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

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**[ABSTRACT]** 5-Hydroxytryptamine 2C (5-HT₂C) 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-HT₂C receptor, and the subsequent bioassay-guided isolation led to identification of several saikosaponins as the active constituents with 5-HT₂C 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-HT₂C receptor agonistic activity. Saikosaponin a (3), the main saponin in *B. chinense*, showed obviously agonistic activity on 5-HT₂C receptor with an EC₅₀ 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-HT₂C) receptor; Anti-obesity; Saikosaponins

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**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-HT₂C) receptor appears to play the greatest role in the regulation of appetite [⁵-⁷]. Many synthesized compounds have been reported to have 5-HT₂C receptor agonistic activity and inhibitory effects on appetite [⁸-¹⁰]. To our best knowledge, natural anti-obesity compounds targeting 5-HT₂C 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, coumarins, flavonoids, polyacetylenes are the major chemical constituents of *B. chinense* with immunodulatory, anti-inflammatory, anti-ulcer, anti-oxidant, and hepatoprotective activities [¹⁶].
In this investigation, the 70% EtOH extract of the roots of *B. chinense* was initially revealed with agonistic activity on 5-HT<sub>2C</sub> 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<sub>2C</sub> receptor agonistic activity were isolated. Saikosaponin a (3), as the main saponin, showed obvious 5-HT<sub>2C</sub> agonistic activity with an EC<sub>50</sub> value of 21.08 ± 0.33 μmol·L<sup>-1</sup> *in vitro* and *in vivo* inhibitory effects on food intake at 3.0 and 6.0 mg·kg<sup>-1</sup> 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 electrospray ionization mass spectroscopy (HRESIMS) was performed on a UPLC-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<sub>254</sub> (0.25 mm, Merck). Fractions were monitored by TLC and the spots were visualized by heating silica gel plates sprayed with 10% H<sub>2</sub>SO<sub>4</sub> in EtOH; Semipreparative Waters Alliance 2695 apparatus with an Agilent ZORBAX SB-C<sub>18</sub> (5 μm, 9.4 mm × 250 mm) column (Agilent, Torrance, CA, USA) was used for high performance liquid chromatography (HPLC) separation.

The 5-HT<sub>2C</sub> 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) was used as positive control. Other reagents were of analytical grade and obtained from GIBCO (Shanghai, China).

The roots of *Bupleurum chinense* DC. were purchased from Jhuacun 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<sub>2C</sub> agonistic activity *in vitro* with the rate of 174.79% at 333 μg·mL<sup>-1</sup>. The Fr. BC was subjected to MCI CHP-20P gel CC (490 g, 5 cm × 45 cm), eluted with EtOH-H<sub>2</sub>O (10 : 90, 50 : 100, 0/70 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<sub>2</sub>O (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<sub>2</sub>O (30 : 70→100 : 0) to give five fractions Fr. BC-3-1-1–5. Fr. BC-3-1-1 (1.2 g) was purified by semi-prep. HPLC using MeCN–H<sub>2</sub>O (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–CH<sub>3</sub>Cl<sub>2</sub> (5 : 95) and then semi-prep. HPLC with MeCN–H<sub>2</sub>O (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<sub>2</sub>O (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<sub>2</sub>O (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<sub>2</sub>O, 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–CH<sub>3</sub>Cl<sub>2</sub> (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–CH<sub>3</sub>Cl<sub>2</sub> (1 : 9) then purified by semi-prep. HPLC with MeCN–H<sub>2</sub>O (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–CH<sub>3</sub>Cl<sub>2</sub> (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<sub>2</sub>O (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–CH<sub>3</sub>Cl<sub>2</sub> (20 : 80) to provide Fr. BC-3-3-1 and 16.
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