Bioorganic & Medicinal Chemistry Letters 26 (2016) 4799–4803

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and hypoglycemic activity of 9-*O*-(lipophilic group substituted) berberine derivatives

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ARTICLE INFO

Article history:
Received 6 June 2016
Revised 28 July 2016
Accepted 10 August 2016
Available online 11 August 2016

Keywords:
Berberine derivatives
Synthesis
Cytotoxicity
Hypoglycemic activity
Structure–activity analysis

ABSTRACT

A series of 9-*O*-(lipophilic group substituted) berberine derivatives were synthesized and evaluated for their cytotoxicity and hypoglycemic activity against HepG2 cells. All the results indicated that most of the synthesized compounds exhibited lower cytotoxicity and a certain degree of hypoglycemic activity. Especially the compounds **5g** and **5h** displayed dramatically increased hypoglycemic activity compared with berberine, and the cytotoxicity maintained or even lower than berberine, indicating that they are potential candidates for new anti-type 2 diabetes mellitus drugs.

Diabetes, one of the most common chronic metabolic disorder diseases in the world, has been characterized with high glucose concentration in the blood, which is termed hyperglycemia. The cases of diabetes are estimated to be 171 million worldwide in 2000, and it is anticipated that the number will become over two fold by 2030.[1] Diabetes can be divided into type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T2DM is the most common one, occupying 90% of diabetics. Compared with T1DM, which is attributed to an absolute deficiency of insulin, T2DM is accompanied with insulin resistance and impaired insulin secretion.[2] T1DM is usually treated with intravenous injection of insulin, while T2DM is treated with oral hypoglycemic agents, including insulin secretagogues (sulfonylureas, glinides, meglitinides), incretin based medicine (exenatide, dipeptidyl peptidase 4 inhibitors, liraglutide, pramlintide, bromocriptine and insulin), insulin sensitizers (biguanides and thiazolidinediones), and other medicines (alpha-glucosidase inhibitors and sodium-glucose cotransporter 2 inhibitors) to maintain proper blood glucose level. However, current anti-diabetic drugs usually have side effects, such as sulfonylureas always cause hypoglycemia and gastrointestinal reaction, biguanides are associated with gastrointestinal complaints[3] and lactic acidosis, thiazolidinediones lead to peripheral edema, and alpha glucosidase inhibitors cause intestinal disorder. All these disadvantages lead us to search therapeutic compound with enhanced hypoglycemic activity.

Berberine (BBR, **1**, Fig. 1), an isoquinoline alkaloid extracted from a traditional Chinese herb *Coptischinensis*,[4] has been extensively used in traditional Chinese medicine and Ayurvedic.[5] In accordance with literatures, BBR has been applied in the treatment of bacterial diarrhea[6] and bacteriostasis.[7] Other pharmacological effects of BBR, such as anti-hyperlipidemic action,[8] anti-inflammatory,[9] anticancer,[10] and antileishmanial[11] have also been reported. Especially, researchers recently find that BBR is associated with glucose homeostasis and hypoglycemic activity in T2DM.[12] Although BBR shows capacity for the treatment of T2DM, its hypoglycemic effect is not effective enough to replace existing anti-diabetic agents in clinic. It is a current trend to improve hypoglycemic effect of BBR by a variety of strategies. One of them is to improve the bioavailability by changing the formulations. To name only a few, Meng et al. developed an amorphous solid dispersion of BBR with sodium caprate, which showed a superior hypoglycemic effect compared to pure BBR.[13] Lv et al. found that both BBR and co-administration with sodium caprate orally could significantly decrease fasting blood glucose and improve glucose tolerance in diabetic rats.[14] Pierro et al. proved that it was more effective in reducing glycosylated hemoglobin by the association of BBR and

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http://dx.doi.org/10.1016/j.bmcl.2016.08.027
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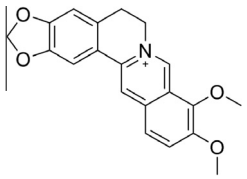


Figure 1. Chemical structure of berberine.

silymarin compared to BBR alone.[15] The more significant strategy is using BBR as a lead compound to modify its structure, thus to obtain new synthetic anti-diabetic drugs, which is also a current hot research area. Chen et al. synthesized a series of 9-*O*-glycosyl-berberine derivatives.[16] Shan et al. designed a BBR analogue, pseudo BBR, to improve glucose-lowering efficacy by prolonging the retention time in HL-7702 liver cells and C2C12 muscle cells.[17] Ding et al. synthesized 8,13-substituted BBR analogues with significant glucose-lowering effect.[18]

Researches had proved that introduction of lipophilic group was a useful method to change the pharmacological activities of BBR. Park et al. synthesized a series of 13-(substituted benzyl) BBR and berberrubine derivatives, and found that they improved the potent antifungal activity against Candida species.[19] Lo et al. found that the lipophilic substitute of 9-*O*-alkyl- and 9-*O*-terpenyl BBR derivatives played an important role in the anti-cancer activity.[20] Based on these researches, in this work, we intended to enhance hypoglycemic effect of BBR by structural modification with lipophilic group. A series of novel 9-*O*-(lipophilic group substituted) BBR derivatives **5a**–**5k**[21] were designed and synthesized based on berberrubine, which was an active metabolite of BBR after first-pass metabolism with enhanced water solubility and biological activity compared with berberine owing to the hydroxyl group at C-9. In these derivatives, several lipid soluble moieties were added through —COO— bond at C-9, meanwhile the hypoglycemic pharmacophores of BBR were maintained, such as methylenedioxy substituted aromatic ring, the cationic nitrogen, and methoxyl group at C-10 (Fig. 2). Therefore, the hypoglycemic activities of final products were worthy to be investigated.

The detailed synthetic routes of 9-*O*-substituted BBR derivatives **5a**–**5k** were outlined in Scheme 1. Briefly, berberine chloride **1** was heated at 190 °C in vacuum oven under reduced pressure for

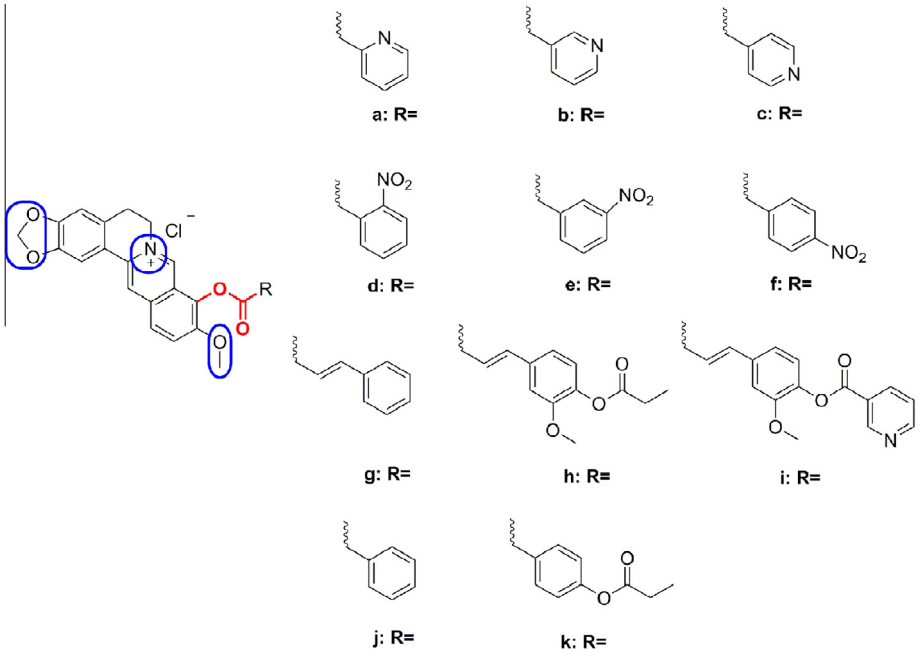
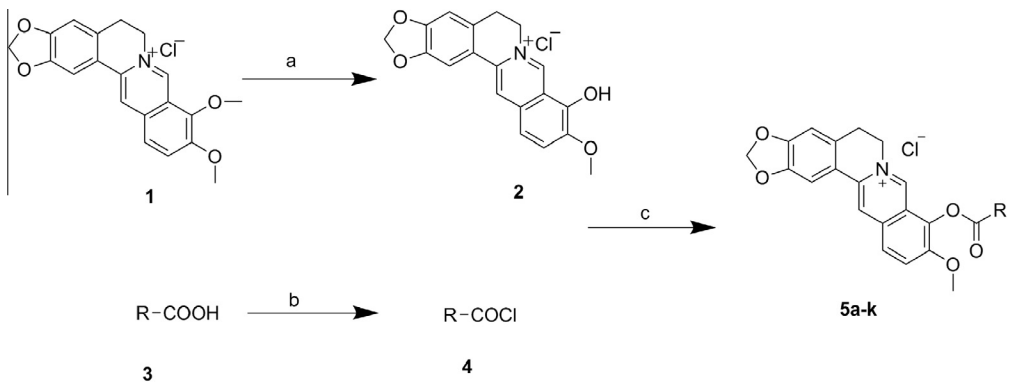


Figure 2. Structure of 9-*O*-(lipophilic group substituted) BBR derivatives. The hypoglycemic pharmacophores of BBR were related to the groups indicated by blue rectangles.



Scheme 1. Synthesis of 9-*O*-(lipophilic group substituted) BBR derivatives. Reagents and conditions: (a) 190 °C in a vacuum; (b) dichlorosulfoxide, pyridine, CH_2Cl_2, 75 °C reflux; (c) pyridine, acetonitrile.

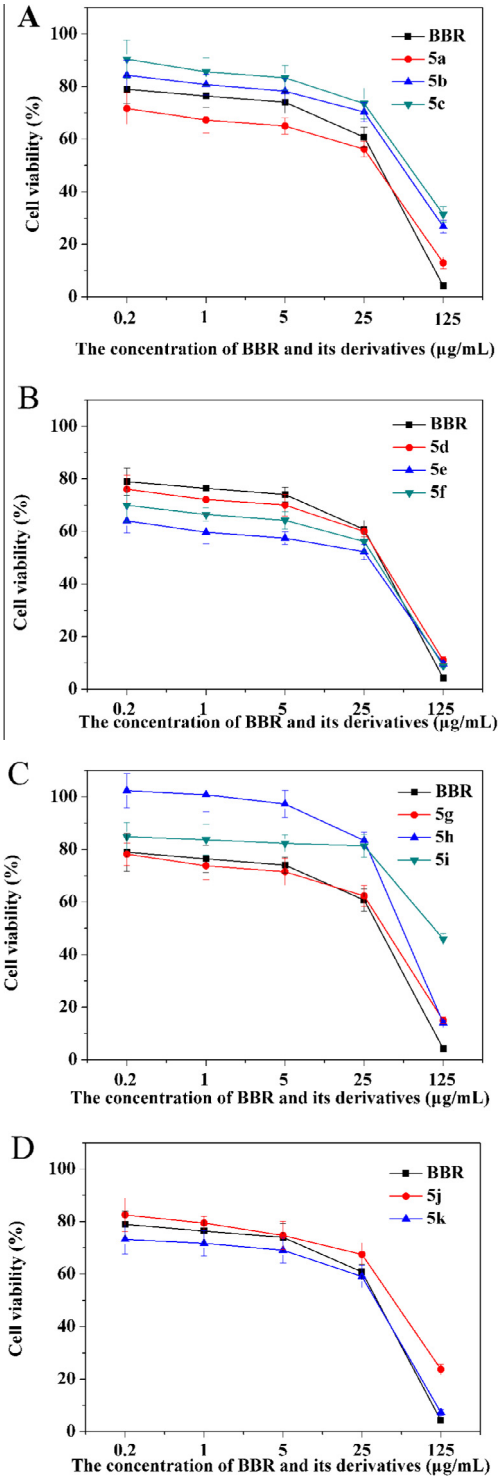
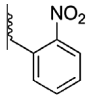
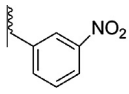
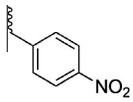
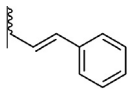
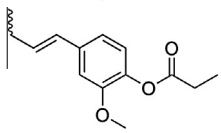
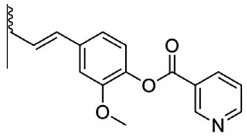
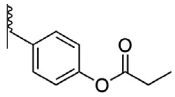


Figure 3. The effect of (A) 9-*O*-pyridine carboxylic acid berberrubine esters (**5a**, **5b** and **5c**); (B) 9-*O*-nitrobenzoic acid berberrubine esters (**5d**, **5e** and **5f**); (C) 9-*O*-cinnamic acid berberrubine esters (**5g**, **5h** and **5i**); and (D) 9-*O*-benzoic acid berberrubine esters (**5j** and **5k**) on the HepG2 cell viability. Data is expressed as means ± SD; $n = 8$.

0.5–1 h to obtain berberrubine **2**.[20] Thionyl chloride was dripped slowly into acid **3** dissolving in anhydrous CH_2Cl_2. The mixture solution was stirred under refluxing for 1–2 h until the completion of reaction and the mixture was concentrated under reduced pressure to give acyl chloride **4**. Acyl chloride **4a**–**4k** was added into berberrubine **2** dissolved in anhydrous CH_2Cl_2 (pyridine as catalyst). The reaction mass was stirred for 2–4 h in room temperature, and the progress of the reaction was monitored with thin-layer chromatography (TLC). After filtrating, washing and drying, the crude product was gained. It was recrystallized twice from methyl alcohol to give the refined product. The structure of BBR derivatives **5a**–**5k** was characterized by ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, infrared spectroscopy (IR) and LC–MS spectroscopy, respectively.

All these synthesized compounds were tested for their cytotoxicity in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at different concentrations (0.2, 1, 5, 25, and 125 μg/mL, respectively), and the results were demonstrated in Figure 3. The viability of HepG2 cell showed that BBR and its derivatives exerted cytotoxicity in a dose-dependent manner. The half maximal inhibitory concentration (IC_{50}), a cytotoxicity index of compound, was calculated and the results were demonstrated in Table 1. Compounds **5b** and **5c** exhibited reduced cytotoxicity than BBR at each dosage (Fig. 3A). The cytotoxicity of 9-*O*-nitrobenzoic acid berberrubine esters (compounds **5d**, **5e** and **5f**) were significantly stronger than BBR (Fig. 3B), which may be caused by

Table 1
The half maximal inhibitory concentrations (IC_{50}) of BBR and BBR derivatives on HepG2 cells

Compounds	R=	IC_{50} (μg/mL)
BBR		46.90
5a		41.64
5b		72.15
5c		82.47
5d		46.88
5e		27.93
5f		37.86
5g		52.26
5h		73.53
5i		91.55
5j		65.93
5k		42.89

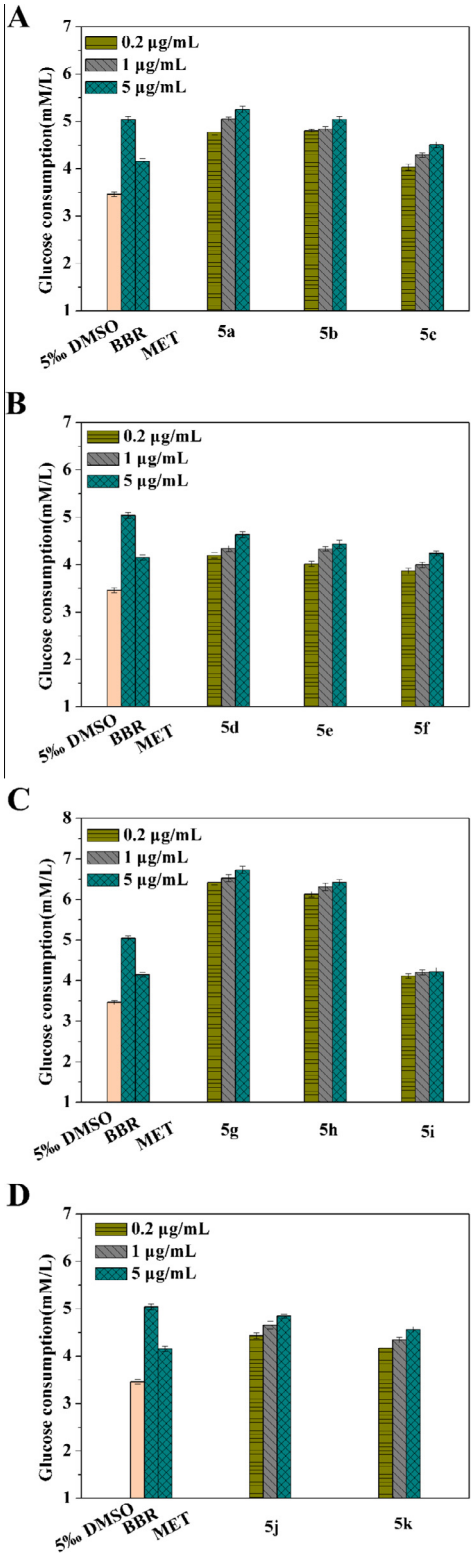


Figure 4. The effect of (A) 9-*O*-pyridine carboxylic acid berberrubine esters (**5a**, **5b** and **5c**); (B) 9-*O*-nitrobenzoic acid berberrubine esters (**5d**, **5e** and **5f**); (C) 9-*O*-cinnamic acid berberrubine esters (**5g**, **5h** and **5i**); and (D) 9-*O*-benzoic acid berberrubine esters (**5j** and **5k**) on glucose consumption of HepG2 cells after 48 h treatment. Data is expressed as means ± SD; $n = 8$.

the introduction of nitro group. While 9-*O*-cinnamic acid berberrubine esters (compounds **5h** and **5i**) had lower cytotoxicity compared with BBR (Fig. 3C), especially compound **5h** exhibited almost none cytotoxicity when the concentration was lower than

Table 2
Data of glucose consumption activity in vitro for all compounds with a concentration of 5 μg/mL (means ± SD, $n = 8$)

Compound	GC (mM)	Percentage increase in GC[a] (%)	Percentage increase in GC[b] (%)
0.5% DMSO	3.46 ± 0.05	\	\
5a	5.25 ± 0.07	51.7	4.2
5b	5.04 ± 0.06	45.7	0
5c	4.51 ± 0.06	30.2	−10.6
5d	4.63 ± 0.06	33.8	−8.1
5e	4.43 ± 0.08	28.0	−12.1
5f	4.24 ± 0.05	22.4	−15.9
5g	6.73 ± 0.09	94.5	33.6
5h	6.42 ± 0.08	85.5	27.4
5i	4.22 ± 0.10	21.8	−16.3
5j	4.85 ± 0.04	40.0	−3.9
5k	4.56 ± 0.06	31.7	−9.6
BBR	5.04 ± 0.07	45.6	\
MET	4.15 ± 0.06	20.0	−17.6

[a] The percentage increase in GC compared with 0.5% DMSO.
[b] The percentage increase in GC compared with BBR.

5 μg/mL and the IC_{50} values of compounds **5h** and **5i** were nearly 1.57 and 1.95 fold of BBR, respectively (Table 1). In addition, compound **5i** showed reduced cytotoxicity at concentration 125 μg/mL compared with BBR.

Glucose consumption (GC) is considered as a main index of hypoglycemic effect. The GC values of HepG2 cells were measured to screen the hypoglycemic activities of the BBR derivatives. Metformin (MET) was used as positive control and the negative control was 0.5% DMSO. HepG2 cells were treated with BBR (5 μg/mL) and its derivatives with different concentrations (0.2, 1, 5 μg/mL), MET (5 μg/mL) and 0.5% DMSO, respectively, for 48 h. As depicted in Figure 4, the GC values of compounds **5a**–**5k** were higher than the negative control group (0.5% DMSO), suggested that the compounds **5a**–**5k** behaved a certain degree of hypoglycemic activity and with a concentration-dependent manner. Compound **5a**, especially, **5g** and **5h** had superior hypoglycemic activity amid all synthesized compounds than BBR (Fig. 4). 9-*O*-nitrobenzoic acid berberrubine esters (compounds **5d**, **5e** and **5f**) (Fig. 4B) showed poorer hypoglycemic activities than BBR. On the contrary, 9-*O*-cinnamic acid berberrubine esters (compounds **5g** and **5h**) (Fig. 4C) were far superior to BBR in hypoglycemic activity. However, compound **5i**, exceptional compound among 9-*O*-cinnamic acid berberrubine esters (Fig. 4C), had the worst activity in the midst of the synthesized compounds. Compounds **5a** and **5b** amidst 9-*O*-pyridine carboxylic acid berberrubine esters (Fig. 4A) were favorable compared with BBR and compound **5j**. Interestingly, 9-*O*-nitrobenzoic acid berberrubine esters presented different activities (**5d** > **5e** > **5f**) as the different positions of the nitro group of benzene ring (*ortho*-position > *meta*-position > *para*-position) which was similar to 9-*O*-pyridine carboxylic acid berberrubine esters (**5a** > **5b** > **5c**). As shown in Table 2, BBR and MET at 5 μg/mL induced an increase of GC in HepG2 cells by 45.6%, 20% compared with the negative control group (0.5% DMSO), respectively. Meanwhile, all synthesized compounds also exhibited a certain extent of hypoglycemic activity. Amidst which, compounds **5a**, **5g** and **5h** increased by 51.7%, 94.5% and 85.5%, respectively. It is regrettable that a large proportion of compounds manifested unfavorable hypoglycemic activity when compared with BBR, but compound **5a**, especially compounds **5g** and **5h** increased by 33.6% and 27.4%, respectively.

Protein tyrosine phosphatase 1B (PTP-1B), a negative regulator of the insulin signaling pathway,[22] was suggested mechanism for the hypoglycemic action of BBR.[23] In order to investigate the effect of the introduced lipophilic group, docking analysis was performed. The automated molecular docking calculations were

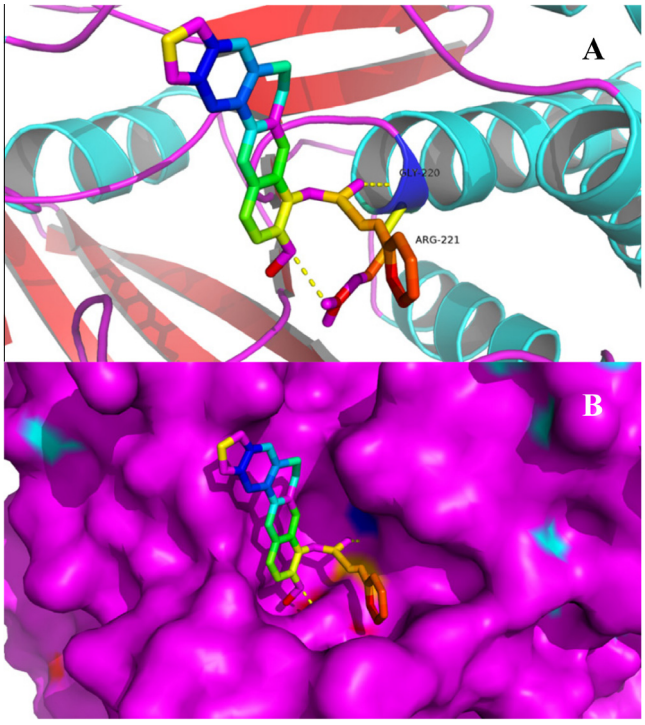


Figure 5. Putative binding of compound **5g** with PTP 1B protein. (A) The protein is represented by ribbon; compound **5g** is displayed by multicolor sticks and hydrogen bonding interactions between the **5g** and PTP 1B residues are shown as yellow dashed lines. (B) Putative binding of compound **5g** with reference inside PTP 1B active pocket. The protein is displayed by purple setting, compound **5g** was displayed by multicolor sticks and hydrophobic interaction between the **5g** and PTP 1B residues were indicated with yellow dashed lines.

carried out using AutoDock 4.0. Before starting the docking process, the 3D coordinates of PTP-1B were retrieved from the Protein Data Bank (PDB: 1g7f) (http://www.rcsb.org/pdb/home/home.do). The active site of the protein was defined using AutoGrid. The grid size was set to 42 × 40 × 40 points with a grid spacing of 0.375 Å based on the binding position of ligand. The binding model was exemplified by the interaction of compound **5g** with PTP-1B. As shown in Figure 5, the oxygen atom of the carbonyl group in compound **5g** formed hydrogen-bonding interaction with amino acid residue glycine-220 (GLY-220). Hydrophobic interaction existed between the benzene ring of esterification moieties and arginine-221 (ARG-221). The docking analysis reveals that the introduced ester group plays an important role in hypoglycemic activity.

For the sake of further exploring the relationship between the substituted groups on introduced aromatic ring and hypoglycemic activity, structure–activity analysis was implemented. We speculate that hypoglycemic activity is related to electron cloud density of ester group. 9-*O*-nitrobenzoic acid berberrubine esters (compounds **5d**, **5e** and **5f**) which have the lightest electron cloud density due to the induction effect of electron-withdrawing group, have weaker activity. On the contrary, 9-*O*-cinnamic acid berberrubine esters (compounds **5g** and **5h**) which have the thickest electron cloud density because of the large conjugation effect between vinyl and benzene ring, exhibit the highest activity. However, compound **5i** showed lower activity than BBR, which may be due to the steric–hindrance effect. It is worth noting that 9-*O*-pyridine carboxylic acid berberrubine esters (compounds **5a** and **5b**) which has been introduced with a pyridine ring whose acid dissociation constant (pK_a) is 5.2,[24] have superior activity compared with 9-*O*-benzoic acid berberrubine ester (compound **5j**) which has been introduced with a benzoic ring. Therefore, it is speculated that the introduction of aromatic nucleus with basic group or electron-donating group bearing C-9 to BBR is positive related to hypoglycemic activity. In addition, steric–hindrance effect is also worthy to be considered.

In summary, we designed and synthesized 11 BBR derivatives bearing 9-OH moiety of berberrubine, and evaluated their cytotoxicity and hypoglycemic activity on HepG2 cell lines. Compounds **5a**, **5g** and **5h** have more potent in hypoglycemic effect than BBR and compounds **5b**, **5c**, **5h**, and **5i** have relatively lower cytotoxicity, compounds **5g** and **5j** have the similar biocompatibility compared with BBR. All the results indicate that compounds **5g** and **5h** were remarkable, showed a superior hypoglycemic effect than that of BBR significant, simultaneously the cytotoxicity maintained and even lower than BBR. This work provides useful information to the relationship of structural modification with hypoglycemic activity of BBR derivatives and compounds **5g** and **5h** could be potential candidates for new anti-T2DM drugs.

Acknowledgments

This work was founded by National Natural Science Foundation of China (81301258), Hunan Provincial Natural Science Foundation of China (2016JJ2161), Specialized Research Fund for the Doctoral Program of Higher Education of China (20130162120078), the Fundamental Research Funds for the Central Universities of Central South University and ShenghuaYuying Project of Central South University.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.08.027.

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