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Hydroxytyrosol nicotinate, a new multifunctional hypolipidemic and hypoglycemic agent

Yun-Dong Xie[a], Zi-Zhang Chen[a], Na Li[b], Wen-Fang Lu[c], Yan-Hong Xu[a], Yuan-Yuan Lin[a], Li-Hua Shao[a], Qiu-Tang Wang[a], Li-Ying Guo[a], Yu-Qiong Gao[a], Guang-De Yang[a], Yi-Ping Li[a], Xiao-Li Bian[a,*]

[a] College of Pharmacy, Xi'an Jiaotong University, No.76 Yanta West Road, Xi'an, Shaanxi Province, 710061, People's Republic of China
[b] College of Pharmacy, Shaanxi University of Chinese Medicine, Shiji Ave., Xi'an-Xianyang New Ecomic Zone, Shaanxi Province, 712046, People's Republic of China
[c] Pharmacy Department, Xianyang Central Hospital, No.78 Renmin East Road, Xianyang, Shaanxi Province, 712000, People's Republic of China

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ABSTRACT

Hydroxytyrosol (**HT**) is a natural polyphenol antioxidant that exists in olive oil. In the study of multifunctional hypolipidemic of nicotinic derivatives, we found that hydroxytyrosol nicotinate (**HT-N**) incorporation of niacin with **HT** displayed α-glucosidase inhibitory activities *in vitro*, such as yeast α-glucosidase (IC_{50} = 117.72 μM) and rat intestinal α-glucosidases maltase (IC_{50} = 31.86 μM) and sucrase (IC_{50} = 22.99 μM), and had a good control of postprandial blood glucose (PBG). **HT-N** shown significantly hypoglycemic action by 16.9% and protection of pancreatic tissue in type 2 diabetic mellitus (T2DM) mouse model. **HT-N** also shown a potent antioxidant activity and property of anti-glycation *in vitro*, which were benefit for ameliorating diabetic complications. Moreover, **HT-N** exhibited much significant hypolipidemia, lowering plasma triglyceride (TG), total cholesterol (TC), and malonaldehyde (MDA) by 34.6%, 45.8% and 32.1% respectively, in hyperlipidemic mice induced by Triton WR 1339. The results indicated that **HT-N** has hypolipidemic, hypoglycemic and antioxidant actions. All these properties could be conducive to amelioration of oxidative stress, hyperlipidemia, and diabetes that **HT-N** may serve as a multifunctional potential therapeutic strategy in diabetic patients with hyperlipidemia.

1. Introduction

The prevalence of diabetes mellitus is increasing at a surprising rapidity worldwide which is part of the most major public health problems. It is reported that the type 2 diabetes mellitus (T2DM) patients are more liable to be dyslipidemia than the ordinary people [1]. The dyslipidemia of diabetes mellitus plays a main role in the development of atherosclerosis. It is well known that the elevated triglyceride (TG) is the most common form of dyslipidemia among patients with T2DM [2]. High TG levels were always regarded as a cardiovascular disease (CVD) risk factor, including the patients with diabetes mellitus. Although many recent studies have shown the relationship between high TG levels and the risk factor of CVD in patients with coronary heart disease, clinical observations to patients with diabetic dyslipidemia have failed to verify CVD risk decrease with TG lowering drugs in combination with statins which is the foundation of therapy of dyslipidemia in diabetes mellitus [3]. For T2DM patients with high TG, the most common setting is that fibrates should be used as the first-line agent, which appears to involve the activation of peroxisome proliferator activated receptor-α (PPAR-α) to modulate lipid metabolism [4]. However, studies to date have not demonstrated an overall benefit of fibrates for reduction of cardiovascular or total mortality [5]. The previous literature demonstrated that the fibrates in patients with T2DM is not tolerated and failed to meliorate high TG [6].

Niacin, a kind of water-soluble B-complex vitamin, is a potent agent for lowering plasma TG in pharmacological doses [7]. Niacin has been previously shown to lessen coronary atherosclerosis and reduce the rate of coronary mortality [8]. To solve the residual risk in dyslipidemic diabetic patients, clinical and experimental surveillances have demonstrated that the combination of niacin with other hypolipidemic agents may offer potent effects in lowering plasma TG and other lipids [9]. The recent study revealed that lipid-modifying doses of niacin can be applied safely to patients with stable and controlled in T2DM. Niacin treatment may be regarded as an alternative to fibrates and/or statins agents in patients with dyslipidemic diabetes [6]. However, niacin has clinically slight negative impact on glycemia that it can lead to an increase in blood glucose [10]. Moreover, niacin has more adverse effects in clinical application, such as flushing, itchy, gastrointestinal

* Corresponding author.
E-mail address: bianxl@mail.xjtu.edu.cn (X.-L. Bian).

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irritation, stomach ulcers, and so on. Therefore, it is unwilling to apply to patients with T2DM.

Furthermore, as is well known that sustainable hyperglycemia is the feature of diabetic mellitus, which leads to an activation of oxidative stress with an overproduction of reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, hydrogen peroxide, and peroxynitrite [11,12]. ROS has been shown to refer to the formation of advanced glycation end-products (AGEs). Excessive production of ROS accelerates non-enzymatic protein glycation reactions. Increased glycation and the build-up of tissue AGEs have been implicated in diabetes mellitus and led to diabetic complications. Recently, there were more and more attentions have been paid on the contribution of AGEs to diabetic mellitus. In the issue, both high-FBG and AGEs are regarded as the pathological cause of diabetic complications. It has been stated that antioxidants and reactive free radical scavengers stop these processes [13]. Additionally, the recent researches have shown that compounds combination antioxidant and antiglycation activities are more efficient in administration diabetic mellitus and its complications [14].

HT (3, 4-dihydroxy phenylethyl alcohol) is the major natural phenolic antioxidant present in olives and olive oil. The natural antioxidant phenolic molecules have antioxidant and α-glucosidase inhibition activities [15]. Moreover, **HT** has shown a diverse biological activities such as scavenging ROS, inhibition of lipid peroxidation, cardiopreventive property, hypolipidemic and hypoglycemic activities [16]. In order to address the insufficient effect on increasing FBG of niacin used in dyslipidemic diabetes, we planned to introduce **HT** pharmacophore to niacin and successfully designed and synthesized compound **HT-N** (Chart 1). We expected that the new compound **HT-N** can not only reduce lipid profiles and FBG, but also decrease the formation of AGEs and oxidative stress as well as lessen the adverse effects.

The hybrid compound **HT-N** is a case in multi-target agents that only one chemical entity is found able to adjust more than one target at the same time. At present, it is meaningful to apply this kind of agents to administrate the dyslipidemic diabetes. In this paper, we reported the conclusive results of antioxidant, anti-glycation, and α-glucosidase inhibition *in vitro* as well as hypolipidemic and hypoglycemic activities *in vivo* of **HT-N**.

2. Experimental protocols

2.1. Chemistry

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a Bruker AV400 spectrometer, chemical shifts were recorded in ppm, tetra-methylsilane was used as the internal standard and coupling constants (*J*) were given in Hertz. Mass spectra were obtained on Shimadzu HPLC-MS-QP2010 instrument. The progress of the reactions was monitored by TLC using Qingdao Haiyang Chemical Co. Ltd, HG/T2354-92 Silica Gel GF254. Purifications of compounds were made by flash column chromatography using Qingdao Haiyang Chemical Co. Ltd, silica gel (100–200 mesh). Melting points were recorded using Tech. Instrument Co. Ltd, XT-4 melting point apparatus.

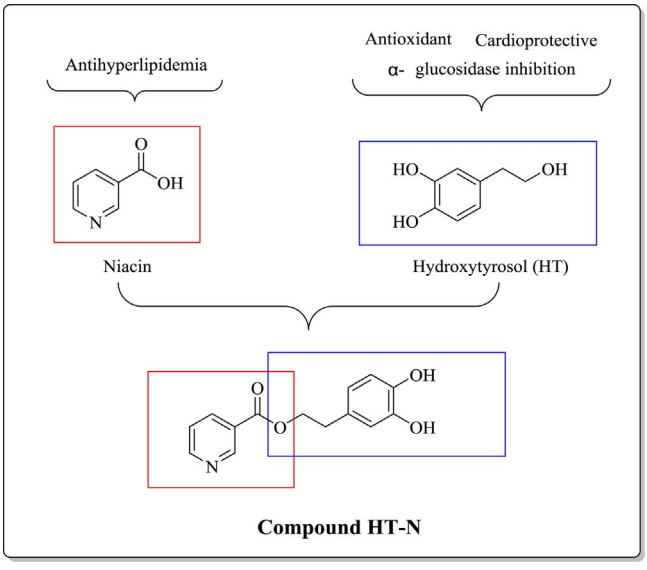

Chart 1. Design of compound **HT-N** and structures of Niacin and Hydroxytyrosol (**HT**).

Compound **1a** was prepared according to the literature procedure (Mp: 55–57 °C) [17]. Niacin (1.00 g, 8.12 mmol) was dissolved in dichloromethane (CH_2Cl_2) solution and reacted with oxalyl chloride (1.05 mL, 10.58 mmol) under ice-bath for 12 h to give **1b**. **1a** (2.17 g, 6.49 mmol) was dissolved in CH_2Cl_2, and reacted with **1b** by dropwise adding under ice-bath for 12 h. Then the crude product was in ethanol solution was mixed with palladium over charcoal (Pd-C) and the mixture was hydrogenated under the atmosphere of hydrogen with magnetic stirring. After 24 h at r.t. the catalyst was filtered off and solvent was evaporated in vacuum, and purified by column chromatography (chloroform-methanol, 20:1) to yield **HT-N** as white solid (1.26g, Yield: 74.91%). Mp: 145.1 ~ 146.2 °C. EI-MS (m/z): 259.1 (M^+). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (d, J = 1.7 Hz, 1H), 8.86 ~ 8.79 (m, 2H), 8.73 (s, 1H), 8.26 (m, 1H), 7.58 (m, 1H), 6.65 ~ 6.69 (m,2H), 6.55 (m, 1H), 4.42 (t, J = 6.8 Hz, 2H), 2.86 (t, J = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.10, 154.12, 150.39, 145.61, 144.27, 137.24, 129.01, 126.17, 124.40, 120.05, 116.72, 116.01, 66.45, 34.19.

2.2. Biological evaluation

2.2.1. Antioxidant activities

The antioxidant activities of **HT-N** were evaluated towards scavenging free radicals (DPPH· and OH·) and inhibiting lipid peroxidation. The assay procedures were referred to the previous literatures.

DPPH radical scavenging activity assay [18]: An aliquot (0.5 mL) of 0.08 g/mL DPPH· solution was mixed with 0.5 mL anhydrous alcohol, 0.5 mL sample (anhydrous alcohol reagent or antioxygen) and 0.1 M acetate sodium acetate buffer solution (pH 5.5). Then the solution should be mixed well and keep in the dark place at r.t. for 30 min. The detective wavelength was 517 nm. The DPPH radical scavenging result was expressed according to the following formula:

DPPH radical scavenging activity (%) = [(Ao-A1)/Ao)] × 100.

Wherein, Ao was the absorbance at 517 nm of the solvent control reaction and A1 is the absorbance at 517 nm of varying concentrations of samples. Each absorbance was rectified by using blank solution (distilled water instead of the samples). All tests were carried out as parallel for three times.

Hydroxyl radical scavenging activity assay: the hydroxyl radical scavenging activity of target compound **HT-N** was calculated by the Fenton reaction [19]. Hydroxyl radicals were produced in the Fe^{2+}–Phenanthroline–H_2O_2 system (Fenton reaction). The reaction solution contained 0.5 mL phenanthroline (2.1 mM), 1.0 mL KH_2PO_4-NaOH buffer (50 mM, pH 7.4), 0.5 mL freshly prepared 0.03% H_2O_2 (v/v), $FeSO_4$, 0.5 mL ascorbic acid (100 mM), 0.5 mL solvent, and 0.5 mL varying concentrations of compound **HT-N**. 10 mL tubes covering reaction solutions were incubated at 37 °C for 60 min. After that, the absorbance of the solution was measured and the detective wavelength was 536 nm with an appropriate blank group (without target compound and replaced by an equal amount of distilled water). The hydroxyl radical scavenging activity was calculated as follows:

Hydroxyl radical scavenging activity (%) = [1-(A_{s0} -A_{s1}) / (A_{b0} -A_{b1})] × 100.

Wherein, A_{s0} was the absorbance at 536 nm of the sample reaction which does not contain the H_2O_2 solution and A_{s1} was the absorbance at 536 nm of the sample reaction which contains the H_2O_2 solution and A_{b0} was the absorbance at 536 nm of the control reaction which does not contain the H_2O_2 solution and A_{v1} was the absorbance at 536 nm of