Bioorganic & Medicinal Chemistry 23 (2015) 422–428

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 26 December 2014

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/

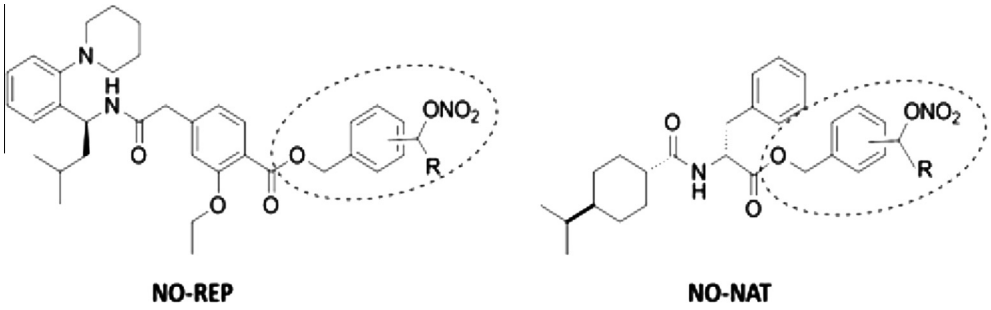


Figure 1. General structures of multifunctional NO-donor/insulin secretagogue derivatives NO-Rep and NO-Nat.

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

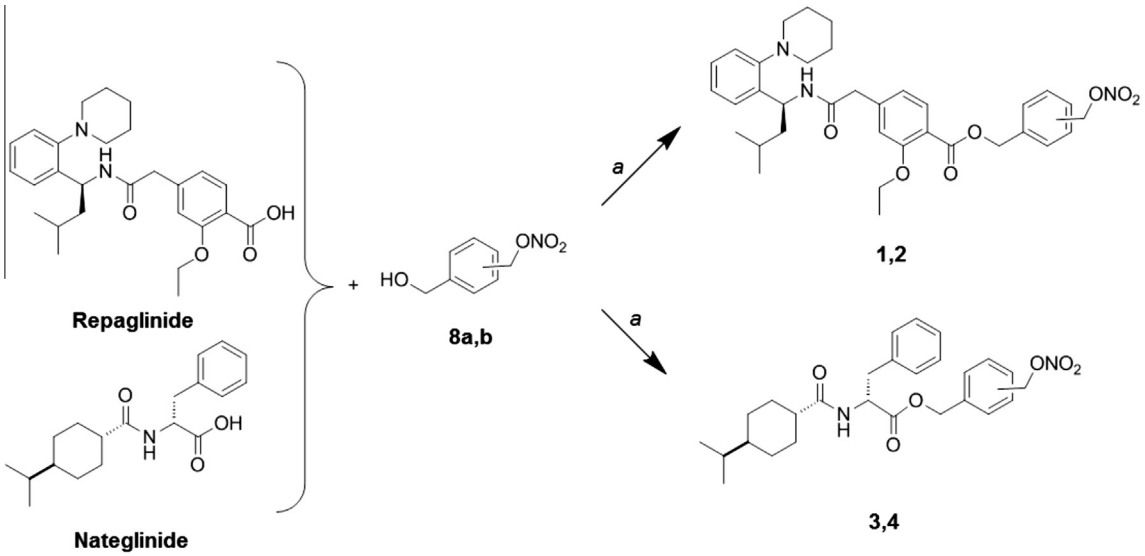
2.1. Chemistry

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.