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Synthesis and Biological Investigations of Nitric Oxide Releasing Nateglinide and Meglitinide Type II Antidiabetic Prodrugs: In-Vivo Antihyperglycemic Activities and Blood Pressure Lowering Studies

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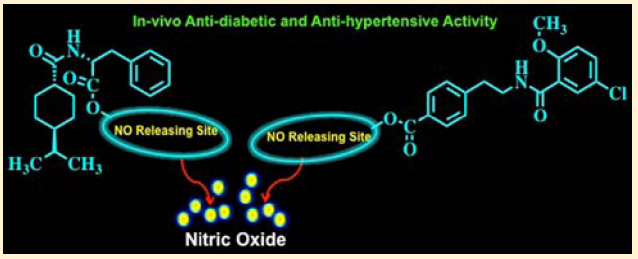
Supporting Information

ABSTRACT: A new group of hybrid nitric oxide-releasing type II antidiabetic drugs possessing a 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**13** and **18**), 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**14** and **19**), or nitrooxyethyl (**15** and **20**) moiety attached to the carboxylic acid group of the type II antidiabetic drugs nateglinide and meglitinide were synthesized. These prodrugs, based on the beneficial properties of nitric oxide (NO), were designed to reduce the risk of adverse cardiovascular events in diabetic patients. Ester prodrugs (**13**−**15** and **18**−**20**) exhibited appreciable oral antihyperglycemic activity comparable to the parent drugs in nonfasted diabetic rats. Systolic and diastolic blood pressure profiles validated the beneficial hypotensive properties of these prodrugs. These prodrugs released NO (1.3−72.2% range) upon incubation with either phosphate buffer solution at pH 7.4 or in the presence of serum. This new type of hybrid NO donor prodrug represents an attractive approach for the rational design of type II antidiabetic drugs with a reduced risk of contraindicated cardiovascular events.



INTRODUCTION

Diabetes mellitus is a disease in which there is either inadequate insulin production (type I) or inability to effectively use insulin (type II). The rising prevalence of type II diabetes, and associated adverse cardiovascular risks, is now considered a major public health challenge. In this regard, diabetic patients are at high risk for macrovascular complications such as myocardial ischemia, stroke, adverse cerebrovascular events, and hypertension.[1−8] Ultimately, >65% of diabetic patients die from heart disease or stroke. In diabetic patients, critical cardiovascular complications[9−12] are often attributed to endothelial dysfunction[13,14] that is associated with a deficient biosynthesis of beneficial vasodilator substances such as NO and prostacyclin (PGI_2). It is widely accepted that a reduction in the bioavailability of NO is the central mechanism involved in endothelial dysfunction. This subphysiological level of NO in diabetic individuals increases the prevalence of severe thrombosis, atherosclerosis, cardiac inflammation, hypertension, and remodeling.[8,14] Accordingly, in order to reduce adverse cardiovascular risks, diabetic patients are regularly prescribed additional drugs targeting hypertension, platelet aggregation, and dyslipidaemia (ACE-inhibitors, calcium channel blockers, aspirin, statins, etc.).[8,15,16]

The use of multitargeted drugs represents an attractive medicinal chemistry drug design strategy that offers an alternative to using a multiple drug regimen that may not be the best pharmacological approach. The concept of developing a hybrid multitargeted prodrug that will release each component of the hybrid in vivo, to furnish the desired pharmacological effects at the same prodrug dose, warrants further exploration. In this regard, a NO-glibenclamide prototype has been reported where the hypoglycemic effect is enriched by additional NO-vasorelaxing and antiplatelet effects that are potentially useful to circumvent diabetes-related cardiovascular disorders.[16] More recently, in vitro insulin release, antiaggregatory, and vasorelaxant properties of some NO-tolbutamide analogues have been described.[17]

NO is an efficient vasodilation agent that induces vascular smooth muscle relaxation, and it reduces the risk of blood clot formation (thrombosis) by inhibiting platelet aggregation and adhesion.[18] A slow release of NO can provide NO to circumvent the endogenous NO deficiency (endothelial dysfunction) in diabetic patients. In view of the beneficial properties of NO, modification of type II antidiabetic drugs by incorporating a NO donor moiety offers an attractive drug design strategy to simultaneously reduce cardiovascular side effects observed in diabetic patients being prescribed type II antidiabetic drugs. A hybrid prodrug, or designed multiple ligand (DML), can provide a variety of potential advantages

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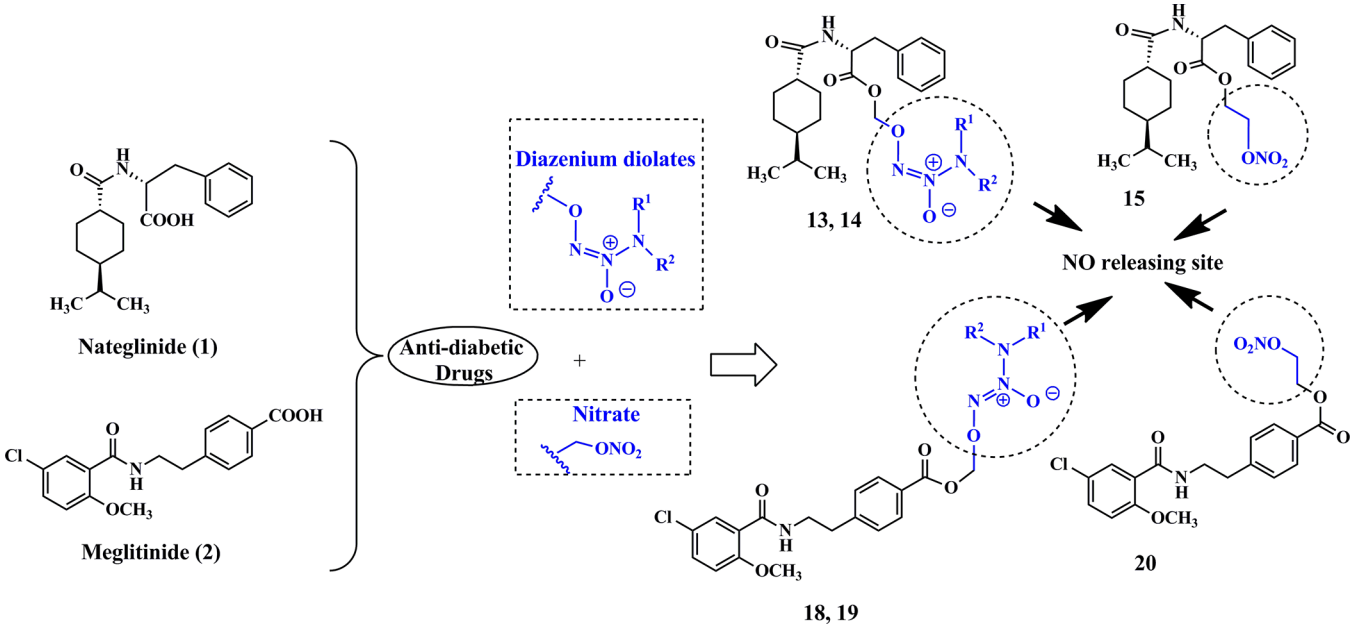
Chart 1. Design of NO-Releasing Type II Antidiabetic Hybrid Prodrugs



relative to the administration of two individual drugs having the same pharmacological actions. For example, (i) different relative metabolic rates between patients can result in highly complex pharmacokinetic (PK) and pharmacodynamic (PD) relationships for multicomponent drugs leading to unpredictable variability among patients that requires extensive and expensive clinical monitoring of each drug,[19] (ii) there is a lower risk of drug−drug interactions in comparison to cocktails or multicomponent drugs,[20] (iii) patient compliance may be better, and (iv) there is a potential to enhance efficacy and safety. Accordingly, coupling a NO donor moiety provides an opportunity to tune the absorption, distribution, metabolism, excretion (ADME), and PK and PD properties of a type II diabetic agent where the deficiency of NO is the cause of contraindicated side effects associated with diabetes.

In this study, we modified the type II antidiabetic drugs nateglinide (**1**, Nat) and meglitinide (**2**, Meg), which belong to a family of drugs that unlike sulfonylureas stimulate first-phase insulin release in a glucose-sensitive manner that theoretically reduces the risk of hypoglycemic events.[21−27] As part of our ongoing program to design improved drugs, we now describe the synthesis, in vivo antihyperglycemic activity, and blood pressure studies of a new group of prototypical NO-releasing type II antidiabetic hybrid prodrugs that retain the pharmacological activity of the parent antidiabetic component but also confer the beneficial biological actions of NO (Chart 1). This new group of hybrid prodrugs **13** (Nat-PYR), **14** (Nat-DEA), **15** (Nat-NOE), **18** (Met-PYR), **19** (Met-DEA), and **20** (Met-NOE) were prepared by conjugating the carboxyl group present in Nat and Meg to NO releasing 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (PYR), 1-(*N*,*N*-diethylamino)-diazen-1-ium-1,2-diolate (DEA), or nitrooxyethyl (NOE) moieties.

RESULTS AND DISCUSSION

Chemistry. The syntheses of O^2-chloromethyl-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**9**) and O^2-chloromethyl-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**10**) were carried out according to the reported procedures[28,29] as illustrated in Scheme 1. The first type II antidiabetic drug (2*R*)-2-(*trans*-4-isopropylcyclohexanecarboxamido)-3-phenylpropanoic acid (nateglinide, **1**) was synthesized using a methodology similar to that reported in ref 30 (Scheme 1). Thus, the reaction of *trans*-4-isopropylcyclohexanecarboxylic acid (**11**) with *para*-toluenesulphonyl chloride in the presence of Et_3N afforded the mixed anhydride **12**, which without isolation using a one-pot reaction was condensed with D-phenylalanine to furnish Nat (**1**, 79% yield). The (*R*)-antipode of Nat was selected for use in this study since it is reported to be a 70-fold more potent hypoglycemic agent than the (*S*)-antipode.[24] The second type II antidiabetic drug 4-(2-(5-chloro-2-methoxybenzamido)-ethyl)benzoic acid (meglitinide, **2**) was prepared by elaboration of 5-chloro-*o*-anisic acid (**16**) to the acid chloride **17**.[31] Subsequent reaction of **17** with 4-(2-aminoethyl)benzoic acid hydrochloride in the presence of NaOH afforded Meg[26] (**2**, 57% yield) as illustrated in Scheme 1.

The stable target NO donor ester prodrugs (**13**, **14**, **18**, and **19**) were synthesized by converting Nat and Meg to the respective sodium salt by treatment with Na_2CO_3 in the polar aprotic solvent hexamethylphosphoramide (HMPA) at 25 °C. The sodium salt of each drug obtained, upon reaction with a O^2-chloromethyldiazen-1-ium-1,2-diolate **9** or **10** in HMPA, furnished the respective NO donor ester pro-drug (**13**, **14**, **18**, or **19**) in good yield (61−80% range). The corresponding 2-nitrooxyethyl ester hybrid prodrugs (**15** and **20**) were synthesized by reaction of the carboxyl group of either Nat (**1**) or Meg (**2**) with 2-nitrooxyethyl bromide in the presence of K_2CO_3 in DMF as illustrated in Scheme 1.

Antihyperglycemic Activity. The in vivo antihyperglycemic activities for the hybrid NO-donor prodrug derivatives of Nat **13**−**15** and Meg **18**−**20** were determined using streptozotocin (STZ) induced nonfasting diabetic rats (n = 4 per group) for comparison with the parent drugs using an oral dose of 63.01 μmol/kg. The blood glucose level (BGL) was measured at 1, 3, 6, and 24 h postdrug administration, and the % reduction in the blood glucose level (BGL_{red}) relative to the initial BGL at 0 h just prior to drug administration was

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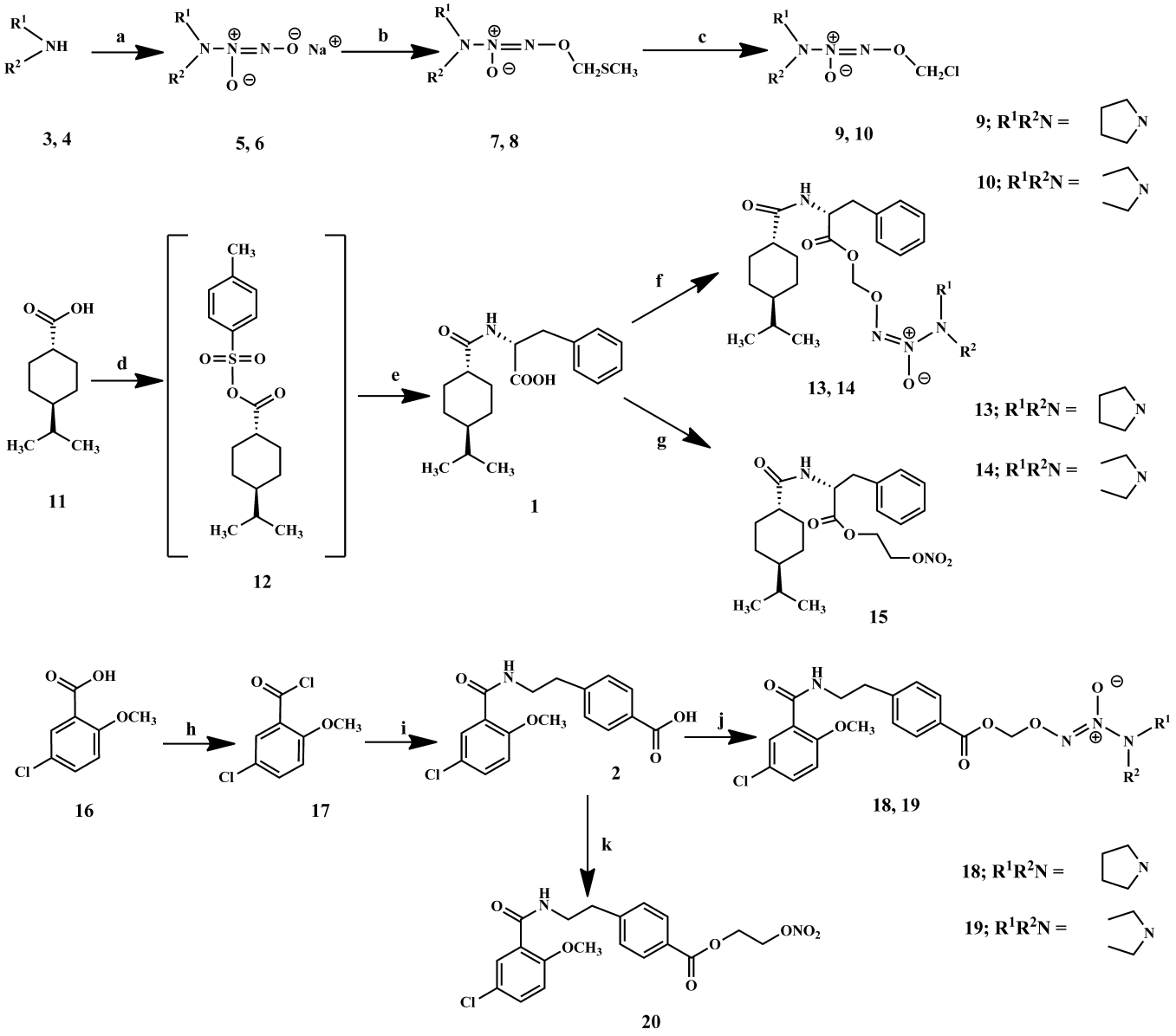
Scheme 1. Synthesis of NO-Ester Prodrugs[a]



[a]Reagents and conditions: (a) NO oxide (40 psi), NaOMe, MeOH, ether, 25 °C; (b) $ClCH_2SCH_3$, DMF/THF, Na_2CO_3, KI, 25 °C, argon atmosphere, 48 h; (c) SO_2Cl_2, DCM, 25 °C, 30 min; (d) *p*-toluene sulfonyl chloride, Et_3N, DCM, 25 °C, 5 h; (e) D-phenylalanine, 25 °C, 12 h; (f) Na_2CO_3, compound **9** or **10**, HMPA, 25 °C, 65 h for compound **13** and 48 h for compound **14**; (g) 2-nitrooxyethyl bromide, K_2CO_3, DMF, 25 °C, 24 h; (h) thionyl chloride, catalytic amount of dry DMF, 25 °C, 30 min; (i) 4-(2-aminoethyl)benzoic acid hydrochloride, NaOH, acetone, 2 h; (j) Na_2CO_3, compound **9** or **10**, HMPA, 25 °C, 33 h for compound **18** and 31 h for compound **19**; (k) 2-nitrooxyethyl bromide, K_2CO_3, DMF, 25 °C, 12 h.

calculated (see data in Figures 1 and 2, and Table S1 (Supporting Information)).

In the Nat group of compounds **13**–**15**, Nat-PYR having a 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate NO-donor moiety showed a lower reduction in the BGL at 1 h (BGL_{red} = 8.1%; $p < 0.05$) and 3 h (BGL_{red} = 22.6%; $p < 0.05$) compared to the parent drug Nat (BGL_{red} = 13.8% at 1 h; 26.4% at 3 h; 30.6% at 6 h) as illustrated in Figures 1a and b. In this regard, Nat-PYR did not reduce the BGL at 6 h since it showed a BGL similar to that of the control BGL at time 0. Nat-PYR induced a significant hyperglycemic effect at 24 h postdrug administration where the BGL was increased 48.7% relative to the BGL at time 0 prior to drug administration. The mechanism for this unexpected hyperglycemic effect at 24 h postdrug administration is not known, and it is distinctly different from all other compounds **14**–**15**, **18**–**20**, Nat, Meg, and vehicle alone evaluated in this study, which did not induce a hyperglycemic or hypoglycemic effect at 24 h. In comparison, Nat-DEA having a 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate NO-donor moiety showed a longer duration of action like that of Nat lasting 6 h and provided a greater reduction in BGLs (BGL_{red} = 21.1% at 1 h; 23.5% at 3 h; 34.1% at 6 h; $p < 0.05$) than Nat. Nat-NOE, a 2-nitrooxyethyl ester pro-drug, exhibited a longer 6 h duration of action like Nat-DEA and Nat, but Nat-NOE was less potent (BGL_{red} = 8.2, 13.4 and 20.5% at 1, 3, and 6 h, respectively) than either Nat-DEA or Nat.

Antidiabetic (hypoglycemic) structure–activity data were also acquired for a similar group (Meg-PYR; Meg-DEA, Meg-

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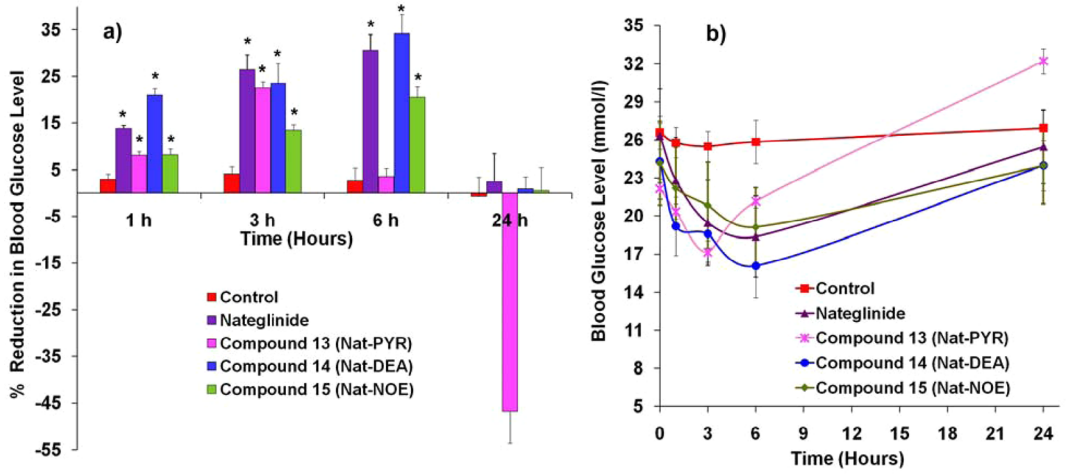

Figure 1. (a) Percentage reduction of BGL in STZ induced nonfasting diabetic rats after oral administration (63.01 μmol/kg) of compounds **13**, **14**, and **15** at 1, 3, 6, and 24 h postdrug administration. (b) Change in BGL in STZ induced nonfasting diabetic rats after oral administration (63.01 μmol/kg) of compounds **13**, **14**, and **15** at 1, 3, 6, and 24 h postdrug administration. Values are the mean % changes (n = 4 animals in each group). The level of significance represents the difference in the BGL at time 0 prior to drug administration, relative to that at the indicated postdrug administration times, for each animal (the difference is significant when $^*p < 0.05$).

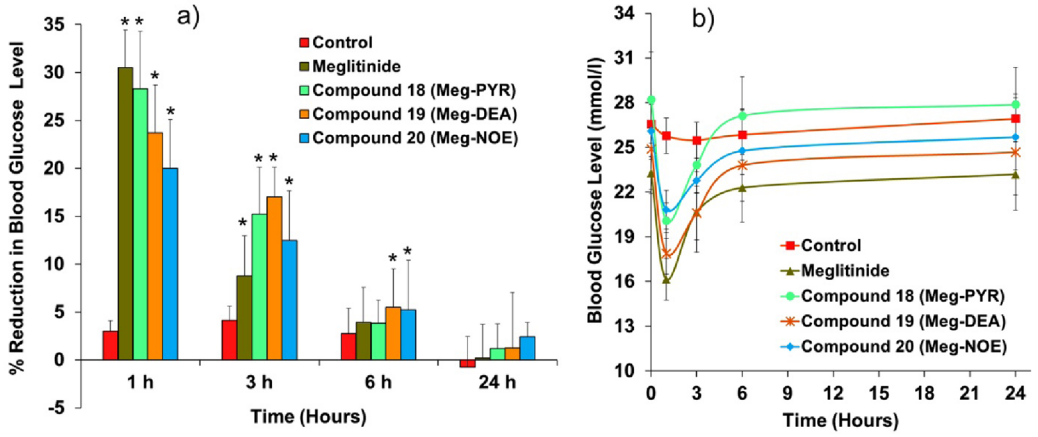

Figure 2. (a) Percentage reduction of BGL in STZ induced nonfasting diabetic rats after oral administration (63.01 μmol/kg) of compounds **18**, **19**, and **20** at 1, 3, 6, and 24 h postdrug administration. (b) Change in BGL in STZ induced nonfasting diabetic rats after oral administration (63.01 μmol/kg) of compounds **18**, **19**, and **20** at 1, 3, 6, and 24 h postdrug administration. Values are the mean % changes (n = 4 animals in each group). The level of significance represents the difference in the BGL at time 0 prior to drug administration, relative to that at the indicated postdrug administration times, for each animal (the difference is significant when $^*p < 0.05$).

NOE) of hybrid Meg NO-donor prodrugs (see data in Figures 2a and b). Meg-PYR, Meg-DEA, and Meg-NOE showed a lower reduction in BGLs (BGL_{red} in the 20.0 to 28.3% range) than the parent drug Meg (BGL_{red} = 30.5%) at 1 h but a larger reduction in BGLs (BGL_{red} in the 12.5 to 17.0% range) than Meg (BGL_{red} = 8.8%) at 3 h postdrug administration. Meg-PYR, Meg-DEA, Meg-NOE, and Meg exhibit a short duration of action since weak (Met-DEA and Met-NOE) hypoglycemic activity was observed at 6 h, and no hypoglycemic activity was observed (Met-Pyr, Met-DEA, Met-NOE, Meg, and vehicle control) at 24 h postdrug administration. Table S1 listing the structure–activity data described (numerical data ± standard deviation) is provided in Supporting Information.

Blood Pressure Studies. Systolic blood pressure (BP_{sys}, mm Hg), diastolic blood pressure (BP_{dia}, mm Hg), and heart rate (HR, beats min^{-1}) were measured at 1, 3, and 6 h time intervals following oral administration of either the vehicle alone (control group, n = 6) or NO donor prodrugs **13**–**15** and **18**-**20** (63.01 μmol/kg po, n = 3) to C57 black mice. A minimum of three consecutive measurements were made for each mouse at each time interval and the mean value is the average of these three measurements. The data obtained for BP_{sys}, BP_{dias}, BP_{mean} (average of BP_{sys} and BP_{dia}) and HR are illustrated in Figure 3a–d. Table S2 listing numerical data ± standard deviation is provided as Supporting Information.

All of the NO-donor Meg-PYR (90.3), Meg-DEA (96.8), Meg-NOE (105.8), Nat-PYR (108.4) and Nat-DEA (113.0) prodrugs showed a significant reduction ($^*p < 0.05$) in BP_{sys} (mm Hg), except for Nat-NOE (129.9), relative to the control group (126.5) at 1 h postdrug administration (see data in Figure 3a). In comparison, only the Nat-PYR (106.2) and Nat-DEA (98.1) conjugates showed a significant reduction in BP_{sys} relative to the control group (118.3 mmHg)) at 3 h postdrug administration. The mechanism responsible for these transient changes in BP_{sys} at 3 h is not known since the BP_{sys} profile changed at 6 h postdrug administration where BP_{sys} for Nat-NOE, Meg-PYR, Meg-DEA and Meg-NOE (103.4 to 106.9 mmHg range), but not that for Nat-PYR (134.3) or Nat-DEA

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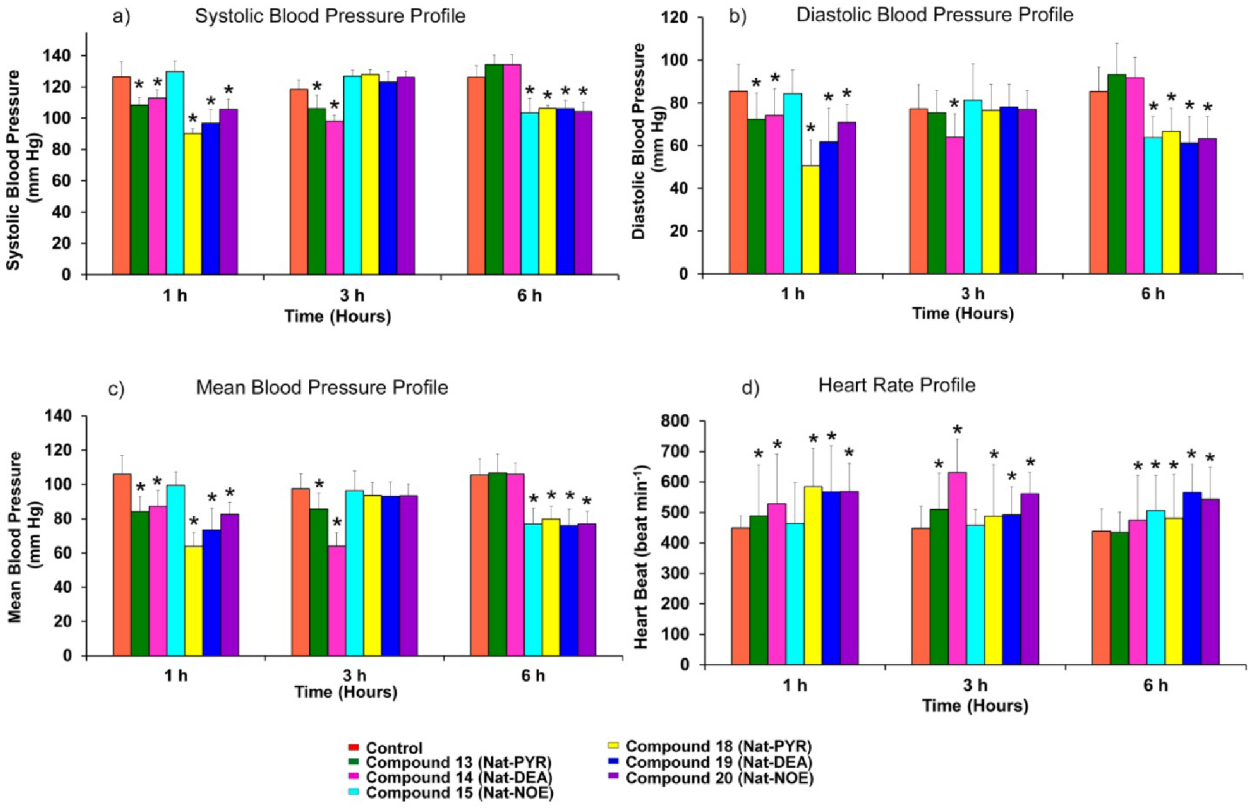

Figure 3. BP_{sys}, BP_{dia}, BP_{mean}, and HR in conscious mice following oral administration of either vehicle alone (control group, mean ± SD, $n = 6$) or test compounds **13**–**15** and **18**–**20** (63.01 μmol/kg po; $n = 3$). Data are presented as the group mean ± SD. Statistical significant differences between control groups and drug treatment groups at each time interval are denoted as $^*p < 0.05$.

(134.4), was significantly lower ($^*p < 0.05$) than that for the control group (126.2 mmHg).

The BP_{dia} structure–activity data acquired for the Nat and Meg hybrid NO-donor conjugates (**13**–**15**, **18**–**20**) presented in Figure 3b indicates that the BP_{dia} profile for this group of compounds follows a very similar profile to that previously described for BP_{sys} at 1, 3, and 6 h postdrug administration (compare profiles in Figure 3a and Figure 3b for each respective hybrid NO-donor conjugate). The only difference observed is for the Nat-PYR conjugate **13** which shows a significant reduction ($^*p < 0.05$) in BP_{sys}, but not BP_{dia} ($^*p > 0.05$), at 3 h postdrug administration. Accordingly as expected, BP_{mean} (average of BP_{sys} and BP_{dia}) follows a similar profile (see Figure 3c) to that observed for BP_{sys} (Figure 3a) and BP_{dia} (Figure 3b).

The HR of mice was determined and the data are shown in Figure 3d (complete data is provided in Table S2 as Supporting Information). The HR at 1 h (454–568 range), 3 h (458–631 range) and 6 h (435–566 range) was generally higher than that for control mice (449, 448, and 439 beats min^{-1} at 1, 3, and 6 h postdrug administration, respectivcly). One plausible explanation for the increase in HR, for those compounds in which an elevated HR occurred, may be a homeostatic mechanism to compensate for the reduction in $BP_{sys,}$ BP_{dia} and BP_{mean}.

Nitric oxide Release. The diazen-1-ium-1,2-diolate and nitrooxy moieties used to prepare the hybrid prodrug conjugates of Nat **13**–**15** and Meg **18**-**20** are well established NO donor groups. Therefore, it was of interest to determine the percentage of NO released from the hybrid ester prodrugs **13**–**15** and **18**–**20** upon incubation in either phosphate-buffered saline (PBS at pH 7.4) or PBS at pH 7.4 in the presence of rat serum that contains nonspecific esterases. The indirect quantitation of NO as nitrite anion using the Griess reaction (see data in Table 1) was determined for incubation times of 1, 3, 4, 16, and 24 h. The rate of NO release from diazen-1-ium-1,2-diolates can be controlled by chemical modification such as attachment of an alkyl substituent to the O^2-position.[32] These O^2-substituted-diazen-1-ium-1,2-diolates are stable compounds that hydrolyze slowly even in acidic solution.[33] When compounds **13**, **14**, **18**, and **19** were

Table 1. Percentage (%) of NO Released from Compounds 13–15 and 18–20

	% NO released[a]							
	PBS (pH 7.4)				PBS (pH 7.4) + Serum			
compd	1 h	4 h	6 h	24 h	1 h	3 h	6 h	24 h
13	6.7	6.8	52.7	59.9	40.2	47.2	50.1	65.3
14	4.4	5.1	58.8	61.3	44.9	50.2	57.2	72.2
15	6.7	7.5	7.7	9.2	1.3	1.9	3.9	6.3
18	5.1	5.7	7.2	8.9	37.6	51.7	59.2	60.5
19	4.2	6.0	6.1	6.5	54.5	55.9	61.5	63.6
20	4.1	7.2	7.7	9.4	2.1	2.3	2.6	3.8

[a]Percentage of NO released (mean value, $n = 3$) relative to a theoretical maximum release of 2 mol of NO/mol of test compounds (**13**, **14**, **18**, and **19**) and 1 mol of NO/mol of test compounds (**15** and **20**) was determined using the Griess reaction. Variation from the mean % value was ≤ 0.02%. Incubations were carried out for 1, 4, 6, and 24 h in 2.4 mL of phosphate buffer solution (PBS, pH 7.4) or for 1, 3, 6, and 24 h in PBS buffer (2.4 mL) containing 90 μL of rat serum at 37 °C.

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incubated in PBS buffer (pH 7.4), the percentage of NO released varied from a low of 4.1% to 6.7% at 1 h up to a maximal release of 59.9% (Nat-Pyr), 61.3% (Nat-DEA), 8.9% (Meg-Pyr), and 6.5% (Meg-DEA) for a 24 h incubation. In this regard, the Meg-Pyr and Meg-DEA conjugates, like Nat-NOE and Meg-NOE, release a much lower % of NO. In contrast, incubation of Meg-Pyr and Meg-DEA in PBS at pH 7.4 in the presence of rat serum that contains nonspecific esterases increased the amount of NO released. Accordingly, the extent of NO release from compounds **13**, **14**, **18**, and **19** was substantially higher (37.6−54.5% range) at 1 h reaching maximal release (60.5−72.2% range) for 24 h incubations. This increase in % NO release indicates that nonspecific esterases present in rat serum cleave the hybrid prodrug esters more effectively than PBS at pH 7.4. A plausible mechanism for the ester hydrolysis of hybrid ester prodrugs containing a diazen-1-ium-1,2-diolate moiety was described in an earlier study.[34] In contrast to compounds **13**, **14**, **18**, and **19** containing a diazen-1-ium-1,2-diolate NO-donor moiety, the extent of NO released upon incubation of the hybrid nitrooxyethyl ester prodrugs (Nat-NOE, 6.7 to 9.2% range; Meg-NOE, 4.1 to 9.4% range) was lower for incubations of 1, 3, 4, 6, and 24 h in both PBS buffer at pH 7.4 and in PBS buffer containing rat serum (Nat-NOE, 1.3−6.3% range; Meg-NOE, 2.1−3.8% range).

Vascular Relaxation. Nat-DEA (1 μM) caused rapid and complete reversal, and Nat-NOE (1 μM) caused less rapid but near complete reversal of phenylephrine-induced tone in isolated rat mesenteric arteries, which was abolished by ODQ (10 μM; n = 3), a selective inhibitor of soluble guanylyl cyclase.[35] Representative traces of the effects of Nat-DEA and Nat-NOE are shown in Figure 4. The ability of Nat-DEA and Nat-NOE to reverse phenylephrine-induced tone in isolated mesenteric arteries demonstrates that these compounds can act directly on vascular smooth muscle cells to cause relaxation (vasodilation) of resistance vessels. Abolition of the response by ODQ indicates that Nat-DEA acts via activation of soluble guanylyl cyclase and supports the contention that Nat-DEA reduces blood pressure via the generation of NO.

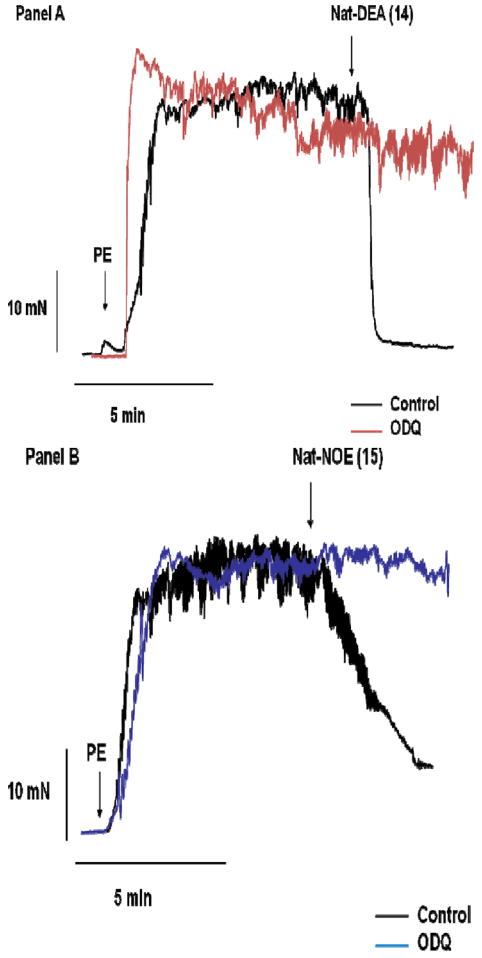

Figure 4. Vascular relaxation (panel A) by Nat-DEA (1 μM) and (panel B) by Nat-NOE (1 μM) of phenylephrine-induced tone in rat mesenteric arteries in the presence and absence of the selective inhibitor of soluble guanylyl cyclase inhibitor ODQ (10 μM).

CONCLUSIONS

A new group of hybrid NO-releasing type II antidiabetic ester prodrugs possessing a PYR, DEA, or NOE moiety attached to the carboxylic acid group of the type II oral antidiabetic drugs Nat and Meg were synthesized. In vivo biological studies indicated that this group of hybrid prodrugs (i) exhibit antihyperglycemic activity with durations of action up to six hours that compares favorably to that of the parent antidiabetic drug Nat or Meg and (ii) reduce BP_{sys}, BP_{dia}, and BP_{mean} for up to six hours postdrug administration. In vitro NO release studies showed that the PYR and DEA moieties are effective NO-donors in phosphate buffer at pH 7.4 in the presence of rat serum. The hybrid Nat-DEA prodrug **14** was identified as a potential lead-compound that (i) exhibited an appreciable reduction in the BGL (BGL_{red} in the 21.1−34.1% range) of diabetic rats up to six hours postdrug administration, (ii) showed a significant reduction in blood pressure up to three hours postdrug administration, and (iii) induced vascular relaxation of mesenteric arteries, which was blocked by ODQ, indicative of soluble guanylyl cyclase activation supporting the contention that Nat-DEA reduces blood pressure via the generation of NO. Accordingly, this new type of hybrid NO donor prodrug constitutes an attractive approach for the rational design of type II antidiabetic drugs with a reduced risk of contraindicated cardiovascular events frequently observed in diabetic individuals.

EXPERIMENTAL SECTION

General. Melting points were measured in capillaries using a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 MHz or AM-300 300 MHz NMR spectrometer using $CDCl_3$ or D_2O as solvent. Chemical shifts are given in parts per million (ppm) with tetramethylsilane (TMS) as an internal reference. Infrared (IR) spectra were recorded as films on NaCl plates using a Nicolet 550 Series II Magna FTIR spectrometer. Mass spectra (MS) were recorded on a Water's Micromass ZQ 4000 mass spectrometer using the ESI ionization mode. The purity of the compounds was established using elemental analysis, which were acquired for C, H, N by the Microanalytical Service Laboratory, Department of Chemistry, University of Alberta. Compounds showed a single spot on Macherey-Nagel Polygram Sil G/UV254 silica gel plates (0.2 mm) using a low, medium, and highly polar solvent system, and no residue remained after combustion, indicating a purity >98%. Column chromatography was performed on a Combiflash R_f system using a gold silica column. All other reagents, purchased from the Aldrich Chemical Co. (Milwaukee, WI) and TCI America, were used without further purification. Compounds **1**,[30] **2**,[26] **5**,[36] **6**,[37] **7**,[28] **8**,[29] **9**,[28] and **10**,[29] were prepared according to literature procedures.

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*(2R)-2-(((trans)-4-Isopropylcyclohexanecarboxamido)-3-phenylpropanoyl)oxy)methoxy)-1-(pyrrolidin-1-yl)diazene oxide (**13**).* The sodium salt of nateglinide was prepared in situ by stirring **1** (0.5 g, 1.57 mmol) with a suspension of Na_2CO_3 (0.166 g, 1.57 mmol) and HMPA (4.5 mL) for 19 h at 25 °C. A solution of O^2-(chloromethyl)-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**9**, 0.282 g, 1.57 mmol) in HMPA (1.5 mL) was added, and the reaction was allowed to proceed for 46 h at 25 °C. Water (15 mL) was added, the mixture was extracted with EtOAc (3 × 25 mL), the organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography using EtOAc/hexane (1:1, v/v) as eluant to furnish **13** (0.58 g, 80%) as a pale yellow viscous liquid, $[\alpha]^{21.0}_D$ = −25.39 (0.500, $CHCl_3$). IR ($CHCl_3$): 3282, 2934, 2813, 1734, 1636, 1269, 1183 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.83 [d, J = 7.3 Hz, 6H, $CH(CH_3)_2$], 0.90−0.99 (m, 3H, one cyclohexyl H-3 and one cyclohexyl H-5, cyclohexyl H-4), 1.34−1.41 (m, 3H, one cyclohexyl H-3 and one cyclohexyl H-5, $CHMe_2$), 1.72−1.83 (m, 2H, one cyclohexyl H-2 and one cyclohexyl H-6) 1.92−1.99 (m, 6H, one cyclohexyl H-2 and one cyclohexyl H-6, pyrrolidinyl H-3, H-4), 2.00−2.05 (m, 1H, cyclohexyl H-1), 3.11 and 3.17 (two dd J = 14. 1, 5.7 Hz, 1H each, CH_2Ph), 3.54−3.59 (m, 4H, pyrrolidinyl H-2, H-5), 4.91−4.94 (m, 1H, NH−CH), 5.70 (d, J = 7.32 Hz, 1H, OCH*H*'O), 5.88 (d, J = 7.32 Hz, 1H, OC*H*H'O), 5.98 (d, J = 7.8 Hz, 1H, N*H*), 7.07 (dd, J = 7.6, 1.8 Hz, 2H, *ortho*-phenyl hydrogens), 7.15−7.29 (m, 3H, *meta*- and *para*-phenyl hydrogens). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 19.6 ($CHMe_2$), 22.9 (pyrrolidinyl C-3, C-4), 28.9 and 29.2 (cyclohexyl C-2, C-3, C-5, C-6), 32.6 ($CHMe_2$), 37.3 (CH_2Ph), 43.1 and 45.3 (cyclohexyl C-4, C-1), 50.6 (pyrroldinyl C-2, C-5), 52.5 (NHCHCO), 87.9 (OCH_2O), 127.0, 128.3, and 129.3 (phenyl *C*H), 135.4 (phenyl C-1), 173.6 (C═O), 176.2 (C═O). ESI-MS: 483 $[M+Na]^+$. Anal. Calcd. for $C_{24}H_{36}N_4O_5$: C, 62.59; H, 7.88; N, 12.16. Found: C, 62.56; H, 7.93; N, 12.22.

*(3R)-3-Benzyl-10-ethyl-1-((trans)-4-isopropylcyclohexyl)-1,4-dioxo-5,7-dioxa-2,8,9,10-tetraazadodec-8-en-9-oxide (**14**).* The sodium salt of nateglinide was prepared in situ by stirring **1** (0.5 g, 1.57 mmol) with a suspension of Na_2CO_3 (0.166 g, 1.57 mmol) and HMPA (4.5 mL) for 19 h at 25 °C. A solution of O^2-(chloromethyl)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**10**, 0.284 g, 1.57 mmol) in HMPA (1.5 mL) was added, and the reaction was allowed to proceed for 29 h at 25 °C. Water (15 mL) was added and the mixture was extracted with EtOAc (3 × 15 mL). The combined EtOAc fractions were dried (Na_2SO_4), the solvent was removed in vacuo, and the residue obtained was purified by silica gel column chromatography using EtOAc/hexane (1:1, v/v) as eluent to furnish compound **14** (0.444 g, 61%) as a white solid; mp 125−126 °C; $[\alpha]^{21.0}_D$ = −13.00 (0.500, $CHCl_3$). IR ($CHCl_3$): 3263, 2916, 2835, 1740, 1682, 1242, 1136 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ 0.86 [d, J = 6.6 Hz, 6H, $CH(CH_3)_2$], 0.98−1.08 (m, 3H, one cyclohexyl H-3, one cyclohexyl H-5, cyclohexyl H-4), 1.13 (t, J = 7.2 Hz, 6H, two CH_2CH_3), 1.37−1.42 (m, 3H, one cyclohexyl H-3, one cyclohexyl-5, $CHMe_2$), 1.77−1.79 (m, 2H, one cyclohexyl H-2, one cyclohexyl H-6), 1.83−1.90 (m, 2H, one cyclohexyl H-2, one cyclohexyl H-6), 1.99−2.01 (m, 1H, cyclohexyl H-1), 3.11 and 3.21 (dd, J = 14.1, 5.7 Hz, 1H each, CH_2Ph), 3.26 (q, J = 7.2 Hz, 4H, two NCH_2), 4.94−4.98 (m, 1H, NH−CH), 5.81 (d, J = 7.2 Hz, 1H, OCH*H*'O), 5.83 (d, J = 7.2 Hz, 1H, OC*H*H'O), 5.98 (d, J = 6.6 Hz, 1H, N*H*), 7.10 (dd, J = 8.4, 1.8 Hz, 2H, *ortho*-phenyl hydrogens), 7.25−7.30 (m, 3H, *meta*- and *para*-phenyl hydrogens); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 11.4 (CH_2CH_3), 19.7 ($CHMe_2$), 28.9 and 29.4 (cyclohexyl C-2, C-3, C-5, C-6), 32.7 ($CHMe_2$), 37.3 (CH_2Ph), 43.1 and 45.4 (cyclohexyl C-4, C-1), 47.9 (CH_2CH_3), 52.4 (NHCHCO), 87.9 (OCH_2O), 127.2, 128.6, and 129.4 (phenyl *C*H), 135.3 (phenyl C-1), 170.4 (C═O), 175.7 (C═O). ESI-MS: 463 $[M + H]^+$. Anal. Calcd. for $C_{24}H_{38}N_4O_5$: C, 62.31; H, 8.28; N, 12.11. Found: C, 62.39; H, 8.35; N, 12.19.

*(2R)-2-Nitrooxyethyl 2-((trans)-4-isopropylcyclohexanecarboxamido)-3-phenylpropanoate (**15**).* A solution of 2-nitrooxyethyl bromide (204 mg, 1.2 mmol), nateglinide (317 mg, 1 mmol) and K_2CO_3 (310 mg, 1.5 mmol) in dry DMF (5 mL) was stirred at 25 °C for 24 h. Water (15 mL) was added, the mixture was extracted with EtOAc (3 × 20 mL), and the EtOAc extracts were washed with brine (15 mL). The organic phase was dried (Na_2SO_4), the solvent was removed in vacuo, and the residue obtained was purified by silica gel chromatography using ethyl acetate−hexane (1:1, v/v) as eluent to furnish compound **15** (0.337 g, 83%) as a light brown solid; mp 114−115 °C; $[\alpha]^{21.0}_D$ = −32.99 (0.500, $CHCl_3$). IR ($CHCl_3$): 3279, 2944, 2822, 1745, 1682, 1621, 1280 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz): δ 0.77 [d, J = 6.6 Hz, 6H, $CH(CH_3)_2$], 0.88−0.95 (m, 3H, one cyclohexyl H-3, one cyclohexyl H-5, cyclohexyl H-4), 1.30−1.34 (m, 3H, one cyclohexyl H-3, one cyclohexyl-5, $CHMe_2$), 1.69−1.71 (m, 2H, one cyclohexyl H-2, one cyclohexyl H-6), 1.72−1.80 (m, 2H, one cyclohexyl H-2, one cyclohexyl H-6), 1.94−1.99 (m, 1H, cyclohexyl H-1), 3.04−3.06 (m, 2H, CH_2Ph), 4.26−4.35 (m, 2H, CO_2CH_2), 4.51−4.53 (m, 2H, CH_2ONO_2), 4.80−4.83 (m, 1H, NH−CH), 5.77 (d, J = 7.8 Hz, 1H, N*H*), 7.05 (dd, J = 8.4, 1.8 Hz, 2H, *ortho*-phenyl hydrogens), 7.15−7.23 (m, 3H, *meta*- and *para*-phenyl hydrogens). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 19.7 ($CHMe_2$), 29.4 and 29.7 (cyclohexyl C-2, C-3, C-5, C-6), 32.7 (CHMe), 37.8 (CH_2Ph), 43.2 and 45.4 (cyclohexyl C-4, C-1), 53.2 (NHCHCO), 60.8 (CO_2CH_2), 70.0 (CH_2ONO_2), 127.3, 128.7, and 129.1 (phenyl *C*H), 135.6 (phenyl C-1), 171.5 (C═O), 175.7 (C═O). ESI-MS: 407 $[M + H]^+$. Anal. Calcd. for $C_{21}H_{30}N_2O_6$: C, 62.05; H, 7.44; N, 6.84. Found: C, 62.13; H, 7.40; N, 6.84.

*2-(((4-(2-(5-Chloro-2-methoxybenzamido)ethyl)benzoyl)oxy)-methoxy)-1-(pyrrolidin-1-yl)diazene oxide (**18**).* The sodium salt of meglitinide was prepared in situ by stirring meglitinide (**2**, 0.334 g, 1 mmol) in a suspension of Na_2CO_3 (0.105 g, 1 mmol) and HMPA (4.5 mL) for 19 h at 25 °C. A solution of O^2-(chloromethyl)-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**9**, 0.216 g, 1.2 mmol) in HMPA (1.5 mL) was added, and the reaction was allowed to proceed for 14 h at 25 °C. Water (15 mL) was added to the reaction mixture, which was extracted with EtOAc (3 × 25 mL). The organic phase was dried (Na_2SO_4), the solvent was removed in vacuo, and the residue obtained was purified by silica gel column chromatography using EtOAc/hexane (1:1, v/v) as eluent to furnish compound **18** (0.372 g, 78%) as a white solid; mp 110−111 °C. IR ($CHCl_3$): 3280, 2987, 2846, 1710, 1612, 1255, 1145 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz): δ 1.95−197 (m, 4H, two pyrrolidinyl H-3, H-4), 3.01 (t, J = 6.9 Hz, 2H, $NHCH_2CH_2$), 3.57−3.61 (m, 4H, pyrrolidinyl H-2, H-5), 3.76 (t, J = 6.6 Hz, 2H, $NHCH_2$), 3.79 (s, 3H, OCH_3); 6.02 (s, 1H, OCH_2O), 6.88 (d, J = 9.0 Hz, 1H, MeO-phenyl H-3), 7.35−7.39 (m, 3H, benzoate H-3, H-5, Cl-phenyl H-4), 7.84 (broad s, 1H, NH), 8.05 (d, J = 8.4 Hz, 2H, benzoate H-2, H-6), 8.17 (d, J = 2.4 Hz, 1H, Cl-phenyl *H*-6). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 22.9 (pyrrolidinyl C-3, C-4), 35.6 ($NHCH_2CH_2$), 40.6 ($NHCH_2$), 50.7 (pyrrolidinyl C-2, C-5), 56.2 (OCH_3), 87.9 (OCH_2O), 112.8 (MeO-phenyl C-3), 122.7 (MeO-phenyl C-1), 126.7 (Cl-phenyl C-5), 127.6 (benzoate C-1), 129.0, 130.3, 131.9, and 132.3 (Cl-phenyl C-6 and C-4, benzoate C-2, C-3, C-5, C-6), 145.5 (benzoate C-4), 155.9 (MeO-phenyl C-2), 164.0 (C═O), 165.0 (C═O). ESI-MS: 477, 479 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{25}ClN_4O_6$: C, 55.40; H, 5.20; N, 11.75. Found: C, 55.40; H, 5.33; N, 11.49.

*1-(((4-(2-(5-Chloro-2-methoxybenzamido)ethyl)benzoyl)oxy)-methoxy)-3,3-diethyltriaz-1-ene 2-oxide (**19**).* The sodium salt of meglitinide was prepared in situ by stirring **2** (0.334 g, 1 mmol) with a suspension of Na_2CO_3 (0.105 g, 1 mmol) in HMPA (4.5 mL) for 19 h at 25 °C. A solution of O^2-(chloromethyl)-1-(*N*,*N*-diethylamino)-diazen-1-ium-1,2-diolate (**10**, 0.218 g, 1.2 mmol) in HMPA (1.5 mL) was then added, and the reaction was allowed to proceed for 12 h at 25 °C. Water (15 mL) was added, the mixture was extracted with EtOAc (3 × 25 mL), and the combined EtOAc extracts were dried (Na_2SO_4). Removal of the solvent in vacuo gave a residue that was purified by silica gel column chromatography using EtOAc/hexane (1:1, v/v) to afford **19** (0.335 g, 70%) as a white solid; mp 90−92 °C. IR ($CHCl_3$): 3199, 2977, 2854, 1725, 1622, 1252, 1132 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz): δ 1.02 (t, J = 7.2 Hz, 6H, two CH_2CH_3), 2.92 (t, J = 6.9 Hz, 2H, $NHCH_2CH_2$), 3.12 (q, J = 7.2 Hz, 4H, NCH_2CH_3), 3.68 (t, J = 6.0 Hz, 2H, $NHCH_2CH_2$), 3.70 (s, 3H, OCH_3); 6.0 (s, 1H, OCH_2O), 6.79 (d, J = 9.0 Hz, 1H, MeO-phenyl H-3), 7.26−7.31 (m, 3H, benzoate H-3, H-5, Cl-phenyl H-4), 7.74 (broad s, 1H, NH), 7.95 (d, J = 8.4 Hz, 2H, benzoate H-2, H-6), 8.09 (d, J = 2.4 Hz, 1H, Cl-

phenyl H-6). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 11.3 (CH_2CH_3), 35.6 ($NHCH_2CH_2$), 40.5 ($NHCH_2CH_2$), 48.1 (CH_2CH_3), 56.1 (OCH_3), 87.8 (OCH_2O), 112.7 (MeO-phenyl C-3), 122.6 (MeO-phenyl C-1), 126.7 (Cl-phenyl C-5), 127.3 (benzoate C-1), 129.0, 130.2, 131.9, and 132.3 (Cl-phenyl C-6 and C-4, benzoate C-2, C-3, C-5, C-6), 145.6 (benzoate C-4), 155.8 (MeO-phenyl C-2), 163.9 (C=O), 164.7 (C=O). ESI-MS: 479, 481 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{27}ClN_4O_6$: C, 55.17; H, 5.68; N, 11.70. Found: C, 55.20; H, 5.68; N, 11.76

*2-Nitrooxyethyl 4-(2-(5-chloro-2-methoxybenzamido)ethyl)-benzoate (**20**).* A solution of 2-nitooxyethyl bromide (204 mg, 1.2 mmol), meglitinide (**2**, 333 mg, 1 mmol) and K_2CO_3 (310 mg, 1.5 mmol) in dry DMF (5 mL) was stirred at 25 °C for 12 h. Water (15 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL) and washed with brine (20 mL). The EtOAc fraction was dried (Na_2SO_4), the solvent was removed in vacuo, and the residue obtained was purified by silica gel column chromatography using ethyl acetate−hexane (1:1, v/v) as eluent to furnish compound **20** (0.359 g, 85%) as a cream colored solid; mp 100−102 °C. IR ($CHCl_3$): 3269, 2955, 2823, 1758, 1649, 1619, 1282 cm^{-1}. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 3.01 (t, J = 6.9 Hz, 2H, $NHCH_2CH_2$), 3.77 (t, J = 6.9 Hz, 2H, $NHCH_2$), 3.79 (s, 3H, OCH_3); 4.62−4.63 (m, 2H, CO_2CH_2), 4.82−4.84 (m, 2H, CH_2ONO_2), 6.88 (d, J = 8.4 Hz, 1H, MeO-phenyl H-3), 7.36−7.39 (m, 3H, benzoate H-3, H-5, Cl-phenyl H-4), 7.83 (broad s, 1H, NH), 8.01−8.03 (d, J = 8.4 Hz, 2H, benzoate H-2, H-6), 8.19 (d, J = 2.4 Hz, 1H, Cl-phenyl H-6). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 35.6 ($NHCH_2CH_2$), 40.5 ($NHCH_2$), 56.1 (OCH_3), 60.7 (CO_2CH_2), 70.4 (CH_2ONO_2), 112.7 (MeO-phenyl C-3), 122.6 (MeO-phenyl C-1), 126.6 (Cl-phenyl C-5), 127.5 (benzoate C-1), 128.9, 129.9, 131.9, and 132.3 (Cl-phenyl C-6 and C-4, benzoate C-2, C-3, C-5, C-6), 145.3 (benzoate C-4), 155.8 (MeO-phenyl C-2), 163.9 (C=O), 165.9 (C=O). ESI-MS: 423, 425 $[M + H]^+$. Anal. Calcd. for $C_{19}H_{19}ClN_2O_7$: C, 53.97; H, 4.53; N, 6.63. Found: C, 53.86; H, 4.49; N, 6.53.

Animals. Male Sprague−Dawley rats, 300−350 g in weight purchased from the University of Alberta Health Science Animal Services Facility, were employed in the streptozotocin-induced diabetic rat model to determine antihyperglycemic activity. Antihypertensive blood pressure measurement studies were carried out using male and female C57BL/6 mice, aged 3−5 months and weighing 22−33 g, purchased from the Charles River Animal Facility. All animal experiments were conducted in accordance with guidelines established by Health Science Laboratory Animal Services (HSLAS), University of Alberta.

Antihyperglycemic Studies. Male Sprague−Dawley rats, 300−350 g in weight, were employed for the determination of antihyperglycemic activity in a streptozotocin-induced diabetic rat model. Streptozotocin was dissolved in citrate buffer (pH 4.5), and a calculated amount of the fresh solution (65 mg/kg ip dose) was injected intraperitoneally into nonfasting rats. Blood glucose was measured after 48 h using glucostrips (One Touch Ultra 2 test strips), and animals showing a blood glucose level within the range of 20−30 mmol/L, which are considered to be diabetic, were selected for determination of antihyperglycemic activity. A solution of the test compound (one of **13**−**15**, **18**−**20**, nateglinide, and meglitinide) dissolved in water containing 1% methyl cellulose or a control solution (water +1% methyl cellulose) not containing the test compound was administered orally to diabetic rats. The reference drugs nateglinide (**1**), meglitinide (**2**) and the test compounds **13**−**15** and **18**−**20** were administered at the same equimolar dose of 63.01 μmol/kg po. Following administration of the experimental drug or control vehicle a blood sample was collected by puncture of the tail vein, and the blood glucose level was measured at 1, 3, 6, and 24 h postdrug administration using a One Touch Ultra 2 Blood Glucose Meter.

In Vivo Blood Pressure Measurement. Noninvasive blood pressure and heart rate measurements were performed according to a protocol approved by the Health Sciences Animal Welfare Committee at the University of Alberta (Edmonton, Canada). Following oral administration of each test compound (63.01 μmol/kg po dose) dissolved in water containing 1% methyl cellulose, changes in BP_{sys}, BP_{dia}, BP_{mean}, and HR in C57 black mice were measured in a conscious state at 1, 3, and 6 h time intervals using our previously reported method.[38]

Nitric Oxide Release Assay. In vitro nitric oxide release was estimated by quantification of nitrite produced by the reaction of nitric oxide with oxygen and water using the Griess reaction upon incubation of the test compound (2.4 mL of 5.0 × 10^{-2} mM) in phosphate buffer solution (PBS) at pH 7.4 (incubation times of 1, 4, 6, and 24 h) and in 2.4 mL of PBS to which 90 μL of serum was added (incubations times of 1, 3, 6, and 24 h) at 37 °C. The amount of nitric oxide released at each time interval was determined for test compounds **13**−**15** and **18**−**20** using the reported procedure.[39]

Vascular Relaxation (Wire Myography). Third order branches of the rat superior mesenteric artery were mounted in a Mulvany-Halpern myograph (model 400A, J.P. Trading, Denmark) for recording of changes in isometric tension as previously described.[40] The endothelial cell layer was removed by gently rubbing the intimal surface of the arteries with a hair prior to mounting. Successful removal of the endothelium was confirmed by the absence of relaxation to acetylcholine (10 μM).

The ability of Nat-DEA (1 μM) and Nat-NOE (1 μM) to cause vascular relaxation via activation of soluble guanylyl cyclase was assessed by adding the drugs to tissues in which tone was raised to 75% of the maximal by phenylephrine (1−3 μM) in the absence and presence of 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 10 μM).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

In vivo antihyperglycemic activity for compounds **13**−**15** and **18**−**20**, and the reference drugs nateglinide and meglitinide; and blood pressure (mm Hg) and heart rate (beats min^{-1}) data at 1, 3, and 6 h postdrug administration. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

NO, nitric oxide; PGI_2, prostacyclin; Nat-PYR, (2*R*)-2-(((*trans*)-4-isopropylcyclohexanecarboxamido)-3-phenylpropanoyl)oxy)methoxy)-1-(pyrrolidin-1-yl)diazene oxide; Nat-DEA, (3*R*)-3-benzyl-10-ethyl-1-((*trans*)-4-isopropylcyclohexyl)-1,4-dioxo-5,7-dioxa-2,8,9,10-tetraazadodec-8-en-9-oxide; Nat-NOE, (2*R*)-2-nitrooxyethyl 2-((*trans*)-4-isopropylcyclohexanecarboxamido)-3-phenylpropanoate; Meg-PYR, 2-(((4-(2-(5-chloro-2-methoxybenzamido)ethyl)benzoyl)oxy)methoxy)-1-(pyrrolidin-1-yl)diazene oxide; Meg-DEA, 1-(((4-(2-(5-chloro-2-methoxybenzamido)ethyl)benzoyl)oxy)methoxy)-3,3-diethyltriaz-1-ene 2-oxide; Meg-NOE, 2-nitrooxyethyl 4-(2-(5-chloro-2-methoxybenzamido)ethyl)benzoate; BGL, blood glucose level; BGL_{red}, reduction in blood glucose level; BP_{sys}, systolic blood pressure; BP_{dia}, diastolic blood pressure; BP_{mean}, mean blood pressure; HR, heart rate

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