19.40. Found: C, 47.13; H, 4.68; N, 19.05.

5.8.6. O^2-Acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate 4-(2-pyridyl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylate (23). Yellow powder (29%); mp 170–171 °C; IR (KBr): 3328 (NH), 1602 (C=C), 1427 (NO_2), 1266, 897 (N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.74 (s, 1H, N*H*), 8.26 (d, $J_{5,6} = 6.3$ Hz, 1H, pyridyl *H*-6), 7.37 (dd, $J_{3,4} = 7.5$, $J_{4,5} = 6.3$ Hz, 1H, pyridyl *H*-4), 7.25 (d, $J_{3,4} = 7.5$ Hz, 1H, pyridyl *H*-3), 6.90 (dd, $J_{5,6} = J_{4,5} = 6.3$ Hz, 1H, pyridyl *H*-5), 5.61 (s, 2H, OCH_2O), 5.34 (s, 1H, *H*-4), 3.95–4.00 (m, 2H, CO_2CH_2), 3.18–3.30 (m, 4H, piperazin-1-yl *H*-2, *H*-6), 2.59 (m, 2H, CH_2CH_2N), 2.44 (m, 4H, piperazin-1-yl *H*-3, *H*-5), 2.38 (s, 3H, C-2 CH_3), 2.19 (s, 3H, C-6 CH_3), 1.96 (s, 3H, $COCH_3$). Anal. Calcd for $C_{22}H_{29}N_7O_8$: C, 50.86; H, 5.63; N, 18.87. Found: C, 50.46; H, 5.39; N, 18.69.

5.8.7. O^2-Acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate 4-(3-pyridyl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylate (24). Yellow powder (19%); mp 75–77 °C; IR (KBr): 3308 (NH), 1763 (CO), 1649 (C=C), 1474 (NO_2), 1273, 742 (N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.55 (s, 1H, N*H*), 8.52 (d, $J_{2,4} = 2.1$ Hz, 1H, pyridyl *H*-2), 8.42 (dd, $J_{5,6} = 4.8$, $J_{4,6} = 1.2$ Hz, 1H, pyridyl *H*-6), 7.74 (ddd, $J_{4,5} = 7.5$, $J_{2,4} = 2.1$, $J_{4,6} = 1.2$ Hz, 1H, pyridyl *H*-4), 7.24 (dd, $J_{4,5} = 7.5$, $J_{5,6} = 4.8$ Hz, 1H, pyridyl *H*-5), 5.79 (s, 2H, OCH_2O), 5.39 (s, 1H, *H*-4), 4.13–4.19 (m, 2H, CO_2CH_2), 3.40–3.49 (m, 4H, piperazin-1-yl *H*-2, *H*-6), 2.56–2.64 (m, 6H, piperazin-1-yl *H*-3, *H*-5, CH_2CH_2N), 2.53 (s, 3H, C-2 CH_3), 2.38 (s, 3H, C-6 CH_3), 2.05 (s, 3H, $COCH_3$). Anal. Calcd for $C_{22}H_{29}N_7O_8$: C, 50.86; H, 5.63; N, 18.87. Found: C, 50.79; H, 5.79; N, 18.65.

5.8.8. O^2-Acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate 4-(4-pyridyl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylate (25). Yellow powder (29%); mp 180–182 °C; IR (KBr): 3053 (NH), 1716 (CO), 1602 (C=C), 1427 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.46 (d, $J_{ortho} = 5.4$ Hz, 2H, pyridyl *H*-2, *H*-6), 7.60 (s, 1H, N*H*), 7.24 (d, $J_{ortho} = 5.4$ Hz, 2H, pyridyl *H*-3, *H*-5), 5.79 (s, 2H, OCH_2O), 5.36 (s, 1H, *H*-4), 4.21–4.27 (m, 2H, CO_2CH_2), 3.39–3.47 (m, 4H, piperazin-1-yl *H*-2, *H*-6), 2.52–2.61 (m, 6H, piperazin-1-yl *H*-3, *H*-5, CH_2CH_2N), 2.51 (s, 3H, C-2 CH_3), 2.36 (s, 3H, C-6 CH_3), 2.05 (s, 3H, $COCH_3$). Anal. Calcd for $C_{22}H_{29}N_7O_8$: C, 50.86; H, 5.63; N, 18.87. Found: C, 50.79; H, 5.61; N, 18.63.

5.8.9. O^2-Acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate 4-(2-trifluoromethylphenyl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylate (26). Yellow powder (26%); mp 70–72 °C; IR (KBr): 3321 (NH), 1763 (CO), 1649 (C=C), 1501 (NO_2), 1320, 769 (N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.53 (d, $J_{3,4} = 7.5$ Hz, 1H, phenyl *H*-3), 7.40–7.44 (m, 2H, phenyl *H*-3, *H*-5), 7.26–7.28 (m, 1H, phenyl *H*-4), 6.45 (s, 1H, N*H*), 5.93 (s, 1H, *H*-4), 5.78 (s, 2H, OCH_2O), 4.21–4.25 (m, 1H, CO_2CHH'), 4.06–4.19 (m, 1H, CO_2CHH'), 3.37 (m, 4H, piperazin-1-yl *H*-2, *H*-6), 2.54–2.56 (m, 6H, piperazin-1-

yl *H*-3, *H*-5, CH_2CH_2N), 2.48 (s, 3H, C-2 CH_3), 2.33 (s, 3H, C-6 CH_3), 2.12 (s, 3H, $COCH_3$). Anal. Calcd for $C_{24}H_{29}F_3N_6O_8$: C, 49.15; H, 4.98; N, 14.33. Found: C, 48.99; H, 4.88; N, 14.13.

5.8.10. O^2-Acetoxymethyl-1-(4-ethylpiperazin-1-yl)diazen-1-ium-1,2-diolate 4-(benzofurazan-4-yl)-1,4-dihydro-2,6-dimethyl-3-nitropyridine-5-carboxylate (27). Yellow powder (28%); mp 85–88 °C; IR (KBr): 3314 (NH), 1763 (CO), 1649 (C=C), 1501 (NO_2), 1266, 803 (N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.70 (d, $J_{6,7} = 9.0$ Hz, 1H, benzofurazan-4-yl *H*-7), 7.45 (d, $J_{5,6} = 6.6$ Hz, 1H, benzofurazan-4-yl *H*-5), 7.35 (dd, $J_{6,7} = 9.0$, $J_{5,6} = 6.6$ Hz, 1H, benzofurazan-4-yl *H*-6), 6.66 (s, 1H, N*H*), 5.81 (s, 1H, *H*-4), 5.80 (s, 2H, OCH_2O), 4.16 (t, $J = 6.3$ Hz, 2H, CO_2CH_2), 3.38–3.45 (m, 4H, piperazin-1-yl *H*-2, *H*-6), 2.53–2.70 (m, 6H, piperazin-1-yl *H*-3, *H*-5, CH_2CH_2N), 2.55 (s, 3H, C-2 CH_3), 2.36 (s, 3H, C-6 CH_3), 2.13 (s, 3H, $COCH_3$). Anal. Calcd for $C_{23}H_{28}N_8O_9$: C, 49.28; H, 5.04; N, 19.99. Found: C, 49.18; H, 4.99; N, 19.72.

5.9. In vitro calcium channel antagonist and agonist assays

Smooth muscle calcium channel antagonist activity was determined as the micromolar (μM) concentration of the test compound required to produce 50% inhibition of the muscarinic receptor-mediated (carbachol, 0.167 μM) Ca^{2+}-dependent contraction (tonic response) of guinea pig ileum longitudinal smooth muscle (GPILSM) using the procedure previously reported.[34] The IC_{50} (± SEM, $n = 3$) was determined graphically from the dose–response curve. Calcium channel agonist activity on GPILSM (in the absence of 0.167 μM carbachol) was calculated as the micromolar (μM) concentration of the test compound required to induce a 50% increase in the contractile response, relative to the response produced by carbachol (0.167 μM). However, no smooth muscle calcium channel agonist response was observed for any of the drugs tested (**18**–**27**) at the highest test compound concentration employed (44.66 μM). The cardiac calcium channel agonist effect was calculated as the percentage increase (positive inotropic effect) on contractile force of isolated guinea pig left atrium (GPLA) relative to its basal contractile force in the absence of test compound. The positive inotropic EC_{50} value (± SEM, $n = 3$) was determined graphically from the dose–response curve.

5.10. In vitro nitric oxide release assay

In vitro nitric oxide release in phosphate buffer, pig liver esterase, or guinea pig serum, was determined by quantification of nitrite produced by the reaction of nitric oxide with oxygen and water using the Griess reaction. Nitric oxide release data were acquired for test compounds (**18**–**27**), and the reference compounds O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**6**), and O^2-sodium 1-[4-(2-hydroxyethyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (**10**) using the reported procedures.[13]

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