Annphenone from *Artemisia vestita* Inhibits HepG2 Cell Proliferation

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Artemisia vestita is a common traditional Tibetan medicinal plant which has been widely studied for its antiinflammatory activity. However, little is known about its antitumor activity. The present study performed a bioassay-guided isolation to isolated annphenone from *A. vestita*, which showed potent and specific antiproliferative activity against HepG2 cells with the half maximal inhibitory concentration (IC₅₀) value of 2.0 ± 0.4 $\mu\text{g/mL}$. Cell cycle analysis showed that annphenone arrested HepG2 cells in G₀/G₁ phase. Immunocytochemistry detection suggested that annphenone could inhibit the expression of β -catenin and induce its localization transfer, thereby reducing the expression of cyclin D1 protein. Molecular docking study indicated annphenone was a possible ligand of asialoglycoprotein receptor (ASGP-R), which was related with its selectivity for HepG2 cells. Therefore, annphenone was a potential specific antiproliferative agent against hepatocellular carcinoma cells.

Key Words: Annphenone, *Artemisia vestita*, Antiproliferative, HepG2 cell.

INTRODUCTION

The hepatocellular carcinoma (HCC) is one of the cancer types of highest incidence in the world¹. Most patients diagnosed with hepatocellular carcinoma have low recovery rates and conventional therapies currently available are rarely beneficial². Effective and specific drugs to treat hepatocellular carcinoma are urgent needed. The sources of these drugs include both synthetic and natural compounds. Large quantities of compounds were reported that had potent antitumor activities, including anti hepatocellular carcinoma capability. The anticancer mechanisms of these compounds are quite different. One of these mechanisms is related with the regulation of β -catenin and Wnt signaling pathway. β -Catenin, encoded by the CTNNB1 gene, is a subunit of the cadherin protein complex and has been implicated as an integral component in the Wnt signaling pathway in humans. Its abnormal expression may cause accumulation of β -catenin and activation of cyclin D1, resulting to unusual proliferation of cells and induction of tumors³. Cyclin D1 is one of the most critical proteins that regulate the cell cycle, which can promote cells to convert from G₁ phase to S phase through the combination and activation of cell cycle related kinase CDK4 or DK64.

Many compounds showed significant antitumor activities in *in vitro* experiments. However, their activities cannot be

displayed in *in vivo* experiments, because these compounds usually cannot reach the targeted tissues and organs. Therefore, in recent years, targeted drug therapy has attracted more and more attention. The asialoglycoprotein receptor (ASGP-R) is a calcium-dependent lectin and is expressed exclusively by parenchymal hepatocytes⁵. The human ASGP-R consists of two homologous subunits, designated H1 and H2, both of which are single-spanning membrane proteins with a calcium-dependent D-Gal/D-GalNAc recognition domain⁶. In 2000, the X-ray crystal structure of the carbohydrate recognition domain (CRD) of the major H1-subunit was solved⁷. Most of the hepatoma cells surfaces have ASGP-R. Therefore, people have designed many ASGP-R-mediated drug deliver systems to transport antitumor drugs to hepatoma cells by introduction ligands, such as D-galactose or N-acetyl-D-galactosamine, into the drugs molecular structures.

Artemisia vestita is a traditional Tibetan medicine and distributed at wasteland and river beaches of China. Most of the previous studies focused the antiinflammatory activities of *A. vestita*^{8,9}, but there are rare reports on the antitumor activity of *A. vestita*. Many antitumor compounds were isolated from *Artemisia* species¹⁰. Therefore, some natural antitumor compounds should also be found in *A. vestita*.

In this study, we have isolated the antitumor components of *A. vestita* under the guide of bioassay using the human

hepatoma cell line HepG2 as screening tool, which was established in 1979 and has been used to examine various mechanisms of hepatotoxicity¹¹. From the isolated compounds (Fig. 1), we find a compound, annphenone, which showed potent and specific antiproliferative activity against HepG2 cells. We evaluated the effect of annphenone on the cell cycle and expression of β -catenin and cyclin D1. Furthermore, we investigated the interaction of annphenone as a possible ligand with the ASGP-R by docking simulation. The purpose of the study was to preliminarily explain the possible antiproliferative mechanism of annphenone.

Fig. 1. Chemical structures of natural compounds isolated from *A. vestita*

EXPERIMENTAL

All chemicals (reagent grade) used were purchased from Aldrich (USA). Separation of the compounds by column chromatography was carried out with HP-20 (250–850 μ m, Mitsubishi Chemical, Japan), Sephadex LH-20 column (25–100 μ m, Mitsubishi Chemical, Japan), silica gel column (100–200 mesh, Qingdao Oceanic Chemical Plant, China), C₁₈ column (4.6 mm $\times$ 250 mm, 5 μ m, Kromasil). Thin layer chromatography (TLC) was run on the silica gel coated aluminum sheets (silica gel 60 GF₂₅₄, E. Merck, Germany) and visualized in ultraviolet (UV) light (254 nm). Melting points (uncorrected) were determined with an XT4 MP apparatus (Taike Corp., Beijing, China). ¹H and ¹³C NMR spectra (300 MHz) were recorded on a ¹H-Varian-Mercury-300 spectrometer at 25 °C, using tetramethylsilane (TMS) as the internal standard. ESI-MS were recorded with a Mariner System 5304 mass spectrometer. EI-MS spectra were recorded with a Finnigan Trace MS spectro-

meter. Elementary analyzes were performed on a CHN-O-Rapid instrument within ± 0.4 % of the theoretical values.

Extraction and isolation: The aerial parts of *Artemisia vestita* were obtained from Tibet pharmaceutical factory of Tibet University and identified as *Artemisia vestita* Wall. by Dr. Ciren Dunzhu (Tibet Tibetan medicine college). A voucher specimen (20050801) has been deposited at the Herbarium of China Pharmaceutical University.

The dried aerial part of the *A. vestita* (3 kg) was extracted twice with 75 % ethanol by reflux (54 L/kg, 2 h for the first time; 36 L/kg, 1 h for the second time). The extracts were combined and concentrated under reduced pressure to 12 L and then was suspended in water to afford the aqueous solution (30 L). The solution was applied on a chromatography with a macroporous resin adsorption column (HP-20), eluting with water, 30 % ethanol, 60 % ethanol and 90 % ethanol, respectively. The four fractions were collected and evaporated to dryness under reduced pressure to afford fractions **I–IV** (**I**, 30.5 g; **II**, 60.6 g; **III**, 90.6 g; **IV**, 33.1 g)¹². For further activity-guided isolation, fraction **II**, which showed the highest antiproliferation activity, was subjected on a gradient chromatography with a silica gel column by eluting with dichloromethane/methanol (100/0, 99:1, 98:2, 95:5, 90:10 and 70:30) to give 6 fractions (**II.1–II.6**). For another screen of antiproliferative activity, **II.4** showed higher activity and was continued conducted a silica gel column chromatography with dichloromethane/methanol as eluent to give quercetin (yellow needle crystals, 100 mg, purity 95.3 % by HPLC), patuletin (yellow crystals, 25 mg, purity 97.2 % by HPLC), annphenone (white powders, 70 mg, purity 96.2 % by HPLC) and 2,4-dihydroxy-6-methoxyacetophenone (white powders, 10 mg, purity 95.1 % by HPLC). Also from **II.2** we isolated scopoletin (pale yellow needle crystals, 3 g, purity 97.8 % by HPLC) and umbelliferone (white powder crystals, 1 g, purity 96.7 % by HPLC); from **II.3** we got pectolinarigenin (yellow needle crystals, 50 mg, purity 97.1 % by HPLC), apigenin (yellow needle crystals, 15 mg, 97.9 %), cirsiol (yellow powder, 20 mg, purity 96.8 % by HPLC), 5,7,3',4'-tetrahydroxy-6,5'-dimethoxyflavone (yellow needle crystals, 15 mg, 96.5 % by HPLC) and 7-methoxycoumarin (colourless needle crystals, 80 mg, 96.8 % by HPLC).

The structures of these isolated compounds were identified by melting points, ¹H, ¹³C NMR, ESI-MS and EI-MS spectra and elementary analysis after comparing with previous data.

Antiproliferative assay: **I–IV**, **II.1–II.6** and compound **1–11** were tested their anti-proliferative activities against HepG2 cell line by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl- tetrazolium bromide] method¹³. Compound **1–11** were also evaluated their antiproliferative activities against B16-F10 and A549 cell lines. Target tumor cell lines were grown to log phase in RPMI 1640 medium supplemented with 10 % fetal bovine serum. After diluting to 2×10^4 cells/mL with the complete medium, 100 μ L of the obtained cell suspension was added to each well of 96-well culture plates. The subsequent incubation was permitted at 37 °C, 5 % CO₂ atmosphere for 24 h before the cytotoxicity assessments. Tested samples at pre-set concentrations were added to the wells with 5-fluorouracil co-assayed as positive reference. After 48 h

exposure period, 40 μ L of PBS containing 2.5 mg/mL of MTT was added to each well. After 4 h, 100 μ L extraction solution (10 % SDS-5 % isobutyl alcohol-0.01 M HCl) was added. After an overnight incubation at 37 °C, the optical density was measured at a wavelength of 570 nm on an ELISA microplate reader. In all experiments three replicate wells were used for each sample concentration. Each assay was carried out at least three times.

Cell cycle analysis: We evaluated the effect of annphenone on the cell cycle. HepG2 cell line was grown to log phase in RPMI 1640 medium supplemented with 10 % fetal bovine serum. After diluting to 1×10^4 cells/mL with the complete medium, 100 mL of the obtained cell suspension was added to each well of 6-well culture plates and adherent for 4 h. Annphenone of different concentrations were added to the wells. After 48 h exposure period, 70 % ethanol was added to each well. After an overnight incubation at 4 °C, cell suspension was centrifuged for 5 min at 1000 rpm. A 100 μ L PBS solution (containing 2 % Triton X-100 and 50 μ g/mL RNase A) was added. A 100 μ L PI solution was added to each well and dark stained for 0.5 h at 4 °C. A 200 μ L PBS solution was added to suspend cells. Then samples were analyzed by flow cytometer. In all experiments three replicate wells were used for each sample concentration. Each assay was carried out at least three times.

Immunocytochemistry analysis: The expression of β -catenin and cyclin D1 were detected by immunocytochemistry. HepG2 cells were cultured on the microscopic glass pre-placed in 6-well plates and adherent for 4 h. Annphenone of different concentrations were added. After 48 h exposure period, samples were washed twice by PBS solution and fixed by 80 % acetone for 15 min at 4 °C. Staining procedure was according to the instructions of SABC and DAB kits, taking PBS instead of first antibody as negative control. These brown cells, of which colours were deeper than the background, in the cytoplasm or nucleus were positive cells.

Docking study: Molecular docking of annphenone into the three-dimensional X-ray structure of H1-CRD of ASGP-R (PDB code: 1DV8)⁷ was carried out using the LigandFit Dock protocol of Discovery Studio 3.1. In addition, we fabricated 2-hydroxy-4-O- β -D-galactopyranosyloxy-6-methoxyacetophenone (**12**) as reference to discuss the binding model.

RESULTS AND DISCUSSION

Isolation of annphenone: Previous studies of *Artemisia vestita* were mainly focused on the immunosuppressive activities^{8,9}. However, *Artemisia* species are widely spread genus and contain plentiful active compounds, such as terpenoids, flavonoids, coumarins, acetylenes, caffeoylquinic acids and sterols, shown to have antimalarial, antiviral, antitumor, antipyretic, antihemorrhagic, anticoagulant, antianginal, antioxidant, antihepatitis, antiulcerogenic, antispasmodic and anticomplementary activities¹⁰. In recent studies, some active compounds with antitumor activity were also found in *A. vestita*, such as jaceosidin^{14,15} and cirsilinol^{12,16}. Therefore, the present study was to evaluate the antitumor activity of *A. vestita* and its active compositions.

The crude ethanol extract of *A. vestita* was purified by macroporous adsorption resin and 4 fractions (named **I-IV**) were yielded. Then we examined the effects of the 4 fractions on HepG2 cells. The results showed that fraction **II** had the highest antiproliferative activity with the half maximal inhibitory concentration (IC_{50}) value of 358 μ g/mL (Table-1). To further identify the active constituents in *A. vestita*, fraction **II** was subjected on a gradient chromatography with a silica gel column by eluting with dichloromethane/methanol to give 6 fractions (named **II.1-II.6**). For another screen of antiproliferative activity, **II.4** showed higher activity (IC_{50} = 150 μ g/mL) against HepG2 cells. Therefore, it was continued conducted a silica gel column chromatography with dichloromethane/methanol as eluent to give annphenone (**11**). Along together, we also isolated quercetin (**3**), patuletin (**6**) and 2,4-dihydroxy-6-methoxyacetophenone (**10**) from **II.4**, scopoletin (**7**) and umbelliferone (**8**) from **II.2**, pectolinarigenin (**1**), apigenin (**2**), cirsiolol (**4**), 5,7,3',4'-tetrahydroxy-6,5'-dimethoxyflavone (**5**) and 7-methoxycoumarin (**9**) from **II.3**.

TABLE-1
ANTIPROLIFERATIVE ACTIVITY OF FRACTIONS
AND COMPOUNDS ISOLATED FROM *A. vestita*
AGAINST HepG2, B16-F10 AND A549 CELLS

Fraction or compound	$IC_{50} \pm SD$ (μ g/mL)		
	HepG2	B16-F10	A549
I	> 1 000	NM*	NM
II	358 $\pm$ 16	NM	NM
III	> 1 000	NM	NM
IV	> 1 000	NM	NM
II.1	> 1 000	NM	NM
II.2	683 $\pm$ 25	NM	NM
II.3	439 $\pm$ 13	NM	NM
II.4	150 $\pm$ 20	NM	NM
II.5	814 $\pm$ 28	NM	NM
II.6	> 1 000	NM	NM
1	22.6 $\pm$ 0.2	16.6 $\pm$ 0.3	25.7 $\pm$ 0.2
2	34.8 $\pm$ 0.2	59.0 $\pm$ 0.2	38.9 $\pm$ 0.3
3	95.1 $\pm$ 0.8	88.5 $\pm$ 1.4	76.3 $\pm$ 2.9
4	168 $\pm$ 13	138 $\pm$ 14	144 $\pm$ 25
5	114 $\pm$ 14	116 $\pm$ 13	122 $\pm$ 19
6	33.4 $\pm$ 1.4	26.6 $\pm$ 2.3	23.2 $\pm$ 0.7
7	553 $\pm$ 31	264 $\pm$ 23	111 $\pm$ 17
8	257 $\pm$ 15	146 $\pm$ 28	105 $\pm$ 26
9	635 $\pm$ 34	448 $\pm$ 21	516 $\pm$ 25
10	121 $\pm$ 19	223 $\pm$ 16	232 $\pm$ 27
11	2.0 $\pm$ 0.4	135 $\pm$ 15	152 $\pm$ 10
5-FU	0.33 $\pm$ 0.03	0.45 $\pm$ 0.11	0.25 $\pm$ 0.08

*NM = not measured; 5FU = 5-Fluorouracil

The name of annphenone came from *Artemisia annua* and it was found in many *Artemisia* genus plants, such as *A. annua*¹⁷, *A. santolinifolia*¹⁸ and *A. sacrorum*¹⁹. However, its isolation from *A. vestita* was first reported in this study. Compounds **4-10** were also first reported in *A. vestita*.

Antiproliferative activity of annphenone: Annphenone is an interesting natural product and found to have many biological activities. Shin *et al.*²⁰, reported that annphenone exhibited antioxidant properties by inhibiting ROS generation and thus protecting cells from H₂O₂-induced cell damage. They suggested that annphenone extracted from *Artemisia* species

could be used as an ingredient for health food and beverage products and cosmetics²¹. In our observation, annphenone also showed potent anti-proliferative activity against HepG2 cells with an IC_{50} value of 2.0 $\mu\text{g/mL}$, which was closed to that of 5-fluorouracil (Table-1). However, its antiproliferative effect on B16-F10 and A549 cells was moderate ($IC_{50} = 135 \mu\text{g/mL}$ for B16-F10; $IC_{50} = 152 \mu\text{g/mL}$ for A549). Therefore, the anti-proliferative activity of annphenone was specific. The isolated flavones (**1-6**) and acetophenone (**10**) derivatives showed moderate anti-proliferative activities. The activities of coumarin derivatives (**7-9**) were relatively weak.

We have carried out cell cycle analysis of annphenone using flow cytometry and the results were shown in Fig. 2. It can be seen that annphenone induced HepG2 cells of G_0/G_1 phase to increase and those cells of S or G_2/M phase to decrease. The effect was significant when the annphenone concentration was 4 $\mu\text{g/mL}$. These findings indicated an impairment of cell division and annphenone posed an effect on the cell cycle.

Fig. 2. Effects of annphenone on cell cycle progression of HepG2 cells. The measurement were carried out by flow cytometry. HepG2 cells were treated with different concentrations of annphenone for 48 h. The percentage of cells in each cycle phase was indicated

We also studied the effect of annphenone on the expression of cyclin D1 and β -catenin by immunocytochemistry. As shown in Fig. 3(A), cyclin D1 was expressed in the nucleus and cytoplasm, whereas β -catenin in the control group was expressed mainly in the nucleus. After exposure to annphenone, β -catenin subcellular localization gradually transferred from the nucleus into the cytoplasm in a significant dose-dependent manner. In the control group, the percentage of positive cells that expressed cyclin D1 protein was $20.5 \pm 3.8 \%$. When exposure to annphenone of 0.25, 0.5, 1.0 and 2.0 $\mu\text{g/mL}$, the positive cells percentages decreased to 16.8 ± 4.0 , $16.5 \pm 3.2 \%$ ($p < 0.05$), $11.8 \pm 1.5 \%$ ($p < 0.01$) and $9.5 \pm 2.2 \%$ ($p < 0.01$), respectively. And the percentage of positive cells expressed β -catenin protein in control group was $13.8 \pm 3.6 \%$. After exposure annphenone of 0.25, 0.5, 1, 2 $\mu\text{g/mL}$, the positive cells percentages decreased to 12.5 ± 3.5 , 11.4 ± 3.3 and

Fig. 3. Effect of annphenone on the expressions of cyclin D1 and β -catenin in HepG2 cells: (A) Photographs of immunocytochemistry ($\times 200$) and (B) Rates of positive cells. The concentrations of annphenone were 0, 0.25, 0.5, 1.0 and 2.0 $\mu\text{g/mL}$, respectively. These brown cells, of which colours were deeper than the background, in the cytoplasm or nucleus were positive cells. The positive cell rates were calculated by counting the ratio between positive cells and total cells ($n = 10$). * $p < 0.05$ vs control group, ** $p < 0.01$ vs. control group

$9.5 \pm 2.4 \%$ ($p < 0.01$) and $8.2 \pm 2.2 \%$ ($p < 0.01$), respectively [Fig. 3(B)].

The base of tumor is the disorder of cell growth and apoptosis. The destruction of normal cell cycle is one of the key mechanisms for tumor development. There are two important phase transition points in the cell cycle: G_1/S and G_2/M , of which the G_1/S transition point is most important. That is because once the cell cycle changes from G_1 phase into S phase, cells can quickly conduct division independent outside information. Cyclin D1 is a key regulatory protein of cell cycle. When cyclin D1 is over-expressed, G_1/S *trans*-phase process will be accelerated, resulting in uncontrolled cell proliferation and even tumor genesis. Studies have found that 30 % of the hepatocellular carcinoma existed cyclin D1 gene amplification and 58-70 % cells existed cyclin D1 protein over-expression. The expression level of cyclin D1 is closely related to malignant progression of tumors²². This study showed that annphenone significantly inhibited expression of cyclin D1 and simultaneously inhibited HepG2 cells to enter S phase and arrested the HepG2 cells in G_0/G_1 phase. These finding indicated that cyclin D1 and cell cycle was indeed closely related.

β -Catenin is a multifunctional protein. In cell junction it interacted with cadherins and was involved in the formation of adhesive tape. β -Catenin is also a downstream component of the Wnt signaling pathway. Free β -catenin can enter the nucleus and plays a role in gene transcription as a signal transduction molecule. Study confirmed that β -catenin gene was mutated in many human tumors, such as bladder cancer, colorectal cancer and liver cancer. The mutated β -catenin protein cannot bind to glycogen synthase kinase-3 to be

degraded, resulting to the accumulation of β -catenin in cytoplasm. Free β -catenin proteins then enter the nucleus, act on the corresponding target genes, prompt the expression of corresponding proteins, such as cyclin D1, MMP27 and CD44 and eventually induce tumorigenesis²³⁻²⁶.

The present study found that annphenone significantly inhibited HepG2 cell growth. We also found that annphenone arrest HepG2 cells in G₀/G₁ phase, so that HepG2 cell growth slowed down. Immunocytochemical results showed that β -catenin expression was reduced and subcellular localization transferred from the nucleus into the cytoplasm. At the same time, the expression level of β -catenin target gene product, cyclin D1 protein, markedly decreased. These results suggest that annphenone might significantly inhibit β -catenin expression and induce localization transfer, thereby affecting cyclin D1 protein expression to arrest cells in G₀/G₁ phase, so that pose its antiproliferative effect on HepG2 cells growth.

Interaction with ASGP-R: As shown in Table-1, annphenone showed specific antiproliferative activity against HepG2 cells. However, compound **10** (without glucose) did not have the selectivity. It suggested that the glucose group must play the crucial role during the anti-proliferative process against HepG2 cells.

The ASGP-R, a lectin, is expressed exclusively by parenchymal hepatocytes and most of the hepatoma cell surfaces have ASGP-R. It can specifically identify, bind and endocytose molecules with D-Gal/D-GalNAc glycosyl in the blood circulation. Therefore, people have designed many ASGP-R-mediated drug deliver systems to transport drugs to hepatoma cells. Generally, D-galactose or N-acetyl-D-galactosamine is the priority ligand. D-Glucose has the extremely molecular structure and is also a candidate for the ASGP-R ligands, though its activity is usually lower than D-galactose or N-acetyl-D-galactosamine²⁷. For example, β -sitosterol- β -D-glucoside is natural ligand to ASGP-R and was used to modify cationic liposomes to promote transfection in the gene therapy of hepatitis B²⁸.

In our observation, the specific anti-proliferative activity of annphenone against HepG2 cells was possibly attributed to the interaction of glucosyl with ASGP-R on the cell surfaces. To study the possible interaction, we conducted molecular docking to insert annphenone into the three-dimensional X-ray structure of H1-CRD of ASGP-R. The binding mode of annphenone and H1-CRD is depicted in Fig. 4(A). In the binding model, amino hydrogen of Asn264 forms hydrogen bond with the 2- and 3-oxygen. 4-Hydroxyl hydrogen forms hydrogen bond with amino nitrogen of Asp266 and Tyr272 and 9-hydroxyl hydrogen forms hydrogen bond with amino nitrogen of Asp241. Positive π -stacking interactions can be observed between benzene ring of annphenone and benzopyrrole of Trp243. Electrostatic interactions may exist between annphenone and Gln239, Glu252, His256 and Arg270. In addition, annphenone may have van der Waals interactions with His246 and Asp265.

In order to investigate the difference between glucosyl and galactosyl in the binding model with ASGP-R, we fabricated 2-hydroxy-4-O- β -D-galacto-pyranosyloxy-6-methoxyacetophenone (**12**) as reference compound. The binding mode of compound **12** and H1-CRD is depicted in Fig. 4(B). It is

Fig. 4. Molecular docking study: 2-Dimesional ligand interaction diagram of annphenone (A) and compound **12** (B) with H1-CRD of ASGP-R using Discovery Studio program with the essential amino acid residues at the binding site are tagged in circles. The purple circles show the amino acids which participate in hydrogen bonding, electrostatic or polar interactions, blue circles show the amino acids which participate in π - π interaction and the green circles show the amino acids which participate in the van der Waals interaction. (C) Overlap of the conformations of annphenone and compound **12** interacted with H1-CRD of ASGP-R

observed that the interactions between compound **12** and H1-CRD are nearly the same and the difference is only at the conformation. We put the two molecules in the same picture to overlap them [Fig. 4(C)] and the root-mean-square deviation (RMSD) is 0.6843, suggesting that the difference of the two conformation was small.

Therefore, annphenone could be the possible ligand of ASGP-R. Its interaction with ASGP-R is attributed to the glucosyl group, of which binding model is similar to that of galactosyl. The specific antiproliferative activity of annpheone against HepG2 cells was possibly related to the interaction between glucosyl group and ASGP-R in the surfaces of HepG2 cells.

Conclusion

The present study isolated annphenone from *Artemisia vestita*, which showed specific anti-proliferative activity against HepG2 cells. The antiproliferative function of annphenone might be caused by its effect on the inhibition of β -catenin expression and induction of localization transfer, thereby affecting cyclin D1 protein expression to arrest cells in G₀/G₁ phase. Annphenone was a possible ligand of ASGP-R and its specific antiproliferative activity against HepG2 cells was possibly related to the interaction between glucosyl group and ASGP-R. Overall, annphenone is a promising potential specific antitumor agent.

Supplementary information

The supplementary information includes the structure information of compounds **1-11** (including state of matter, melt point, ESI-MS, ¹H and ¹³C NMR, elementary analysis, structure formula and NMR spectra).

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