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Synthesis and structure–activity relationships of berberine analogues as a novel class of low-density-lipoprotein receptor up-regulators

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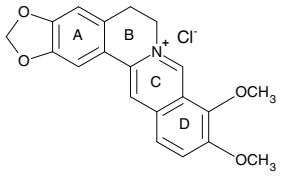
ABSTRACT

Berberine (BBR, **1**) is a novel cholesterol-lowering agent that up-regulates low-density-lipoprotein receptor (LDLR) expression through a mechanism different from that of statins. Because of the unique mode of action and good safety record, BBR provoked our interest to do structure modification at different domains for its cholesterol-lowering activity. Nineteen BBR analogues with substituents on the benzene ring D were synthesized in the present study. The analysis of structure–activity relationship (SAR) indicated that the two methoxyl groups in an ortho-distribution on this benzene ring afforded a good activity. Among the 19 analogues, compound **8j** bearing a methoxyl at both 10- and 11-position showed an increased LDLR up-regulatory activity in respect to BBR, and therefore has been selected as a promising cholesterol-lowering drug candidate for further evaluation.

The expression of liver low-density-lipoprotein receptor (LDLR) regulates homeostasis of human plasma LDL cholesterol (LDL-c). Increased hepatic LDLR expression results in improved clearance of plasma LDL-c through a receptor-mediated endocytosis,[1,2] and is strongly associated with a decreased risk of cardiovascular diseases in humans.[3,4] We found that Berberine (BBR, **1**, Fig. 1), an alkaloid originally isolated from Huanglian (*Coptis chinensis*), up-regulated LDLR expression through a post-transcriptional mechanism of stabilizing LDLR mRNA.[5,6] This biological effect of BBR was independent of the function of sterol regulatory element binding proteins (SREBP), indicating a novel anti-lipid mechanism different from that of statins.[5,6] BBR showed a promising cholesterol-lowering activity in animals as well as in hypercholesterolemic patients with no sign of clinical side-effects.[5,7] In addition, combination of BBR with statins largely increased the cholesterol-lowering effect.[8]

BBR has been extensively used in China as a nonprescription drug to treat diarrhea caused by bacteria since 1950s with a confirmed safety.[9–12] The recent finding of its unique action mode and clinical effects on cholesterol provoked our strong interest to explore the structure–activity relationship (SAR) for the cholesterol-lowering effect. In the present study, we initiated the SAR analysis by first focusing on the modification of the benzene ring D of BBR, on which 19 analogues with various substituents were designed and synthesized.



Berberine (BBR, **1**)

Figure 1. Chemical structure of berberine (1).

All of these analogues were synthesized through a five-step process (Scheme 1), in which intramolecular cyclization was the key step. The starting materials were 3,4-(methylenedioxy) phenylethylamine (**2**) and the substituted benzaldehydes (**3**), both were commercially available. Dehydration[13] of **2** and **3** at 100 °C gave an oleaginous intermediate **4**, which was directly reduced with $NaBH_4$ in refluxing methanol to give the intermediate **5** in a two-step yield of 60-80%. The mixture of **5** and glyoxal was directly treated with $CuSO_4$ and HCl[14] in formic acid to obtain the black solid intermediate **6**. To obtain a single counterion and to get rid of formate and sulfate, intermediate **6** was dissolved in the solvent of methanol and water, and treated with CaO to give the intermediate **7** in a 25–45% two-step yield. Finally, the acidification of

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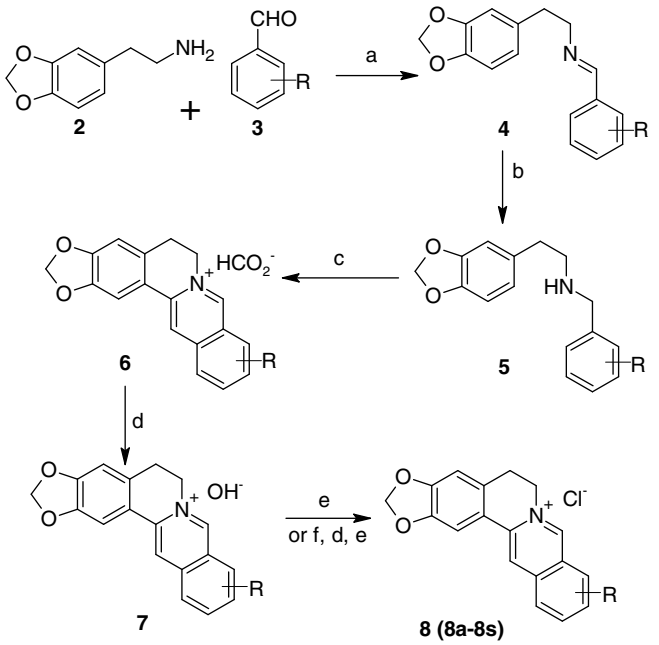


Scheme 1. Reagents and conditions for the chemical synthesis: (a) 100 °C, 8 h; (b) $NaBH_4$, methanol, reflux, 5 h; (c) glyoxal, formic acid, $CuSO_4$, HCl, 100 °C, 5 h; (d) methanol, H_2O, CaO, rt, 2 h; (e) ethanol, HCl, rt, 0.5 h; (f) DMF, K_2CO_3, R^5Br, rt, 2 h.

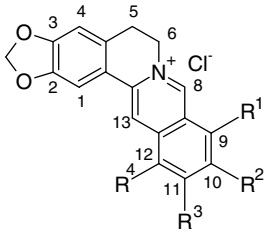
intermediate **7** with HCl in ethanol afforded the crude product of **8a–8e, 8i–8m, 8r**, and **8s** in a yield of 80–95%. Compounds **8f–8h** and **8n–8q** were obtained in a reaction of intermediate **7** with K_2CO_3 and bromoalkane in DMF, followed by treatment with CaO and HCl in ethanol, respectively, in a 65–77% three-step yield. The final compounds in **8** series (**8a–8s**) were purified with silica gel column chromatography using methanol/chloroform (1:20) as the eluent.

Next, the analogues were screened to examine their effect in up-regulating LDLR expression. Human liver HepG2 cells were treated with the study compounds for 8 h, and the cellular mRNA was extracted to measure the intracellular LDLR expression with a specific real time RT-PCR assay.[5] Structures of the analogues **8a–8s** and their LDLR up-regulatory effect are shown in Table 1. BBR is presented in this table as a lead compound designated as compound number **1**.

The SAR study was first focused on the modification of the benzene ring D at the 9- and 10-position with a variety of substituents. Instead of the methoxyl at both the positions in BBR, compounds **8a–8d** possessing a substituent of ethoxyl or propoxyl group at the 9- or/and 10-position were made and tested. The results showed that the analogues with an increased size of substituents at the 9- or 10-position showed an activity on LDLR lower than that of the lead compound. Then, we removed the methoxyl at the 9-position and retained the side chain at the 10-position with a series of substituents (compounds **8e–8h**). Compound **8e** bearing hydroxyl at the 10-position had an activity similar to that of BBR; however, attachment of other substituents with alkoxyl (compounds **8f–8h**) at the 10-position resulted in a partial loss of the activity. Furthermore, compound **8i** with no side chains on the benzene ring D exhibited a decreased activity on LDLR.

In another variation of moving the side chain from the 9-position to 11-position, analogues with different sizes of the side chain substituents at the 10- and 11-position (compounds **8j–8q**) were synthesized. Compound **8j**[15] bearing a methoxyl group at both 10- and 11-position afforded the most potent activity in up-regulating LDLR expression with about 1.6-fold increase over that of BBR. Other compounds with hydroxyl, ethoxyl, or propolxy at the

Table 1
Up-regulation of LDLR expression by BBR and its analogues



Compound	R^1	R^2	R^3	R^4	LDLR mRNA (fold of control [a])
1 (BBR)	OCH_3	OCH_3	H	H	5.3 ± 0.4
8a	OCH_3	OC_2H_5	H	H	2.35 ± 0.32
8b	OCH_3	OC_3H_7	H	H	2.3 ± 0.27
8c	OC_2H_5	OC_2H_5	H	H	2.13 ± 0.19
8d	OC_3H_7	OC_3H_7	H	H	2.21 ± 0.2
8e	H	OH	H	H	5.16 ± 0.52
8f	H	OCH_3	H	H	2.0 ± 0.18
8g	H	OC_2H_5	H	H	2.9 ± 0.3
8h	H	OC_3H_7	H	H	2.13 ± 0.22
8i	H	H	H	H	2.95 ± 0.33
8j	H	OCH_3	OCH_3	H	8.5 ± 0.91
8k	H	OH	OCH_3	H	1.6 ± 0.11
8l	H	OCH_3	OH	H	2.2 ± 0.18
8m	H	OC_2H_5	OH	H	1.8 ± 0.2
8n	H	OC_2H_5	OCH_3	H	2.17 ± 0.19
8o	H	OC_3H_7	OCH_3	H	1.94 ± 0.2
8p	H	OCH_3	OC_2H_5	H	2.1 ± 0.2
8q	H	OCH_3	OC_3H_7	H	2.35 ± 0.25
8r	OCH_3	OCH_3	OCH_3	H	3.16 ± 0.32
8s	H	OCH_3	OCH_3	OCH_3	3.01 ± 0.25

[a] Human liver HepG2 cells were cultured in the EMEM medium containing 0.5% of LPDS, and were then incubated with BBR or its analogues (7.5 μg/mL) for 8 h at 37 °C. Up-regulation of LDLR expression was determined by the real-time RT-PCR method.[5] Abundance of LDLR mRNA in the untreated cells was defined as 1, and the levels of LDLR mRNA from BBR or analogues treated cells were defined as fold of the untreated control. The data shown were mean ± SD of 3 separate experiments.

10- or 11-position (compounds **8k–8q**) exhibited a reduced activity. We therefore deduced that the methoxyl group at the 10- and 11-position was the optimal combination for the structure in their activity on LDLR; and minor alteration of the groups at these positions appeared to cause activity loss. As the methoxyl appeared to be a positive domain for LDLR, we then added additional methoxyl at the 11-position of BBR or the 12-position of compound **8j**, and thus got the analogues with three methoxyl groups on the benzene ring D (compounds **8r** and **8s**). However, the result showed that addition of the third methoxyl group at these positions resulted in a partial loss of the activity. It was probably due to the unfavorable steric obstacle among the three methoxyl substitutions. The SAR information revealed that the potency in up-regulating LDLR was sensitive to the size of the substituents. Replacement of the two vicinal methoxyl with substituents that have sizes bigger than that of methoxyl (such as ethoxyl or propoxy groups), or addition

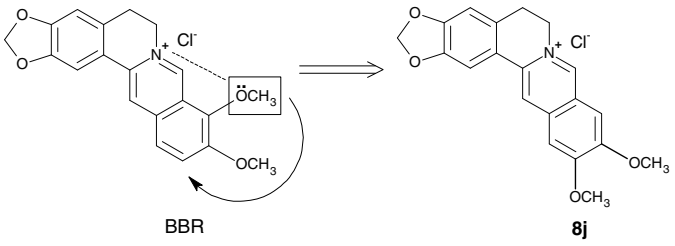


Figure 2. The intramolecular electrostatic effect of BBR and **8j**.

of an extra methoxyl group decreased the up-regulatory effect on LDLR expression.

Compound **8j** is an isomer of BBR, and is called pseudoberberine. It showed a higher activity on LDLR than BBR did. The factor that might contribute to the increased activity of **8j** is presumably the change of the electropositivity at the nitrogen atom. The quaternary ammonium ion at the 7-position of BBR and **8j** plays an important role in their binding affinity with the biological molecules.[16] However, in the BBR structure, the methoxyl at the 9-position could form an electrostatic attraction with the quaternary ammonium ion at the 7-position, and therefore decrease the electropositivity of BBR (Fig. 2). Accordingly, the potential of receiving electron donation from its biological targets at the 7-position is decreased. Switching the methoxyl group from the 9-position (BBR) to the 11-position (**8j**) separates the quaternary ammonium ion from the methoxyl group with an increased distance. This modification eliminates electrostatic effect between positions 7 and 9 of BBR, and retains the electropositivity of quaternary ammonium ion as is. Thus, as compared to BBR, **8j** had an enhanced activity of interacting with the biological targets and subsequently stabilized LDLR mRNA.

In conclusion, we have synthesized nineteen BBR analogues with a variety of side-chain substituents on the benzene ring D, paralleled with which was the biological examination of their activity on LDLR. The results suggested that the compound with a methoxyl group at the 10- and 11-position showed the most potent activity; and the methoxyl group at either 9- or 11-position might provide a beneficial shoring effect for the 10-position to obtain an optimal steric conformation. These results are of interest to establish the SAR of BBR, and they provide the basis for further chemical investigation. As **8j** showed an increasing activity in LDLR expression, it was selected as a potential cholesterol-lowering drug candidate for the next-step evaluation in animal experiments.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.005.

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15. Analytical data for selected final compound **8j**. Mp 242–244 °C; ^{1}H NMR (400 MHz, DMSO-d_6,) δ3.19 (t, 2H, J = 6.4 Hz, 5-CH_2), 3.99 (s, 3H, 11-OCH_3), 4.07 (s, 3H, 10-OCH_3), 4.76 (t, 2H, J = 6.4 Hz, 6-CH_2), 6.17 (s, 2H, –OCH_2O–), 7.09 (s, 1H, 4-CH), 7.57 (s, 1H, 1-CH), 7.71 (s, 1H, 12-CH), 7.73 (s, 1H, 9-CH), 8.76 (s, 1H, 13-CH), 9.52 (s, 1H, 8-CH); MS (m/z): 336 M^+; Anal. calcd for $C_{20}H_{18}NO_4Cl \cdot 1.5\ H_2O$ (in %): C, 60.23; H, 5.30; N, 3.51. Found: C, 60.26; H, 5.23; N, 3.15.
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