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Synthesis and in vitro evaluation of 12-(substituted aminomethyl) berberrubine derivatives as anti-diabetics

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ABSTRACT

By introducing various amino methyl groups into 12-position of berberrubine, a series of 12-(substituted aminomethyl) berberrubine derivatives were synthesized and evaluated for their anti-diabetic activity against type 2 diabetes mellitus. The results indicated that most of the prepared compounds exhibited moderate to good anti-diabetic activity, which were comparable to or even better than the berberine, the positive control rosiglitazone and insulin. Especially, compound **3b** with an *N*-methyl piperazine-4-methyl group at C-12, exerted the most powerful anti-diabetic activity.

Type 2 diabetes mellitus (T2DM), formerly known as non-insulin-dependent diabetes, accounts for most cases of diabetes worldwide and is a well-recognized public health problem today. It is a leading cause of death in developed countries and there is substantial evidence that it has been shown to be an epidemic of significant proportions in many developing and newly industrialized countries. According to the International Diabetes Federation, an estimated 366 million people worldwide have diabetes in 2011 and this number is projected to rise to 552 million by 2030.[1]

T2DM is a complex heterogeneous group of metabolic disorders characterized by increased levels of blood glucose due to impaired insulin action and/or insulin secretion.[2] The partial failure of the insulin producing β cells of the pancreas may lead to various complications, including hyperglycemia, excessive urine production, compensatory thirst, increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism.[3] The enormous and escalating economic and social costs of T2DM present a major challenge for attempts to reduce the risk of developing the condition as well as for energetic management of the established disease.

Current treatment options for T2DM include insulin, insulin sensitizers, secretagogues, alpha glucosidase inhibitors, incretins, pramlintide, and bromocriptine. However, current medicines for T2DM usually cause various side effects.[4] For instance, insulin sensitizer biguanides caused gastrointestinal complaints and the major side effects of another sensitizer thiazolidinediones (TZDs) included weight gain and peripheral edema. Secretagogue agents sulfonylureas and glinedes are always associated with weight gain and hypoglycemia. Alpha glucosidase inhibitors also reduce gastrointestinal complaints, such as bloating, abdominal cramps, flatulence et al. Another method for treatment of T2DM is incretin based therapies, including exenatide, liraglutide, dipeptidyl peptidase 4 (DPP 4) inhibitors, pramlintide, bromocriptine and insulin. However, these types of drugs also lead a series of side effects, such as common gastrointestinal complaints, body weight's change, headache and hypotension.

Berberine (BBR, **1**, Fig. 1), an isoquinoline alkaloid, is the major pharmacological component of the Chinese herb Coptis chinensis (Huang-Lian, a common herb in traditional Chinese medicine). As a botanic drug, berberine or berberine-containing herbs have been used to treat intestinal infections, particularly bacterial diarrhea, for thousands of years in China.[5] Recently, many research groups have showed a strong impact of BBR on glucose homeostasis and marked anti-diabetic effects on both human beings and rodent models, especially, with type 2 diabetes mellitus.[6–8] The action mechanism of anti-diabetic effect of BBR has been widely investigated.[9] The up-to-date reports argued that the anti-diabetic activity of BBR is related to activation of AMP-activated protein kinase (AMPK), acute initiation of glucose transport activity of glucose transporter GLUT1, and improvement of insulin signal transduction in insulin-resistant myotubes in cultured cells.[10,11]

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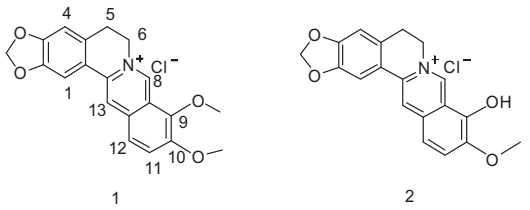


Figure 1. Structures of berberine (**1**) and berberrubine (**2**).

As berberine shows marked anti-diabetic effect, we set up to study the synthesis and anti-diabetic activity of the berberine derivatives using berberine as the lead compound in order to find active hypoglycemic agents. In our earlier studies, we have studied a new shortcut synthesis of 8-oxocoptisine, a natural protoberberine alkaloid starting from readily available and inexpensive berberine hydrochloride.[12] Furthermore, in our research to the anti-diabetic activity of berberine derivatives, we designed and synthesized a series of berberrubine derivatives and accidentally found that most of the 12-(substituted aminomethyl) berberrubine derivatives (Fig. 2) owing good anti-diabetic activities, which are better than that of berberine in the cell studies in vitro.

In this study, berberrubine derivatives were synthesized by means of the introduction of various aminomethyl groups into 12-position of berberrubine to improve the anti-diabetic activity. First, the commercially available berberine hydrochloride was readily converted into the berberrubine (**2**)[13] in a vacuum at 180 °C, and then the appropriate aliphatic amino or aryl amine with formaldehyde were added, stirred at 80 °C or room temperature to obtain the 12-C amino methylation berberrubine derivatives. Finally, salification of them with hydrochloric acid in ethanol gave the products **3a**–**j**[14] (Scheme 1).

All of the synthetic 12-(substituted aminomethyl) berberrubine derivatives and berberine were evaluated for their anti-diabetic activities against type 2 diabetes mellitus in 3T3-L1 adipocytes[15] and L6 myotubes[16] using rosiglitazone and insulin as positive control respectively. In 3T3-L1 adipocytes, berberine stimulated glucose uptake to a similar magnitude as did rosiglitazone, but the mechanism was not fully elucidated,[17] thus we take the rosiglitazone as positive control. The L6 myotubes has been widely used to investigate the mechanism of insulin- and exercise-stimulated glucose transport,[18] and Cheng et al. found that berberine stimulated glucose transport in myotubes.[19] And then in the cell studies, the 3T3-L1 adipocytes is selected to investigate the insulin-resistant reversal activity while the L6 myotubes is selected to investigate the glucose transport activity. The relative sensitization rates of the compounds to 3T3-L1 adipocytes and the sensitization rates of the compounds to L6 myotubes are summarized in Table 1.

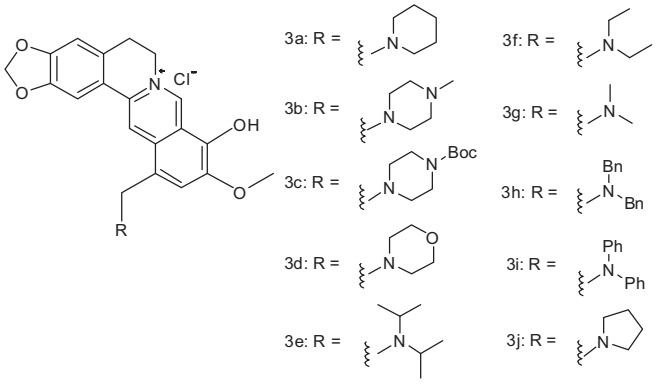
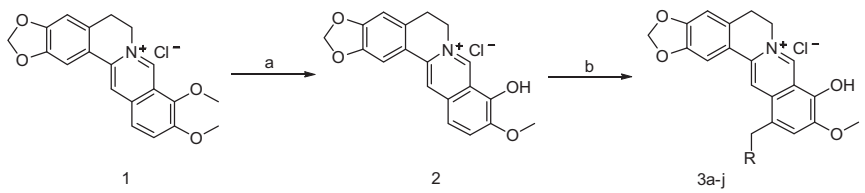


Figure 2. Structures of 12-(substituted aminomethyl) berberrubine derivatives.

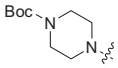
Comparing the 12-(substituted aminomethyl) berberrubine derivatives and berberine at different concentrations in 3T3-L1 adipocytes and L6 myotubes, the insulin-resistant reversal activity and glucose transport activity of the berberrubine derivatives are mostly comparable to or even better than the berberine. And comparing berberrubine derivatives and the positive control rosiglitazone at the concentration of 10 μmol/ml in 3T3-L1 adipocytes, all of the experimental compounds have a certain degree of insulin-resistant reversal activity, Therein the insulin-resistant reversal activities of compounds **3a**–**e** are better than the positive control rosiglitazone, and they are concentration-dependent. Among them the compound **3b** shows the best activity and the sensitization reaches 1.26 fold of rosiglitazone. Even the concentration is lowered to 1 μmol/ml, it still shows similar activity as the positive control. In the L6 myotubes, most of the compounds show middle to high activity to increase the transport of glucose compared to insulin. Especially, the compounds **3g** and **3h** are superior to positive control at the concentration of 10 μmol/ml. And the activities of compounds **3b** and **3g** are similar to or even better than the positive control at the concentration of 1 μmol/ml.

The results indicate that the introducing various aminomethyl groups into 12-position of berberrubine can remarkably improve the insulin-resistant reversal activity and stimulate glucose transport activity against type 2 diabetes mellitus. This modification is likely to enhance their ability binding to the target of drug action mainly through hydrophobic effect, conjugation effect and hydrogen bond on 9-hydroxyl. Since compounds **3a**–**d** differ structurally from compounds **3e**–**j** by the presence of a nitric heterocyclic six-membered ring group, the nitric heterocyclic six-membered ring group is perceived to be important for the enhanced insulin-resistant reversal activity. And in the L6 myotubes, the difference that **3e**–**g** show in the presence of the insulin-resistant reversal activity or glucose transport activity indicates that the single straight chain hydrocarbon substituted aminomethyl is likely important for the enhanced insulin sensitization as well. Among them, the compound **3g** shows the best glucose transport activity. And with the chain hydrocarbon substituted aminomethyl gets shorter from **3e** to **3g**, their insulin-resistant reversal activity is decreasing, while their glucose transport activity is increasing. If substituent is changed to benzyl and phenyl substituted aminomethyl, the compounds **3h** and **3i** show good glucose transport activity for the conjugation system might bind to the



Scheme 1. Synthesis of 12-(substituted aminomethyl) berberrubine derivatives. Reagents and conditions: (a) 180 °C in a vacuum (98% yield); (b) aliphatic amino or aryl amine, HCHO, HCl, C_2H_5OH (65–85% yield).

Table 1
Relative sensitization rates of 12-(substituted aminomethyl) berberrubine derivatives to 3T3-L1 adipocytes and sensitization rates of 12-(substituted aminomethyl) berberrubine derivatives to L6 myotubes

Compound	R =	Concentration (μmol/ml)	Relative sensitization rate [a] (%)	Sensitization rate [b] (%)
3a		10	102.93	43.53
		1.0	41.02	15.78
		0.10	8.33	
3b		10	126.29	48.55
		1.0	90.23	41.90
		0.10	−1.09	
3c		10	107.30	52.51
		1.0	3.14	23.32
		0.10	−43.33	
3d		10	115.14	57.22
		1.0	68.72	26.16
		0.10	28.22	
3e		10	112.25	46.08
		1.0	28.02	17.97
		0.10	−64.34	
3f		10	75.63	48.83
		1.0	23.48	5.81
		0.10	2.03	
3g		10	51.59	78.50
		1.0	−17.72	51.30
		0.10	−16.79	
3h		10	65.58	70.02
		1.0	−9.51	28.39
		0.10	−17.34	
3i		10	96.66	53.05
		1.0	62.08	12.43
		0.10	33.38	
3j		10	73.15	40.73
		1.0	−12.10	7.95
		0.10	−22.09	
Berberine		10	74.23	43.28
		1.0	13.27	21.30
		0.10	−2.93	
Insulin		1.0		48.21

[a] The relative sensitization rates of the compounds to 3T3-L1 adipocytes in vitro. Sensitization rate = [1 − (sample's OD − blank's OD)/(negative's OD − blank's OD)] × 100%, relative sensitization rate = the sensitization rate of sample/the sensitization rate of rosiglitazone × 100%. Each sample is diluted into three concentrations in the screening.
[b] The sensitization rates of the compounds to L6 myotubes in vitro. Sensitization rate = [(negative's OD − sample's OD)/(negative's OD − blank's OD)] × 100%. Each sample is diluted into two concentrations in the screening.

target through conjugation effect. However, it remains to be further investigated that whether their target is AMPK as berberine and the structural change of the group is effective to generate more potent anti-diabetic agents.

In conclusion, the 12-(substituted aminomethyl) berberrubine derivatives have a certain degree of stimulating glucose uptake for the insulin-resistant 3T3-L1 adipocytes and L6 myotubes. It indicates that these compounds can be used in the treatment caused by non-insulin dependent (type 2 diabetes mellitus) DM, obesity, fatty liver disease and their complications. Structural features as mentioned above can provide useful information for the modification of berberrubine derivatives with an enhanced activity in the future.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.032. These data include MOL files and InChiKeys of the most important compounds described in this article.

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13. Berberine hydrochloride (**1**, 20 g, 62.11 mmol) was placed in the round bottom flask and in a vacuum, stirred at 180 °C for 20 min to give the crude product, which was purified using flash silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) to give 22 g of the red solid compound berberrubine (**2**, 98% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.22 (s, 1H), 8.06 (s, 1H), 7.56 (d, 1H, J = 8.0 Hz), 7.43 (s, 1H), 6.96 (s, 1H), 6.85 (d, 1H, J = 8.0 Hz), 6.10 (s, 2H), 4.70 (t, 2H, J = 6.0 Hz), 3.78 (s, 3H), 3.23 (t, 2H, J = 6.0 Hz). MS (ESI+) m/z: 322 $[M+H]^+$.
14. *General procedure for synthesis of the compounds* ***3a–j***: Hydrochloride berberrubine (322 mg, 1.0 mmol) was dissolved in 5.0 ml of the anhydrous ethanol, aliphatic amino or aryl amine (5.0 mmol) and 0.4 ml formaldehyde aqueous solution (37%, 5.0 mmol) were added, stirred at room temperature or

at 80 °C for 24 h. Concentrated under reduced pressure to give the crude products, which were purified using flash silica gel column chromatography (CH_2Cl_2/MeOH = 20:1) to give the dark red solid, salification of them with hydrochloric acid in ethanol gave the products **3a–j**.

*12-(Piperidine-1-ylmethyl)-berberrubine (**3a**)*: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.18 (s, 1H), 8.16 (s, 2H), 7.26 (s, 1H), 7.22 (s, 1H), 6.74 (s, 1H), 6.06 (s, 2H), 4.38 (t, 2H, J = 6.0 Hz), 3.93 (s, 3H), 3.61 (s, 2H), 3.08 (t, 2H, J = 6.0 Hz), 2.41 (s, 4H), 1.56 (m, 6H). MS (ESI+) m/z: 420 $[M+H]^+$.

*12-(N-Methyl piperazine-4-methyl)-berberrubine (**3b**)*: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.20 (s, 1H), 8.09 (s, 2H), 7.24 (s, 1H), 7.22 (s, 1H), 6.75 (s, 1H), 6.07 (s, 2H), 4.39 (t, 2H, J = 6.0 Hz), 3.92 (s, 3H), 3.66 (s, 2H), 3.08 (t, 2H, J = 6.0 Hz), 2.45 (s, 8H). MS (ESI+) m/z: 435 $[M+H]^+$.

*12-(Morpholinomethyl)-berberrubine (**3d**)*: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.22 (s, 1H), 8.10 (s, 2H), 7.27 (s, 1H), 7.21 (s, 1H), 6.75 (s, 1H), 6.07 (s, 2H), 4.40 (t, 2H, J = 6.0 Hz), 3.93 (s, 3H), 3.70 (t, 4H, J = 4.4 Hz), 3.66 (s, 2H), 3.09 (t, 2H, J = 6.0 Hz), 2.50 (t, 4H, J = 4.4 Hz). MS (ESI+) m/z: 421 $[M+H]^+$.

*12-(N,N-Diethyl amino-methyl)-berberrubine (**3f**)*: ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.10 (s, 1H), 7.97 (s, 1H), 7.65 (s, 1H), 7.10 (s, 1H), 6.97 (s, 1H), 6.15 (s, 2H), 4.46 (t, 2H, J = 6.0 Hz), 3.73 (s, 3H), 3.54 (s, 2H), 2.57 (m, 4H), 1.05 (m, 6H). MS (ESI+) m/z: 408 $[M+H]^+$.

*12-(Dimethylamino-methyl)-berberrubine (**3g**)*: ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.07 (s, 1H), 7.98 (s, 1H), 7.64 (s, 1H), 7.13 (s, 1H), 6.96 (s, 1H), 6.10 (s, 2H), 4.47 (t, 2H, J = 6.0 Hz), 3.72 (s, 3H), 3.56 (s, 2H), 3.03 (t, 2H, J = 6.0 Hz), 2.16 (s, 6H). MS (ESI+) m/z: 379 $[M+H]^+$.

*12-(Pyrrolidin-1-ylmethyl)-berberrubine (**3j**)*: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.17 (s, 1H), 8.01 (s, 2H), 7.26 (s, 2H), 6.74 (s, 1H), 6.05 (s, 2H), 4.36 (t, 2H, J = 6.0 Hz), 3.93 (s, 3H), 3.78 (s, 2H), 3.05 (t, 2H, J = 6.0 Hz), 2.55 (s, 4H), 1.68 (s, 4H). MS (ESI+) m/z: 406 $[M+H]^+$.

15. 3T3-L1 adipocytes are cultured in six-well plates with high glucose Dulbecco's modified Eagle medium (DMEM) containing 10% Fetal bovine serum (FBS) and phenolsulfonphthalein (PSP), differentiated for four days in the high glucose DMEM supplemented with 10% FBS, PSP, Ins, Dex, IBMX when the confluence degree reaches 90%. Then the cells are digested and inoculated in 24-well plates, cultured in high glucose DMEM supplemented with 10% FBS and PSP for two days. The cells are induced insulin resistance in the high glucose DMEM supplemented with 10% FBS, PSP, low concentration of Ins, high concentration of Dex for four days, and switch to the high glucose DMEM supplemented with the sample but no PSP, meanwhile we set up the blank control (normal cells after being induced differentiation) and the negative control (insulin-resistant cells after being induced differentiation) containing no samples. The cells are continued to be cultivated for 50 h. Then we take 10 μl supernatant to the enzyme label plate, examine the glucose level via Kit assay (Chengdu Mike Technology Company), and determine the OD (optical density) value in 490 nm. This experiment adopts the positive control rosiglitazone (purchased from GSK).
16. L6 myotubes are cultured in 24-well plates with high glucose DMEM containing 10% FBS, PSP for 24 h, and differentiated in the high glucose DMEM supplemented with 2% FBS and PSP. The differentiation medium is charged every 48 h. The cells are disposed overnight with high glucose DMEM supplemented with no serum after being differentiated for 6–8 days, and then disposed for 4–6 h in the DMEM supplemented with no serum, no glucose. At last, the cells are disposed in the 180 μl serum-free DMEM containing 20 mmol glucose for 16–24 h. Then 10 μl supernatant is taken to the enzyme label plate. We examine the glucose level via Kit assay (Chengdu Mike Technology Company), and determine the OD value in 490 nm. This experiment adopts the positive control insulin (purchased from the SIGMA).
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