Bioorganic & Medicinal Chemistry Letters 19 (2009) 6004–6008

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and biological evaluation of berberine analogues as novel up-regulators for both low-density-lipoprotein receptor and insulin receptor

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ARTICLE INFO

Article history:
Received 23 July 2009
Revised 13 September 2009
Accepted 16 September 2009
Available online 19 September 2009

Keywords:
Berberine
Low-density-lipoprotein receptor
Insulin receptor
Structure–activity relationship
Metabolic syndrome

ABSTRACT

Berberine (BBR) is a natural compound with up-regulating activity on both low-density-lipoprotein receptor (LDLR) and insulin receptor (InsR). This one-drug-multiple-target characteristic might be suitable for the treatment of metabolic syndrome. In searching for up-regulators effective for both LDLR and InsR expression, the structure–activity relationship (SAR) analysis for BBR analogues was done. Fourteen BBR analogues were designed, synthesized and biologically evaluated. SAR analysis revealed that appropriate modifications on the phenyl ring A or D of BBR might retain the up-regulatory activities on the expression of both LDLR and InsR. Among these compounds, compound **13a** bearing 9-methoxy and 10-hydroxyl on the ring D showed promising activities on either LDLR or InsR gene expression. The 10-hydroxyl of **13a** could be an arm to connect proper chemical groups for optimizing drug-bioavailability in vivo. Thus, **13a** could be considered to be a parent compound to make pro-drugs for either blood lipids or glucose.

Berberine (BBR, **1**, Fig. 1) is an isoquinoline natural product extracted from *Coptis chinensis*, and has been extensively used as a nonprescription drug to treat diarrhea in China for decades with a confirmed safety. Because of its poor solubility and bioavailability in oral administration, most of the BBR remains in intestine.[1,2] Our previous work has identified BBR as a new drug for hyperlipidemia causing reduction of cholesterol and triglyceride in hyperlipidemic patients.[3,4] The results were confirmed by other groups.[5–7] BBR increases low-density-lipoprotein receptor (LDLR) expression by stabilization of LDLR mRNA through activation of extracellular signal-regulated kinase (ERK) pathway.[4,8,9] In addition, we have recently discovered insulin receptor (InsR) to be another key target of BBR with significant benefit of antagonizing insulin resistance in type 2 diabetes.[10] It activated the promoter of InsR gene through a protein kinase C (PKC) pathway, increased the expression of InsR in liver and muscle cells, and therefore enhanced glucose consumption.[10] Clinical studies have shown a significant hypoglycemic effect of BBR in patients with type 2 diabetes.[11,12] Our findings suggest that BBR is an one-drug-multiple-target compound with great potential for the treatment of metabolism syndrome, which represents a group of metabolic disorders including insulin resistance, obesity, dyslipidaemia, hypertension and low level of high-density-lipoprotein (HDL). There currently are no approved medicines for this complicated disease.[13]

The structure–activity relationship (SAR) analysis of BBR for the up-regulation of LDLR mRNA expression was elucidated in our laboratory.[14,15] Among these analogues, pseudoberberine (**2**) bearing 10,11-dimethoxy and compound **3** with 10-hydroxy on the ring D are the representative compounds (Fig. 1), and exhibited potent activity on LDLR mRNA expression (Table 1).[14] As the lipid and sugar metabolism are dynamically associated, we then turned our attention of chemical modifications to agents effective in lowering both blood lipid and glucose. Therefore, SAR studies for the up-regulatory activities on both LDLR and InsR mRNA expression were conducted by concentrating the modifications on the phenyl ring A mainly through *de novo* synthesis.

In the present study, we retained the active fragment of the ring D, that is, 9,10-dimethoxy of **1**, or 10,11-dimethoxy of **2**, or 10-hydroxy of **3**, respectively (Fig. 1), and replaced the methylenedioxy moiety at the 2,3-position of the ring A with various side chain substituents including hydrogen, methoxy, hydroxyl and benzyloxy groups. On the basis of this strategy, twelve new BBR analogues (**13b–m**) were designed and *de novo* synthesized. The non-active trimethoxy substitution was also employed in the ring D, from which compound **13n** was created. In addition, compound **13a** (thalifendine) with 9-methoxy and 10-hydroxyl on the ring D was also synthesized according to the pervious methods.[14,15]

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doi:10.1016/j.bmcl.2009.09.059

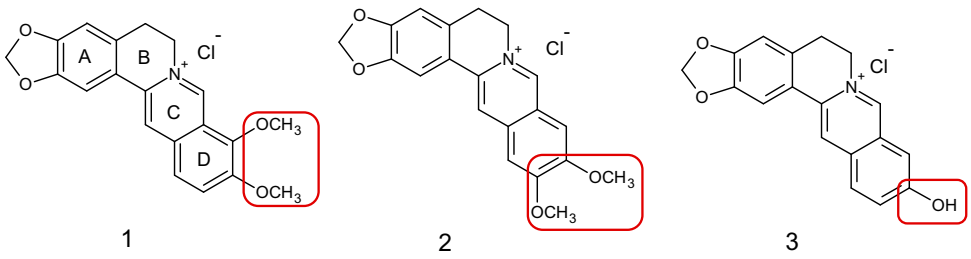
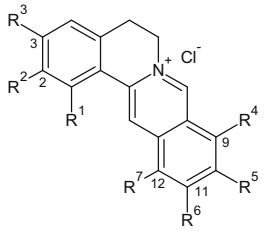


Figure 1. Structures of the compounds **1**–**3**.

Table 1
Structures and up-regulation of LDLR and InsR expression by BBR analogues



Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	LDLR mRNA[a]	InsR mRNA[a]
1	H		OCH_2O	OMe	OMe	H	H	1.9 ± 0.1	2.3 ± 0.4
2	H		OCH_2O	H	OMe	OMe	H	3.1 ± 0.8	1.2 ± 0.1
3	H		OCH_2O	H	OH	H	H	1.9 ± 0.2	1.4 ± 0.1
13a	H		OCH_2O	OMe	OH	H	H	1.6 ± 0.2	1.7 ± 0.2
13b	H	H	OH	OMe	OMe	H	H	0.8 ± 0.1	1.2 ± 0.1
13c	H	H	OCH_2Ph	OMe	OMe	H	H	1.3 ± 0.1	1.3 ± 0.1
13d	H	OCH_2Ph	OMe	OMe	OMe	H	H	1.5 ± 0.2	1.7 ± 0.1
13e	OMe	OMe	OMe	OMe	OMe	H	H	1.1 ± 0.1	1.2 ± 0.1
13f	H	H	OH	H	OMe	OMe	H	0.9 ± 0.1	0.7 ± 0.1
13g	H	H	OCH_2Ph	H	OMe	OMe	H	1.0 ± 0.1	0.8 ± 0.1
13h	OMe	OMe	OMe	H	OMe	OMe	H	1.1 ± 0.1	0.8 ± 0.1
13i	H	OH	OMe	H	OMe	OMe	H	1.1 ± 0.1	0.8 ± 0.1
13j	H	OMe	OMe	H	OMe	OMe	H	1.1 ± 0.1	1.1 ± 0.1
13k	H	OMe	OH	H	OMe	OMe	H	1.0 ± 0.1	0.6 ± 0.1
13l	H	OMe	OMe	H	OH	H	H	1.0 ± 0.1	0.9 ± 0.1
13m	H	OMe	OH	H	OH	H	H	1.4 ± 0.2	1.2 ± 0.1
13n	H	OMe	OH	H	OMe	OMe	OMe	1.0 ± 0.1	1.0 ± 0.1

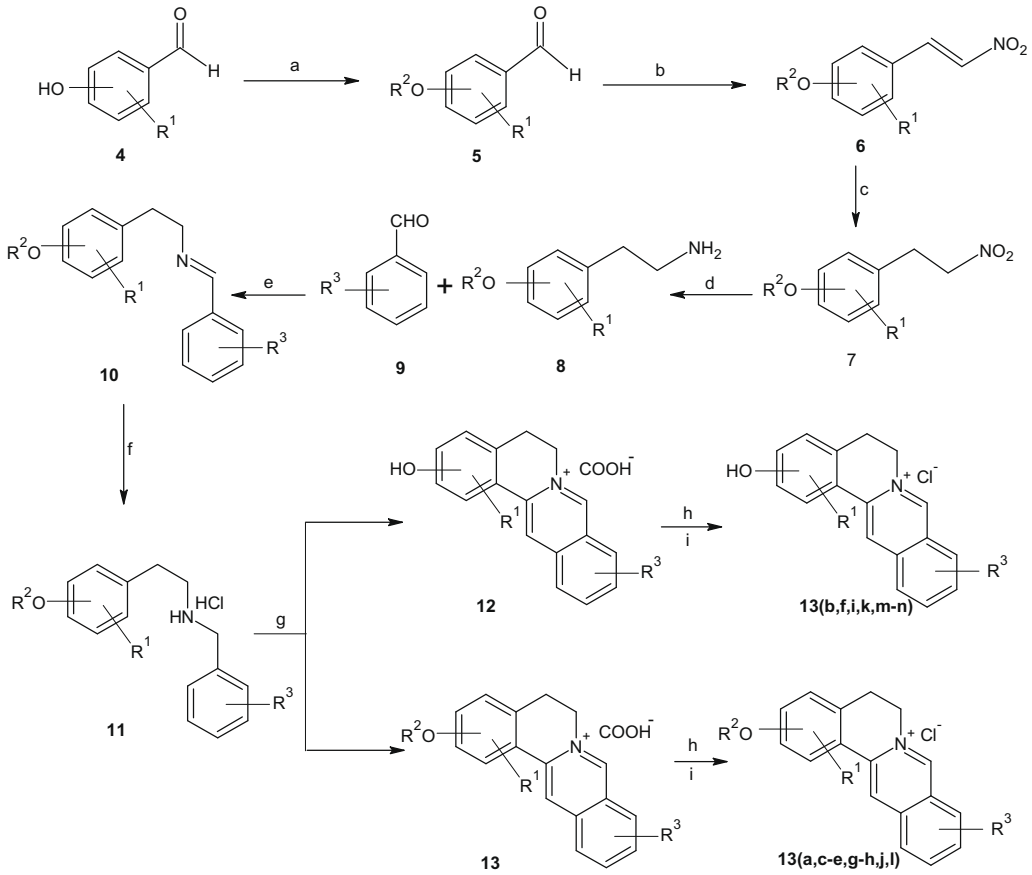
[a] Human liver HepG2 cells were cultured in the MEM medium, and incubated with BBR or its analogues (10 μg/mL) for 8 h. Up-regulation of LDLR or InsR mRNA expression was determined with real-time RT-PCR method. The level of LDLR or InsR mRNA in the untreated cells was defined as 1, and the levels of LDLR or InsR mRNA from the cells treated with study analogues were defined as a fold of the untreated control. The data shown were mean ± SD of three separate experiments.

The fourteen BBR analogues (**13a–n**) were synthesized as described in Scheme 1. It includes three synthetic processes employing different starting materials.[16,17] Compound **13a** was obtained directly by using homopiperonylamine (**8**) and 2-methoxy-3-hydroxybenzaldehyde (**9**) as starting materials, using the method reported previously.[14]

The desired products **13b**, **13f**, **13i**, **13k** and **13m–n** were synthesized through an eight-step process, using commercially available derivatives of hydroxyl benzaldehyde (**4**) as starting materials.[14] The hydroxyl was protected with a benzyl group using benzyl bromide and K_2CO_3 as the base. Then, the intermediate **5** underwent condensation with nitromethane in the presence of methylamine. Another key intermediate **8** was obtained through a two-step reduction (58–65% yield) in which the double bond was reduced by $NaBH_4$ and the nitro group was reduced by Zinc powder respectively. Using zinc powder or Pd/C hydrogen (10%) alone as the reducing agent, resulted in lower yields of **8** due to the poor solubility of α,β-unsaturated nitro compound **6** in methanol or ethanol. The final products were obtained through dehydration, reduction and intramolecular cyclization from substituted phenylethylamines (**8**) and benzaldehydes (**9**).[14] During the course of the intramolecular cyclization of **11** under an acid condition, the de-protection of benzyl group occurred simultaneously and the desired products were then obtained directly.

The desired compounds **13c–e**, **13g–h**, **13j** and **13l** were synthesized with derivatives of benzaldehyde (**5**) as starting materials using a seven-step sequence including condensation, reduction, dehydration, reduction and cyclization.[14] The final products in the **13** series were purified with flash column chromatography using methanol–dichloromethane as the eluent.

Next, the fourteen study analogues were examined in human liver HepG2 cells for their up-regulatory activities on both LDLR and InsR mRNA expression. The up-regulatory effect was quantitatively measured with specific real-time RT-PCR assay.[4] Structures of the analogues and their LDLR and InsR up-regulatory effect are shown in Table 1.



Scheme 1. Synthesis of the study compounds (**13a**–**n**). Reagents and conditions: (a) BnBr, K_2CO_3, DMF, 4 h; (b) CH_3NO_2, CH_3NH_2, methanol, 4 h; (c) $NaBH_4$, 1,4-dioxane, reflux, 2 h; (d) Zn, HCl; (e) 100 °C, 8 h; (f) $NaBH_4$, methanol, reflux, 5 h; (g) glyoxal, formic acid, $CuSO_4$, HCl, 100 °C, 5 h; (h) methanol/H_2O, CaO, rt, 2 h; (i) ethanol/HCl, rt, 0.5 h.

First, the SAR analysis was done for the activity on LDLR mRNA expression. Replacing methoxy at the 10-position of the ring D of BBR with hydroxyl, compound **13a** afforded a promising activity on LDLR expression. Introducing different groups at the 2- or/and 3-position of BBR, compounds **13b–e** were obtained. Replacement of the methylenedioxy moiety of the ring A in BBR with a hydroxyl at the 3-position, the obtained compound (**13b**) showed no activity. Replacing hydroxyl with benzyloxy at the 3-position, the compound (**13c**) showed a weak activity. The compound **13d** bearing a benzyloxy at the 2-position and a methoxy at the 3-position exhibited a good activity on LDLR expression. Three methoxy groups at the 1, 2, and 3 positions of ring A (**13e**) afforded no activity on LDLR mRNA expression.

At the 2- or/and 3-position, replacing the methylenedioxy of compound **2** with various substituents, such as hydroxyl, methoxy and benzyloxy groups, all of the analogs (**13f–k**) lost the up-regulatory effect on LDLR expression. Similarly, the methylenedioxy of compound **3** was replaced with hydroxyl or methoxy at positions 2 and 3. The compound **13m**, possessing a 2-methoxy and 3-hydroxyl, showed an activity lower than its parent compound **3** did. Compound **13n** bearing methoxy groups at the 9, 10 and 11 positions of ring D lost the activity completely.

Second, SAR analysis for the activity on InsR mRNA expression was carried out as well (Table 1). SAR results showed that the up-regulatory effect of the study compounds (**13a–n**) on InsR mRNA expression was basically consistent with that for LDLR expression. Compounds **13a** and **13d** showed up-regulatory activities for both LDLR and InsR mRNA expression; especially, compound **13a** exhibited good activities for both receptors.

The up-regulation of LDLR and InsR mRNA expression by **13a** were further validated with flow cytometric analysis for the protein profile on the surface of human HepG2 liver cells (Fig. 2A and B). The results showed that either LDLR or InsR protein expression was increased on the surface of the **13a**-treated hepatocytes. Although the up-regulatory activities of **13a** on LDLR and InsR expression was not as high as that of BBR, the hydroxyl at the 10-position provides an opportunity to prepare pro-drugs by connecting **13a** with acids or alcohols. Therefore, **13a** could be an ideal form of BBR to improve the bioavailability and therapy efficacy in vivo.[18]

In conclusion, we have synthesized fourteen BBR analogues with substituents on the ring A or D and studied the biological effect of their activities on LDLR and InsR up-regulation. The results suggested that introducing suitable substituents at the ring A or D might retain the up-regulatory activities for both LDLR and InsR. As **13a** retains good up-regulatory activities in both receptors and possesses a free arm for chemical modification, it might be selected as a parent structure to make pro-drug candidates for their potential in the treatment of both hyperlipidemia and hyperglycemia.

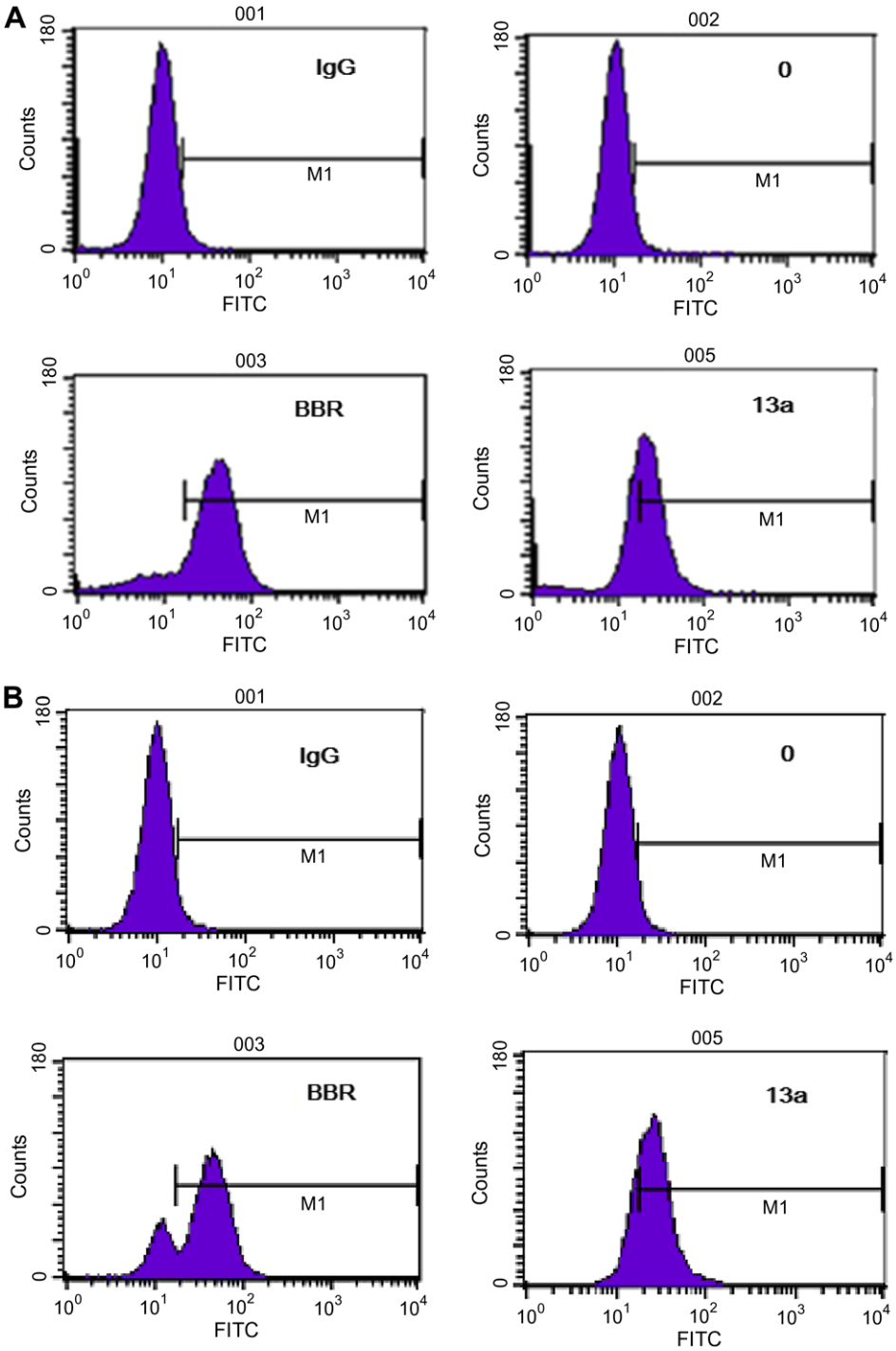


Figure 2. Up-regulation of LDLR or InsR protein expression by **13a** in the liver cells. HepG2 cells were untreated or treated with 10 μg/mL of BBR or **13a** for 12 h. Cells were analyzed by flow cytometry for cell surface LDLR (A) or InsR (B) protein expression using a rabbit-polyclonal antibody against LDLR or InsR. Normal rabbit IgG was used as control for the background staining. Fluorescence intensities on cell surface were analyzed in a FACSCalibur system. The count on *Y*-axis is the absolute number of cells; and FL1 in the *X*-axis represents the fluorescent intensity of the LDLR or InsR protein expressed on the HepG2 cell surface.

Acknowledgment

This work was supported by the 'Innovative Drug R&D' (11th 5-Year Plan) of the Ministry of Science and Technology, PR China.

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