Bioorganic & Medicinal Chemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and evaluation of multi-functional NO-donor/insulin-secretagogue derivatives for the treatment of type II diabetes and its cardiovascular complications

Maria Digiacomo [†], Alma Martelli [†], Lara Testai [†], Annalina Lapucci, Maria C. Breschi, Vincenzo Calderone [*], Simona Rapposelli [*]

Dipartimento di Farmacia, Università di Pisa, Via Bonanno, 6, 56126 Pisa, Italy

ARTICLE INFO

Article history:
Received 4 September 2014
Revised 12 December 2014
Accepted 17 December 2014
Available online xxxx

Keywords:
Hybrid drugs
NO-releasing drugs
NO-donor
Insulin-secretagogue drug
Hypoglycemic
Cardioprotection

ABSTRACT

Although there is a significant effort in the discovery of effective therapies to contrast both the pathological endocrine and metabolic aspects of diabetes and the endothelial dysfunction associated with this disease, no hypoglycemic drug has been proven to defeat the cardiovascular complications associated with type II diabetes. The aim of this research was to design new compounds exhibiting a double profile of hypoglycemic agents/NO-donors. The synthesis of molecules obtained by the conjunction of NO-donor moieties with two oral insulin-secretagogue drugs (repaglinide and nateglinide) was reported. NO-mediated vasorelaxing effects of the synthesized compounds were evaluated by functional tests on isolated endothelium-denuded rat aortic rings. The most potent molecule (**4**) was tested to evaluate the hypoglycemic and the anti-ischemic cardioprotective activities. This study indicates that **4** should represent a new insulin-secretagogue/NO-donor prodrug with an enhanced cardiovascular activity, which may contrast the pathological aspects of diabetes and endowed of cardioprotective activity.

1. Introduction

Type II diabetes mellitus is a multifactorial disease characterized by a combination of insulin resistance and reduced insulin secretion. The impact of this disease on social health is closely linked to the co-existence of both metabolic and cardiovascular disorders.[1] It is well-known that the endothelial dysfunction is responsible for important macro-vascular problems such as myocardial ischemia, hypertension and peripheral vasculopathy. Lately, heart diseases and stroke are the main causes of death and disability among people with type 2 diabetes.[2]

Structural and functional alterations of vascular structures, such as the glycation of wall components of blood vessels, are strongly involved in the pathogenesis of cardiovascular complications associated with diabetes. One of the major vascular alterations is the endothelial dysfunction, resulting in a relevant imbalance in the production of endothelium-derived endogenous factors pivotally involved in the regulation of the cardiovascular function. In particular, it is well known that diabetes is associated with a significant reduction in the biosynthesis and release of endothelial nitric oxide (NO).[3]

NO is an important endothelium-derived mediator endowed of vasodilator, anti-platelet, anti-proliferative, permeability-decreasing and anti-inflammatory properties.[4] An impairment of endothelium-dependent vasorelaxation caused by a reduced NO activity, worsens the diabetic metabolic alterations (dyslipidaemia, glycation end-products, oxidative stress), thus resulting in a dramatically prevalence of atherosclerosis, thrombosis, vascular inflammation and remodeling, hypertension, coronaropathy and stroke.[5,6]

Therefore, in order to reduce the cardiovascular risk, diabetic patients usually follow additional pharmacological treatments targeting hypertension, platelet aggregation, and dyslipidaemia.[6,7]

In the last years, the development of new 'chimeras', with the double pharmacodynamic profile of hypoglycemic agents and, at the same time, of slow NO donors, has been first reported by us and then widely investigated.[8–10] NO-sulfonylureas and NO-meglitinides[2,8,11] are interesting examples of drugs able to contrast both the endocrine and the cardiovascular complications of diabetes mellitus. These 'chimeric drugs' conserved the antidiabetic properties, due to their insulin-secretagogue activity; moreover, they were endowed of additional NO-releasing property. Such an

* Corresponding authors. Tel.: +39 050 2219589; fax: +39 050 2219577 (V. Calderone); tel.: +39 050 2219582; fax: +39 050 2219577 (S. Rapposelli).
E-mail addresses: calderone@farm.unipi.it (V. Calderone), simona.rapposelli@farm.unipi.it (S. Rapposelli).
[†] These authors contributed equally.

http://dx.doi.org/10.1016/j.bmc.2014.12.043
0968-0896/

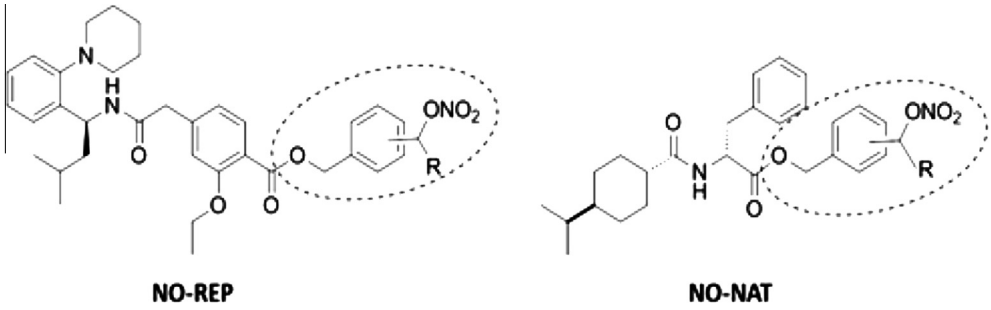


Figure 1. General structures of multifunctional NO-donor/insulin secretagogue derivatives NO-Rep and NO-Nat.

ability to release exogenous NO was considered as an intriguing pharmacological approach, aimed at counterbalancing the reduced availability of endogenous NO in the diabetic patients, and thus at attenuating the diabetes-associated cardiovascular complications. Indeed, the meglitinides repaglinide (REP) and nateglinide (NAT) are non-sulfonylurea insulin secretagogue drugs, effective in treating type 2 diabetes.[12] Like sulfonylureas, these drugs stimulate insulin secretion by blocking the ATP-sensitive potassium channels (KATP) in pancreatic β cells, thus improving overall glycemic control. REP and NAT inhibit also KATP channels of cardiomyocytes, and vascular smooth muscle cells,[13,14] and this may contribute to the onset of cardiovascular complications.[15]

As a further development of our work on the design of new NO-donor antidiabetics,[8] we describe a new class of NO-donor hybrids obtained by coupling REP and NAT with appropriate NO-releasing moieties,[16] aiming at improving the pharmacological profile (Fig. 1). In particular, this paper describes the synthesis of new multifunctional insulin-secretagogue/NO-donor derivatives and the evaluation of both the NO-mediated vasorelaxing effects on isolated rat aortic rings and the in vivo hypoglycemic properties. Moreover, the cardioprotective and hypoglycemic activities of the most active compound **4** (namely, NO-NAT) were evaluated.

2. Results and discussion

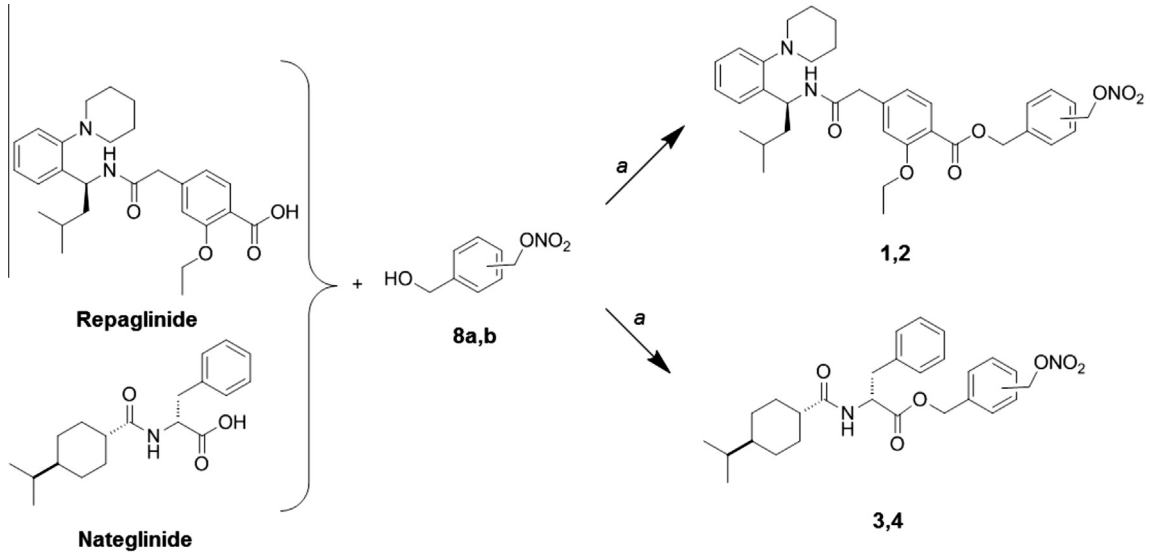
2.1. Chemistry

The new compounds were prepared by the coupling of the carboxylic function of REP and NAT with a NO-donor moiety through an in vivo hydrolysable ester bond. The NO-releasing groups involved in this study have already been used for the synthesis of other class of NO-donor drugs.[16] In particular 3-nitrooxymethylbenzyl alcohol-(**8a**), 4-nitrooxymethylbenzyl alcohol-(**8b**), 3-[1-(nitrooxy)ethyl]benzyl alcohol, and 4-[1-(nitrooxy)ethyl]benzyl alcohol were chosen as linker groups because of their different NO-releasing rate.[16] The NO donor-meglitinide derivatives NO-REP (**1**,**2**) and NO-NAT (**3**,**4**), were synthesized by condensation of REP or NAT, respectively, with the appropriate nitrooxymethylbenzyl alcohol (**8a**,**b**)[17] in the presence of DCC and a catalytic amount of DMAP in DCM (Scheme 1). The *meta*- and *para*-[(1-nitrooxy)ethyl]benzyloxy derivatives **5** and **6** were prepared, as shown in Scheme 2. The 3-(1-hydroxyethyl)benzyl alcohol (**9a**) or 4-(1-hydroxyethyl)benzyl alcohol (**9b**), obtained by reduction of the appropriate acetyl benzoic acid, was condensed with REP in the presence of DCC and a catalytic amount of DMAP affording the product (**10**,**11**). The subsequent reaction of the alcohol **10**,**11** with HCl in toluene gave the corresponding chloride and the nitration with $AgNO_3$ afforded to the products **5** and **6**. Compound **7** was obtained starting from NAT and following the same synthetic procedure described above for compounds **5** and **6**.

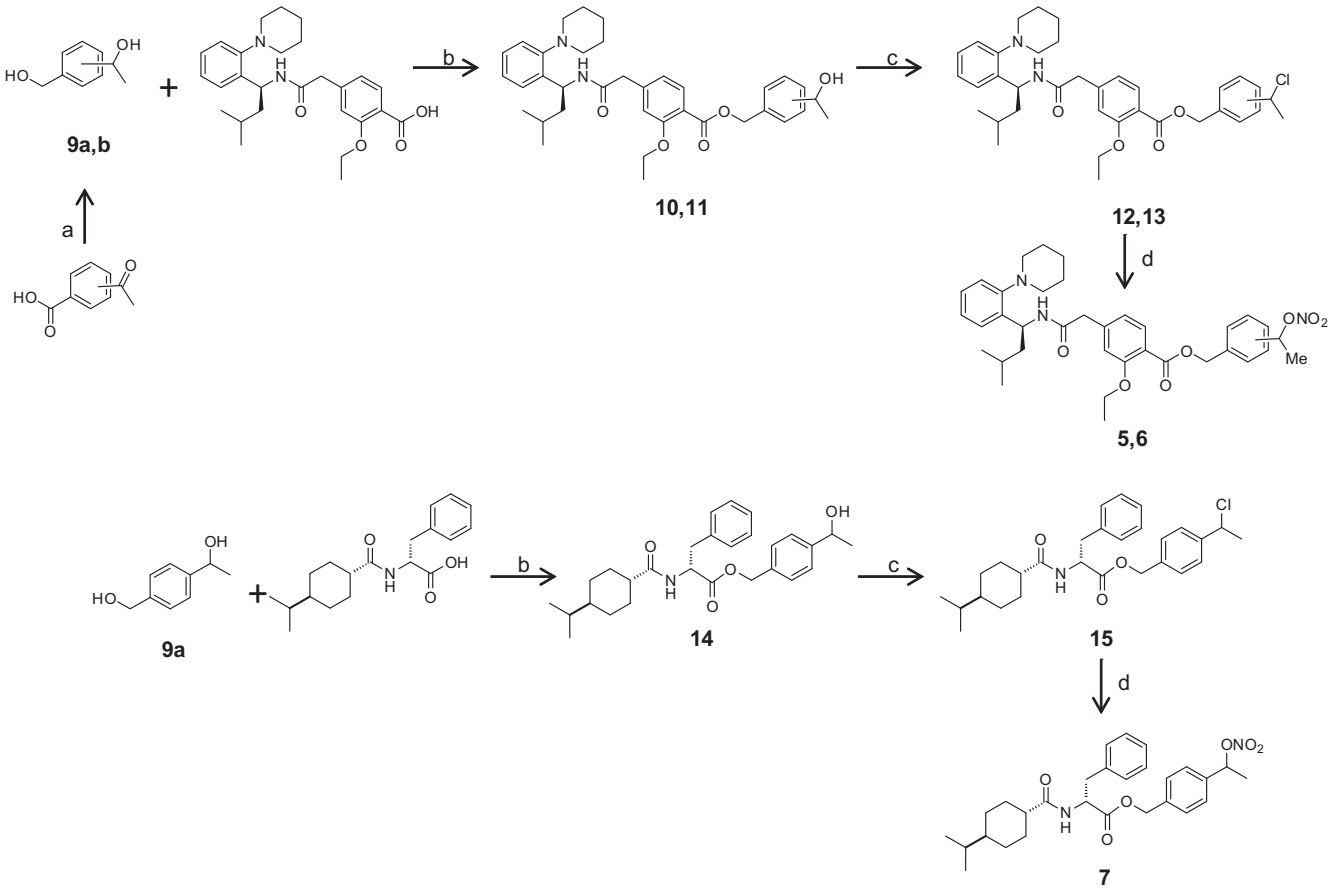
2.2. Pharmacology

2.2.1. Evaluation of NO-releasing properties

The prodrugs **2** and **4** induced almost full vasorelaxing effects (E_{max} = 94 ± 4 and 93 ± 1, for **2** and **4**, respectively; Table 1) which were strongly inhibited by 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) 1 μM, an inhibitor of guanylate cyclase, as expected in



Scheme 1. Synthetic route for the preparation of the NO-releasing/insulin secretagogues hybrids **1**–**4**. Reagents and conditions: (a) DCC, DMAP, DCM, 3 h, rt.



Scheme 2. Synthetic route for the preparation of the NO-releasing/meglitinides hybrids **5**–**7**. Reagents and conditions: (a) $LiAlH_4$ 1 M, THF, 0 °C (yield >90%); (b) DCC, DMAP, DCM, 3 h, rt (yield >90%); (c) HCl_{concd}, 20 h, rt (yield: 60–80%); (d) $AgNO_3$, MeCN, 4 h, rt (yield 16–25%).

Table 1
The NO-mediated vasorelaxing efficacy (E_{max}%) and potency values (pIC_{50}) of **1**–**7**

Compd	pIC_{50}	E_{max}
1	4.88 ± 0.03	79 ± 4
2	5.09 ± 0.03	94 ± 1
3	n.c.[a]	45 ± 2
4	5.30 ± 0.04	93 ± 4
5	n.c.[a]	26 ± 6
6	5.30 ± 0.04	84 ± 3
7	4.63 ± 0.03	58 ± 6

The parameters of efficacy and potency are expressed as mean ± SEM.
[a] The pIC_{50} value was not calculable (n.c.) because of the low vasorelaxing efficacy (lower than 50%).

the case of an NO-mediated effect. The shifting of the nitrooxymethyl-group from the *para* (**2**,**4**) to the *meta* position (**1**,**3**) in both NAT and REP derivatives reduced the vasorelaxing efficacy; such a reduction is more marked in the NAT-derivatives with respect to the REP-derivatives (Table 1). The same effect has been also observed for **5**–**7** compounds in which a methyl group has been added at the *alpha*-position of nitrooxy-group.

2.2.2. Evaluation of hypoglycemic properties

Since the essential issue for an insulin-secretagogue/NO-donor hybrid is the retention of its hypoglycemic activity, we selected the NO-NAT derivative **4**, which showed the best vasorelaxing profile, to further investigate its activity on the glucose metabolism. As shown in Figure 2, when compared to the normoglycemic animals, the diabetic rats showed a significantly higher increase of glycemic levels, following the administration of glucose. The glycemic pattern shown by diabetic animals submitted to acute pre-treatment with NAT or with an equivalent dose of **4** was almost superimposable to that exhibited by normoglycemic animals, indicating that NAT and the hybrid compound **4** are endowed with almost equivalent insulin-secretagogue activity (Fig. 2).

2.2.3. Cardio-protective anti-ischemic effects

In order to evaluate if the new multifunctional insulin-secretagogue/NO-donor derivative **4** was provided of additional beneficial cardiovascular activity, the effect induced by **4** on Langendorff-perfused diabetic rat hearts subjected to an ischemia-reperfusion cycle was evaluated. As indicated in Figure 3, the ischemia/reperfusion injury in the hearts from diabetic rats (decrease of RPP and presence of ischemic areas) was almost equivalent to that observed in hearts from normoglycemic animals. Pre-treatment with nateglinide determined a slight and not significant improvement of the parameters. Pre-treatment with **4** promoted a cardio-protective effect, determining an evident (albeit not statistically significant) reduction of the ischemic areas and a marked and a statistically significant (P <0.01) improvement of the post-ischemic recovery of functional parameter (RPP).

3. Conclusion

In the last decade, the development of 'chimeric drugs' has been one of the most exciting fields of investigation. The addition of NO-releasing properties has been a widely used strategy in the design of such 'chimeras', in order to improve the overall

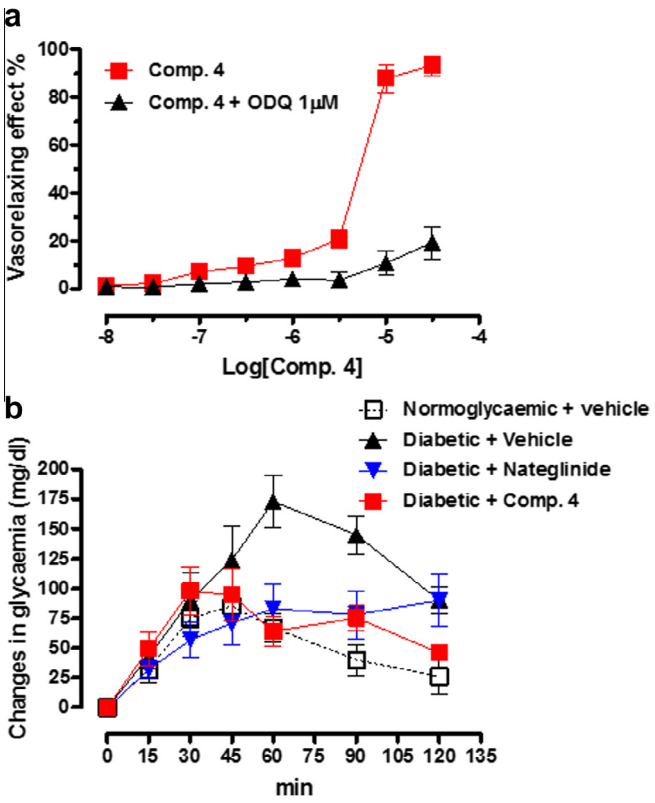


Figure 2. (a) Vasorelaxing effect (%) of **4** alone and in the presence of ODQ. (b) Hypoglycemic effects of **4**. Non-fasting glycemic levels recorded in normoglycemic animals and in streptozotocin-induced diabetic rats after intraperitoneal administration of Nateglinide (50 mg/kg, ip), or an equimolar dose of **4** (75.8 mg/kg ip) or vehicle.

pharmacotherapeutic impact of a given drug and/or to reduce the adverse effects.[9,10] Since the reduced biosynthesis of endogenous NO is a typical feature in diabetes (leading to the well-known diabetes-associated cardiovascular complications),[1] the addition of NO-releasing properties to antidiabetic agents seems to be a winning strategy for projecting 'chimeric' drugs able to target both the metabolic dysfunction and, at the same time, the vascular complications.[2,8] Indeed, this work reported effective NO-antidiabetic molecules, combining the hypoglycemic activity of meglitinides and NO-mediated cardiovascular properties, due to the conjugation of the insulin secretagogue drugs NAT and REP with opportunely spaced nitrooxy function, which is one of the most versatile and reliable NO-releasing moiety. In particular, the NO-NAT derivative **4**, selected as the lead compound in this experimental work, was endowed of satisfactory hypoglycemic effects, comparable to those exhibited by the 'parent drug' NAT. Moreover, the presence of the NO-releasing feature conferred on this dual drug vasorelaxing and cardioprotective effects, which seem to be an extremely useful improvement of the pharmacological profile. Besides the improved cardiovascular profile, it is noteworthy that the risk of excessive hypoglycaemia (i.e., the typical side-effect of insulin-secretagogues) is likely to be lower in meglitinides than in sulfonylureas.[18] However, no specific test has been carried out in this study. The evaluation of the possible toxicity of the new NO-meglitinides reported in this study, as well as the investigation of further NO-mediated useful effects, such as the antiplatelet and the antihypertensive ones, will be the object of future experimental work.

4. Experimental section

4.1. Chemistry

^{1}H NMR spectra of all compounds were obtained with a Gemini 200 spectrometer operating at 200 MHz (for compounds **6**, **9**–**15**) or with a Bruker TopSpin 3.2 400 MHz spectrometer (for compounds **1**–**5**, **7**). ^{13}C NMR spectra were fully decoupled. The following abbreviations are used: singlet (s), doublet (d), triplet (t), multiplet (m) and broad signal (br s). Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Analytical TLCs were carried out on 0.25 mm layer silica gel plates containing a fluorescent indicator; spots were detected under UV light (254 nm). Column

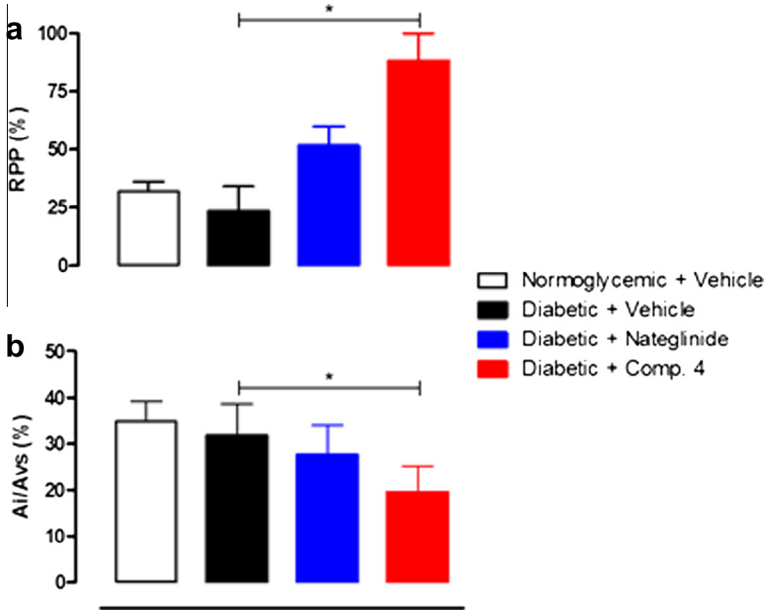


Figure 3. (a) Functional recovery (RPP %) and (b) morphological evidence of myocardial injury (Ai/Avs %) recorded in Langendorff-perfused hearts exposed to ischemia-reperfusion. The hearts were isolated from normoglycemic and diabetic rats, submitted to different pharmacological treatments. The data are expressed as mean ± SEM.

Please cite this article in press as: Digiacomo, M.; et al. *Bioorg. Med. Chem.* (2015), http://dx.doi.org/10.1016/j.bmc.2014.12.043

chromatography was performed using 70–230 mesh silica gel. Infrared (IR) spectra were obtained using a Agilent 660 with ATR Pike spectrophotometer. Data are presented as frequency of absorption (cm^{-1}). Mass spectra were obtained on a Hewlett-Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. Evaporation was performed in vacuo (rotating evaporator); sodium sulfate was always used as drying agent. Elemental analyses were performed in our analytical laboratory and agreed with the theoretical values to within ±0.4%.

4.1.1. 3-Nitrooxymethyl-benzoate of repaglinide 1

A solution of repaglinide (300 mg, 0.66 mmol), 3-(nitrooxymethyl)-benzylalcohol **8a** (121 mg, 0.66 mmol), DCC (164 mg, 0.79 mmol) and DMAP (7 mg) in CH_2Cl_2 (13 mL) was stirred at room temperature for 3 h. The suspension was then filtered off and the solvent evaporated to give a crude residue which was purified by column chromatography eluting with hexane/AcOEt (7:3). The solid obtained was then mashed with hexane provided compound **1** (162 mg, 0.26 mmol, 39%). ^{1}H NMR ($CDCl_3$): δ 0.91 (d, 6H, J = 4.0 Hz, CH_3), 1.37 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 1.49–1.70 (m, 9H, CH_2, CH), 2.54–2.65 (m, 2H, CH_2N), 2.86–2.97 (m, 2H, CH_2N), 3.53 (s, 2H, CH_2CONH), 3.94–4.07 (m, 2H, OCH_2CH_3), 5.30–5.38 (m, 1H, CHNH), 5.34 (s, 2H, CH_2O), 5.43 (s, 2H, CH_2ONO_2), 6.69 (d, 1H, J = 8.6 Hz, Ar), 6.78–6.86 (m, 2H, Ar), 7.05–7.07 (m, 2H, Ar), 7.16–7.19 (m, 2H, Ar), 7.37–7.48 (m, 4H, Ar), 7.78 (d, 1H, J = 7.7 Hz, NH). ^{13}C NMR ($CDCl_3$): δ 14.73 (CH_3), 22.62 (CH_3), 22.86 (CH), 24.22 (CH_2), 25.43 (CH_2, pip), 26.84 (CH_2, pip), 44.32 (CH_2CO), 46.77 (CH_2O), 49.90 (CHNH), 64.60 (CH_2N, pip), 66.02 (OCH_2), 74.64 ($CHONO_2$), {113.91, 118.70, 120.92, 122.90, 125.17, 127.48, 128.03, 128.65, 128.78, 129.15, 129.23, 132.44, 132.61, 137.25, 138.75, 141.70, 152.61, 159.12} (Ar), 166.03 (COO), 168.81 (CONH) ppm. Anal. ($C_{35}H_{43}N_3O_7$) C, H, N Calcd: 68.07 (C); 6.97 (H); 6.81 (N). Found: 68.28 (C); 7.23 (H); 6.77 (N).

4.1.2. 4-Nitrooxymethyl-benzoate of repaglinide 2

The repaglinide 4-(nitrooxymethyl)benzoate was synthesized from 4-(nitrooxymethyl)benzylalcohol **8b** (121 mg, 0.66 mmol) following the same procedure described above for the preparation of the repaglinide 3-(nitrooxymethyl)benzoate (**1**). This compound was purified by column chromatography eluting with hexane/AcOEt (7:3) and then by precipitation with *n*-hexane from AcOEt to give compound **2** (154 mg, 0.25 mmol, 38%). FTIR (neat) ν 3273 (NH), 2931 (Ar), 1699 (CO), 1640 (NO_2), 1277 (NO_2), 1252 (OEt) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.91 (d, 6H, J = 4.0 Hz, CH_3), 1.37 (t, 3H, J = 8.0 Hz, OCH_2CH_3), 1.49–1.59 (m, 9H, CH_2, CH), 2.57–2.65 (m, 2H, CH_2N), 2.86–2.98 (m, 2H, CH_2N), 3.53 (s, 2H, CH_2-CONH), 3.95–4.10 (m, 2H, OCH_2CH_3), 5.30–5.39 (m, 1H, CHNH), 5.34 (s, 2H, CH_2O), 5.43 (s, 2H, CH_2ONO_2), 6.73–6.74 (m, 1H, Ar), 6.82–6.86 (m, 2H, Ar), 7.05–7.08 (m, 2H, Ar), 7.18–7.20 (m, 2H, Ar), 7.40 (d, 2H, J = 8.0 Hz, AA′XX′), 7.49 (d, 2H, J = 8.0 Hz, AA′XX′), 7.78 (d, 1H, J = 8.0 Hz, NH). ^{13}C NMR ($CDCl_3$): δ 14.79 (CH_3), 22.66 (CH_3), 22.88 (CH), 24.25 (CH_2), 25.46 (CH_2, pip), 26.87 (CH_2, pip), 44.38 (CH_2CO), 46.79 (CH_2O), 49.96 (CHNH), 64.63 (CH_2N, pip), 66.98 (OCH_2), 74.55 ($CHONO_2$), {113.90, 118.74, 120.94, 122.95, 125.20, 127.82, 128.05, 128.51, 129.32, 132.11, 132.48, 137.90, 138.78, 141.70, 152.64, 159.15} (Ar), 166.04 (COO), 168.78 (CONH) ppm. Anal. ($C_{35}H_{43}N_3O_7$) C, H, N. Calcd: 68.07 (C); 6.97 (H); 6.81 (N). Found: 67.82 (C); 6.68 (H); 6.45 (N).

4.1.3. 3-Nitrooxymethylbenzoate of nateglinide 3

A solution of nateglinide (200 mg, 0.63 mmol) in CH_2Cl_2 (12 mL), 3-(nitroxymethyl)benzylalcohol **8a** (115 mg, 0.63 mmol), DCC (156 mg, 0.75 mmol) and DMAP (6 mg) was stirred at room temperature for 3 h. The mixture was then filtered off and the organic layer evaporated to give a crude residue which was purified by column chromatography eluting with hexane/AcOEt (7:3). The product was then crystallized from AcOEt/hexane obtained compound **3** (98 mg, 0.20 mmol, 32%). Mp: 93–95 °C, ^{1}H NMR ($CDCl_3$): δ 0.85 (d, 6H, J = 4.0 Hz, CH_3), 0.94–1.04 (m, 3H, cyclohexyl), 1.25–1.42 (m, 3H, cyclohexyl), 1.75–2.03 (m, 5H, cyclohexyl), 3.07–3.17 (m, 2H, CH_2Ph), 4.90–4.96 (m, 1H, CHNH), 5.11–5.18 (m, 2H, CH_2O), 5.42 (s, 2H, CH_2ONO_2), 5.87 (d, 1H, J = 8.0 Hz, NH), 6.95–7.05 (m, 2H, Ar), 7.21–7.39 (m, 7H, Ar). ^{13}C NMR ($CDCl_3$): δ 19.85 (CH_3), {29.02, 29.10, 29.63, 29.88} (cyclohexyl), 32.89 (CH), 38.03 (CH_2Ph), 43.34 (CH), 45.60 (CHCO), 52.89 (CHCOO), 66.74 (CH_2O), 74.48 (CH_2ONO_2), {127.23, 128.66, 129.14, 129.24, 129.36, 129.45, 129.67, 132.87, 135.87, 136.09} (Ar), 171.76 (CONH), 175.78 (COO) ppm. [α] (in MeOH 1.0%) = −1.7. Anal. ($C_{27}H_{34}N_2O_6$) C, H, N. Calcd: 67.20 (C); 7.10 (H); 5.80 (N). Found: 67.08 (C); 6.87 (H); 5.55 (N).

4.1.4. 4-(Nitroxymethyl)benzoate of nateglinide 4

The nateglinide 4-(nitrooxymethyl)benzoate was synthesized from 4-(nitrooxymethyl)benzylalcohol **8b** (231 mg, 1.26 mmol) following the same procedure described above for the preparation of the nateglinide 3-(nitrooxymethyl)benzoate (**3**). This compound was purified by column chromatography eluting with AcOEt/hexane (8:2) and then by precipitation with *n*-hexane from AcOEt to give compound **4** (140 mg, 0.29 mmol, 23%). Mp: 115–117 °C, IR (neat): ν 3299 (NH), 2928 (Ar), 1737 (CO), 1640 (NO_2), 1277 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.85 (d, 6H, J = 4.0 Hz, CH_3), 0.90–1.06 (m, 3H, cyclohexyl), 1.26–1.42 (m, 3H, cyclohexyl), 1.75–1.78 (m, 2H, cyclohexyl), 1.83–1.89 (m, 2H, cyclohexyl), 1.96–2.04 (m, 1H, CH), 3.06–3.17 (m, 2H, CH_2Ph), 4.90–4.95 (m, 1H, CHNH), 5.12 (d, 1H, J = 14.0 Hz, CH_2O), 5.17 (d, 1H, J = 14.0 Hz, CH_2O), 5.43 (s, 2H, CH_2ONO_2), 5.89 (d, 1H, J = 8.0 Hz, N*H*CH), 6.99–7.01 (m, 2H, Ar), 7.21–7.23 (m, 3H, Ar), 7.32 (d, 2H, J = 8.0 Hz, AA′XX′), 7.39 (d, 2H, J = 8.0 Hz, AA′XX′) ppm. ^{13}C NMR ($CDCl_3$): δ 19.89 (CH_3), {29.15, 29.22, 29.75, 30.00} (cyclohexyl), 33.01 (CH), 38.11 (CH_2Ph), 43.46 (CH), 45.72 (CHCO), 53.00 (CHCOO), 66.79 (CH_2O), 74.51 (CH_2ONO_2), {127.36, 128.78, 129.13, 129.51, 129.57, 132.81, 135.98, 136.77} (Ar), 171.86 (CONH), 175.90 (COO) ppm. MS (m/z): 482 (M^+, 70%), 439 (M^+−$(CH_3)_2CH$, 100%). Anal. ($C_{27}H_{34}N_2O_6$) C, H, N. Calcd: 67.20 (C); 7.10 (H); 5.80 (N). Found: 67.14 (C); 6.99 (H); 5.67 (N).

4.1.5. 3-[1-(Nitrooxy)ethyl]benzoate of repaglinide 5

$AgNO_3$ (201 mg, 1.19 mmol) was added to a stirred solution of 3-[1-(chloroethyl]benzoate of repaglinide (**12**) (184 mg, 0.30 mmol) in CH_3CN (1.1 mL). Stirring was continued over 4 h at rt in the dark, and then the precipitate (silver chloride) was filtered off and the solvent was evaporated. The crude product was purified by column chromatography eluting with hexane/AcOEt (7:3) to give **5** (30 mg, 0.05 mmol, 16% yield) as a white solid: ^{1}H NMR ($CDCl_3$): δ 0.91 (d, 6H, J = 4.0 Hz, CH_3), 1.22–1.26 (m, 2H, CH_2), 1.37 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 1.50–1.70 (m, 10H, CH_2, CH, CH_3), 2.55–2.65 (m, 2H, CH_2N), 2.86–2.97 (m, 2H, CH_2N), 3.53 (s, 2H, CH_2CONH), 3.97–4.06 (m, 2H, OCH_2CH_3), 5.30–5.42 (m, 1H, CHNH), 5.34 (s, 2H, CH_2O), 5.94 (q, 1H, J = 4.0 Hz, $CHCH_3$), 6.71 (d, 1H, J = 8.0 Hz, Ar), 6.81–6.86 (m, 2H, Ar), 7.05–7.07 (m, 2H, Ar), 7.16–7.22 (m, 2H, Ar), 7.33–7.46 (m, 4H, Ar), 7.79 (d, 1H, J = 8.0 Hz, NH) ppm. ^{13}C NMR ($CDCl_3$): δ 14.79 (CH_3), 20.52 (CH_3), 22.68 (CH_3), 22.91 (CH_2), 24.26 (CH), 25.47 (CH_2, pip), 26.90 (CH_2, pip), 29.85 (CH), 44.43 (CH_2CO), 46.80 (CH_2O), 64.66 (CH_2N, pip), 66.19 (OCH_2), 81.91($CHONO_2$), {113.95, 120.98, 122.82, 125.26, 125.98, 126.03, 127.75, 127.77, 128.09, 128.82, 129.21, 132.50, 137.20, 138.77, 139.17, 141.67, 152.66, 159.16} (Ar), 165.96 (COO), 168.70 (CONH). Anal. ($C_{31}H_{36}N_2O_7$) C, H, N. Calcd: 68.44 (C); 7.18 (H); 6.65 (N). Found: 68.57 (C); 7.46 (H); 6.98 (N).

4.1.6. 4-[1-(Nitrooxy)ethyl]benzoate of repaglinide 6

$AgNO_3$ (827 mg, 4.87 mmol) was added to a stirred solution of of 4-[1-(chloroethyl]benzoate of repaglinide (**11**) (752 mg, 1.24 mmol) in CH_3CN (10 mL). Stirring was continued over 4 h at rt in the dark, and then the precipitate (silver chloride) was filtered off and the solvent was evaporated. The crude product was purified by column chromatography eluting with hexane/AcOEt (7:3) to give **6** (156 mg, 0.25 mmol, 20% yield): ^{1}H NMR ($CDCl_3$): δ 0.91 (d, 6H, J = 6.7 Hz, CH_3); 1.25–1.64 (m, 12H, CH_2, CH, CH_3); 1.37 (t, 3H, J = 7.0 Hz, OCH_2CH_3); 2.55–2.65 (m, 2H, CH_2N); 2.86–2.97 (m, 2H, CH_2N); 3.53 (s, 2H, CH_2CONH); 3.94–4.06 (m, 2H, OCH_2CH_3); 5.30–5.42 (m, 1H, CHNH); 5.33 (s, 2H, CH_2O); 5.93 (q, 1H, J = 6.7 Hz, $CHCH_3$); 6.70 (d, 1H, J = 8.1 Hz, Ar); 6.81–6.85 (m, 2H, Ar); 7.02–7.10 (m, 2H, Ar); 7.16–7.50 (m, 6H, Ar); 7.79 (d, 1H, J = 7.7 Hz, NH). ^{13}C NMR ($CDCl_3$): δ 14.87 (CH_3), 22.75 (CH_3), 22.97 (CH_3), 24.39 (CH_2), 25.63 (CH_2, pip), 26.99 (CH_2, pip), 34.15 (CH), 44.94 (CH_2CO), 46.93 (CH_2O), 49.97 (CHNH), 55.26 (CH_2N, pip), 58.57 (OCH_2), 64.87 ($CHONO_2$), {113.54, 114.23, 120.99, 122.84, 125.19, 125.61, 126.41, 126.48 127.27, 127.78, 128.03, 128.42, 132.35, 138.90} (Ar), 159.50 (COO) 169.00 (CONH). Anal. ($C_{31}H_{36}N_2O_7$) C, H, N. Calcd: 68.44 (C); 7.18 (H); 6.65 (N). Found: 68.72 (C); 7.39 (H); 6.74 (N).

4.1.7. 4-[1-(Nitrooxy)ethyl]benzoate of nateglinide 7

$AgNO_3$ (991 mg, 5.84 mmol) was added to a stirred solution of of 4-[1-(chloroethyl]benzoate of nateglinide (**15**) (700 mg, 1.49 mmol) in CH_3CN (8 mL). Stirring was continued over 4 h rt in the dark, and then the precipitate (silver chloride) was filtered off and the solvent was evaporated. The crude product was purified by column chromatography eluting with hexane/AcOEt (7:3) to give **7** (185 mg, 0.37 mmol, 25% yield) as a white solid: mp: 80–82 °C, ^{1}H NMR ($CDCl_3$): δ 0.85 (d, 6H, J = 4.0 Hz, $2CH_3$), 0.91–1.06 (m, 3H, cyclohexyl), 1.33–1.42 (m, 3H, cyclohexyl), 1.63 (d, 3H, J = 4.0 Hz, CH_3), 1.75–1.78 (m, 2H, cyclohexyl), 1.83–1.88 (m, 2H, cyclohexyl), 1.96–2.03 (m, 1H, $CHCH_3$), 3.07–3.17 (m, 2H, CH_2Ph), 4.90–4.96 (m, 1H, CHNH), 5.12 (d, 1H, J = 14.0 Hz, CH_2O), 5.17 (d, 1H, J = 14.0 Hz, CH_2O), 5.89 (d, 1H, J = 8.0 Hz, NHCH), 5.94 (q, 1H, J = 4.0 Hz, $CHONO_2$), 6.96–7.01 (m, 2H, Ar), 7.21–7.22 (m, 3H, Ar); 7.31 (d, 2H, J = 8.2 Hz, AA′XX′); 7.38 (d, 2H, J = 8.2 Hz, AA′XX′) ppm. ^{13}C NMR ($CDCl_3$): δ 19.91 (CH_3), 20.54 (CH_3), {29.08, 29.16, 29.68, 29.94} (cicloesil), 32.95 (CH), 38.04 (CH_2Ph), 43.40 (CH), 45.66 (CH-CONH), 52.91 (CHCOO), 66.79 (CH_2O), 81.71 ($CHONO_2$), {126.67, 127.28, 128.69, 129.17, 129.52, 135.92, 136.11, 139.29} (Ar), 171.80 (CONH), 175.80 (COO) ppm. Anal. ($C_{28}H_{36}N_2O_6$) C, H, N. Calcd: 67.34 (C); 6.91 (H); 5.82 (N). Found: 67.58 (C); 6.95 (H); 6.05 (N).

4.1.8. 3-(1-Hydroxyethyl)benzyl alcohol 9a

A solution of 3-acetylbenzoic acid (500 mg; 3.05 mmol) in THF (3 mL) was added to a solution of $LiAlH_4$ 1 M in THF (230 mg; 6.09 mmol) cooled at 0 °C. The mixture was stirred at 0 °C for 12 h, then water (1.7 mL) and NaOH 1 M (0.4 mL) was added, and the resulting suspension filtrated. The solvent was evaporated to give **9a** (432 mg, 2.84 mmol, 93% yield) as a yellow oil: ^{1}H NMR ($CDCl_3$): δ 1.46 (d, 3H, J = 6.5 Hz, CH_3); 4.63 (s, 2H, CH_2OH); 4.85 (q, 1H, J = 6.5 Hz, CH); 7.20–7.35 ppm (m, 4H, Ar).

4.1.9. 4-(1-Hydroxyethyl)benzyl alcohol 9b

A solution of 4-acetylbenzoic acid (1 g; 6.10 mmol) in THF (6 mL) was added to a solution of $LiAlH_4$ 1 M in THF (691 mg; 18.29 mmol) cooled at 0 °C. The mixture was stirred at 0 °C for 12 h, then water (5 mL) and NaOH 1 M (1.3 mL) was added, and the resulting suspension filtrated. The solvent was evaporated to give **9b** (899 mg, 5.92 mmol, 97% yield) as a yellow oil: ^{1}H NMR ($CDCl_3$): δ 1.48 (d, 3H, J = 6.4 Hz, CH_3); 4.66 (s, 2H, CH_2OH); 4.88 (q, 1H, J = 6.4 Hz, CH); 7.29–7.38 ppm (m, 4H, Ar).

4.1.10. 3-[1-(Hydroxyethyl]benzoate of repaglinide 10

To a solution of 3-(1-hydroxyethyl)benzyl alcohol (**9a**) (168 mg, 1.11 mmol) in CH_2Cl_2 (22 mL) was added repaglinide (500 mg, 1.11 mmol), DCC (273 mg, 1.32 mmol) and DMAP (11 mg). The resulting suspension was stirred at rt for about 4 h, then the precipitate was removed by filtration and the filtrate was concentrated, to give **10** (583 mg, 0.99 mmol, 90% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$): δ 0.90 (d, 6H, J = 6.2 Hz, CH_3); 1.37 (t, 3H, J = 7.0 Hz, OCH_2CH_3); 1.48–1.89 (m, 12H, CH_2, CH, CH_3); 2.54–2.65 (m, 2H, CH_2N); 2.86–2.97 (m, 2H, CH_2N); 3.52 (s, 2H, CH_2CONH); 3.94–4.10 (m, 2H, OCH_2CH_3); 4.91 (q, 1H, J = 6.2 Hz, $CHCH_3$); 5.28–5.38 (m, 1H, CHNH); 5.33 (s, 2H, CH_2O); 6.68 (d, 1H, J = 8.2 Hz, Ar); 6.80–6.84 (m, 2H, Ar); 7.05–7.44 (m, 8H, Ar); 7.70–7.80 (m, 1H, NH). Anal. ($C_{36}H_{46}N_2O_5$) C, H, N. Calcd: 73.69 (C); 7.90 (H); 4.77 (N). Found: 73.58 (C); 7.95 (H); 5.05 (N).

4.1.11. 4-[1-(Hydroxyethyl]benzoate of repaglinide 11

To a solution of 4-(1-hydroxyethyl)benzyl alcohol (235 mg, 1.55 mmol) in CH_2Cl_2 (5 mL) was added repaglinide (700 mg, 1.55 mmol), DCC (383 mg, 1.86 mmol) and DMAP (15 mg). The resulting suspension was stirred at rt for about 4 h, then the precipitate was removed by filtration and the filtrate was concentrated, to give **11** (817 mg, 1.39 mmol, 90% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$): δ 0.90 (d, 6H, J = 6.3 Hz, CH_3); 1.37 (t, 3H, J = 6.6 Hz, OCH_2CH_3); 1.16–1.80 (m, 12H, CH_2, CH, CH_3); 2.54–2.65 (m, 2H, CH_2N); 2.86–2.97 (m, 2H, CH_2N); 3.52 (s, 2H, CH_2CONH); 3.93–4.05 (m, 2H, OCH_2CH_3); 4.91 (q, 1H, J = 6.3 Hz, $CHCH_3$); 5.29–5.42 (m, 1H, CHNH); 5.31 (s, 2H, CH_2O); 6.71 (d, 1H, J = 8.4 Hz, Ar); 6.79–6.83 (m, 2H, Ar); 7.01–7.10 (m, 2H, Ar); 7.15–7.23 (m, 2H, Ar); 7.35–7.44 (m, 4H, Ar); 7.77 (m,1H, NH). Anal. ($C_{36}H_{46}N_2O_5$) C, H, N. Calcd: 73.69 (C); 7.90 (H); 4.77 (N). Found: 73.47 (C); 8.02 (H); 4.89 (N).

4.1.12. 3-[1-(Chloroethyl]benzoate of repaglinide 12

HCl_{concd} (0.32 mL) was added at room temperature to a stirred suspension of 3-[1-(hydroxyethyl]benzoate of repaglinide (**10**) (356 mg, 0.61 mmol) in toluene (3 mL). The resulting solution was stirred for 20 h at room temperature. Then, the solvent was evaporated to afford **12** as a colorless oil (295 mg, 0.48 mmol, 80% yield): ^{1}H NMR ($CDCl_3$): δ 0.87–0.99 (m, 6H, CH_3); 1.20–2.11 (m, 15H, CH_2, CH, CH_3); 2.62–2.70 (m, 2H, CH_2N); 3.07–3.43 (m, 2H, CH_2N); 3.67–3.82 (m, 2H, CH_2CONH); 4.00–4.17 (m, 2H, OCH_2-CH_3); 4.60–4.71 (m, 1H, CHNH); (q, 1H, J = 7.0 Hz, $CHCH_3$); 5.30 (s, 2H, CH_2O); 6.70–6.73 (m, 1H, Ar); 6.90–7.68 (m, 10H, Ar); 7.89–8.00 (m,1H, NH). Anal. ($C_{36}H_{45}Cl\ N_2O_4$) C, H, N. Calcd: 71.44 (C); 7.49 (H); 4.63 (N). Found: 71.56 (C); 7.71 (H); 4.80 (N).

4.1.13. 4-[1-(Chloroethyl]benzoate of repaglinide 13

HCl_{concd} (1 mL) was added at room temperature to a stirred suspension of 4-[1-(hydroxyethyl]benzoate of repaglinide (**11**) (1.12 g, 1.91 mmol) in toluene (7 mL). The resulting solution was stirred for 20 h at room temperature. Then, the solvent was evaporated to afford **13** as a colorless oil (752 mg, 1.24 mmol, 65% yield): ^{1}H NMR ($CDCl_3$): δ 0.85–0.99 (m, 6H, CH_3); 1.30–2.08 (m, 15H, CH_2, CH, CH_3); 2.58–2.68 (m, 2H, CH_2N); 3.00–3.08 (m, 2H, CH_2N); 3.75 (s, 2H, CH_2CONH); 3.94–4.08 (m, 2H, OCH_2CH_3); 4.60–4.71 (m, 1H, CHNH); 5.08 (q, 1H, J = 6.8 Hz, $CHCH_3$); 5.28 (s, 2H, CH_2O); 6.71–6.74 (d, 1H, J = 6.2 Hz, Ar); 6.89 (d, 1H, J = 7.7 Hz, Ar); 7.08 (s, 1H, Ar); 7.17–7.66 (m, 8H, Ar); 7.83–7.86 (m, 1H, NH). Anal. ($C_{36}H_{45}Cl\ N_2O_4$) C, H, N. Calcd: 71.44 (C); 7.49 (H); 4.63 (N). Found: 71.22 (C); 7.19 (H); 4.53 (N).

4.1.14. 4-[1-(Hydroxyethyl]benzoate of nateglinide 14

To a solution of 4-(1-hydroxyethyl)benzyl alcohol (335 mg, 2.21 mmol) in CH_2Cl_2 (5 mL) was added nateglinide (700 mg, 2.21 mmol), DCC (547 mg, 2.65 mmol) and DMAP (22 mg). The

resulting suspension was stirred at rt for about 4 h, then the precipitate was removed by filtration and the filtrate was concentrated, to give **14** (966 mg, 2.14 mmol, 97% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$): δ 0.84 (d, 6H, J = 6.8 Hz, $2CH_3$), 0.96–1.01 (m, 2H, CH_2), 1.29–1.58 (m, 4H, CH_2, 2CH), 1.50 (d, 3H, J = 6.4 Hz, CH_3); 1.65–2.00 (m, 5H, $2CH_2$, CH), 3.04–3.21 (m, 2H, CH_2Ph), 4.86–4.94 (m, 1H, CHNH), 5.06–5.19 (m, 3H, CH_2O, CHOH), 5.89 (d, 1H, J = 7.9 Hz, NH), 6.95–7.02 (m, 2H, Ar), 7.07–7.41 (m, 7H, Ar). Anal. ($C_{28}H_{37}NO_4$) C, H, N. Calcd: 74.47 (C); 8.26 (H); 3.10 (N). Found: 74.36 (C); 8.11 (H); 2.98 (N).

4.1.15. 4-[1-(Chloroethyl]benzoate of nateglinide 15

HCl_{concd} (1.2 mL) was added at room temperature to a stirred suspension of 4-[1-(hydroxyethyl]benzoate of nateglinide (**14**) (1.04 g, 2.31 mmol) in toluene (10 mL). The resulting solution was stirred for 20 h at room temperature. Then, the solvent was evaporated to afford **15** as a colorless oil (1.02 mg, 2.17 mmol, 94% yield): ^{1}H NMR ($CDCl_3$): δ 0.83 (d, 6H, J = 6.6 Hz, $2CH_3$), 0.97–1.01 (m, 2H, CH_2), 1.25–1.42 (m, 4H, CH_2, 2CH), 1.58–2.00 (m, 8H, $2CH_2$, CH, CH_3), 3.10–3.24 (m, 2H, CH_2Ph), 4.81–4.96 (m, 1H, CHNH), 5.04–5.20 (m, 3H, CH_2O, CHCl), 5.94 (d, 1H, J = 7.7 Hz, NH), 6.94–7.00 (m, 2H, Ar), 7.10–7.44 (m, 7H, Ar). Anal. ($C_{28}H_{36}ClNO_3$) C, H, N. Calcd: 71.55 (C); 7.72 (H); 2.98 (N). Found: 71.68 (C); 7.84 (H); 3.09 (N).

4.2. Pharmacology

4.2.1. Evaluation of NO-releasing properties

As a preliminary pharmacological investigation, the NO-mediated vasorelaxing effects of the synthesized compounds were evaluated by functional tests on isolated endothelium-denuded rat aortic rings.

As concerns the vasorelaxing responses, increasing concentrations (10 nM–30 μM) of the tested compounds were added cumulatively to aortic rings pre-contracted by KCl 30 mM. The index of potency was expressed as pIC_{50}, representing the negative Logarithm of the concentration of the tested compound evoking an half-reduction of the contractile tone induced by KCl.

The index of efficacy (E_{max}) indicated the maximal vasorelaxing response induced by the highest concentration (30 μM) of the tested compounds and expressed as a % of the contractile tone induced by KCl. The pIC_{50} value could not be calculated for those compounds, exhibiting E_{max} < 50%. The inhibition of guanylate cyclase by ODQ 1 μM was used as a tool to correlate the vasorelaxing activity with the release of NO.

4.2.2. Evaluation of hypoglycemic properties

Male Wistar rats (250–300 g) were treated with streptozotocin (50 mg/kg ip). After 4 days, animals showing a fasting glycemic level of 100–150 mg/dl (representative of mild type 2 diabetes) were used for the study. Normoglycemic male Wistar rats (showing fasting glycemia between 30 and 40 mg/dl) were used as reference animals. Diabetic animals were deprived of food for 24 h, and then they received Nateglinide (50 mg/kg, ip), or an equimolar dose of **4** (75.8 mg/kg ip) or vehicle. 10 min after drug administration, the animals received glucose (1 g/kg ip). While, normoglycemic animals were deprived of food for 24 h, and then they received only vehicle. 10 min after the drug or vehicle administration, all the animals received glucose (1 g/kg ip). The glycemic levels were recorded for two h (at intervals of 0, 15, 30, 45, 60, 90 and 120 min following the glucose administration) by tail puncture and the use of commonly used sensors (New Glucocard G sensor and Glucocard Gmeter, Arkray, A. Menarini diagnostics), and reported as increase from the basal one. The corresponding areas under curve (AUC) were also calculated.

4.2.3. Evaluation of cardio-protective anti-ischemic effects

Male Wistar rats (250–300 g) were treated with streptozotocin (50 mg/kg ip). After 4 days, animals showing a fasting glycemic level of 100–150 mg/dl (representative of mild type 2 diabetes) were used for the study. 11 days after the administration of streptozotocin, diabetic animals were treated with Nateglinide (12 mg/kg/day, ip), or an equimolar dose of **4** (18.2 mg/kg/day ip) or vehicle, for 7 days. Vehicle-treated normoglycemic male Wistar rats (showing fasting glycemia between 30 and 40 mg/dl) were used as reference animals. On the day of the experiment, the animals were heparinised and anaesthetised with sodium pentobarbital. The hearts were rapidly removed and perfused in a Langendorff apparatus. After 30 min of equilibration time, the hearts were submitted to a ischemia/reperfusion cycle (30 min and 120 min, respectively). The inotropic and chronotropic parameters were monitored with a latex balloon inserted in the left ventricle through the mitral valve and expressed as rate pressure product (RPP = left ventricle developed pressure x heart rate). The RPP value recorded at the end of reperfusion (120th min) is reported as a % of the pre-ischemic one. After 120 min of reperfusion, 2 mm-wide slices of left ventricles were treated with triphenyltetrazolium chloride, in order to allow a planimetric evaluation of damaged areas, expressed as ischemic area % of the whole area (Ai/Atot).

Acknowledgment

This study has been partially supported by NICOX SpA.

Supplementary data

Supplementary data (^{1}H NMR and ^{13}C NMR spectra) of final compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.12.043.

References and notes

1. Schalkwijk, C. G.; Stehouwer, C. D. *Clin. Sci.* **2005**, *109*, 143.
2. Kaur, J.; Bhardwaj, A.; Huang, Z.; Narang, D.; Chen, T. Y.; Plane, F.; Knaus, E. E. *J. Med. Chem.* **2012**, *55*, 7883.
3. Drexler, H.; Hornig, B. *J. Mol. Cell. Cardiol.* **1999**, *31*, 51.
4. Kawashima, S. *Endothelium* **2004**, *11*, 99.
5. Torlone, E.; Rambotti, A. M.; Perriello, G.; Botta, G.; Santeusanio, F.; Brunetti, P.; Bolli, G. B. *Diabetologia* **1991**, *34*, 119.
6. Storey, A. M.; Perry, C. J.; Petrie, J. R. *Br. J. Diabetes Vasc. Dis.* **2001**, *1*, 22.
7. Scheen, A. J. *Acta Clin. Belg.* **2003**, *58*, 3184.
8. Calderone, V.; Rapposelli, S.; Martelli, A.; Digiacomo, M.; Testai, L.; Torri, S.; Marchetti, P.; Breschi, M. C.; Balsamo, A. *Bioorg. Med. Chem.* **2009**, *17*, 5426.
9. Martelli, A.; Rapposelli, S.; Calderone, V. *Curr. Med. Chem.* **2006**, *13*, 609.
10. Martelli, A.; Breschi, M. C.; Calderone, V. *Curr. Pharm. Des.* **2009**, *15*, 614.
11. Tamboli, Y.; Lazzarato, L.; Marini, E.; Guglielmo, S.; Novelli, M.; Beffy, P.; Masiello, P.; Fruttero, R.; Gasco, A. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3810.
12. DeFronzo, R. A. *Ann. Intern. Med.* **1999**, *131*, 281.
13. Dabrowski, M.; Wahl, P.; Holmes, W. E.; Ashcroft, F. M. *Diabetologia* **2001**, *44*, 747.
14. Stephan, D.; Winkler, M.; Kuhner, P.; Russ, U.; Quast, U. *Diabetologia* **2006**, *49*, 2039.
15. Quast, U.; Stephan, D.; Bieger, S.; Russ, U. *Diabetes* **2004**, *53*, S156.
16. Calderone, V.; Digiacomo, M.; Martelli, A.; Minutolo, F.; Rapposelli, S.; Testai, L.; Balsamo, A. *J. Pharm. Pharmacol.* **2008**, *60*, 189.
17. Breschi, M. C.; Calderone, V.; Digiacomo, M.; Macchia, M.; Martelli, A.; Martinotti, E.; Minutolo, F.; Rapposelli, S.; Rossello, A.; Testai, L.; Balsamo, A. *J. Med. Chem.* **2006**, *49*, 2628.
18. Guardado-Mendoza, R.; Prioletta, A.; Jiménez-Ceja, L. M.; Sosale, A.; Folli, F. *Arch. Med. Sci.* **2013**, *9*, 936.