Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2017, **15**(6): 0467–0473
Chinese Journal of Natural Medicines

Bioassay-guided isolation of saikosaponins with agonistic activity on 5-hydroxytryptamine 2C receptor from *Bupleurum chinense* and their potential use for the treatment of obesity

SUN Chang-Li[1, 2], GENG Chang-An[1], HUANG Xiao-Yan[1], MA Yun-Bao[1], ZHENG Xiao-Hong[1, 2], YANG Tong-Hua[1, 2], CHEN Xing-Long[1, 2], YIN Xiu-Juan[1, 2], ZHANG Xue-Mei[1], CHEN Ji-Jun[1*]

[1] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China;*

[2] *University of Chinese Academy of Sciences, Beijing 100049, China*

Available online 20 Jun., 2017

[ABSTRACT] 5-Hydroxytryptamine 2C (5-HT_{2C}) receptor is one of the major targets of anti-obesity agents, due to its role in regulation of appetite. In the present study, the 70% EtOH extract of the roots of *Bupleurum chinense* was revealed to have agonistic activity on 5-HT_{2C} receptor, and the subsequent bioassay-guided isolation led to identification of several saikosaponins as the active constituents with 5-HT_{2C} receptor agonistic activity *in vitro* and anti-obesity activity *in vivo*. The new compound, 22-oxosaikosaponin d (**1**), was determined by extensive spectroscopic analyses (HR-ESI-MS, IR, and 1D and 2D NMR). The primary structure-activity relationship study suggested that the intramolecular ether bond between C-13 and C-28 and the number of sugars at C-3 position were closely related to the 5-HT_{2C} receptor agonistic activity. Saikosaponin a (**3**), the main saponin in *B. chinense*, showed obviously agonistic activity on 5-HT_{2C} receptor with an EC_{50} value of 21.08 ± 0.33 μmol·L^{-1} *in vitro* and could reduce food intake by 39.1% and 69.2%, and weight gain by 13.6% and 16.4%, respectively, at 3.0 and 6.0 mg·kg^{-1} *in vivo*. This investigation provided valuable information for the potential use of *B. chinense* as anti-obesity agent.

[KEY WORDS] *Bupleurum chinense*; 5-hydroxytryptamine 2C (5-HT_{2C}) receptor; Anti-obesity; Saikosaponins

[CLC Number] R284 **[Document code]** A **[Article ID]** 2095-6975(2017)06-0467-07

Introduction

Obesity as an increasingly chronic condition leads to high morbidity and mortality [1]. The prevalence of serious health problems such as coronary heart disease, hypertension, stroke, diabetes, and infectious diseases is closely related to the severity of obesity [2-4]. The anti-obesity pharmacotherapy is often focused on neurotransmitter receptors, of which 5-hydroxytryptamine 2C (5-HT_{2C}) receptor appears to play the greatest role in the regulation of appetite [5-7]. Many synthesized compounds have been reported to have 5-HT_{2C} acceptor agonistic activity and inhibitory effects on appetite [8-10]. To our best knowledge, natural anti-obesity compounds targeting 5-HT_{2C} receptor are rarely reported.

Bupleurum chinense, belonging to the genus *Bupleurum* of the family Umbelliferae, is a famous traditional Chinese medicine (TCM), which was originally documented in the oldest Chinese material medicinal monographs “Shennong’s Herbal”. The roots of *B. chinense*, recorded as “Chai-Hu” in every edition of “Chinese Pharmacopoeia”, have the action of dispelling exogenous evils, invigorating splenic yang, and are widely used to treat fever and hypochondriasis [11]. Furthermore, *B. chinense* is prescribed in many ancient formulas (*e.g.*, Xiao-Chai-Hu-Tang and Xiao-Yao-San) as the principle drugs for treating chronic hepatitis and depression [12-15]. Previously phytochemical research has suggested that saikosaponins, lignans, courmarins, flavonoids, polyacetylenes are the major chemical constituents of *B. chinense* with immunodulatory, anti-inflammatory, anti-ulcer, anti-oxidant, and hepatoprotective activities [16].

[Received on] 30-Oct.-2016

[Research funding] This work was supported by the National Science Foundation of China (No. 81573322), the Hundred-Talent Program of CAS, the CAS "Light of West China" Program, and the Youth Innovation Promotion Association of CAS.

[*Corresponding author] Tel: 86-871-65223265, Fax: 86-871-65227197; E-mail: chenjj@mail.kib.ac.cn

These authors have no conflict of interest to declare.

In this investigation, the 70% EtOH extract of the roots of *B. chinense* was initially revealed with agonistic activity on 5-HT_{2C} receptor, indicating the potential use for the treatment of obesity. In order to elucidate its anti-obesity activity and the main active compounds, bioassay-guided isolation led to the active fraction with significant anti-obesity activity *in vivo*, from which a series of saikosaponins with 5-HT_{2C} receptor agonistic activity were isolated. Saikosaponin a (**3**), as the main saponin, showed obvious 5-HT_{2C} agonistic activity with an EC_{50} value of 21.08 ± 0.33 μmol·L^{-1} *in vitro* and *in vivo* inhibitory effects on food intake at 3.0 and 6.0 mg·kg⁻by 39.1% and 69.2%, and weight gain by 13.6% and 16.4%, respectively. [1]

Material and Methods

General procedures

The high resolution electrospary ionization mass spectroscopy (HRESIMS) was performed on a UFLC-MS-IT-TOF apparatus (Shimadzu, Kyoto, Japan). The nuclear magnetic resonance (NMR) experiments were performed on AVANCE III-600 spectrometer (Bruker, Bremerhaven, Germany) with tetramethylsilane (TMS) as the internal standard. Column chromatography (CC) was performed on MCI-gel CHP20P (75–150 μm; Mitsubishi Chemical Co., Chigasaki, Japan), silica gel (200–300 mesh, Qingdao Marine Chemical Co., Qingdao, China), and Rp-18 (40–63 μm, Merck, Shanghai, China). Thin layer chromatography (TLC) was performed on HSGF254 (0.2 mm, Qingdao Marine Chemical Co.) or Rp-18 F_{254} (0.25 mm, Merck). Fractions were monitored by TLC and the spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH; Semipreparative Waters Alliance 2695 apparatus with an Agilent ZORBAX SB-C_{18} (5 μm, 9.4 mm × 250 mm) column (Agilent, Torrance, CA, USA) was used for high performance liquid chromatography (HPLC) separation.

The 5-HT_{2C} agonistic assay in *vitro* was measured in HEK293 cell line (HD Biosciences Co. Ltd., Shanghai, China). Dulbecco's modified Eagle's media (DMEM), dialyzed fetal bovine serum (FBS), and 96-well plates used for cell culture were obtained from GIBCO, Shanghai, China. The cells were dyed by HDB Wash Free Fluo-8 Calcium Assay kit (HD Biosciences Co. Ltd., Shanghai, China). Flex Station 3 Benchtop Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) was used in calcium test with excitation wavelength at 485 nm, emission wavelength at 525 nm, and emission cut-off at 515 nm. 5-HT (Alfa Aesar, Shanghai, China) was used as positive control. Other reagents were of analytical grade and obtained from GIBCO (Shanghai, China).

Male Sprague-Dawley rats (purchased from the Laboratory Animal Center of Kunming Medicinal University, Kunming, China) weighting 200–220 g at the beginning of the experiments were used in acute food intake and 7-day body weight loss assays. The animals were housed in cages with a 12 h/12 h light/dark cycle at a constant room temperature (23 ± 2 ºC) and humidity (55% ± 10%). Water and standard laboratory chow were available *ad libitum*. Animal experiments were conducted according to the current ethical regulations for animal care and use and were reviewed and approved by the Animal Ethics Committee of Kunming Institute of Botany, Chinese Academy of Sciences.

Plant materials

The roots of *Bupleurum chinense* DC. were purchased from Jvhuacun medicinal herbal market (Kunming, China) and authenticated by Dr. Prof. LEI Li-Gong (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (No. 20140510) was deposited in the Laboratory of Anti-virus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and bioassay-guided isolation

The dried and powdered roots of *B. chinense* (1.0 kg) were extracted with 70% EtOH (5 L) under reflux thrice, 3 h each. The total extract was filtered and evaporated to give fraction (Fr.) BC (102 g), which showed obvious 5-HT_{2C} agonistic activity *in vitro* with the rate of 174.79% at 333 μg·mL^{-1}. The Fr. BC was subjected to MCI CHP-20P gel CC (490 g, 5 cm × 45 cm), eluted with EtOH-H_2O (10 : 90, 50 : 50, 100 : 0, *V*/*V*, each 2.0 L) to afford water fraction (BC-1, 51 g), 50% EtOH fraction (BC-2, 23 g) and EtOH fraction (BC-3, 19 g). Fr. BC-3 showed the highest activity *in vitro* and thus was applied for further investigation.

Fr. BC-3 (19 g) was separated by silica gel CC (200 g, 6 cm × 50 cm), eluted with MeOH–EtOAc–H_2O (2 : 8 : 0.2, 3 : 7 : 0.3, 10 : 0 : 0) to afford three fractions, Frs. BC-3-1, BC-3-2, and BC-3-3. Fr. BC-3-1 (4.7 g) was subjected to an RP-18 gel CC and eluted with MeOH-H_2O (30 : 70→100 : 0) to give three fractions Fr. BC-3-1-1–3. Fr. BC-3-1-1 (1.2 g) was purified by silica gel CC, eluted with MeOH–$CHCl_3$ (5 : 95) and further purified by semi-prep. HPLC using MeCN–H_2O (40 : 60) to afford Compounds **6** (24 mg) and **4** (31 mg). Fr. BC-3-1-2 (2.4 g) was submitted on silica gel CC eluted with MeOH–$CHCl_3$ (5 : 95) and then semi-prep. HPLC with MeCN–H_2O (40 : 60) to yield compounds **13** (25 mg) and **5** (11 mg). Fr. BC-3-1-3 (75 mg) was separated by semi-prep. HPLC with MeCN–H_2O (42 : 58) to yield compound **2** (8 mg). Fr. BC-3-2 (7.0 g) was separated by an RP-18 gel CC with MeOH-H_2O (30 : 70→100 : 0) to afford five fractions Fr. BC-3-2-1–4. Fr. BC-3-2-1 (210 mg) was purified by semi-prep. HPLC (MeCN–H_2O, 35 : 65) to yield Compounds **9** (39 mg) and **7** (121 mg). Fr. BC-3-2-2 (1.2 g) was performed on silica gel CC with MeOH-$CHCl_3$ (10 : 90) to give compound **3** (310 mg). Fr. BC-3-3-3 (0.5 g) was submitted on silica gel CC, eluted with MeOH–$CHCl_3$ (1 : 9) then purified by semi-prep. HPLC with MeCN–H_2O (35 : 65) to afford compounds **12** (16 mg) and **1** (7 mg), and **14** (13 mg) respectively. Fr.BC-3-3-4 (0.9 g) was isolated by silica gel CC with MeOH–$CHCl_3$ (10 : 90) to get compound **11** (14 mg) and Fr. BC-3-3-4-1. Fr.BC-3-3-4-1 was separated by semi-prep. HPLC with MeCN–H_2O (40 : 60) to get compounds **15** (8 mg) and **16** (11 mg). Fr.BC-3-3 (0.6 g) was conducted on silica gel CC with MeOH-$CHCl_3$ (20 : 80) to provide Fr. BC-3-3-1 and

compound **10** (25 mg). Fr.BC-3-3-1 was purified by semi-prep. HPLC with MeCN–H_2O (25 : 75) to afford Compound **8** (46 mg).

Acid hydrolysis of compound 1 and GC analysis

Compound **1** (2 mg) were refluxed with 2 mol·L^{-1} HCl (1,4 dioxane/H_2O 1 : 1, 2 mL) on a water bath for 2 h. After cooling, the reaction mixture was neutralized with 1 mol·L^{-1} NaOH and filtered. The filtrate was extracted with $CHCl_3$ (3 × 5 mL). The aqueous layer was evaporated to dryness. The dried residue was dissolved in 1 ml of anhydrous pyridine and treated with L-cysteine methyl ester hydrochloride (1.5 mg), stirred at 60 ºC for 30 min. The supernatants (4 μL) were analyzed by GC under the following condition: detection, H_2 flame ionization detector; column, HP-5 (30 m × 0.25 mm), 0.25 μm; carrier gas, N_2; injection temperature, 250 ºC; detection temperature, 280 ºC; and column temperature, 160–280 ºC, with the rate of 5 ºC/min. The configurations of D-glucose and D-fucose for compound 1 were determined by comparison of the retention times of the corresponding derivatives with those of standard D-glucose and D-fucose giving a single peak at 24.33 min and 23.52 min, respectively.

Identification

22-oxosaikosaponin d (compound **1**): white amorphous powder; (+) HR-ESI-MS *m/z* 817.435 8 $[M + Na]^+$ Cacld. for $C_{42}H_{66}O_{14}$, 817.434 5. $[\alpha]_D^{20}$: +28.08 (*c* 0.15, MeOH); IR (KBr): ν_{max} 3 446, 2 927, 2 859, 1 705, 1 639, 1 450, 1 384, 1 706, 1 027 cm^{-1}; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data (Table 2).

5-HT$_{2C}$ receptor agonistic assay in vitro

The HEK293 cells stably expressing the human 5-HT$_{2C}$ receptor were maintained in DMEM containing 10% FBS. The cells were plated at a density of 4 × 10^4 cells/100 μL/well in pre-Matrigel-coated 96-well black wall/clear bottom plates. Following an overnight incubation at 37 ºC in a moist atmosphere containing 5% CO_2, the cells were dyed by 100 μL of HDB Wash Free Fluo-8 Calcium Assay kit at 37 ºC for 1 h and then transferred to the Flex Station 3 Benchtop Multi-Mode Microplate Reader. The test samples in 50 μL loading buffer were added to the cell on the Microplate Reader to determine the agonistic effect. The raw data from time sequence recording was normalized to the percentage response obtained from 5-HT on the same plate and analyzed to fit the four-parameter logistic equation to assess the drug's agonistic rate or EC_{50} value.

Evaluation of anti-obesity activity in vivo

Acute food intake assay in vivo

Fr. BC-3 and saikosaponin a (**3**) with 5-HT$_{2C}$ agonistic activity were evaluated for the ability of reducing food consumption during a 24 h of period, which began at the onset of the dark cycle. After a 4-day adaption of the environment, the rats were randomly divided into different groups. The rats (n = 6/group) were administered test samples (3.0 and 9.0 mg·kg^{-1} for BC-3 and 3.0 and 6.0 mg·kg^{-1} for saikosaponin a (**3**)) or vehicle (5% dimethyl sulfoxide (DMSO) + 95% H_2O) by intraperitoneal injection (ip). The administrated animals were then placed in individual cages with quantitative food for 24 h (12 h of dark cycle and then 12 h of light cycle). The average food consumption was measured at 3, 6, 12, and 24 h after administration and the reduction rate of food intake was calculated as follows:

$$\text{Inhibitory rate (\%)} = (W_{\text{vehicle}} - W_{\text{tested}}) / W_{\text{vehicle}} \times 100$$

7-Day body weight loss assay in vivo

The 7-day body weight loss assay was conducted to study the potential weight gain inhibitory activity of Fr. BC-3 and saikosaponin a (**3**). The rats were adapted for 4 days, divided into different groups randomly, and then placed in individual cages. The rats (n = 6/group) were administered with the test samples (3.0 and 9.0 mg·kg^{-1} for BC-3 and 3.0 and 6.0 mg·kg^{-1} for saikosaponin a (**3**) or vehicle (5% DMSO + 95% H_2O) by ip per 24 h for 7 days and weight was measured prior to treatment and during the treatment daily. Food and water were available *ad libitum* throughout the study. The weight gain was converted to percent change as follows:

$$\text{Increase rate (\%)} = (W_{\text{during the treatment}} - W_{\text{prior to treatment}}) / W_{\text{prior to treatment}} \times 100$$

The weight gain inhibitory rate of the test group was calculated by the following formula:

$$\text{Inhibitory rate (\%)} = \text{increase rate (\%) of vehicle group} - \text{increase rate (\%) of test group}$$

Statistical analysis

All the in *vitro* experiments were replicated three times. The data were presented as means ± standard deviation. One-way analysis of variance (ANOVA) and two-tailed *t*-test were used to determine statistical significance in animal test. A P value of < 0.05 was considered statistically significant.

Results and Discussion

5-HT$_{2C}$ agonistic activity of the fractions of B. chinense in vitro and the potential anti-obesity effects of the active fraction (BC-3) in vivo

The 5-HT$_{2C}$ receptor agonistic activities of the fractions are summarized in Table 1. The 70% EtOH extract of *B. chinense* showed 5-HT$_{2C}$ receptor agonistic activity with the rate of 174.79% at 333 μg·mL^{-1}. The extract was separated into three fractions (BC-1, BC-2, and BC-3), among which Fr. BC-3 showed obvious activity while the other two fractions showed no activity under the test concentrations.

Table 1 The 5-HT$_{2C}$ receptor agonistic rates of the fractions of *B. chinense in vitro*

Fractions	Agonistic rate (%)
BC	174.79 ± 15.31
BC-1	–1.45 ± 4.48
BC-2	1.18 ± 1.15
BC-3	177.56 ± 14.90

Note: 5-HT was used as positive control with EC_{50} of 0.46 ± 0.13 nmol·L^{-1}; All the samples were tested at the concentration of 333 μg·mL^{-1} and the values are expressed as means ± SD of three independent experiments

In order to evaluate the potential use of *B. chinense* for the treatment of obesity, Fr. BC-3 was assayed for the acute food intake and 7-day weight gain inhibitory effects *in vivo*. Fr. BC-3 showed a dose-dependent reduction in food consumption with the rate of 29.9% ($P < 0.05$) at 9.0 mg·kg^{-1} after administration for 24 h (Fig.1A). In the 7-day weight gain inhibitory effect testing, the weight increase of the rats was restrained in a dose-dependent manner with a statistically significant reduction after 5 days at 9.0 mg·kg^{-1} ($P < 0.05$). The overall reduction in body weight compared with vehicle at day 7 were 4.5% and 11.6% ($P < 0.05$) at 3.0 and 9.0 mg·kg^{-1}, respectively (Fig.1B).

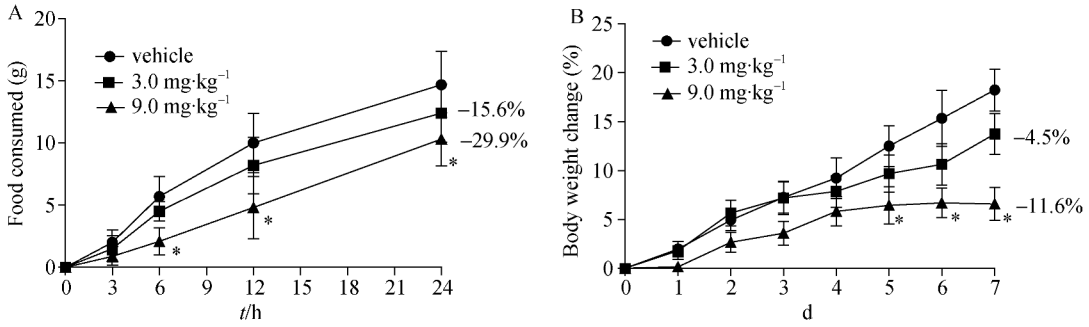

Fig. 1 Acute food intake (A) and 7-day weight gain inhibitory effects (B) of the active fraction (BC-3) on the rats. The data are given as means ± SD (n = 6). Values indicated percent reduction in consumed food (A) and weight gain (B) from vehicle dosed animals. $^{*}P < 0.05$ *vs* vehicle

The structures of the compounds isolated from the active fraction (BC-3) and their 5-HT$_{2C}$ receptor agonistic activities in vitro

In order to elucidate the active compounds with 5-HT$_{2C}$ receptor agonistic and potential anti-obesity activities, 16 saikosaponins, including a new one, were obtained from Fr. BC-3. The new compound, 22-oxosaikosaponin d (**1**), was determined by extensive spectroscopic analyses (HRESIMS, 1D and 2D NMR). The structures of the known compounds (**2**–**16**) were determined by comparison of their NMR spectra with those in the literatures and elucidated as 3-*O*-*β*-D-fucopyranosylsaikogenin f (**2**) [17], saikosaponin a (**3**) [17-18], 6"-*O*-acetylsaikosaponin a (**4**) [20], 3",6"-di-*O*-acetylsaikosaponin a (**5**) [19], saikosaponin d (**6**) [17], saikosaponin e (**7**) [18], saikosaponin c (**8**) [19], saikosaponin b$_1$ (**9**) [18], saikosaponin b$_2$ (**10**) [18], 3"-*O*-acetylsaikosaponin b$_2$ (**11**) [17], 6"-*O*-acetylsaikosaponin b$_2$ (**12**) [17], saikosaponin g (**13**) [20], hydroxysaikosaponin a (**14**) [21], saikosaponin b$_3$ (**15**) [18], and 6"-*O*-acetylsaikosaponin b$_3$ (**16**) [17] (Fig. 2).

Compound **1** was obtained as white amorphous powder with molecular formula $C_{42}H_{46}O_{14}$ on the basis of pseudomolecular ion peak at *m/z* 817.435 8 $[M + Na]^+$ (caclcd. for 817.434 5). The ^{1}H NMR spectrum showed seven methyls at δ_H 0.79 (3H, s, H-30), 0.83 (3H, s, H-29), 0.92 (3H, s, H-24), 1.00 (3H, s, H-25), 1.02 (3H, s, H-27), 1.43 (3H, d, J = 6.4 Hz, H-6'), 1.54 (3H, s, H-26), two anomeric proton signals of sugars at δ_H 4.98 (1H, d, J = 7.8 Hz, H-1') and 5.34 (1H, d, J = 7.8 Hz, H-1"), and two olefinic proton signals at δ_H 5.67 (1H, dd, J = 10.3, 2.9 Hz, H-11), 6.08 (1H, d, J = 10.3 Hz, H-12) (Table 2). The ^{13}C and DEPT NMR spectra displayed 42 signals with seven methyls, ten methylenes, seventeen methines, and eight quaternary carbons, of which twelve signals were clearly assigned to two sugar moieties and the left thirty ones to a triterpene aglycone. These ^{13}C and DEPT NMR spectroscopic data were similar with those of saikosaponin d (**6**) [17]. The main difference was the presence of one more carbonyl group and the absence of a methylene at C-22. The location of the carbonyl group was confirmed at C-22 by the HMBC spectrum, which showed ^{1}H-^{13}C long-range correlations between the resonances of δ_H 4.89 (1H, m, H-16), 2.32 (1H, m, H-18) and 3.88 (1H, d, J = 7.9 Hz, H-28) wth δ_C 214.2 (s, C-22). Acid hydrolysis of Compound 1 with 2 mol·L^{-1} HCl in dioxane-H_2O (1 : 1) yield D-glucose and D-fucose as carbohydrate moieties, which were determined by GC analysis of their corresponding trimethylsilated L-cysteine adducts. The *β*- configuration of the two sugars were deduced by the coupling constants ($^3J_{1,2}$ > 7 Hz). The sequence and linkage positions of the sugars were verified by detailed 2D NMR analysis. In the HMBC spectrum, the correlations of δ_H 4.30 (1H, dd, J = 11.6, 4.6 Hz, H-3) with δ_C 106.0 (d, C-1'), δ_H 4.98 (1H, d, J = 7.8 Hz, H-1') with δ_C 81.4 (d, C-3) and δ_H 4.04 (1H, m, H-3') with δ_C 106.4 (d, C-1"), δ_H 5.34 (1H, d, J = 7.8 Hz, H-1") with δ_C 85.1 (d, C-3') were observed, which hinted that a *β*-glucopyranosyl and *β*-fucopyranosyl attached at C-3 and C-3', respectively. Therefore, the structure of Compound 1 was elucidated as 3*β*, 16*α*-dihydroxy-13*β*, 28*β*-epoxy-22-oxo-olean-11-ene 3-*O*-(*β*-D-glucopyranosyl)-(1→3)-*β*-D- fucopyranoside and named as 22-oxosaikosaponin d.

The 5-HT$_{2C}$ receptor agonistic activities of the compounds were evaluated and are summarized in Table 3. Compounds **1**–**7** with intramolecuar ether bond at C-13 and C-28 in aglycone and one or two sugars at C-3 possessed potent 5-HT$_{2C}$ receptor agonistic activity with EC_{50} values of 13.51–33.71 μmol·L^{-1}, but Compound **8** with three sugars at C-3 showed no activity. In addition, the compounds without intramolecuar ether bond at C-13 and C-28 in aglycone (**9**–**16**) showed decreased or no activity in the test. The primary

structure-activity relationship study suggested that the 5-HT_{2C} receptor agonistic activity of saikosaponins was closely related to the intramolecular ether bond between C-13 and C-28 and the number of sugars at C-3 position.

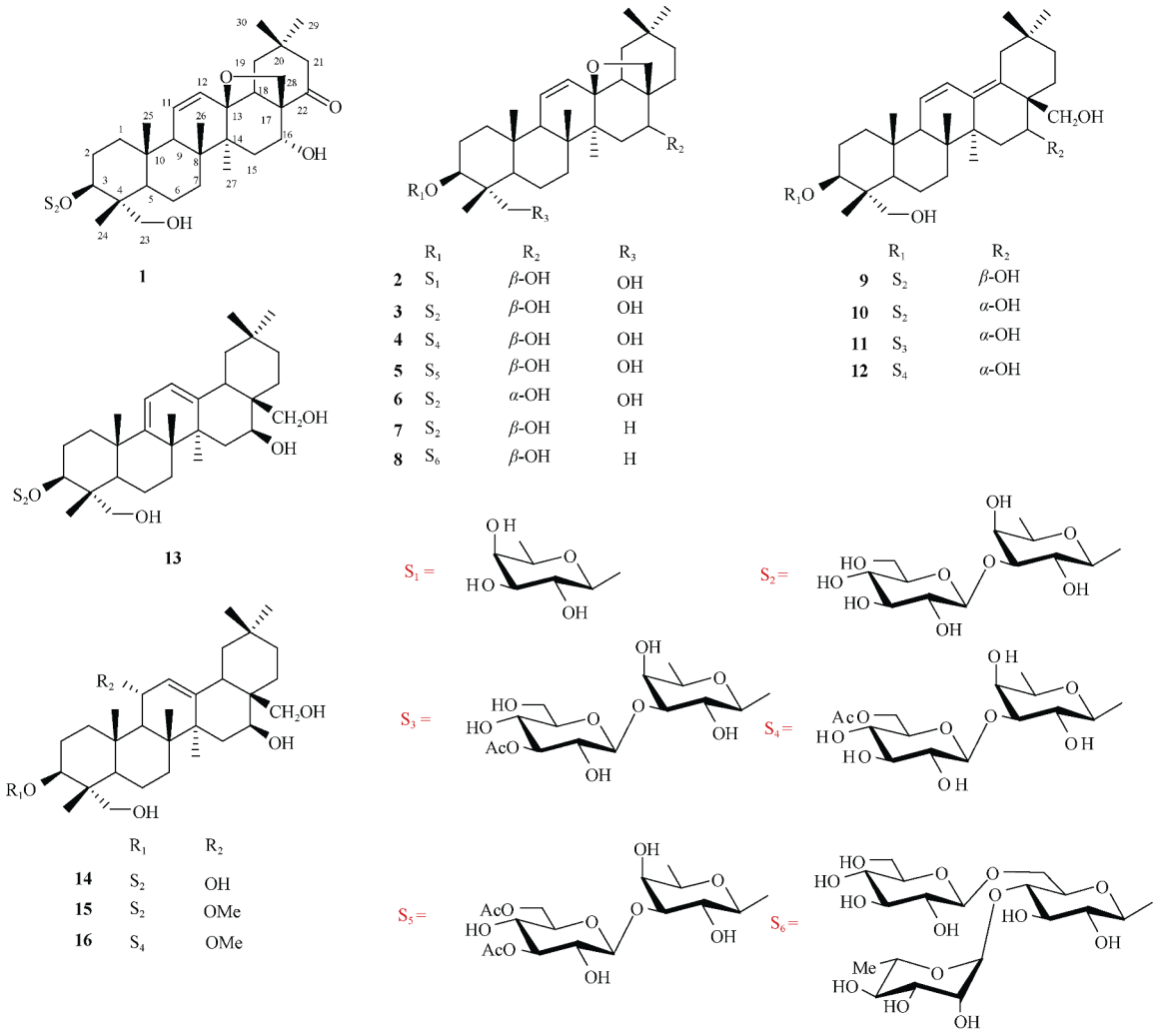

Fig. 2 The structures of compounds 1–16 isolated from Fr. BC-3

Table 2 ^{1}H (600 MHz) and ^{13}C (150 MHz) spectral data for compound 1 (δ, *J* in Hz, C_5D_5N)

Pos.	δ_H	δ_C	Pos.	δ_H	δ_C
1	1.88[a]	38.6	22		214.2
	1.10 m		23	4.37 d(10.8)	63.8
2	2.34[a]	26.0		3.69 d(10.8)	
	2.07[a]		24	0.92 s	12.9
3	4.30 dd (11.6, 4.6)	81.4	25	1.00 s	18.5
4		43.6	26	1.54 s	20.3
5	1.72[a]	47.1	27	1.02 s	15.8
6	1.85[a]	17.6	28	3.88 d (7.9)	74.6
	1.56[a]			3.51 d (7.9)	
7	2.02[a]	34.9	29	0.83 s	33.1
	1.92[a]		30	0.79 s	22.9
8		43.6	Fuc-		
9	2.05[a]	52.6	1'	4.98 d (7.8)	106.0
10		36.3	2'	4.23[a]	71.4
11	5.67 dd (10.3, 2.9)	129.3	3'	4.04[a]	85.1

Continued

Pos.	δ_H	δ_C	Pos.	δ_H	δ_C
12	6.08 d (10.3)	133.3	4'	4.13 d (2.8)	72.1
13		84.5	5'	3.66 m	70.9
14		55.9	6'	1.43 d (6.4)	17.2
15	1.76[a]	35.6	Glc-		
	1.19[a]		1"	5.34 d (7.8)	106.6
16	4.89 m	75.1	2"	4.03[a]	75.7
17		55.0	3"	4.01[a]	78.7
18	2.32 m	55.3	4"	4.53 m	71.7
19	1.76[a]	35.6	5"	4.27[a]	78.3
	1.19[a]		6"	4.56 dd (11.7, 2.0)	62.6
20		31.4		4.40 dd (11.7, 5.3)	
21	1.47[a]	38.4			
	1.36 m				

[a] Overlapped

Table 3 The 5-HT$_{2C}$ receptor agonistic rates and EC$_{50}$ values of the compounds isolated from the Fr. BC-3 (means ± SD, n = 3)

Compounds	Agonistic rate (%)	EC$_{50}$ (μmol·L^{-1})	Compounds	Agonistic rate (%)	EC$_{50}$ (μmol·L^{-1})
1	178.67 ± 7.91	13.51 ± 1.29	**9**	153.63 ± 1.23	147.41 ± 4.41
2	172.53 ± 10.01	18.38 ± 1.99	**10**	1.45 ± 5.11	-
3	171.39 ± 9.63	21.08 ± 0.33	**11**	2.10 ± 3.73	-
4	175.74 ± 12.37	23.48 ± 0.97	**12**	15.77 ± 4.80	-
5	167.44 ± 7.35	27.93 ± 4.18	**13**	167.64 ± 13.27	142.00 ± 5.02
6	161.07 ± 9.87	16.25 ± 2.09	**14**	8.74 ± 2.71	-
7	163.70 ± 5.24	33.71 ± 4.57	**15**	0.65 ± 2.43	-
8	9.33 ± 2.51	-	**16**	3.21 ± 1.37	-

Note: 5-HT was used as positive control with EC$_{50}$ of 0.52 ± 0.21 nmol·L^{-1}; All the samples were tested at the concentration of 333 μg·mL^{-1} in agonistic rates test and the ones with obvious activity were further tested for EC$_{50}$ values

The potential anti-obesity assays of saikosaponin a (3) in vivo.

To further identify the anti-obesity activity of the 5-HT$_{2C}$ receptor agonistic saikosaponins, saikosaponin a (**3**) was selected for the *in vivo* test due to its obvious activity *in vitro* (EC$_{50}$ = 21.08 ± 0.33 μmol·L^{-1}) and high content in *B. chinense*. The 24-h food intake and 7-day weight gain inhibitory effects are indicated in Fig. 3. In the acute food intake test, saikosaponin a (**3**) showed a dose-dependent reduction in food consumption with the rates of 39.1% (P < 0.01) at 3.0 mg·kg^{-1} and 69.2% (P < 0.01) at 6.0 mg·kg^{-1} at 24 h after administration (Fig. 3A). The weight gain of the rats was restrained in all the days in the experiment, and the inhibitory rate over control at day 7 was 13.6% (P < 0.01) and 16.4% (P < 0.01) at 3.0 and 6.0 mg·kg^{-1}, respectively. The normal weight gain of rats was totally reversed at 6.0 mg·kg^{-1} in the test (Fig. 3B).

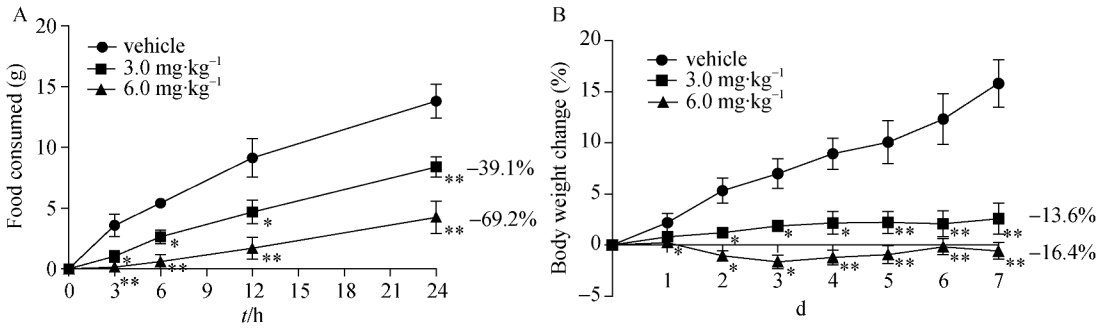

Fig. 3 Acute food intake (A) and 7-day weight gain inhibitory effects (B) of saikosaponin a (3) on rats. The data are given as means ± SD (n = 6). Values indicated percent reduction in consumed food (A) and body weight change (B) from vehicle dosed animals. $^{*}P$ < 0.05, $^{**}P$ < 0.01 *vs* vehicle

Conclusion

5-HT_{2C} receptor agonists are interesting candidates for searching appetite-inhibition and anti-obesity drugs. As a well-known traditional Chinese medicine (TCM), the roots of *B. chinense* have been revealed with various activities, but its 5-HT_{2C} receptor agonistic activity has not been reported. The total extract of *B. chinense* showed obvious agonistic activity on 5-HT_{2C} receptor in the present study. Bioassay-guided fractionation led to the isolation of active fraction (BC-3), which exhibited obvious appetite and weight gain inhibitory activities in animal testing. A series of saikosaponins were isolated from BC-3, among which Compounds **1**–**7** exhibited obvious 5-HT_{2C} receptor agonistic activity and the primary structure-activity relationship study suggested that the intramolecular ether bond between C-13 and C-28 and the number of sugars at C-3 position were closely related to the 5-HT_{2C} receptor agonistic activity. Saikosaponin a (**3**) could reduce food consumption and weight gain significantly at 3.0 and 6.0 mg·kg^{-1} *in vivo*. The investigation provided valuable information for the potential use of *B. chinense* as an anti-obesity agent.

References

[1] Haslam DW, James WP. Obesity [J]. *The Lancet*, 2005, **366**(9492): 1197-1209.

[2] Eckel RH. Obesity and heart disease [J]. *Circulation*, 1997, **96**(9): 3248-3250.

[3] Huttunen R, Syrjanen J. Obesity and the risk and outcome of infection [J]. *Int J Obesity*, 2013, **37**(5): 333-340.

[4] Ogden CL, Carroll MD, Flegal KM. Epidemiologic trends in overweight and obesity [J]. *Endocrin Metab Clinics*, 2003, **32**(4): 741-760.

[5] Nonogaki K, Strack AM, Dallman MF, *et al*. Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT_{2C} receptor gene [J]. *Nat Med*, 1998, **4**(10): 1152-1156.

[6] Tecott LH., Sun LM., Akana SF., *et al*. Eating disorder and epilepsy in mice lacking 5-HT_{2C} serotonin receptors [J]. *Nature*, 1995, **374**(6522): 542-546.

[7] Vickers SP, Benwell KR, Porter RH, *et al*. Comparative effects of continuous infusion of mCPP, Ro 60-0175 and d-fenfluramine on food intake, body weight and locomotor activity in rats [J]. *Brit J Pharmacol*, 2000, **130**(6): 1305-1314.

[8] Smith BM, Smith JM, Tsai JH, *et al*. Discovery and structure - activity relationship of (1*R*) – 8 – chloro - 2, 3, 4, 5 – tetrahydro - 1- methyl - 1*H* – 3 - benzazepine (Lorcaserin), a selective serotonin 5-HT_{2C} receptor agonist for the treatment of obesity [J]. *J Med Chem*, 2008, **51**(2): 305-313.

[9] Wacker DA, Varnes JG, Malmstrom AE., *et al*. Discovery of (*R*) – 9 – Ethyl - 1, 3, 4, 10b – tetrahydro – 7 - trifluoromethylprazino [2, 1-*α*] isoindol-6(2*H*)-one, a selective, orally active agonist of the 5-HT_{2C} receptor [J]. *J Med Chem*, 2007, **50**(6): 1365-1379.

[10] Fevig JM, Feng JX, Rossii KA, *et al*. Synthesis and SAR of 2, 3, 3a, 4 – tetrahydro - 1*H*-pyrrolo [3,4-*c*] isoquinolin -5 (9b*H*) - ones as 5-HT_{2C} receptor agonists [J]. *Bioorg Med Chem Lett*, 2013, **23**(1): 330-335.

[11] Hikino H, Kiso Y. Natural products for liver diseases [J]. *Econ Med Plant*, 1988, **2**(1): 39-67.

[12] Tajiri H, Kozaiwa K, Ozaki Y, *et al*. Effect of sho-saiko-to (Xiao-Chai-Hu-Tang) on HBeAg clearance in children with chronic Hepatitis B virus infection and with sustained liver disease [J]. *Am J Chin Med*, 1991, **19**(2): 121-129.

[13] Chang JS, Wang KC, Chen MC, *et al*. Sho-Saiko-To (Xiao-Chai-Hu-Tang) and crude saikosaponins inhibit Hepatitis B virus in a stable HBV-producing cell line [J]. *Am J Chin Med*, 2007, **35**(2): 341-351.

[14] Liu XJ, Zhou YZ, Li ZF, *et al*. Anti-depressant effects of xiaoyaosan on rat model of chronic unpredictable mild stress: a plasma metabonomics study based on NMR spectroscopy [J]. *J Pharm Pharmacol*, 2011, **64**(4): 578-588.

[15] Tian JS, Peng GJ, Gao X.X, *et al*. Dynamic analysis of the endogenous metabolites in depressed patients treated with TCM formula xiaoyaosan using urinary ^{1}H NMR-based metabolomics [J]. *J Ethnopharmacol*, 2014, **158**(2): 1-10.

[16] Ashour ML, Wink M. Genus Bupleurum: a review of its phytochemistry, pharmacology and modes of action [J]. *J Pharm Pharmacol*, 2011, **63**(3): 305-321.

[17] Ding JK, Fujino H, Kasai R, *et al*. Chemical evaluation of *Bupleurum* species in Yunnan, China [J]. *Chem Pharm Bull*, 1986, **34**(3): 1158-1167.

[18] Ishii H, Nakamura M, Seo S, *et al*. Isolation, characterization, and nuclear magnetic resonance spectra of new saponins from the roots of *Bupleurum falcatum* L. [J]. *Chem Pharm Bull*, 1980, **28**(8): 2367-2383.

[19] Seto H, Kawai H, Otake N, *et al*. Structures of new saponins isolated from *Bupleurum kunmingense* [J]. *Agric Biol Chem*, 1986, **50**(6): 1613-1620.

[20] Shimizu K, Amagaya S, Ogihara Y. New derivatives of saikosaponins [J]. *Chem Pharm Bull*, 1985, **33**(8): 3349-3355.

[21] Ebata N, Nakajima K, Hayashi K, *et al*. Saponins from the root of *Bupleurum falcatum* [J]. *Phytochemistry*, 1996, **41**(3): 895-901.

Cite this article as: SUN Chang-Li, GENG Chang-An, HUANG Xiao-Yan, MA Yun-Bao, ZHENG Xiao-Hong, YANG Tong-Hua, CHEN Xing-Long, YIN Xiu-Juan, ZHANG Xue-Mei, CHEN Ji-Jun. Bioassay-guided isolation of saikosaponins with agonistic activity on 5-hydroxytryptamine 2C receptor from *Bupleurum chinense* and their potential use for the treatment of obesity [J]. *Chin J Nat Med*, 2017, **15**(6): 467-473