

Virtual Screening–Molecular Docking–Activity Evaluation of *Ailanthus altissima* (Mill.) Swingle Bark in the Treatment of Ulcerative Colitis

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Abstract

Background: The dried bark of *Ailanthus altissima* (Mill.) Swingle (BAA), which is a traditional Chinese medicine, is widely used in Chinese folklore for the treatment of ulcerative colitis. Consequently, the objective of this study was to explore the therapeutic basis of BAA for the treatment of ulcerative colitis (UC) based on Virtual Screening–Molecular Docking–Activity Evaluation technology.

Methods: By searching the Traditional Chinese Medicine (TCM) Systems Pharmacology (TCMSP) Database and Analysis Platform, 89 compounds from the chemical components of BAA were obtained. Then, after preliminarily screening the compounds based on Lipinski's rule of five and other relevant conditions, the AutoDock Vina molecular docking software was used to evaluate the affinity of the compounds to UC-related target proteins and their binding modes by scoring function to identify the best candidate compounds. Additionally, further verification of the compound's properties was achieved through in vitro experiments.

Results: Twenty-two compounds obtained from the secondary screening were molecularly docked with UC-related target proteins (IL-1R, TLR, EGFR, TGFR, and Wnt) using AutoDock Vina. The free energy of the highest scoring compounds binding to the active cavity of human IL-1R, TLR, EGFR, TGFR, and Wnt proteins was -8.7 , -8.0 , -9.2 , -7.7 , and -8.5 kcal/mol, respectively. Potential compounds, dehydrocrebanine, ailanthone, and kaempferol, were obtained by scoring function and docking mode analysis. Furthermore, the potential compound ailanthone (1, 3, and 10 μM) was found to have no significant effect on cell proliferation, while ailanthone (10 μM) reduced the level of proinflammatory factors caused by lipopolysaccharide (LPS).

Conclusion: Among the active components of BAA, ailanthone plays a major role in its anti-inflammatory properties. The present study shows that ailanthone has advantages in cell proliferation and inhibition of inflammation, but further animal research is needed to confirm the pharmaceutical potential thereof in the future.

Background

Ulcerative colitis (UC), a nonspecific inflammatory bowel disease with an unknown etiology [1], often occurs in the colon and rectum and is diagnosed by colonoscopy [2, 3]. Patients with recurrent and prolonged UC are prone to cancer, and UC has been classified as a refractory disease by the World Health Organization (WHO) [4]. Western medicine does not effectively control UC flares, and cannot prevent its recurrence. Considering the long treatment cycle [5], adverse drug effects [6], high treatment costs [7, 8], and other disadvantages, Traditional Chinese Medicine (TCM) has a significant effect in the clinical treatment of UC [9].

Bark of *Ailanthus altissima* (Mill.) Swingle (BAA), which is also known as chun root bark, citrus white bark, ailanthus bark, and kucha bark, is commonly used in TCM. It has a bitter, astringent, and cold taste [10]. BAA can clear heat, dry dampness, astringe the intestines, and stop bleeding, and it has been used for chronic dysentery, enteritis, diarrhea, gastric and duodenal ulcers, blood in the stool, nocturnal emission, leucorrhea, and other diseases [11, 12]. Modern medical research has found that BAA and its preparations have a good curative effect on UC. A 100%-pure decoction of BAA has been shown to have inhibitory effects on *Shigella flexneri*, *Shigella sonnei*, *Bacillus typhi*, and *Escherichia coli* in vitro, and *Staphylococcus aureus* is moderately sensitive to it [13, 14]. BAA extract can also reduce the activity of nitric oxide (NO) free radicals and nitric oxide synthase (NOS) [15, 16]—inflammatory factors of UC [17].

Molecular docking technology docks known small-molecule active substances with related target proteins, which can elucidate the mechanism of action between active ingredients of TCM and targets at the molecular level [18, 19]. At present, molecular docking technology has played an important role in elucidating the mechanism of action between active ingredients of TCM and related target proteins in many diseases, such as cardiovascular disease [20] and cancer [21]. Research on the treatment of UC is in the initial stage, and the pharmacodynamic substances that are effective against UC have not been fully elucidated [22]. The molecular docking virtual screening technology used to screen the pharmacodynamic substances (active component groups) of BAA effective in the treatment of UC is relatively simple and can obtain more accurate results.

The aim of this study was to identify all of the known chemical components of BAA through the Traditional Chinese Medicine Systems Pharmacology (TCMSP) Database and Analysis Platform, combined with the five principles of drug-like molecules (Lipinski rule) and the standard conditions, such as oral bioavailability and drug similarity, to preliminarily screen for compounds that could be developed into therapeutics. We set human IL-1R, TLR, EGFR, TGFR, and Wnt proteins as the targets of UC, and AutoDock Vina molecular docking software was used to sort the bioactive compounds of BAA by scoring function. Additionally, the primary-screening compound group and key targets of disease pathogenesis were also explored. The affinity and binding mode of the proteins were then used to screen for active ingredients with potential therapeutic effectiveness against UC. In addition, potential compounds were further tested in vitro.

Materials And Methods

Construction of Ligand Library (Compound)

The TCMSP Database and Analysis Platform were used to establish a preliminary-screening compound group of TCM (<https://old.tcmsp-e.com/tcmsp.php>)[23]. The small-molecule data were downloaded in mol2 format from the TCMSP database according to the small molecule MOLID number. The small molecule was then imported into ChemBio3D Ultra 14.0 (Cambridgesoft, Cambridge, MA, USA) for energy minimization. The Minimum RMS Gradient was set at 0.001, and the small molecule was saved in mol2 format. The optimized small molecule was imported into AutoDockTools (Scripps Research Institute, La Jolla, CA, USA) for hydrogenation, and the charge was calculated and assigned, in addition to the rotatable bond being set. The file was saved in "pdbqt" format.

Preparation Of Receptors (Proteins)

UC-related target protein data came from Protein Data Bank (PDB, <http://www.rcsb.org/>)[24]. The most relevant UC target proteins, including human IL-1R, TLR, EGFR, TGFR, and Wnt, were set as the receptor proteins. High resolution was preferred, and the original ligand had high structural similarity to the active ingredient to be docked. Pymol (DeLano Scientific LLC, Palo Alto, CA, USA) was used to remove protein crystal water and original ligands. The protein structure was imported into AutoDockTools (Scripps Research Institute, La Jolla, CA, USA) for hydrogenation, and the charge was calculated and assigned. The type of atom was specified, and the file was saved in "pdbqt" format.

Docking Settings

AutoDock Vina (Scripps Research Institute, La Jolla, CA, USA) was used for docking, and the EGFR (PDB ID: 7LGS) target-related parameters were set as follows: center_x = 29.720; center_y = - 19.100; center_z = 5.833; the size of the grid box was set to 60 × 60 × 60 (the spacing between each grid point was 0.375Å); and the rest of the parameters were the default settings. IL-1R (PDB ID: 5R8J) target-related parameters were set as follows: center_x = 38.545; center_y = 13.206; center_z = 68.747; the size of the grid box was set to 114 × 94 × 110 (the spacing between each grid point was 0.375Å); and the rest of the parameters were the default settings. TLR1 (PDB ID: 7NT7) target-related parameters were set as follows: center_x = 38.062; center_y = 15.372; center_z = - 6.727; the size of the grid box was set to 84 × 106 × 120 (the spacing between each grid point was 0.375 Å); and the rest of the parameters were the default settings. The Wnt-8 (PDB ID: 4FOA) target-related parameters were set as follows: center_x = - 62.066; center_y = - 9.0; center_z = 9.934; the size of the grid box was set to 126 × 126 × 126 (the spacing between each grid point was 0.375Å); and the rest of the parameters were the default settings. TGFR (PDB ID: 5Q1L) target-related parameters were set as follows: center_x = 4.108; center_y = 9.252; center_z = 7.215; the size of the grid box was set to 60 × 60 × 60 (the spacing between each grid point was 0.375Å); and the rest of the parameters were the default settings.

2molecular Docking Between Chemical Components And Target Proteins

The compounds with the highest docking score for each protein were selected to visualize the docking results using Discovery Studio (Accelrys Software Inc., San Diego, USA).

Cck-8 Cytotoxicity Assay

We took the cells in the logarithmic growth phase (NCM460; Raw264.7), counted them, pipetted 200 µL of cell suspension into a 96-well plate, and set the cell density to 5×10^3 cells per well. We divided the samples into blank and control groups, which were both placed in an incubator for routine culture for 24 h. We accurately weighed the test drug aianthone, used DMSO to prepare a mother solution with a concentration of 200 µM, and then sequentially diluted it with DMSO to a concentration of 1, 3, 10, 30, and 50 µM for administration. The next day, in both the blank group and the control group, the medium was replaced with a fresh culture medium. Specifically, in the administration group, the culture medium containing drugs (1, 3, 10, 30, and 50 µM) was used; in the solvent control group, we added 1 µL of DMSO, and they were placed in an incubator for routine culture. After 24 h, 10 µL of CCK8 solution was added to each well, and the cells were placed in an incubator for further incubation for 2.5 h. We measured the absorbance value A of each well at a wavelength of 450 nm with an enzyme-linked immunosorbent assay, calculated the average value of the absorbance of each group, and calculated the inhibition rate according to the following formula: Inhibition rate% = $100\% \times [(A \text{ control group} - A \text{ administration group}) / (A \text{ control group} - A \text{ blank group})]$. The IC₅₀ values of drugs on cells (NCM460; Raw264.7) were calculated with GraphPad Prism software.

Determination Of Anti-inflammatory Activity

According to the CCK-8 cytotoxicity test, the concentration of the drug whose toxicity to Raw264.7 cells was less than the IC_{50} value was selected for anti-inflammatory activity detection, and the drug concentration was selected as 1, 3, and 10 μM . Raw264.7 cells in the logarithmic growth phase were taken and trypsinized; the cells were collected and centrifuged at 1000 rpm/min; the supernatant was discarded, and the cells were resuspended in a fresh culture medium. We counted the cells, adjusted the cell density, pipetted 100 μL of the cell suspension into a 24-well plate, and then added 700 μL of the culture medium to achieve the cell density 1×10^5 cells per well. We set the blank group and the control group and placed them in an incubator for routine culture for 24 h. The test drug ailanthone was precisely weighed, and the mother solution was prepared with DMSO to achieve a concentration of 200 μM , which was sequentially diluted with DMSO to a concentration of 1, 3, and 10 μM for administration. The DMSO content of the administered cells was less than or equal to 1%. The cells were first incubated with drugs for 2 h, then exposed to LPS with a final concentration of 1 $\mu g/mL$, and finally co-cultured for 24 h. After 24 h, the cell supernatant of each experimental group was collected, and the absorbance value was detected by a microplate reader in accordance with the operation instructions of the ELISA kit. The contents of TNF- α , IL-6, and IL-1 β in the supernatant were determined.

Statistical analysis

SSPS software was used for statistical analysis, and $P < 0.05$ was considered statistically significant.

Results

Preliminary Screening of Compounds in BAA

Compounds were searched with the name of BAA as the keyword, and 89 small-molecule chemical components were retrieved from the TCMSP database. Eighty-nine compound libraries were initially constructed. The five principles of drug-like and other related screening conditions were used for secondary screening: 1) molecular weight (MW) less than 500; 2) lipid–water partition coefficient (ALogP) less than 5; 3) number of hydrogen-bond donors (Hdon) less than 5; 4) number of hydrogen-bond receptors (Hacc) less than 10; 5) oral bioavailability (OB) greater than 50; and 6) drug similarity (DL) greater than 0.18. The secondary screening resulted in 22 compounds. As shown in Table 1, these 22 compounds were suggested to have good drug-like properties, and were saved in mol2 format for future use.

Table 1
Compounds that met the initial screening conditions.

Mol ID	Molecule Name
MOL000422	Kaempferol
MOL006276	SMR000232320
MOL006277	Shinjudilactone
MOL006278	Shinjulactone A
MOL006279	Shinjulactone B
MOL006280	Shinjulactone C
MOL006281	Shinjulactone K
MOL006284	Ailanthone
MOL006285	Ailantanol A
MOL006294	2,3,5,6,9,11,12,15,16,1y-decahydro-1H-cyclopentalphenanthren-iy-yl-methylheptane-2,3,4-triol
MOL006300	1-Hydroxy-canthin-6-one
MOL006301	1-Methoxycanthinone
MOL006302	2-Hydroxy-canthin-6-one
MOL006303	4,5-Dihydrocanthin-6-one
MOL006304	4-Hydroxy-canthin-6-one
MOL006306	5-Methoxycanthin-6-one
MOL006308	66762-19-4
MOL006309	Amarolide 11-acetate
MOL006310	Amarolide
MOL006311	Artelin
MOL006314	Canthin-6-one
MOL006315	Dehydrocrebanine

Scoring Function Ranking of Secondary-Screening Chemical Components and Target Proteins.

The 22 compounds obtained from the secondary screening were molecularly docked with human IL-1R, TLR, EGFR, TGFR, and Wnt proteins using AutoDock Vina software. The binding conformation was scored using a scoring function based on shape matching and energy matching. The docking results of the compounds and receptors were sorted in descending order of the total score, and the results are shown in Table 2. The free energy of binding to the active cavity of human IL-1R, TLR, EGFR, TGFR, and Wnt protein of the highest scoring compound obtained by AutoDock Vina software was -8.7 , -8.0 , -9.2 , -7.7 , and -8.5 kcal/mol, respectively. A larger absolute value of the binding constant indicates that lower free energy is required for the compound to bind. It is generally believed that when the absolute value is greater than 7, the combination of the compound and the protein is more likely.

Table 2
Molecular docking function scoring and ranking results.

EGFR	Affinity kcal/mol	IL-1R	Affinity kcal/mol	TGFR	Affinity kcal/mol	TLR1	Affinity kcal/mol	Wnt8	Affinity kcal/mol
MOL006315	-8.7	MOL006284	-8	MOL000422	-9.2	MOL006284	-7.7	MOL006284	-8.5
MOL006301	-8.5	MOL006310	-7.8	MOL006311	-8.9	MOL006277	-7.6	MOL006278	-8.4
MOL006277	-8.4	MOL006277	-7.7	MOL006315	-8.7	MOL006278	-7.6	MOL006279	-8.1
MOL006302	-8.4	MOL006285	-7.7	MOL006303	-8.6	MOL006300	-7.4	MOL006309	-8
MOL006308	-8.4	MOL006309	-7.6	MOL006304	-8.6	MOL006294	-7.2	MOL006310	-7.9
MOL000422	-8.3	MOL006278	-7.5	MOL006301	-8.5	MOL006303	-7.2	MOL006277	-7.7
MOL006304	-8.3	MOL006281	-7.1	MOL006302	-8.5	MOL006310	-7.1	MOL006294	-7.7
MOL006306	-8.2	MOL006315	-7.1	MOL006306	-8.5	MOL006280	-7	MOL006280	-7.3
MOL006311	-8.2	MOL000422	-6.9	MOL006284	-8.4	MOL006301	-6.9	MOL006281	-7.1
MOL006314	-8.2	MOL006311	-6.8	MOL006300	-8.4	MOL006306	-6.9	MOL006311	-7.1
MOL006300	-8.1	MOL006279	-6.7	MOL006314	-8.4	MOL006314	-6.9	MOL006315	-7.1
MOL006284	-8	MOL006280	-6.5	MOL006277	-8.2	MOL006285	-6.8	MOL000422	-7
MOL006303	-8	MOL006276	-6.3	MOL006308	-8.2	MOL006309	-6.8	MOL006285	-7
MOL006310	-8	MOL006294	-6.1	MOL006280	-7.7	MOL006315	-6.8	MOL006276	-6.8
MOL006309	-7.9	MOL006303	-6	MOL006310	-7.7	MOL006304	-6.7	MOL006302	-6.7
MOL006285	-7.8	MOL006304	-6	MOL006278	-7.4	MOL006279	-6.6	MOL006301	-6.4
MOL006279	-7.7	MOL006300	-5.9	MOL006279	-7.4	MOL006311	-6.6	MOL006306	-6.4
MOL006276	-7.6	MOL006302	-5.8	MOL006294	-7.3	MOL000422	-6.5	MOL006300	-6.3
MOL006280	-7.6	MOL006306	-5.8	MOL006276	-7.2	MOL006281	-6.3	MOL006303	-6.2
MOL006294	-7.6	MOL006308	-5.8	MOL006309	-7.2	MOL006276	-6.1	MOL006304	-6.2
MOL006278	-7.4	MOL006314	-5.8	MOL006285	-7.1	MOL006308	-6	MOL006314	-6.2
MOL006281	-6.5	MOL006301	-5.7	MOL006281	-7	MOL006302	-5.9	MOL006308	-6.1

Binding Pattern of the Highest Scoring Compound and Target Protein.

Figure 1 shows that the compounds bind closely to the surface of human IL-1R, TLR, EGFR, TGFR, and Wnt proteins, and there is a good spatial match between them. All of the compounds bind to the active space of the corresponding protein.

Microscopic Binding of the Highest Scoring Compound to Amino Acid Residues Near the Active Site of the Target Protein.

Figure 2 shows that dehydrocrebanine has a noncovalent bond with the residues between the amino acid motifs of threonine (THR) 857 near the active site of EGFR protein, and aianthone interacts with the active site ASP of IL-1R protein. Partic acid (86 amino acid residues) has hydrogen-bonding interactions. Kaempferol forms a cation- π interaction with lysine (LYS) 232 at the active site of the TGFR protein, and aianthone forms a π -alkyl interaction with tryptophan (TYR) 691 near the active site of TLR. Aianthone forms a π -alkyl interaction with the active site of Wnt LYS 210, forming hydrogen-bond interactions.

Inhibitory Effect of Aianthone on Cell Proliferation.

Aianthone was found to be able to combine with L-1R, TLR1, and Wnt proteins at the same time through virtual screening of front molecule docking. It is worth noting that the docking scores were high. However, the computer results could not replace the experiment. The cytotoxicity of aianthone was tested on cells by CCK-8 experiment (NCM460; Raw264.7). The results showed that cell proliferation was not significantly affected at the concentrations of 1, 3, and 10 μ mol of aianthone. This safe dose would be used to observe the anti-inflammatory activity of aianthone (Fig. 3).

Aianthone can inhibit the activity of TNF- α , IL-6, and IL-1 β .

The activity of TNF- α , IL-6, and IL-1 β is closely related to the development of UC. The concentrations of 1, 3, and 10 μ M of ailanthone were used to observe the effect on the activity of TNF- α , IL-6, and IL-1 β in Raw264.7 cells (Fig. 4). The levels of these proinflammatory cytokines decreased after administration of ailanthone (10 μ M).

Discussion

In recent years, there have been many studies on the treatment of UC with TCM [25]. Many components in TCM, such as *Patrinia scabiosaefolia* Fisch [26], *Lindera aggregata* (Sims) Kosterm [27], *Glycyrrhizae* [28], *Angelica sinensis* [29], and BAA [10], have been reported to have therapeutic effects. Particularly significant among these components are flavonoids, glycosides, phenols, polysaccharides, and alkaloids. BAA, a common herbal medicine used in the TCM treatment of UC, has attracted increasing attention. Modern medical research has shown that BAA and its preparations have a good curative effect on UC [10]. At present, research on the treatment of UC is in the initial stage, and its pharmacodynamic substances have not been fully elucidated[30]. The molecular docking virtual screening technology used to screen the pharmacodynamic substances (active component groups) of BAA in the treatment of UC is relatively simple and can obtain more accurate results.

Molecular docking technology docks known small-molecule active substances with related target proteins, which can clarify the mechanism of action between active ingredients of TCM and targets at the molecular level [31, 32]. At present, molecular docking technology plays an important role in elucidating the mechanism of action between active ingredients of TCM and related target proteins in many diseases, such as cardiovascular disease [20] and cancer [21]. Therefore, we conducted preliminary screening with reference to the five principles of drug-like standards and other screening conditions of "drug similarity" and obtained 22 compounds. The 22 compounds obtained from the secondary screening were molecularly docked with human IL-1R, TLR, EGFR, TGFR, and Wnt proteins using AutoDock Vina software, and the binding conformation was scored using a scoring function. The free energy of the highest scoring compound binding to the active cavity of human IL-1R, TLR, EGFR, TGFR, and Wnt proteins was - 8.7, - 8.0, - 9.2, - 7.7, and - 8.5 kcal/mol, respectively. It is generally believed that when the absolute value is greater than 7 kcal/mol, the possibility of the combination of the compound and the protein is high[33]. The larger the absolute value of the binding constant, the lower the free energy required for the compound to bind[34]. The compound with the highest function score can be selected in order to construct a compound-protein binding diagram, which can visually convey the binding mode of the active compound with human IL-1R, TLR, EGFR, TGFR, and Wnt proteins and the interaction with the surrounding amino acid residues. It is worth noting that ailanthone was found to be able to combine with L-1R, TLR1, and Wnt proteins at the same time through virtual screening of front molecule docking, and the binding score was very high. This compound was further tested in vitro. Ailanthone had no significant inhibitory effect on cell proliferation within the concentration range of 1–10 μ M, and 10 μ M concentration significantly inhibited the proinflammatory response induced by LPS.

Conclusions

Briefly, in this study, the effective substances of BAA in treating UC were studied based on Virtual Screening–Molecular Docking–Activity Evaluation technology. We found that ailanthone had good activity in vitro. Although further animal experiments are needed for verification, ailanthone has a high possibility of becoming a drug in the future.

Abbreviations

UC:Ulcerative colitis;TCM:Traditional Chinese Medicine ;

BAA:Bark of *Ailanthus altissima* (Mill.) Swingle;NO:NOS:nitric oxide;nitric oxide synthase;TCMSP:Traditional Chinese Medicine Systems Pharmacology;IC₅₀:half maximal inhibitory concentration;DMSO:Dimethyl sulfoxide;LPS:Lipopolysaccharide

Declarations

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Authors' contributions

Conceptualization, Shanbo Ma ; Methodology, Lun Liu; Software, Xiang Li; Supervision&Validation, Yan-hua Xie; Writing-original draft, Xiaopeng Shi; Writing -review & editing,Si-wang Wang

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Availability of data and materials

The datasets generated during the current study are available in the TCMSP Database (<https://old.tcm-sp-e.com/tcm-sp.php>) & Protein Data Bank (<https://www.rcsb.org/>). The experimental data used to support the findings are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

As this study does not involve animal and patient experiments, the ethical approval and consent to participate are not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Figures

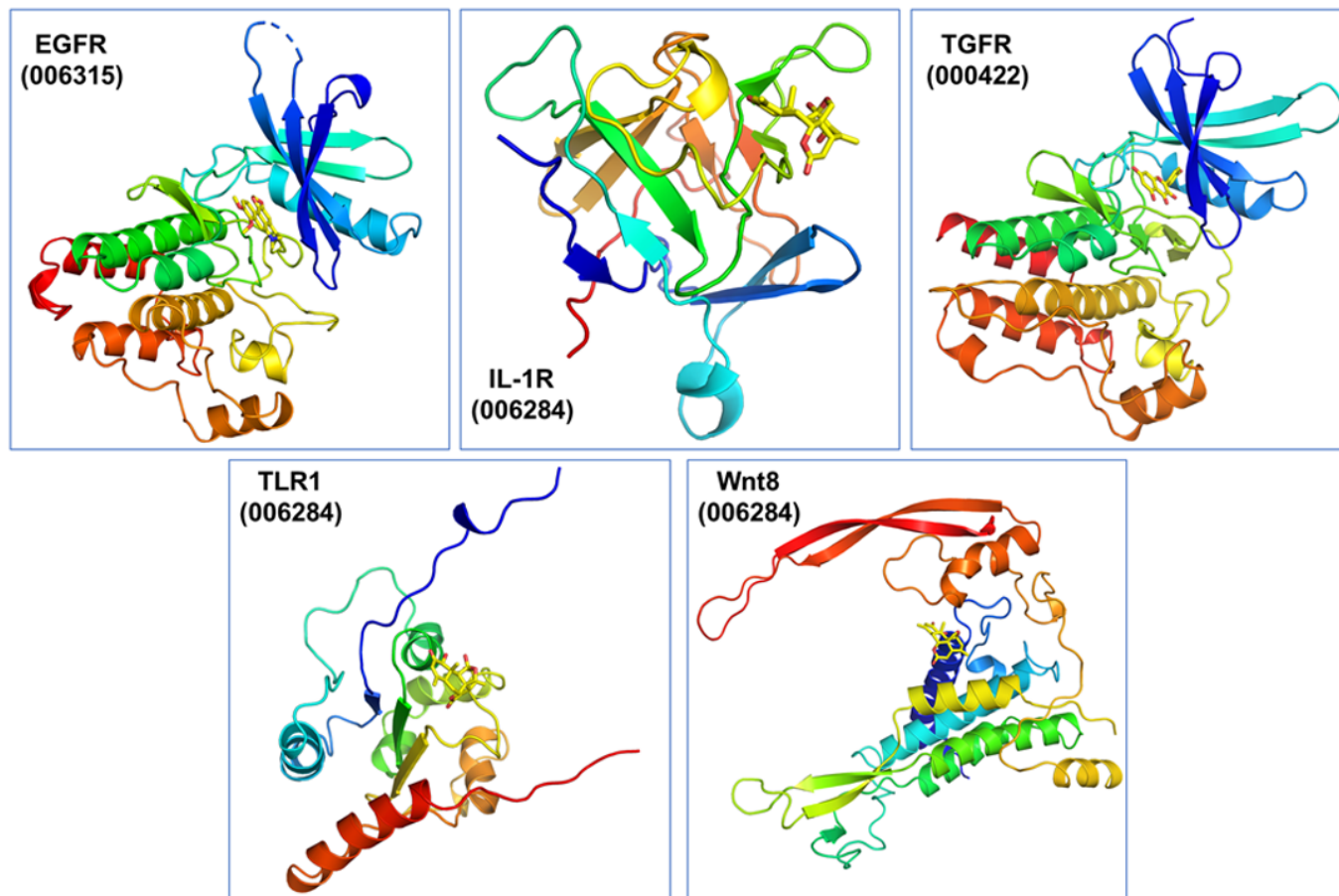


Figure 1

Binding pattern of the highest scoring compounds to human EGFR, IL-1R, TGFR, TLR, and Wnt8 proteins. The color curly structure represents the amino acid spatial sequence of the target protein, and the yellow structure in the protein cavity represents small molecules. The free energy of binding to the active cavity of human EGFR, IL-1R, TGFR, TLR1 and Wnt protein of the highest scoring compound dehydrocrebanine (006315), ailanthon (006284), kaempferol (000422), ailanthon (006284), and ailanthon (006284) is -8.7, -8.0, -9.2, -7.7, and -8.5 kcal/mol, respectively.

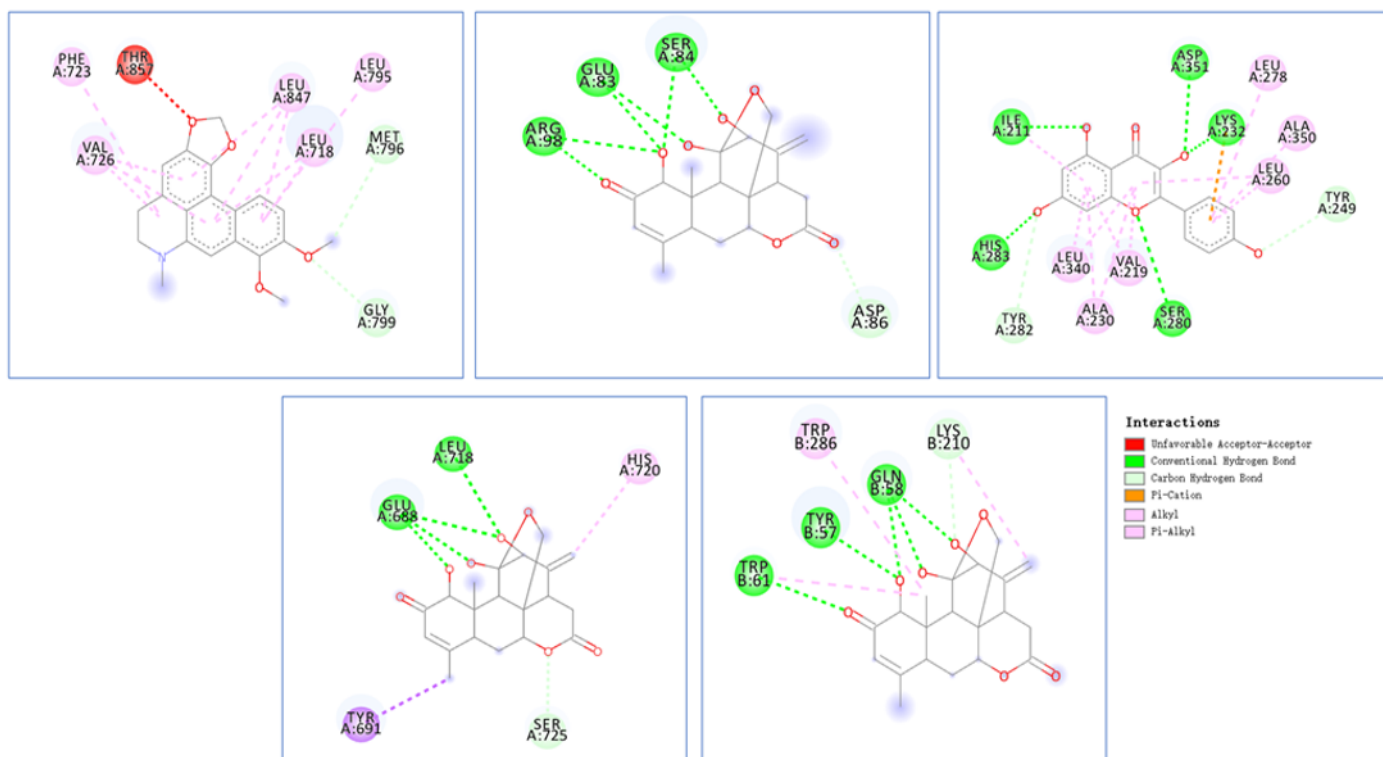


Figure 2

Microscopic binding of the highest scoring compound to amino acid residues near the active site of the target protein. Green circles and lines represent conventional hydrogen bonds; light blue circles and lines represent carbon hydrocarbon bonds; orange circles and lines represent π -cation; pink circles and lines represent alkyl; light pink circles and lines represent π -alkyl. The letter and the number under the amino acid represent the abbreviated name and the primary structure of the sequence of amino acids.

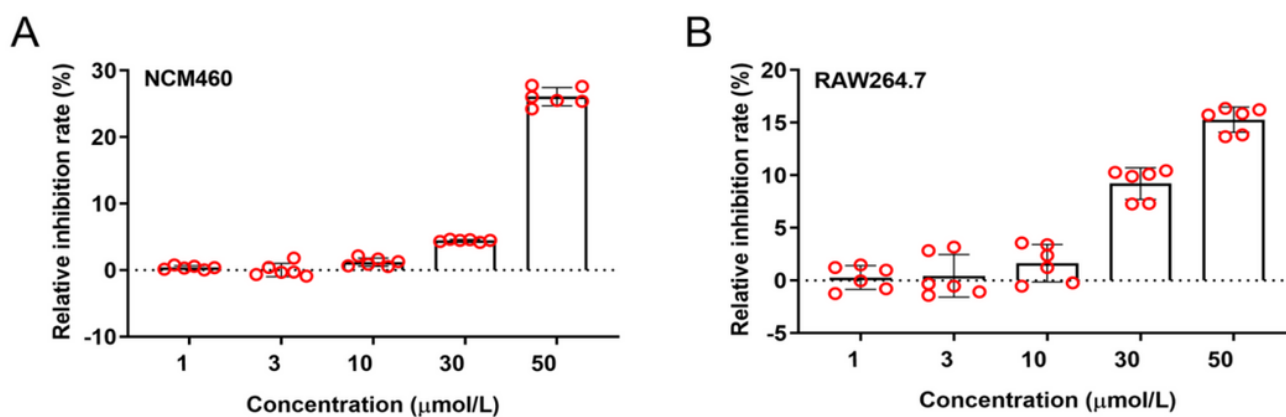


Figure 3

Inhibitory effect of ailanthon on cell proliferation. (A) The relative inhibition rate of ailanthon was tested on NCM460 cells by CCK-8 experiment. (B) The relative inhibition of ailanthon was tested on Raw264.7 cells by CCK-8 experiment. N=6.

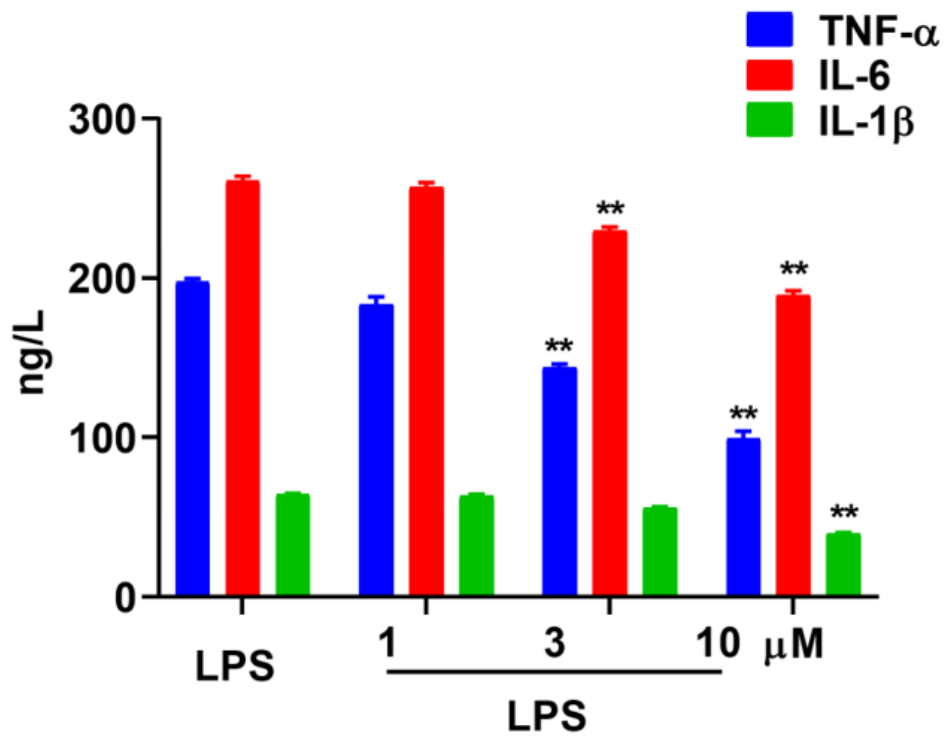


Figure 4

Ailanthone can inhibit the activity of TNF- α , IL-6, and IL-1 β . The activity of the proinflammatory cytokines was observed by the ELISA experiment on Raw264.7 cells. N=6. ** $P<0.01$.