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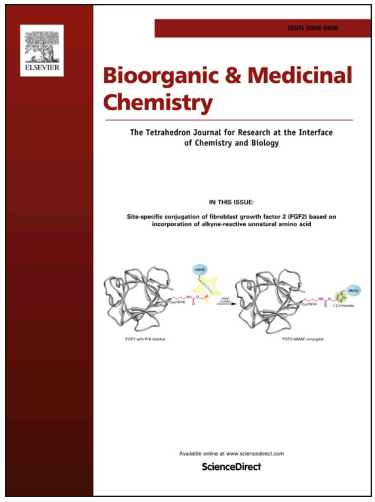

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The design and synthesis of a novel compound of berberine and baicalein that inhibits the efficacy of lipid accumulation in 3T3-L1 adipocytes

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The combination of berberine and baicalein may have a better therapeutic effect against disease. To explore the combined effect of baicalein and berberine in the treatment of obesity, we designed and synthesized a hybrid compound, and its biological activities were evaluated in 3T3-L1 adipocytes. The structures of the berberine-baicalein (BBS) compounds were confirmed by ^{1}H NMR, ^{13}C NMR, ultraviolet spectroscopy and high resolution mass sectrometry (HRMS). The present study showed that the IC_{50} values of the inhibitory effects of baicalein, berberine and BBS against 3T3-L1 cells were 29.81±0.90, 21.84±1.67 and 9.42±0.60 μM, respectively. The expression of mRNAs related to lipolysis and lipogenesis were examined by quantitative real-time PCR. The results showed that BBS could up-regulate the expression of the *Atgl* gene and down-regulate the mRNA expression of *Srebp-1c*, *Fasn*, *Scd1*, and *Acc* in 3T3-L1 adipocytes. These results indicate that BBS may have a stronger effect than baicalein and berberine on the viability of 3T3-L1 preadipocytes. In addition, BBS may have therapeutic effects and pharmacological activities against obesity. This "medicine couple" may be beneficial for studies of traditional Chinese medicine.

Keywords: Obesity; Berberine; Baicalein; Synthesis

1. Introduction

Obesity is an overaccumulation of lipids and a symptom of metabolic disorders. Obesity is a major risk factor for a number of chronic diseases, particularly cardiovascular diseases, type 2 diabetes, obstructive sleep apnea, cancer, osteoarthritis and depression. The World Health organization (WHO) defines overweight as a body mass index (BMI) equal to or greater than 25 and obesity as a BMI equal to or greater than 30.[1] According to research by the WHO published in May 2017, obesity has reached epidemic proportions globally, and at least 2.8 million people die each year from being overweight or obese.[1-3] Obesity is more common in women than men. Authorities consider it one of the most serious public health problems of the 21st century.[4] It is very important for the treatment of obesity to decrease lipid accumulation and reduced triglyceride content.[5]

Until now, hundreds of traditional Chinese herbs and active components have been proven to be effective for treatment against diabetes, dyslipidaemia and obesity. For instance, in *Coptis chinensis*, a popular traditional Chinese medical plant used in the treatment of diabetes and infections, the major active component is berberine (BBR). It was observed that berberine can improve metabolic syndrome, leading to decreased plasma cholesterol and triglyceride levels in hypercholesterolemic patients.[6-9] A study showed that BBR decreased the TG content in 3T3-L1 adipocytes by directly increasing the expression of adipose triglyceride lipase (*Atgl*). In the procession of *Atgl* expression, the effect of BBR could be abolished by Compound C.This suggested that the AMP-activated protein kinase (AMPK) pathway is involved in the effects of BBR on the pormone-sensitive lipase (*p-Hsl*) and *Atgl*.[10] BBR may act as an adjuvant medication to prevent lipid metabolism disorders caused by antipsychotic medications by down-regulating the sterol regulatory element-binding protein (*Srebp*) pathway and up-regulating the phosphorylation of AMPK.[11-12] Many published studies consistently demonstrate that BBR is a strong inducer of Thr-172 phosphorylation in AMPK.[13-18]

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In *Scutellaria baicalensis*, the major active component is baicalein; *Scutellaria baicalensis* is widely used in traditional Chinese medicine. It has previously been shown that extracts of S. baicalensis improved obesity and hypertriglyceridemia in a type 2 diabetic animal model.[19-23] Baicalein may be used as a treatment to prevent metabolic disorders. Its major mechanism is through the up-regulation of AMPK and its related signal pathway.[23-26] Baicalein may be a promising complementary therapy for weight management or obesity.[27] Baicalein inhibits lipid accumulation during adipogenesis in 3T3-L1 cells.[28]

Using prescriptions called "formulae" as combination therapy has a history of 2500 years. The combination of some different traditional Chinese medicine may increase the therapeutic effect.[29-30] Huanghousu was synthesized by magnolol and berberine, it is reported that its efficacy in improving lipid metabolism was better than that of a single component.[31] *Coptis chinensis* and *Scutellaria baicalensis* are a well-known "medicine couple" in China. This combination is believed to produce enhanced therapeutic effect and is very frequently co-prescribed in Chinese Medicinal formulae.[32-33] The process and efficacy of a new compound for synthesizing berberine and baicalein have been questioned. To synthesize highly effective new compounds for the treatment of obesity, we designed and synthesized berberine and baicalein. Here the structures of all compounds in the synthesis process were confirmed by spectroscopy (^{1}H NMR, ^{13}C NMR and HRMS spectra). We evaluated BBS inhibit Lipid accumulation efficacy in 3T3-L1 adipocytes, which measured mRNA expression of lipolysis and lipogenesis associated genes using quantitative real-time PCR.

2. Materials and methods

2.1. Chemicals and reagents

Berberine and baicalein (purity≥98%) were purchased from the China Drugs and Biological Products Inspection Institute (Beijing, China). Phosphate-buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM) and foetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Newborn calf serum (CS) was purchased from Bovogen (Melbourne, Australia). 3-Isobutyl-1-methylxanthine (IBMX), insulin (INS), dexamethasone (DEX) and Oil Red O were purchased from Sigma Aldrich (St Louis, MO). A Cell Counting Kit-8 (CCK8) was bought from Dojindo (Kyushu, Japan). A triglyceride GPO-POD assay kit and a BCA protein assay kit were from Applygen (Beijing, China). RNAiso Plus, the PrimeScript™ RT Reagent Kit with gDNA Eraser (Perfect Real Time), and SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) were purchased from Takara (Tokyo, Japan). The mass spectra were recorded using a DSQ mass spectrometer (Thermo, USA).

2.2. Berberine-baicalein (BBS) synthesis

2.2.1. Synthesis of berberrubine[34]

The synthesis of berberrubine utilized a microwave synthesis method as described in the literature.[34] Berberine (1 g, 2.07 mmol) was dissolved in N,N-dimethylformamide (DMF, 25 ml). Several zeolites were added and refluxed. The reaction mixture was microwave irradiated for 15 min at 400 W. The completion of the reaction was diluted with water (40 ml) and refrigerated until crystallization was complete. The crystals were washed with petroleum ether and evaporated in a vacuum to obtain compound **1** (berberrubine) with a 72% yield. The filtrate was purified using PIPO-02 macroporous resin, filtered and evaporated to isolate compound **1** with a 21% yield. Compound **1** was obtained with 93% yield (purity≥98%).

2.2.2. Synthesis of 9-(6-bromoethyl)berberine hydrochloride

Compound **1** (15 g, 46.55 mmol) in acetonitrile (600 ml) was added to 1,6-dibromoethane (80 ml) and stirred at 86□ for 1 h. The reaction mixture was evaporated to obtain the crude compound, which was washed with acetonitrile and aceticether to afford compound **2** (9-(6-bromoethyl)berberine hydrochloride) with a 93% yield.

9-(6-Bromoethyl)berberine hydrochloride ***2***. Yellow crystals; UV: λmax 202.8, 229.4, 266.3, 346.8, and 430.3nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.80 (1H, s, H-1), 7.09 (1H, s, H-4), 3.57 (2H, t, *J* = 5.8, H-5), 4.96 (2H, t, *J* = 5.8, H-6), 9.76 (1H, s, H-8), 8.00 (1H, d, *J* = 9.1, H-11), 8.19 (1H, d, *J* = 9.1, H-12), 8.95 (1H, s, H-13), 6.17 (2H, s, H-15), 4.05 (3H, s, H-16), 4.28 (2H, t, H-17), 1.84-1.89 (2H, m, H-18 H-21), 1.49-1.51 (2H, m, H-19 H-20), and 3.19 (2H, t, H-22); ^{13}C NMR ($CDCl_3$, 300 MHz) δ: 105.32 (C-1), 150.29 (C-2), 149.71 (C-3), 108.31 (C-4), 130.58 (C-4a), 27.22 (C-5), 56.95 (C-6), 145.18 (C-8), 137.35 (C-8a), 120.12 (C-9), 147.57 (C-10), 120.35 (C-11), 123.21 (C-12), 132.92 (C-12a), 126.57 (C-13), 142.71 (C-14), 121.55 (C-14a), 101.98 (C-15), 55.20 (C-16), 73.97 (C-17), 29.20 (C-18), 24.31 (C-19), 26.22 (C-20), 32.07 (C-21), and 35.11 (C-22); ESI-MS: m/z, $[C_{25}H_{27}BrNO_4]^+$, 484.11$[M-Br]^+$.

2.2.3. Synthesis of berberine-baicalein(BBS)

Compound **2** (0.5 g, 1.03 mmol) was added to DMF (35 ml) and triethylamine (6 ml), Baicalein (0.18 g, 0.67 mmol) and several zeolites and then refluxed under sonication for 30 s. Then, the reaction mixture was microwave irradiated for 10 min at 240 W. At the completion of the reaction, the reaction was cooled to room temperature, and the pH value of the solution was adjusted to 2–3 by adding HCl. The reaction mixture was diluted with water and refrigerated until crystallization was complete. The crystals were washed with distilled water to neutral pH. The crude product was washed with acetic ether and evaporated in a vacuum at 80□ to obtain compound **3**. Compound **3** (BBS) was obtained with a 47% yield (purity≥96%).

Berberine-baicalein ***3***. Yellow solid; UV: λmax 217.7, 271.8, 334.2, and 436.7 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.73 (1H, s, H-1), 7.01 (1H, s, H-4), 3.18 (2H, t, *J* = 5.8, H-5), 4.95 (2H, t, *J* = 5.8, H-6), 9.74 (1H, s, H-8), 7.94 (1H, d, *J* = 9.1, H-11), 8.15 (1H, d, *J* = 9.1, H-12), 8.90 (1H, s, H-13), 6.14 (2H, s, H-15), 4.04 (3H, s, H-16), 4.30 (2H, t, H-17), 1.84-1.92 (2H, m, H-18 H-21), 1.57 (2H, m, H-19 H-20), 4.13 (2H, t, H-22), 8.67 (1H, s, H-23), 12.48 (1H, s, H-24), 6.94 (1H, s, H-27), 6.89 (1H, s, H-30), 8.05-8.06 (2H, m, H-33 H-37), and 7.54-7.61 (3H, m, H-34 H-35 H-36); ^{13}C NMR ($CDCl_3$, 300 MHz) δ: 105.29 (C-1), 150.20 (C-2), 149.61 (C-3), 108.24 (C-4), 130.47 (C-4a), 28.31 (C-5), 56.94 (C-6), 145.06 (C-8), 137.26 (C-8a), 120.12 (C-9), 147.54 (C-10), 120.28 (C-11), 123.16 (C-12), 132.90 (C-12a), 126.50 (C-13), 142.70 (C-14), 121.47 (C-14a), 101.98 (C-15), 55.17 (C-16), 74.03 (C-17), 25.05 (C-18), 24.89 (C-19), 26.23 (C-20), 29.33 (C-21), 68.66 (C-22), 131.84 (C-23), 149.61 (C-24), 105.01 (C-25), 145.95 (C-26), 91.79 (C-27), 153.86 (C-28), 182.13 (C-29), 104.51 (C-30), 162.93 (C-31), 130.69 (C-32), 126.17 (C-33 C-37), 129.00 (C-34 C-6), and 130.03 (C-35); ESI-MS: m/z, $[C_{40}H_{36}NO_9]^+$, 674.24.

2.3. Cell culture and treatment

2.3.1. Cell culture and adipocyte differentiation

3T3-L1 cells were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China) and were maintained in DMEM supplemented with 10% CS and 1% penicillin-streptomycin in a humidified 5% CO_2 incubator at 37□. For the experiments, cells were seeded at a density of 2×10^5 cells/well in 6-well plates with DMEM supplemented with 10% FBS. Two days after confluency was reached, the culture medium was switched to differentiation medium (DMEM, 10% FBS, 0.5 mmol/L IBMX, 1.0 μmol/L DEX and 10 mg/L insulin) for 3 days. Then, the cells were maintained in differentiation medium containing only 10 mg/L of insulin for 2 days. The cells were replenished with DMEM containing 10% FBS every other day. On day 10, 80% of the 3T3-L1 cells had differentiated into mature adipocytes.

2.3.2. Cytotoxicity assay

The cell viability was assayed using the Cell Counting Kit-8 (CCK8) according to the manufacturer's protocol. The cells were seeded in a 96-well plate at a density of 5×10^3 cells/well. The cells were incubated for 24 h and then treated with medium containing various concentrations of berberine, baicalein and BBS (0-80 μM) for 48 h. After incubation, 10 μl of CCK8 solution were added to each well, and the plate incubated for 2 h at 37°C. The absorbance of the solution was then measured at 450 nm using a microplate reader (Thermo Fisher, USA). The IC_{50} value was defined as the half-maximal inhibitory concentration of cell viability, and it was quantitative measured by the logit method.

2.3.3. Oil Red O staining and lipid accumulation

Differentiated adipocytes were rinsed twice with PBS (pH 7.4) and were fixed in 10% buffered formalin for 30 min at room temperature. Then, the cells were washed with distilled water twice and stained with freshly diluted Oil Red O solution (six parts of 0.5% Oil Red O solution and four parts of distilled water) for 1 h at room temperature. Then, the Oil Red O-stained cells were washed twice with distilled water and visualized under thane inverted microscope (TS100, Nikon, Tokyo, Japan). For the quantitative determinations of the accumulated lipids, the Oil Red O-stained cells were washed twice with 60% isopropanol and destained with isopropanol. Then, the absorbance was measured at 510 nm with a spectrophotometer.[35]

2.3.4. Triglyceride quantification

The cells were washed with PBS and dissolved in Cell lysates. The cell lysates were heated at 70℃ for 10 min. The mixture was centrifuged at 2000 rpm for 5 min, and the supernatant was collected. 10 μl of the supernatant and 200 μl of working fluid were mixed together at 37℃ for 10 min. The absorbance was quantified at 550 nm with a spectrophotometer. The protein content of the remainder of the mixture was measured according to the BCA method. The TG values were expressed as TG/protein.

2.3.5. Quantitative Real-Time Polymerase Chain Reaction (qPCR)

The total RNA was extracted from differentiated adipocytes using RNAiso Plus according to the manufacturer's instructions. The RT reaction was carried out using 1 μg of total RNA. The RNA was reverse transcribed into cDNA using a reverse transcription kit. The gene expression levels were measured with qRT-PCR using the CFX96™ Real-Time PCR Detection System (Bio-Rad) and SYBR® Premix Ex TaqTM II. The primers that were used are shown in Table 1. The relative gene expression quantification was normalized to the β-actin mRNA expression using the $2^{-\Delta\Delta Ct}$ analysis method.

Table 1 Primers used for quantitative real-time PCR.

Gene	Forward Primer	Reverse Primer	Annealing Temperature (°C)
Ampk	GTCCTGCTTGATGCACACAT	GTCCTGCTTGATGCACACAT	55.5
HSL	AGACACCAGCCAACGGATAC	GCTGGCACGGAAGAAGATAC	57.4
Atgl	GGAATGGCCTACTGAACCAA	GCAATTGATCCTCCTCTCCA	54.5
Acc	GCCTCCAACCTCAACCACTA	AAGGTCCGGAAAGAGACCAT	56
Srebp-1c	TGCTCCAGCTCATCAACAAC	GGCCAGAGAAGCAGAAGAGA	56
Fasn	CTGAAGAGCCTGGAAGATCG	CGGAGCTTGTGGTAGAAGGA	55.5
Scd1	GCTGGAGTACGTCTGGAGGA	CCGAAGAGGCAGGTGTAGAG	57.4
β-actin	GGAGATTACTGCCCTGGCTCCT	GACTCATCGTACTCCTGCTTGCT	60

Abbreviations: Ampk (PRKAA1), AMP-activated protein kinase; HSL, Hormone-sensitive lipase; Atgl, Adipose triglyceride lipase; ACC, acetyl-CoA carboxylase; Srebp-1c, Sterol regulatory element-binding protein 1; Fasn, Fatty acid synthase; Scd1, Stearoyl-CoA desaturase-1.

2.4. Statistical analysis

The data are expressed as the means ± SD. Statistical analysis was performed using the SPSS 20.0 statistical software. The significant differences between groups were statistically analysed using one-way analysis of variance (ANOVA) followed by a post hoc test (LSD). All differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Optimization of the conditions used for berberrubine synthesis

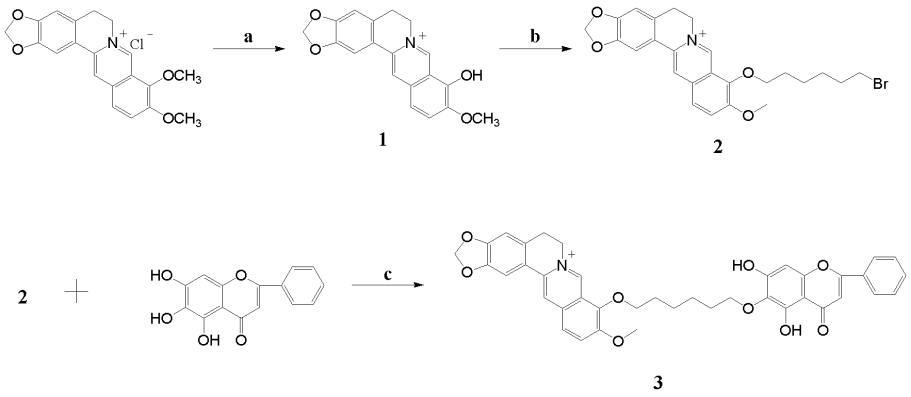
In a previous work, it was reported that berberrubine was obtained by vacuum pyrolysis. Because of uneven heating, the product had a low purity and yield.[36] To obtain high purity berberrubine, berberrubine was synthesized by microwave irradiation under different power conditions, BBR-DMF ratios, and times, which are described in Table 2. Berberine was converted into berberrubine at the critical temperature of 160℃. Microwave radiation was efficient in polar medium. The reaction medium had a high boiling point and used DMF. The optimal protocol is that berberine was dissolved in DMF (25 ml) and irradiated 15 min at 400 W. Under these conditions, berberrubine was obtained with a 93% yield (purity≥98%).

Table 2 The microwave irradiation synthesis of berberrubine under different conditions.

Entr	Ratio(BBR:DMF)	Time(min)	Power(W)	Yield(%)
1	1:50	15	240	80.1
2	1:50	15	400	95.4
3	1:50	15	640	88.5
4	1:50	15	800	83.5
5	1:5	15	400	81.6
6	1:10	15	400	83.2
7	1:15	15	400	93.3
8	1:20	15	400	94.7
9	1:25	15	400	98.4
10	1:30	15	400	94.9
11	1:50	15	400	95.2
12	1:25	5	400	75.9
13	1:25	10	400	87.6
14	1:25	15	400	98.4
15	1:25	20	400	96.8
16	1:25	25	400	97
17	1:25	30	400	96.7

3.2. Design of Berberine-baicalein hybrid compound (BBS)

The synthesis of the berberine-baicalein hybrid compounds was prepared according to the synthetic scheme in Scheme 1. Berberine was microwave irradiated to obtain berberrubine **1**, which carried a hydroxyl group at the 9-position, with 93% yield. Treatment of berberrubine **1** with 1,6-dibromoethane in acetonitrile at 86℃ for 1 h enabled the formation of the key intermediate 9-(6-bromoethyl)berberine hydrochloride **2** with 93% yield. The reaction of 9-(6-bromoethyl)berberine hydrochloride **2** with DMF, triethylamine and baicalein were refluxed and microwave irradiated to produce berberine-baicalein hybrid compound **3** with a 47% yield. The structures of the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, ultraviolet spectroscopy and HRMS spectroscopy. 9-(6-bromoethyl)berberine hydrochloride **2** was synthesized using acetonitrile as the solvent. The crystals of 1,6-dibromethane were removed using acetic ether. The results indicate that two different bioactive molecules were synthesized into a hybrid compound by chemical bonding.



Scheme 1. The synthesis of berberine-baicalein (BBS) compounds. Reagents and conditions: (a) DMF, microwave irradiation (400 W), reflux, 15 min, (93% yield); (b) acetonitrile, 1,6-dibromoethane, 86□, 1 h, (93% yield); (c) DMF, triethylamine, reflux, microwave irradiate (240 W), 10 min, (47% yield).

3.3. Cytotoxicity of BBS in 3T3-L1 Preadipocytes

To examine cell viability, 3T3-L1 cells were treated with various concentrations (1.25, 2.5, 5, 10, 20, 40, and 80 μM) of baicalein, berberine and BBS for 48 h. Then, the cell viability was measured using a CCK8 assay. As shown in Fig. 1, we found that 3T3-L1 cells exhibited different susceptibilities to baicalein, berberine and BBS in a dose-dependent manner. The results indicate that baicalein did not affect cell viability at concentrations between 1.25 and 5 μM, while greater than 10 μM was toxic (Fig. 1A). Compared to the control, the number of cells was reduced by 45% with treatment with 20 μM berberine (Fig. 1B). BBS did not cause significant cytotoxicity below 5 μM (Fig. 1C). We found that baicalein had no effect on cell viability at 100 μM; thus, the inhibitory effect of baicalein on fat accumulation is not due to its cytotoxicity. The IC_{50} values of inhibitory effects of baicalein, berberine and BBS against 3T3-L1 cells were 29.81±0.90, 21.84±1.67 and 9.42±0.60 μM, respectively. Therefore, the nontoxic concentrations of baicalein, berberine and BBS selected for treatment in 3T3-L1 cells were at 2.5 and 5 μM.

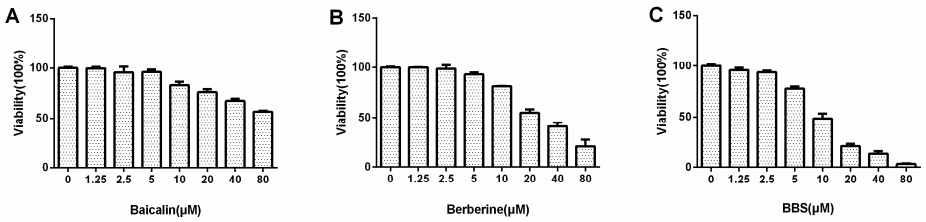


Fig. 1. The effects of baicalein (A), berberine (B), BBS (C) on cell viability. Cell viability was measured using a CCK8 assay. The cells were plated in 96-well plates at a density of 5×10^3 cells/well for 24 h. Then, the cells were treated with different concentrations of baicalein, berberine and BBS for 48 h. The control group of cells (con) was treated with 0.1% DMSO. Each value is the mean±SD of the results from six different plates (n = 6) and is representative of the results from at least three different experiments.

3.4. Effect of BBS on the lipid accumulation and triglyceride content in 3T3-L1 adipocytes

3T3-L1 preadipocytes were differentiated for 10 days into mature adipocytes. To assess the effect of BBS on lipid accumulation in 3T3-L1 adipocytes, Oil Red O staining and its subsequent quantification were performed to

observe the intracellular lipid accumulation. As shown in Fig. 2A, 3T3-L1 cells have a fibroblast-like morphology. In the microscopic observation of Oil Red O staining in 3T3-L1 adipocytes (Fig. 2B), we found that a large number of lipid droplets in 3T3-L1 adipocytes were stained red by Oil Red O staining. The results from the Oil Red O quantification showed that the lipid accumulation in 3T3-L1 adipocytes treated with BBS was significantly lower than the lipid accumulation in the mature adipocyte group (CON) (Fig. 2C). To understand the effect of the BBS compound on TG content in 3T3-L1 adipocytes, we used the Triglyceride GPO-POD Assay Kit. BBS at 2.5 μM and 5 μM significantly decreased the TG contents by 27.3% and 37.4%, respectively, compared to untreated 3T3-L1 adipocytes (CON), as shown in Fig. 2D. These results indicated that the metabolism of TG in 3T3-L1 adipocytes was regulated by BBS.

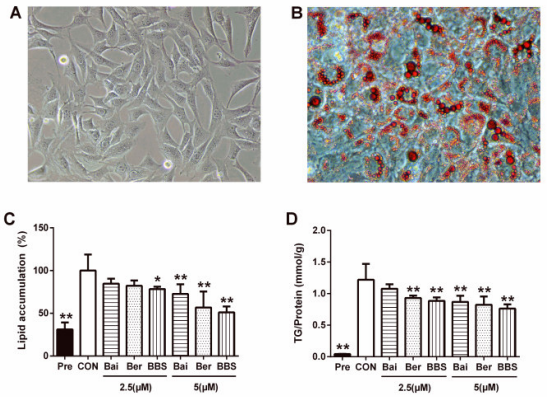


Fig. 2. (A) 3T3-L1 preadipocyte morphology. (B) Oil Red O staining of 3T3-L1 mature adipocytes. (C) A graphical representation of the percentages of intracellular lipids after Oil Red O staining. (D) A TG content assay of 3T3-L1 mature adipocytes after treatment with different concentrations (0, 2.5, and 5 μM) of baicalein, berberine and BBS for 48 h. The TG content was normalized by assessing the total protein levels. The 3T3-L1 mature adipocytes group (CON) and the 3T3-L1 preadipocyte cells (Pre) were treated with 0.1% DMSO. Each value is the mean ± SD of results from at least three different experiments. *$P < 0.05$, **$P < 0.01$ compared with the CON group.

3.5. Effect of BBS on the expression of AMPK and lipolysis-associated genes

AMP-activated protein kinase (*Ampk*) plays a crucial role in energy metabolism and is the master switch of lipid metabolism.[37] ATGL and HSL are pivotal enzymes that limit the rate of lipolysis. To understand the effect of BBS on the lipolytic enzyme in 3T3-L1 adipocytes, 3T3-L1 adipocytes were treated with different concentrations (0, 2.5, and 5 μM) of baicalein, berberine and BBS for 48 h. We examined the mRNA expression of *Ampk*, *Atgl* and *Hsl* using qPCR. As shown in Fig. 3A, when compared with the mature adipocyte group (CON), we found that the expression levels of *Ampk* were up-regulated in the preadipocyte group (Pre) and the groups treated with 5 μM of baicalein and BBS ($P < 0.05$). The *Atgl* mRNA level was significantly increased compared with the CON group (Fig. 3B). The expression of the *Hsl* gene was decreased in response to treatment with 2.5 μM of baicalein and berberine (Fig. 3C). These results indicate that baicalein, berberine and BBS increased adipocyte lipolysis by increasing the expression of the *Atgl* gene. However, there was no significant effect caused by BBS on the expression of *Hsl*.

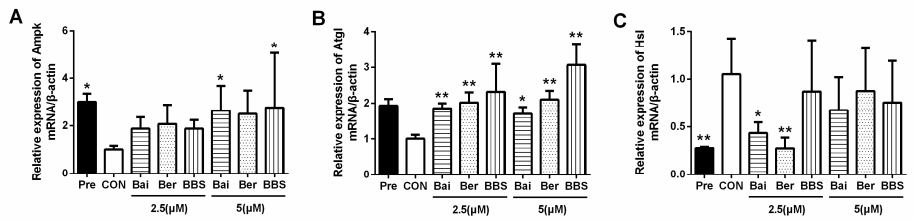


Fig. 3. The effect of baicalein, berberine and BBS on the mRNA expression of *Ampk* (A), *Atgl* (B), and *Hsl* (C) in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with different concentrations (0, 2.5, and 5 μM) of baicalein, berberine and BBS for 48 h. The total RNA was extracted and subjected to qPCR. The mRNA levels were quantified and normalized to that of β-actin. The data are presented as the mean ± SD of three independent experiments. *P < 0.05, **P < 0.01 indicates statistically significant differences compared to the mature adipocyte group (CON). The 3T3-L1 preadipocyte cells were treated with 0.1% DMSO (Pre).

3.6. Effect of BBS on the mRNA expression of lipogenesis associated genes

Srebp-1c is a key transcription factor that controls the expression of the genes of triglyceride synthesis. *Fasn, Acc* and *Scd1* are the genes of the major enzymes controlling the lipogenic pathway.[38] To investigate the effect of baicalein, berberine and BBS on lipogenesis in 3T3-L1 adipocytes, the mRNA expression of the lipogenesis genes (*Srebp-1c, Fasn, Acc,*and *Scd1*) were examined by qPCR. As shown in Fig. 4A, most interestingly, the results revealed that baicalein, berberine and BBS significantly decreased the expression of *Srebp-1c* in a dose-dependent fashion. The expression level of the *Fasn* gene also decreased in response to treatment with 5 μM of baicalein, berberine and BBS (Fig. 4B). The *Scd1* mRNA level was significantly down-regulated compared with the CON group (Fig. 4C). BBS treatment at 5 μM markedly decreased the expression of the *Acc* mRNA level (Fig. 4D). These observations indicate that BBS reduces lipid accumulation by suppressing the expression of of lipogenesis genes. Particularly, BBS exhibited a strong effect on the mRNA expression of lipogenesis-associated genes in 3T3-L1 adipocytes than baicalein and berberine.

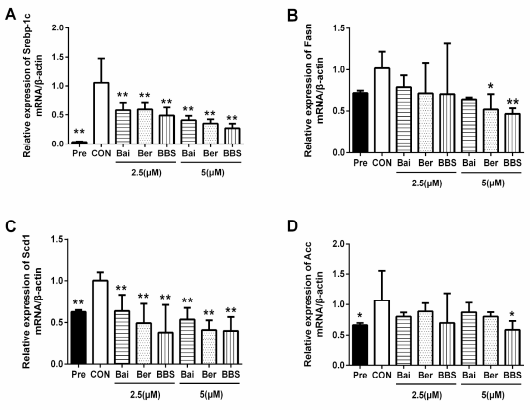


Fig. 4. The effect of baicalein, berberine and BBS on the mRNA expression levels of *Srebp-1c* (A), *Fasn* (B), *Scd1* (C), and *Acc* (D) in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with different concentrations (0, 2.5, and 5 μM) of baicalein, berberine and BBS for 48 h. The total RNA was extracted and subjected to qPCR. The mRNA levels were quantified and normalized to β-actin. The data are presented as the mean ± SD of three independent experiments. *P < 0.05, **P < 0.01 indicates a statistically significant difference to the mature adipocyte group (CON). The 3T3-L1 preadipocyte cells were treated with 0.1% DMSO (Pre).

4. Conclusion

In this study, we successfully designed and synthesized a hybrid compound of berberine and baicalin. We optimized the synthesis process for berberrubine by microwave synthesis using DMF as a reaction medium. This berberrubine synthetic method is simple and suitable for industrial production. Most interestingly, BBS reduced the lipid accumulation and triglyceride content in 3T3-L1 adipocytes. BBS increased adipocyte lipolysis by up-regulating the expression of the *Atgl* gene and reduced lipogenesis by down-regulating the mRNA expression of *Srebp-1c*, *Fasn*, *Scd*, and *Acc* in 3T3-L1 adipocytes. The inhibitory effect of baicalein and berberine on lipid accumulation is consistent with the results reported in the past.[11, 21] Our findings suggest that BBS acts better than baicalein and berberine in reducing lipid accumulation. From these data, we conclude that BBS is a new compound with anti-obesity biological activity. The synthesis of BBS had an obtained purity of 96%. The purity should be improved in upcoming studies. In summary, our work revealed a new compound with therapeutic effects and pharmacological activities on obesity. Our results are beneficial to studies of traditional Chinese medicine.

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Graphical Abstract

The design and synthesis of a novel compound of berberine and baicalein that inhibits the efficacy of lipid accumulation in 3T3-L1 adipocytes

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