Inhibitory Effect of Flavonoid Glycosides from *Chlorophytum comosum* on Nasopharyngeal Carcinoma 5-8F Cells and Its Mechanism

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Abstract [**Objectives**] To study the inhibitory activity of two flavonoid glycosides isolated from *Chlorophytum comosum* Laxum R. Br on human nasopharyngeal carcinoma (NPC) cell line 5-8F *in vitro* and its mechanism. [**Methods**] The flavonoid glycosides were isolated and purified from the ethanol alcoholic extract of the roots of Liliaceae plant *Chlorophytum comosum* by silica gel column chromatography, macroporous resin column chromatography, Sephadex LH-20, and reverse column chromatography (ODS). The inhibitory activity of flavonoid glycosides on human nasopharyngeal carcinoma cells was analyzed by CCK-8 method, and the potential mechanism was preliminarily analyzed by molecular docking. [**Results**] Two flavonoid glycosides were identified as isovitexin 2″-0-rhamnoside and 7-2″-di-O-β-glucopyranosylisovitexin. Two flavonoid glycosides showed promising inhibitory effect on human nasopharyngeal carcinoma cell line 5-8F, with IC_{50} values of 24.8 and 27.5 μmol/L, respectively. Molecular docking results showed that the potential targets of two flavonoid glycosides include CyclinD1, Bcl-2 β-Catenin, ILK, TGF-β, in addition, two glycosides showed higher predicted binding affinity towards CyclinD1, which verifies the cytotoxicity of the two compounds on human nasopharyngeal carcinoma cell line 5-8F *in vitro*. [**Conclusions**] Two flavonoid glycosides are the active molecules in *Chlorophytum comosum* that can inhibit the proliferation of human nasopharyngeal carcinoma cells, and have the potential to be used in the research and development of anti nasopharyngeal carcinoma drugs.

Key words *Chlorophytum comosum* Laxum R. Br., Flavonoid glycosides, 5-8F cells, Antitumor mechanism

1 Introduction

Nasopharyngeal Carcinoma (NPC) is one of the malignant tumors of human head and neck, which showed high incidence rate in many southern provinces of China. In clinic, the disease is mainly treated with combined radiotherapy[1], however, the commonly used chemotherapy drugs in clinical practice have many side effects, leading to the decline of human immune function and poor therapeutic effect[2-3]. In recent years, the development of traditional Chinese medicine has made great progress, and the research on anti-tumor activity of traditional Chinese medicine has also made great breakthroughs. Its efficacy and mechanism of action have been experimentally verified[4-6]. With the improvement and development of network pharmacology and molecular docking technology, the research on molecular mechanism of Chinese medicine to exert clinical efficacy has become visual, efficient and standardized[7-8], providing a guarantee for the research on anti-tumor mechanism of Chinese medicine.

Chlorophytum comosum Laxum R. Br is a plant belonging to *Chlorophytum comosum*, Liliacea, also known as Triangulum, has been listed into the Flora of China of *Chlorophytum comosum* laxiflorum, *Guangxi Plant List of Chinese Chives*, and Main Poisonous Plants in the South of China of *Tuophiopogon japonicus*, mainly grown in southern Guangdong, with the functions of clearing heat, detoxification, swelling and pain[9]. It is mainly used in clinical treatment of snakebite[10], swelling and pain after tibial fracture, adhesion after hand tendon exercise. Its main chemical components include steroids and their glycosides, alkanes, flavonoid glycosides, *etc.*[11]. Studies have shown that flavonoid glycosides have a strong inhibitory effect on the activity of human nasopharyngeal carcinoma cell line 5-8F[12]. Wang *et al.*[13] found that flavonoid glycosides can inhibit autophagy and reverse the radioresistance of nasopharyngeal carcinoma cells. Li Jing *et al.*[14] confirmed that flavonoid glycosides can inhibit the tumor development of nasopharyngeal carcinoma bearing mice. Pyruvoside is a potentially valuable drug molecule in the treatment of nasopharyngeal carcinoma. This study is mainly to isolate flavonoid glycosides from the ethanol extract of *Chlorophytum comosum* grandiflorum, and carry out targeted *in vitro* cytotoxic activity research, explore and analyze its activity mechanism using molecular docking technology, and increase the active ingredients available for screening for the research and development of nasopharyngeal cancer drugs.

2 Materials and methods

2.1 Materials

2.1.1 Instruments. rotary evaporator RE-2000 (Shanghai Yarong Instrument Co., Ltd.), X-4 digital display micro-melting point tester (Beijing Tec Instrument Co., Ltd.), IR tester (EQUZNOXTM55-A590/3 F type), ESI-MS (A. B. Sicence), NMR (Bruker 400 ASCENDTM).

2.1.2 Reagents. petroleum ether (60 - 90 ℃), ethyl acetate, ethanol (95%), Dichloromethane, n-butanol, methanol (all analytical alcohols, purchased from Tianjin Fuyu Fine Chemical Co., Ltd.), silica gel (200 - 300 mesh Qingdao Marine Chemical Plant), ODS column chromatographic filler (Merck, Germany), Sephadex LH-20 column (Pharmacia Biotech AB, Uppsala,

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Switzerland), DMEM medium (Hyclone, USA), fetal bovine serum (FBS, Hyclone, USA), TransDetect Cell Counting Kit (CCK), Article No.: L0001, Shanghai Yuchun Biotechnology Co., Ltd.

2.1.3 Medicinal materials. Dried roots of small flower *Chlorophytum comosum*. The original plant was identified by Teng Xifeng, associate professor of Guangdong Pharmaceutical University, as *Chlorophytum Laxum* R. Br, a small flower of Liliaceae *Chlorophytum comosum*, and stored in Room 608, Science and Engineering Building, Zhaoqing University (CCL-220712).

2.1.4 Cells. Human nasopharyngeal carcinoma cells 5-8F were provided by the Cancer Hospital affiliated to Sun Yat-sen University.

2.2 Methods

2.2.1 Extraction and separation. The root of *Chlorophytum comosum* (2.0 kg) was cut into 1 cm segments, heated and refluxed with 95% ethanol for three times, each time for 3 h, combined with ethanol extract, recovered ethanol, concentrated to obtain ethanol extract (265 g), then dispersed with 700 mL of water to form a suspension, extracted with petroleum ether, dichloromethane, ethyl acetate, and n-butanol in turn (1∶1 *V/V*), extracted three times separately, combined with solutions, concentrated to obtain petroleum ether part (18.4 g), Dichloromethane (39.0 g) ethyl acetate (47.5 g), n-butanol (60.0 g). Took the n-butanol part (58 g), passed through the macroporous resin column, and eluted with (20%, 50%, 75%, and 100%) ethanol: water gradient to obtain four parts (Fr.1-Fr.4). Fr; Fr. 4.1 was separated by silica gel column chromatography again. The sample was separated by silica gel (200 – 300 mesh) column chromatography. The sample was mixed with silica gel (1∶1). The column was packed with sample: silica gel 1∶30, eluted with dichloromethane: ethyl acetate 10∶1 equivalency, TLC detection, the same components were combined, and a total of 6 parts (Fr. D1.1-1.6) were obtained. Fr. D1.3 was eluted with sephadex LH-20 (MeOH) equivalency, and purified to obtain compound 1 (20.0 mg); Fr. D1.4 was eluted and purified with sephadex LH-20 (MeOH) to obtain a compound, which was purified by ODS column chromatography, and then eluted with methanol: water (70∶30) to obtain compound 2 (28.5 mg)

2.2.2 Detection of cell proliferation by CCK8 method. The inhibition rate of two flavonoid glycosides on human nasopharyngeal carcinoma cell line 5-8F was determined by CCK-8 method *in vitro*. 1640 medium for human nasopharyngeal carcinoma cell line 5-8F (with 10% fetal bovine serum, 100 μg/L penicillin and 100 μg/L streptomycin) was cultured in an incubator with 5% CO_2 and 37 ℃. Inoculate 100 μL cell suspension (2 per well × 10^3 cells), pre-cultured for 24 h in a 37 ℃, 5% carbon dioxide incubator according to the experimental requirements. Add different concentrations [(5, 12.5, 25, 50, and 100 μL. The cells were treated with compounds (1 – 2) of M/L)] and cultured for 12 h. 11 μL of CCK solution was added to each well. The cells were cultured for 4 hours in a carbon dioxide incubator. The *OD* at 450 nm was measured with an enzyme marker, and repeated for 6 times. The *OD* value was recorded and the inhibition rate of each compound to be tested was calculated. The IC_{50} was calculated using SPSS software, and the cell control and basic culture control were designed.

2.2.3 Study on the mechanism of anti-nasopharyngeal carcinoma cell line 5-8F. Literature studies show that the proteins or targets related to the proliferation of human nasopharyngeal carcinoma 5-8F cells include TGF-β1 (PDB:ID 1PY5)[12], Bcl-2(PDB:ID 2XA0)[15], β-Catenin (PDB: ID 2GL7)[15], ILK (PDB: ID 3KM W)[16], CyclinD1 (PDB: ID 6P8G) protein[17], respectively, can inhibit the growth of 5-8F in human nasopharyngeal carcinoma cells by inhibiting these protein targets. This experiment will take these six protein targets as the research object, and use Autodock software to dock two flavonoid glycosides components respectively, and preliminarily analyze the molecular mechanism of flavonoid glycosides inhibiting the activity of human nasopharyngeal carcinoma cells.

3 Results and analysis

3.1 Structure identification Compound 1: yellow powder (methanol), mp. 214 – 215 ℃; HR-ESI-MS *m/z* 595.166 4[M + H]$^+$, molecular formula $C_{27}H_{30}O_{15}$, ^{1}HNMR (400 MHz C5D5N) δ (ppm): 7.83 (2H, d, J = 8.3Hz, H-2′, 6′), 7.13 (1H, s, H-8), 7.08 (2H, m, H-3′, 5′), 6.89 (1H, s, H-3), 5.81 (1H, d, J = 7.4 Hz, H-1 of 2″-O-glc), 5.62 (1H, d, J = 6.4Hz, H-6 of 6-C-glc), 5.21 (1H, d, J = 9.3Hz, H-1 of 6-C-glc), 4.05 (1H, d, J = 9.1Hz, H-6 of 2″-O-glc), 4.11 – 4.50 (7H, m); ^{13}CNMR(100 MHz C5D5N) δ(ppm): 160.9 (C-2), 103.8 (C-3), 182.8 (C-4), 163.6 (C-5), 105.9 (C-6), 164.6 (C-7), 94.5 (C-8), 157.1 (C-9), 111.7 (C-10), 121.4 (C-1′), 128.8 (C-2′, 6′), 116.4 (C-3′, 5′), 162.6 (C-4′), 6-C-glc:74.5 (C-1), 80.8 (C-2), 79.0 (C-3), 71.0 (C-4), 82.3(C-5), 62.3 (C-6), 2″-O-glc: 102.9(C-1), 77.5(C-2), 74.9 (C-3), 72.9 (C-4), 70.8 (C-5), 61.7 (C-6). Combined with the spectral data of the literature, compound 1 was identified as isovitexin 2″-0-rhamnoside

Compound 2: yellow powder (methanol), mp. 217 – 219 ℃; HR-ESI-MS *m/z* 757.213 0[M + H]$^+$, molecular formula $C_{33}H_{40}O_{20}$, ^{1}HNMR (400 MHz MeOD) δ (ppm): 7.86(2H, d, J = 8.6 Hz, H-2′, 6′), 6.90(2H, d, J = 8.3Hz, H-3′, 5′), 6.65 (1H, s, H-3), 5.08 (1H, d, J = 7.2 Hz, H-1of 7-O-glc), 5.03 (1H, d, J = 10 Hz, H-1 of 6-C-glc), 4.34 (1H, d, J = 7.0 Hz, H-1 of 2″-O-glc); ^{13}CNMR (100MHz MeOD) δ (ppm):166.1 (C-2), 103.6 (C-3), 183.3 (C-4), 160.8 (C-5), 110.3 (C-6), 163.4 (C-7), 93.8 (C-8), 158.2 (C-9), 106.1 (C-10), 122.2 (C-11), 129.1 (C-2′, 6′), 116.5 (C-3′, 5′), 162.4 (C-4′), 6-C-glc: 72.1 (C-1), 81.4 (C-2), 79.4 (C-3), 70.7 (C-4), 80.4 (C-5), 62.0 (C-6), 7-O-glc:101.7 (C-1), 74.4 (C-2), 77.1 (C-3), 70.5 (C-4), 77.9 (C-5), 61.9 (C-6), 2″-O-glc:104.7 (C-1), 75.2 (C-2), 76.8 (C-3), 70.2 (C-4), 76.7 (C-5), 61.3 (C-6). Com-

bined with literature spectral data, compound 2 was identified as 7, 2″-di-O-β-glucopyranosyliso-vitexin[7]

3.2 Antitumor activity screening The CKK-8 method was used to test the inhibitory activity of two flavonoid glycosides on nasopharyngeal carcinoma cell 5F-8 *in vitro*, and camptothecin was used as the control drug. The results showed that the two flavonoid glycosides showed inhibitory activity on nasopharyngeal carcinoma cell 5F-8, with IC_{50} values of 24.8 and 27.5 μM. These two flavonoid glycosides are potentially active molecules for the treatment of rhinitis and cancer.

3.3 Molecular docking results When the binding free energy is less than 0 kcal/mol, it indicates that compounds with biological activity can spontaneously combine with the target. The smaller the binding free energy, the stronger the binding ability of the compound with the target[18]. In this study, the binding free energy ≤ −5.0 kcal/mol is used as the screening criteria[18]. Two flavonoid glycosides and Bcl-2 β-Catenin, ILK, TGF-β1. The results of molecular docking of the five targets of CyclinD1 are shown in Table 1 and Fig. 1 − 5. The binding free energy is between (between −5.1 and −8.1) kcal/mol, indicating that the two flavonoid glycosides have strong affinity with the five target proteins.

Table 1 Binding free energy of two flavonoid glycosides and five targets

Compound	Relative molecular mass	Binding free energy//kcal/mol				
		2XA0	2GL7	3KMW	6P8G	1PY5
1	594.15	−8.1	−7.3	−6.7	−7.1	−5.1
2	756.20	−7.7	−7.1	−7.6	−7.0	−5.2

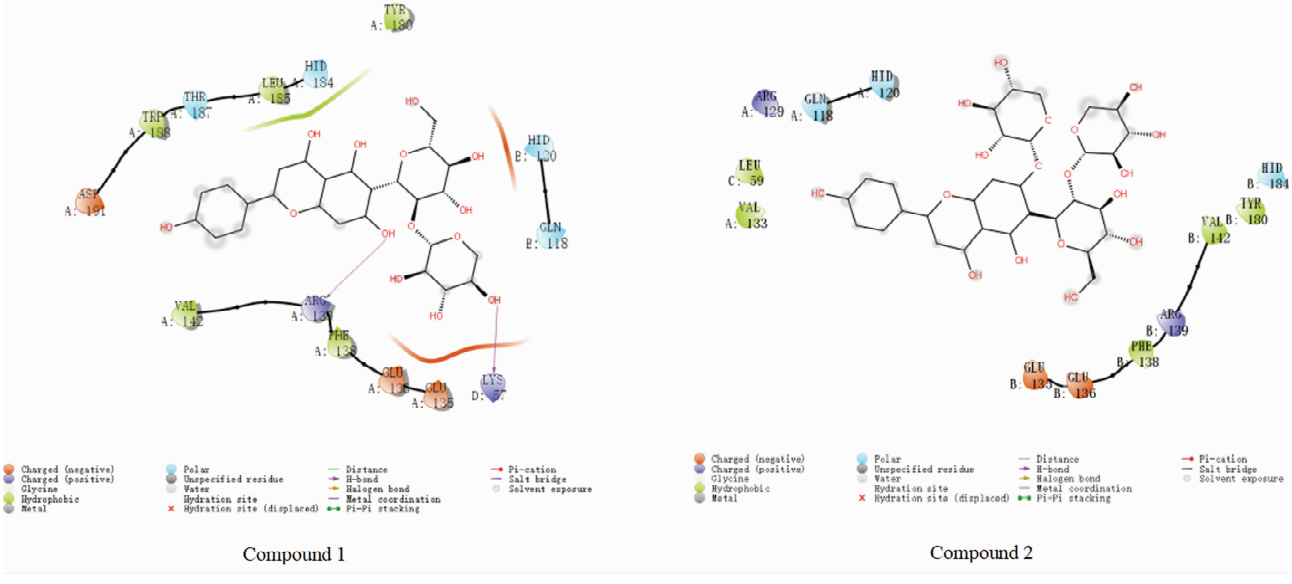

Fig. 1 Schematic diagram for the docking of compounds 1 and 2 with 2XA0

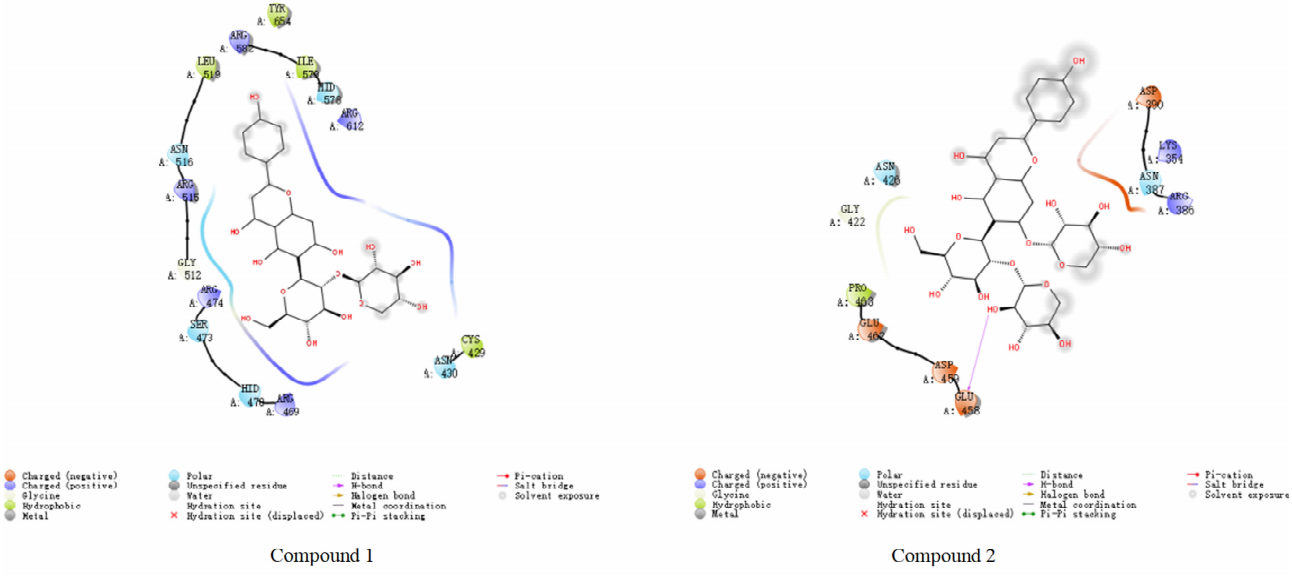

Fig. 2 Schematic diagram for the docking of compounds 1 and 2 with 2GL7

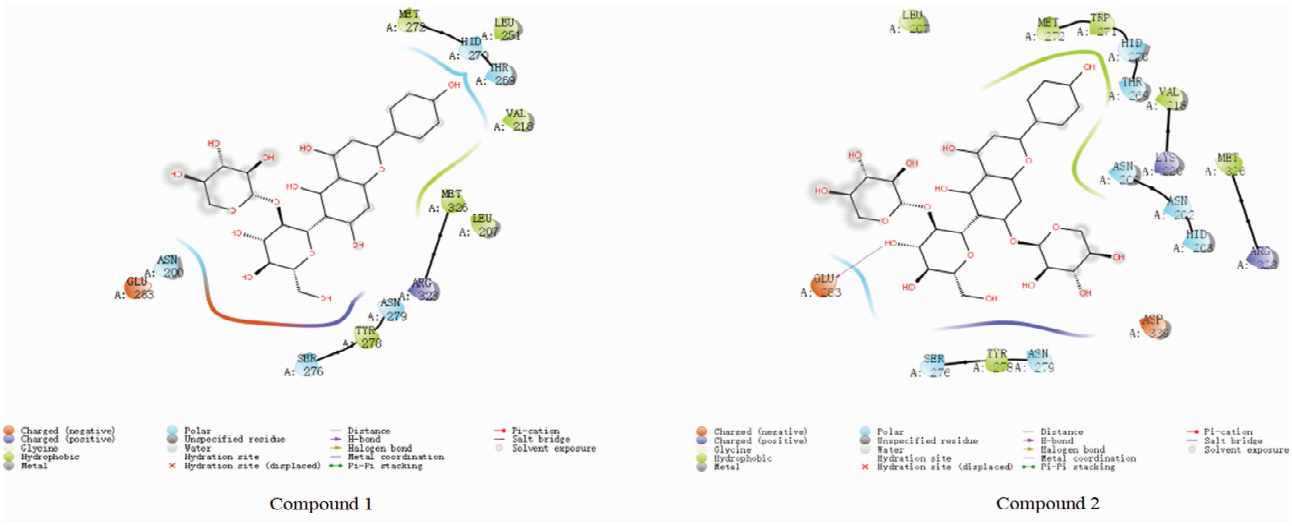

Fig. 3 Schematic diagram for the docking of compounds 1 and 2 with 3KMW

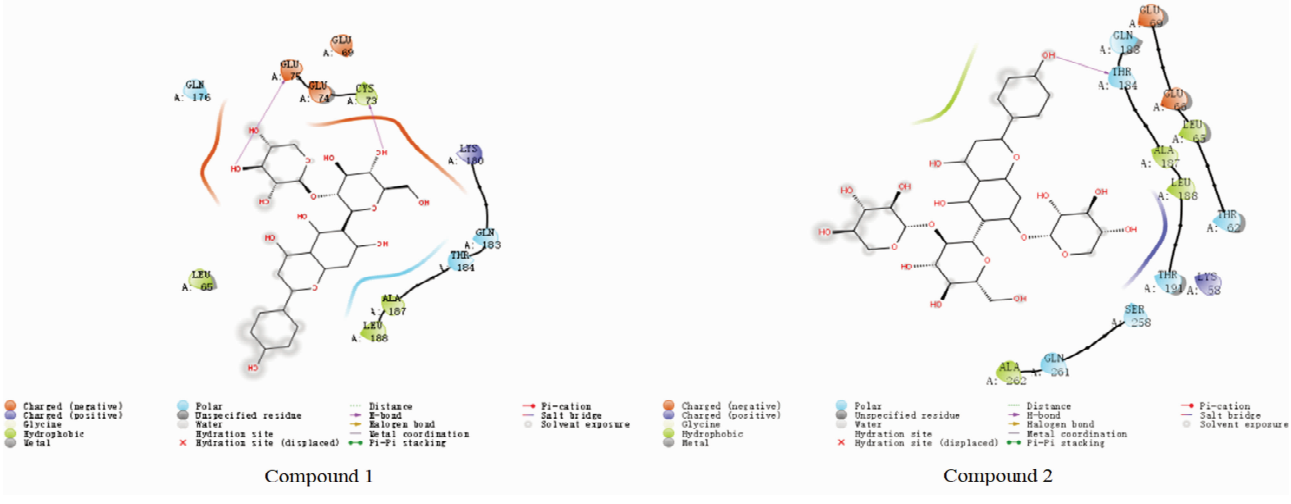

Fig. 4 Schematic diagram for the docking of compounds 1 and 2 with 6P8G

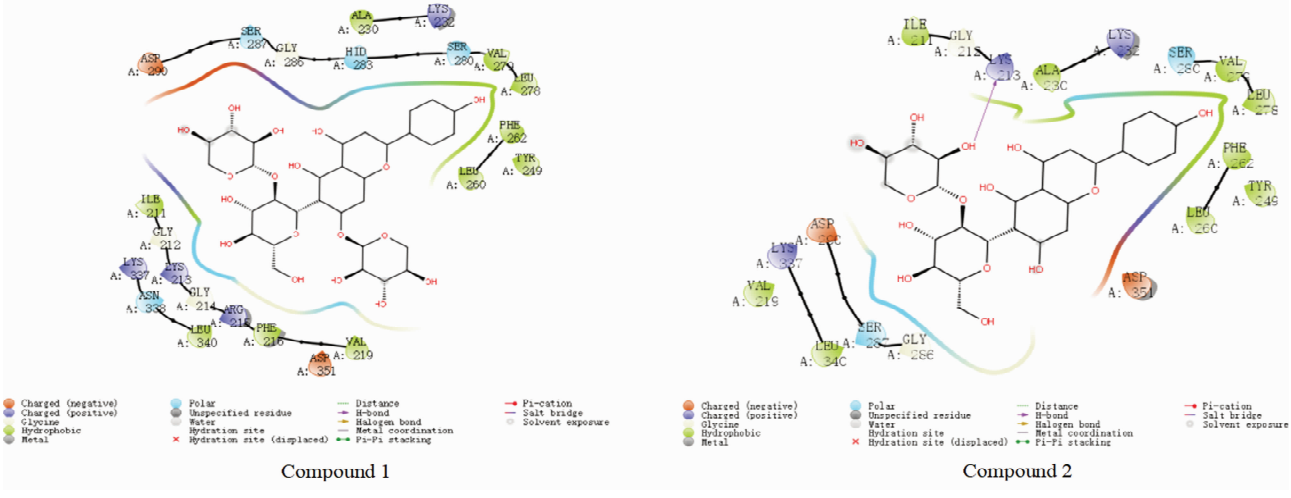

Fig. 5 Schematic diagram for the docking of compounds 1 and 2 with 1PY5

3.4 Analysis of anti-nasopharyngeal carcinoma cell 5-8F mechanism Molecular docking results showed that the binding free energy of the two flavonoid glycosides with BCL-2 and CyclinD1 of human nasopharyngeal carcinoma cell line 5-8F was between (−7.0 and −8.1 kcal/mol), indicating that the compounds may process good affinity with the target, suggesting that

the two flavonoid glycosides might up-regulate the activity of Bax protein by inhibiting the activity of BCL-2 and CyclinD1 of human nasopharyngeal carcinoma cell line 5-8F, thus affecting the cell cycle of 5-8F, Inducing apoptosis[19]; Inhibition of BCL-2 of 5-8F cells may also up-regulate the expression of pro-apoptotic protein NoxAd to promote the apoptosis of nasopharyngeal carcinoma cells[20].

β-Catenin is a key signal molecule in the Wnt signal transduction pathway. The expression of Catenin can inhibit the activity of Wnt pathway, inhibit the proliferation of human nasopharyngeal carcinoma cell line 5-8F, and induce its apoptosis[21]. Two flavonoid glycosides and β-Catenin protein has good affinity, and its binding free energy is −7.3 and −7.1 kcal/mol respectively, suggesting that the two compounds may inhibit the proliferation of human nasopharyngeal carcinoma cell line 5-8F and induce its apoptosis through this pathway.

The binding free energies of the two flavonoid glycosides and ILK were −6.7 and −7.6 kcal/mol, respectively, indicating that the affinity between the compounds and the target was good. By inhibiting the expression of integrin-linked kinase (ILK), it can inhibit the proliferation of human nasopharyngeal carcinoma cells[16], suggesting that the two compounds may also achieve the purpose of inhibiting the proliferation of nasopharyngeal carcinoma cells through this pathway.

4 Conclusions

In this study, two flavonoid glycosides were isolated and identified from *Chlorophytum laxum* R. Br, namely Apigenin-6-C-glucosyl-glucoside and 7-2″-di-O-β-Glucopyranosylisovitexin, antitumor activity study *in vitro* showed that the two flavonoid glycosides showed a certain inhibitory effect on human nasopharyngeal carcinoma cell line 5-8F. Through molecular docking technology, it was found that the two compounds could interact with Bcl-2 β-Catenin, ILK, TGF-β1, and CyclinD1 (PDB: ID 6P8G) with good affinity, which verified the cytotoxicity of two compounds on human nasopharyngeal carcinoma cell line 5-8F *in vitro*. Combined with the research of literature, the cytotoxicity mechanism of two flavonoid glycosides on human nasopharyngeal carcinoma cell line 5-8F was discussed and analyzed, suggesting that the two flavonoid glycosides are active molecules in *Chlorophytum comosum* that can inhibit the proliferation of human nasopharyngeal carcinoma cells. It has the potential to be used in the research and development of anti-nasopharyngeal carcinoma drugs.

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