



Study on The Interaction of Polyoxypregnane Glycosides From *Dregea sinensis* With Immune Proteins*

WANG Zhi¹⁾, SONG Juan^{1,2)}, DAI Rong-Ji¹⁾, DENG Yu-Lin¹⁾, LÜ Fang^{1)**}

¹⁾Beijing Key Laboratory for Separation and Analysis in Biomedicine and Pharmaceuticals, School of Life Science,

Beijing Institute of Technology, Beijing 100081, China;

²⁾Institute of Radiation Medicine, Academy of Military Medical Sciences of Academy of Military Science, Beijing 100850, China)

Abstract Objective Based on chemical structural characteristics, the bioactivity of polyoxypregnane glycosides from *Dregea sinensis*, and the molecular interaction between compounds and targeted proteins were investigated. **Methods** The bioactive screening of 191 polyoxypregnane glycosides (>800 u), and the kinetic evaluation on human immune-related proteins was carried out by molecular docking and SPR experiment. **Results** Seven compounds (6, 18, 23, 30, 78, 79, and 80) and 3 immune-related proteins (IL-2R α , TLR4, and TNF- α) were selected through virtual screening. Compounds 30 and 78 showed the significant binding tendency with IL-2R α and TLR4 in SPR experiment, and K_D values with IL-2R α were 2.41×10^{-6} and 2.14×10^{-6} mol/L, meanwhile K_D values with TLR4 were 1.96×10^{-5} and 5.60×10^{-6} mol/L, respectively. The interactions between targeted proteins and compounds were further characterized by discovery studio. The analysis of molecular docking revealed the binding pocket residues for SPR-positive molecules 6, 18, 30, 78, and 80. **Conclusion** The result indicated these glycosides could bind to targeted proteins through forming hydrogen bonds and Pi-Pi interactions. The study is meaningful for bioactive evaluation of polyoxypregnane glycosides, and provides valuable exploration to the underlying mechanisms of effective compounds with low abundance.

Key words polyoxypregnane glycosides, immune inhibitors, molecular docking, surface plasmon resonance

DOI: 10.16476/j.pibb.2021.0388

Many diseases were in the closest relationship with immune regulation, such as infection, immunodeficiency disease, hypersensitive reaction and cancer^[1]. With the rapid development of drug research, medicinal plants as natural sources of effective immune inhibitors have been widely investigated^[2], and more attention has been paid to the molecular interaction between natural products and targeted proteins^[3-5].

In our previous studies, 191 polyoxypregnane glycosides were identified by nuclear magnetic resonance (NMR) and high-performance liquid chromatography coupled with multistage mass spectrometry (HPLC-MSⁿ) methods^[6-10] from *Dregea sinensis*, an important medicinal plant. Although modern pharmacological studies had reported that polyoxypregnane glycosides possessed various pharmacological effects including anti-tumor,

anti-depression and immunomodulation^[11-15], bioactive studies always remained at screening of crude extract due to the low amount of these compounds in the plant, and further work was rarely carried out.

We attempted to discover immunosuppressive compounds from *Dregea sinensis*, and forecasted the interaction between polyoxypregnane glycosides and immune-related proteins. The research strategy was proposed based on characteristics of chemical structures as following: (1) the construction of

* This work was supported by grants from The National Natural Science Foundation of China (81503353) and National Major Scientific Instruments and Equipments Development Project (2012YQ040140).

** Corresponding author.

Tel: 86-10-68949331, E-mail: lvfangbeijing@bit.edu.cn

Received: December 13, 2021 Accepted: January 25, 2022

database including polyoxypregnane glycosides as ligands and immune-related proteins as target proteins; (2) the virtual screening to filter ligands and target proteins by molecule docking with Discovery Studio (DS); (3) the verification of interaction between selected ligands and selected target proteins by the analysis of surface plasmon resonance (SPR) and *in silico*. According to these strategy, 191 polyoxypregnane glycosides were collected, and some

important cytokines and receptors in immune regulation, *e. g.* interleukin-n (IL-n), interleukin-nR (IL-nR), tumor necrosis factor (TNF) and Toll-like receptors (TLRs) were chosen as target proteins^[16-18]. The top-rank polyoxypregnane glycosides interact with selected target proteins were shown in Figure 1 on the basis of Libdock scores and the orthogonal tests.

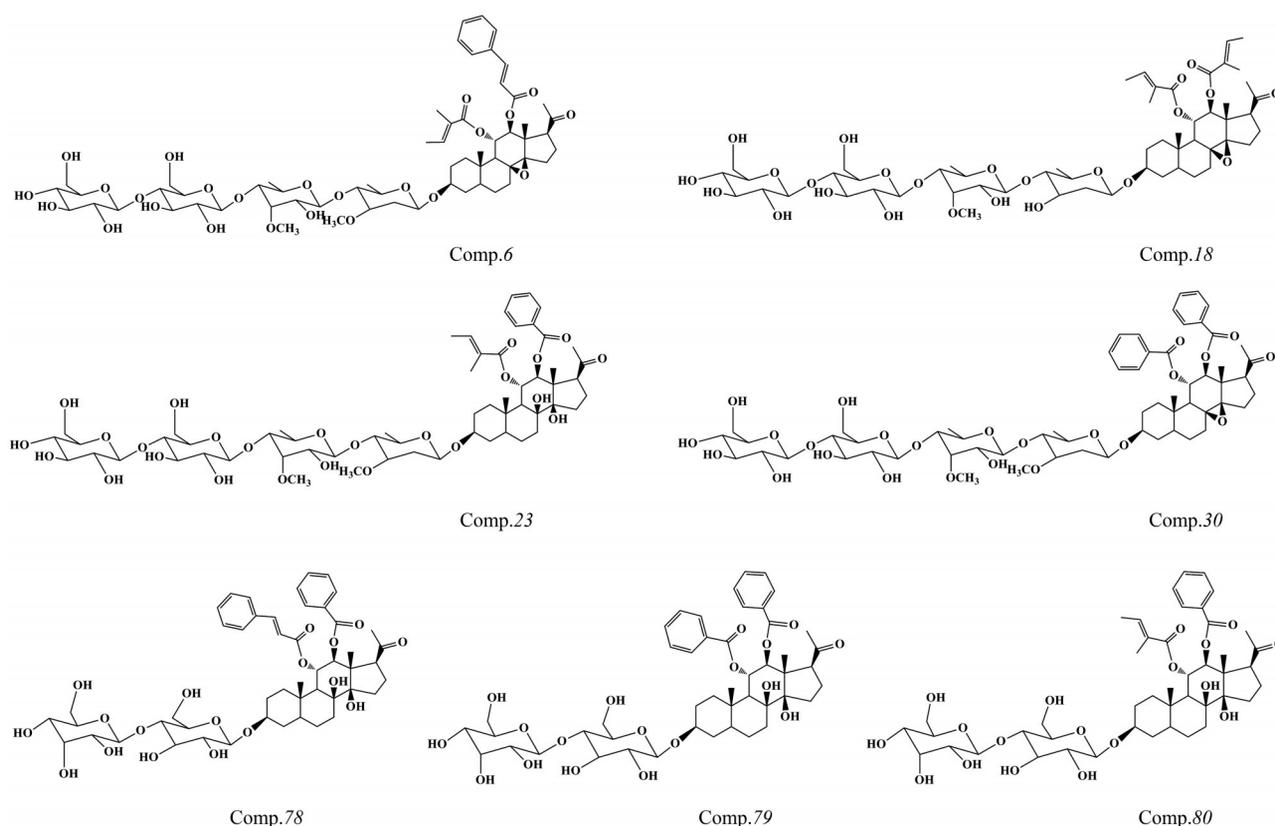


Fig. 1 The structures of top-ranked polyoxypregnane glycosides

Comp.: compound.

1 Materials and methods

1.1 Chemicals and reagents

SH-(PEG)_n-COOH (MW1 000), SH-(PEG)_n-OH (MW 346) and gold coated slides (Plexera) were purchased from Yare biotech (Shanghai, China). EDC-HCL and NHS, DMAP were provided by Aladdin Chemistry. FKBP12 (10268-H08E) was provided by Sino Biological Inc. (Beijing, China). Human recombinant TNF- α (ab155699) was provided by Abcam (Cambridge, UK). Human recombinant IL-2R α (RPB837Hu01) and human recombinant TLR4 (RPA753Hu01) were provide by Cloud-clone

Corp. (Wuhan, China). Buffers and reagents were prepared using Milli Q water (Milipore, USA).

1.2 *In silico* screening of molecules and target proteins

Database of polyoxypregnane glycosides was constructed, and compounds 1–81 were collected from the references^[9-10], compounds 82–138 were collected from the references^[6, 8], compounds 139–191 were collected from the references^[7, 19] (Table S1), and 3D structures of all compounds were transferred by ChemBio Office software (Cambridge, UK). 3D structures of target proteins were obtained from protein data bank (PDB, <http://www.rcsb.org/>)

pdb/home/home. do). The protein molecules were filtered by three criterias: (1) 3D structures without mutation and missing residues around the active site; (2) 3D structures were X-ray crystal structures with high resolution; (3) for protein structures with two chains or more, the chain around the active sites was reserved for docking (Table S2).

The ligands were structurally rearranged in response to the receptor in Discovery Studio 4.0 (Accelrys, San Diego, USA). The interactions of human proteins and ligands were evaluated by molecular dock search algorithm^[20], which hinted the target compounds as inhibitors of proteins (Table S3). The binding molecules were finally selected as the potential active compounds of *Dregea sinensis* on the basis of their higher LibDock score (at least > 100).

1.3 Surface plasmon resonance validation

Validate affinity measurements for binding of the small molecules to TNF- α , IL-2R α and TLR4 were performed on a Plexera PlexArray HT system (Plexera LLC, Woodinville, WA, USA) using a bare gold SPRi chip (Nano-capture gold chips, with a gold layer of 47.5 nm thickness)^[21]. Briefly, proteins (100 nmol/L or 1 000 nmol/L in PBS buffer) were immobilized to the surface of a biochip Graft-to-then-from-COOH *via* PDMS using the SpotBot3 Microarrayer system. Selected polyoxypregnane glycosides were dissolved in PBS buffer at a concentration of 5 mmol/L or 10 mmol/L. The interactions between target proteins and polyoxypregnane glycosides were determined by SPR imaging using the Plexera PlexArray HT system. Realtime binding signals were recorded and the binding data were analyzed using the PLEXERA SPR Date Analysis Module. K_a is the association rate constant for the binding, K_d is the dissociation rate constant for the complex. Affinities were calculated, in the kinetic analysis, from the relation $K_D = K_d/K_a$ in the equilibrium analysis.

1.4 Interaction analysis

CDOCKER module of DS was proposed for analyzing the interaction between selected polyoxypregnane glycosides and proteins, and it was in rigid condition: the receptors were held rigidly while the ligands were allowed to flex during the docking process. CDOCKER was an implementation of a CHARMM based docking tool^[22-23]. For each complex pose, the CHARMM energy (interaction

energy plus ligand strain) and the interaction energy, which indicate ligand binding affinity, were calculated. The structures of identified hits were prepared and docked into the ligand-binding pocket of receptor. Different poses for each test molecule were generated and analyzed on the basis of CDOCKER interaction energy.

2 Results and discussion

The virtual screening had played a guiding role in discovering bioactive molecules, and indicating the interaction between proteins and ligands^[24-26]. The intuitionistic diagram of interaction between ligand molecules and protein molecules was obtained by the computational methods. The possible binding domains of the proteins were defined from PDB site records. The orthogonal test of each ligand-receptor was done to confirm the best poses radius and active pocket site.

In the study, all proteins were ranked after docking with known immunomodulators (Table S4), *e. g.* cyclosporine A^[27] as reference molecules in the primary LibDock process, which was intended to help selection of efficient binding site during the virtual screening. The analysis showed most proteins with binding tendency were immune-related. Combined with the results of cell model test^[8, 10], different PDB protein structures of 3 target proteins (Table S5) were selected for the second round virtual screening against 191 polyoxypregnane glycosides. A cut-off range of docking score had been set as a specific Libdockscores, that is higher than the related reference molecules. Screened ligand molecules were ranked on the basis of their docking score.

To exclude the probability of missing screen or false positives, SPR experiment of the glycosides 6, 18, 23, 30, 78, 79 and 80, isolated in our previous work and ranked in the top ten (Table S6), was carried out to 3 targeted proteins. As a result, compound 6 showed the binding affinity towards TNF- α with K_D value 1.57×10^{-4} mol/L. Compounds 30 and 78 showed the significant binding tendency with IL-2R α and TLR4 in SPR experiment, and K_D values with IL-2R α were 2.41×10^{-6} mol/L and 2.14×10^{-6} mol/L, meanwhile K_D values with TLR4 were 1.96×10^{-5} mol/L and 5.60×10^{-6} mol/L, respectively. In addition, compounds 18 and 80 showed the high binding affinity towards IL-2R α , and K_D values were

4.19×10^{-6} mol/L and 6.50×10^{-6} mol/L, respectively. The binding kinetics and K_D of the candidate

glycosides were showed in Figure 2.

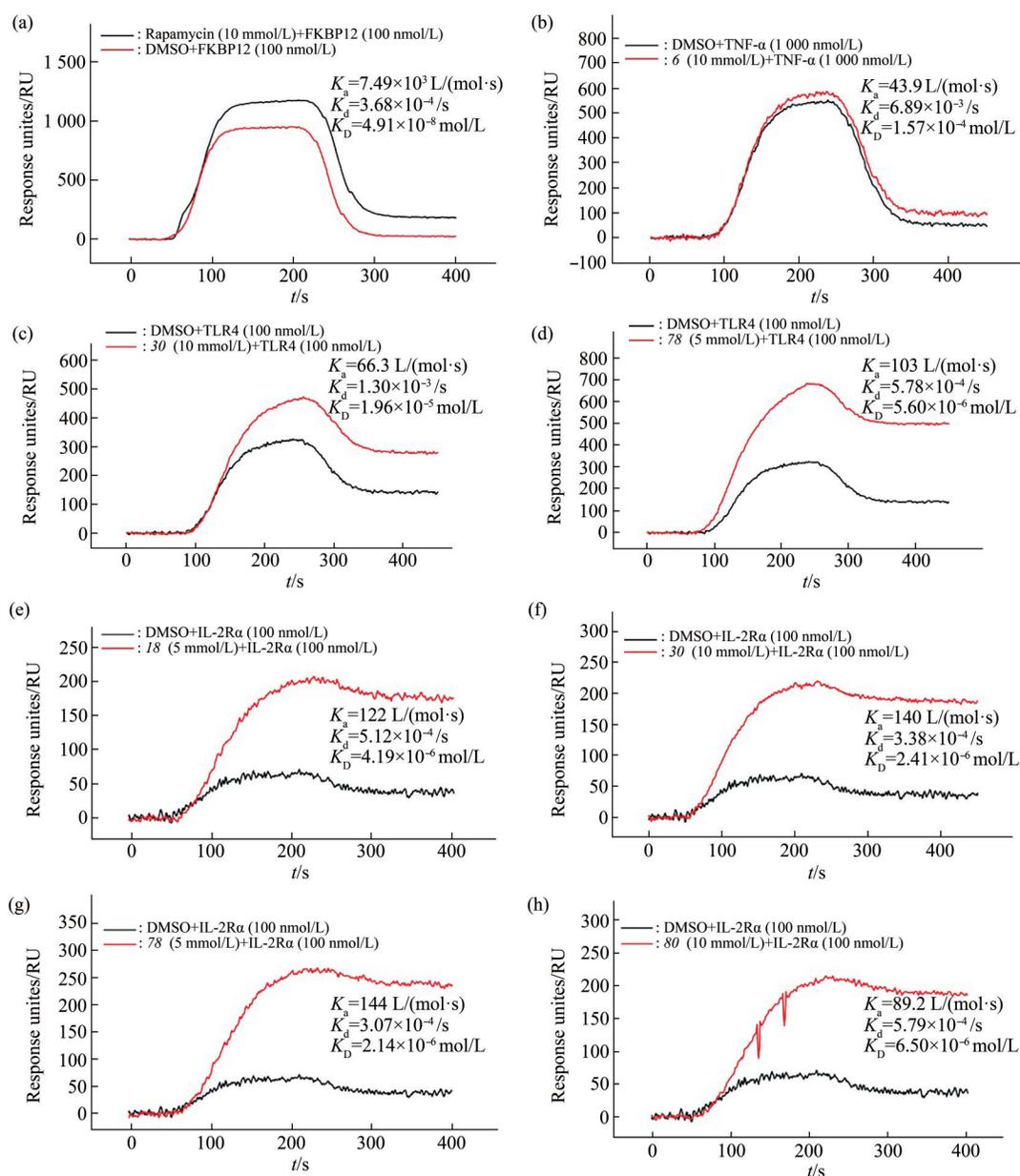


Fig. 2 Kinetic analysis of the binding of selected compounds to the putative targets using the PlexArray®HT instrument

Interaction of rapamycin with FKBP12 as a positive control.

In order to study ligand-protein binding mechanisms, compounds 6, 18, 30, 78, and 80 were further evaluated by CDOCKER module of DS. The CDOCKER interaction energy as an estimation of molecular complex binding affinity was applied in this procedure (Table S7). Based on the analysis of the interactions formed by ligands and IL-2R α (2ERJ), compounds 18, 30, 78 and 80 mainly formed pairs of

hydrogen bonds by the carbonyl-O of the structures with the amide-NH of ASN/ARG/TRP, and formed pairs of Pi-Pi interaction with ARG/TRP/ILE residues. For TLR4 (2Z63), molecules 30 and 78 formed the hydrogen bond mainly by the LYS residue and Pi-Pi interactions by ASN and ILE. Compound 6 had two pairs of hydrogen bonds formed by TRY and GLY of TNF- α (2AZ5). Furthermore, electrostatic

effect and van der Waals effect, and hydrophilic effect between amino acid residues and ligands had a certain contribution to form good binding affinity. In general, the docking simulation performed with CDOCK indicated that the benzoyl group, tigloyl group and cinnamoyl group of chemical structures, and hydroxyl

group of sugar chains played the important part in the molecular binding of polyoxypregnane glycosides with immune-related proteins. The molecular binding of molecules 30 and 78 with 2ERJ and 2Z63 were shown in Figure 3.

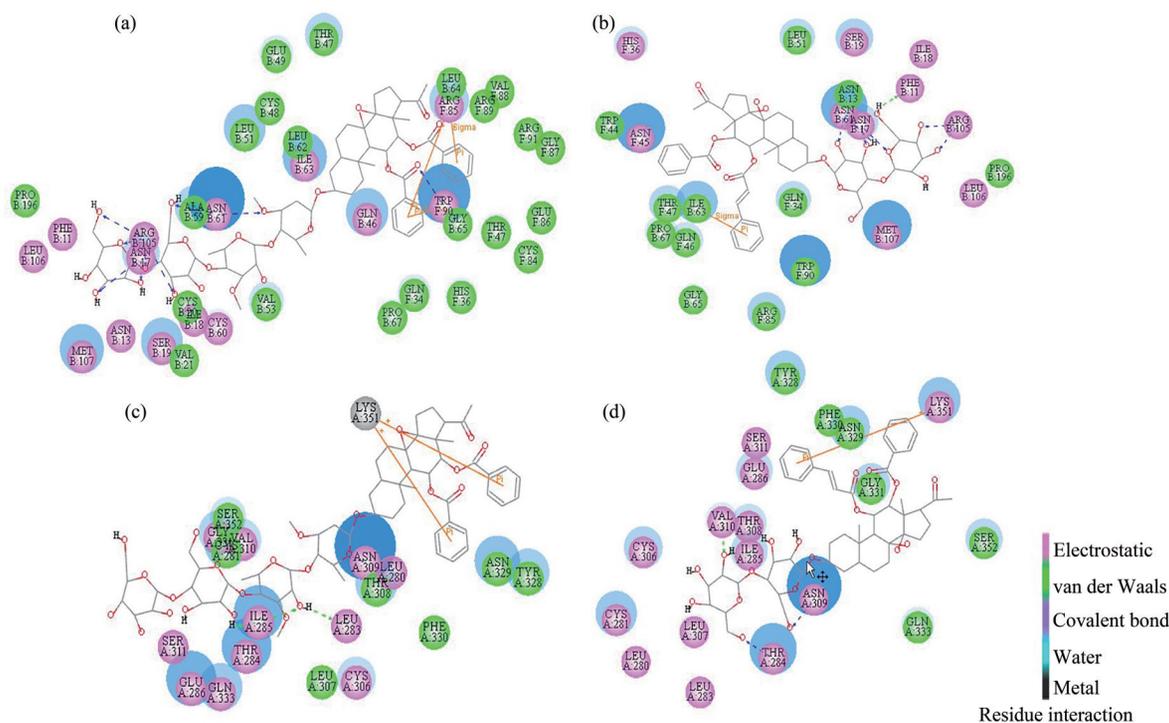


Fig. 3 The inter-molecular interaction of the predicted binding modes of compounds to proteins

(a) 30-2ERJ; (b) 78-2ERJ; (c) 30-2Z63; (d) 78-2Z63.

3 Conclusion

Although there were many studies on immunosuppressants including enzyme complex and small molecules from natural and synthetic sources, the research on the interaction between immune-related proteins and polyoxypregnane glycosides (> 800 u) had not been reported. In this study, the modulation of immune activity *via* the binding of polyoxypregnane glycosides *in silico* and *in SPR* provided information about binding affinity pattern of immune-related proteins in terms of dissociation constant and on and off rates. Five polyoxypregnane glycosides (6, 18, 30, 78, and 80) possessing potential bioactive were screened out. It is the first time to report the study on the binding affinity of polyoxypregnane glycosides with immune-related proteins by molecular docking and SPR experiment,

which provides valuable exploration to the underlying mechanisms of natural products with low abundance.

Supplementary Files are available online (<http://www.pibb.ac.cn> or <http://www.cnki.net>).

PIBB_20210388_Table S1.pdf
 PIBB_20210388_Table S2.pdf
 PIBB_20210388_Table S3.pdf
 PIBB_20210388_Table S4.pdf
 PIBB_20210388_Table S5.pdf
 PIBB_20210388_Table S6.pdf
 PIBB_20210388_Table S7.pdf

References

- [1] Waldmann H. The new immunosuppression. *Curr Opin Chem Biol*, 2003, 7: 476-480
- [2] Newman D J, Cragg G M. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod*, 2012,

- 75(3): 311-335
- [3] Zhou X L, Yu S S, Jing S, *et al.* Computational study on new natural compound inhibitors of pyruvate dehydrogenase kinases. *Int J Mol Sci*, 2016, **17**: 340
- [4] Yang T T, Yang H R, Fan Y, *et al.* Interactions of quercetin, curcumin, epigallocatechin gallate and folic acid with gelatin. *Int J Biol Macromol*, 2018, **118**: 124-131
- [5] Mohammadzadeh-Asl S, Aghanejad A, Yekta R, *et al.* Kinetic and thermodynamic insights into interaction of erlotinib with epidermal growth factor receptor: surface plasmon resonance and molecular docking approaches. *Int J Biol Macromol*, 2020, **63**: 954-958
- [6] Song J, Lv F. Review on chemical constituents and biological activities of *Dregea E. Mey.* *Nat Prod Res Dev*, 2016, **28**(9): 1492-1498
宋娟, 吕芳. 天然产物研究与开发, 2016, **28**(9): 1492-1498
- [7] Jia S H, Song J, Dai R J, *et al.* Structural deduction of pregnane glycosides from *Dregea sinensis* Hemsl by high-performance liquid chromatography and electrospray ionization-tandem mass spectrometry. *Int J Mass Spectrom*, 2017, **415**: 38-43
- [8] Liu X J, Shi Y, Jia S H, *et al.* Six new C-21 steroidal glycosides from *Dregea sinensis* Hemsl. *J Asian Nat Prod Res*, 2017, **19**(8): 745-753
- [9] Song J, Dai R J, Deng Y L, *et al.* Rapid structure prediction by HPLC-ESI-MSⁿ of twenty-five polyoxypregnane tetraglycosides from *Dregea sinensis* with NMR confirmation of eight structures. *Phytochemistry*, 2018, **147**: 147-157
- [10] Song J, Lv F, Dai R J, *et al.* Rapid identification and structural characterization of polyoxypregnane glycosides in *Dregea sinensis* by HPLC-MSⁿ and HRMS. *J Asian Nat Prod Res*, 2021, **23**: 9-19
- [11] Ye Y P, Li X Y, Sun H X, *et al.* Immunomodulating steroidal glycosides from the roots of *Stephanotis mucronata*. *Helv Chim Acta*, 2004, **87**(9): 2378-2384
- [12] Li X Y, Sun H X, Ye Y P, *et al.* C-21 steroidal glycosides from the roots of *Cynanchum chekiangense* and their immunosuppressive activities. *Steroids*, 2006, **71**(1): 61-66
- [13] Ye Y Y, Chen F Y, Sun H X, *et al.* Stemucronoside K, a novel C₂₁ steroidal glycoside from *Stephanotis mucronata*, inhibited the cellular and humoral immune response in mice. *Int Immunopharmacol*, 2008, **8**(9): 1231-1238
- [14] Chen F Y, Ni Y, Ye Y P, *et al.* Comparison of immunosuppressive activity of stephanoside E and its aglycone from *Stephanotis mucronata in vitro*. *Int Immunopharmacol*, 2010, **10**: 1153-1160
- [15] Wang L Y, Chen Z H, Zhou Y, *et al.* Structural revision of periplocosides and periperioxides, natural immunosuppressive agents from the genus *Periploca*. *Phytochemistry*, 2011, **72**(17): 2230-2236
- [16] Locksley R M, Killeen N, Lenardo M J. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell*, 2001, **104**(4): 487-501
- [17] Jin M S, Lee J O. Structures of the toll-like receptor family and its ligand complexes. *Immunity*, 2008, **29**(2): 182-191
- [18] Cecilia G, Dinarello C A, Mantovani A. The interleukin-1 family: back to the future. *Immunity*, 2013, **39**(6): 1003-1018
- [19] Liu Y B, Su E J, Li B J, *et al.* Steroidal glycosides from *Dregea sinensis* var. *corrugate* screened by liquid chromatography-electrospray ionization tandem mass spectrometry. *J Nat Prod*, 2009, **72**(2): 229-237
- [20] Narayanaswamy R, Isha A, Wai L K, *et al.* Molecular docking analysis of selected clinacanthus nutans constituents as xanthine oxidase, nitric oxide synthase, human neutrophil elastase, matrix metalloproteinase 2, matrix metalloproteinase 9 and squalene synthase inhibitors. *Pharmacogn Mag*, 2016, **12**: S21-S26
- [21] Singh V, Singh K, Nand A, *et al.* Small molecule microarray screening methodology based on surface plasmon resonance imaging. *Arab J Chem*, 2019, **12**(8): 2111-2117
- [22] Brooks B R, Brooks C L, Mackerell A D, *et al.* CHARMM: the biomolecular simulation program. *J Comput Chem*, 2009, **30**: 1545-1614
- [23] Gagnon J K, Law S M, Brooks C L. Flexible CDOCKER: development and application of a pseudo-explicit structure-based docking method within CHARMM. *J Comput Chem*, 2016, **37**(8): 753-762
- [24] Usha T, Goyal A K, Lubna S, *et al.* Identification of anti-cancerous targets of eco-friendly waste *Punica granatum* peel by dual reverse virtual screening and binding analysis. *Asian Pac J Cancer Prev*, 2014, **15**(23): 10345-10350
- [25] Usha T, Middha S K, Goyal A K, *et al.* Molecular docking studies of anti-cancerous candidates in *Hippophae rhamnoides* and *Hippophae salicifolia*. *J Biomed Res*, 2014, **28**(5): 406-415
- [26] Yang J M, Shen T W. A pharmacophore-based evolutionary approach for screening selective estrogen receptor modulators. *Proteins*, 2005, **59**: 205-220
- [27] Matsuda S, Koyasu S. Mechanisms of action of cyclosporine. *Immunopharmacology*, 2000, **47**: 119-125

苦绳(*Dregea sinensis*)中多氧化孕烷糖苷与免疫蛋白相互作用的研究*

王智¹⁾ 宋娟^{1,2)} 戴荣继¹⁾ 邓玉林¹⁾ 吕芳^{1)**}

¹⁾ 北京理工大学生命学院, 生物医药成分分离与分析北京市重点实验室, 北京 100081;

²⁾ 军事科学院军事医学研究院辐射医学研究所, 北京 100850)

摘要 目的 基于化学结构特点研究植物苦绳 (*Dregea sinensis*) 中多氧化孕烷糖苷的生物活性, 以及化合物分子与靶蛋白分子间的作用机理。**方法** 本研究采用分子对接和表面等离子体共振方法, 对 191 个多氧化孕烷糖苷类成分 (>800 u) 进行了免疫活性及其与免疫蛋白的动力学评价。**结果** 通过分子对接方法筛选出 7 个配体分子 (6、18、23、30、78、79 和 80) 和 3 个免疫相关蛋白 (IL-2R α 、TLR4 和 TNF- α)。研究表明, 化合物 30 和 78 在 SPR 实验中与靶蛋白 IL-2R α 和 TLR4 具有显著的结合趋势, 与 IL-2R α 的结合常数 K_D 分别为 2.41×10^{-6} 和 2.14×10^{-6} mol/L, 与 TLR4 则分别为 1.96×10^{-5} 和 5.60×10^{-6} mol/L。分子动力学研究进一步表征 SPR 阳性分子 6、18、30、78 和 80 与靶蛋白之间的相互作用基团。**结论** 研究揭示多氧化孕烷糖苷类成分可以通过形成氢键和 Pi-Pi 相互作用与靶蛋白结合。研究内容对快速评价多氧化孕烷糖苷类成分的活性具有重要意义, 为揭示低丰度药效物质的潜在作用机制进行了有益探索。

关键词 多氧化孕烷糖苷, 免疫抑制剂, 分子对接, 表面等离子共振

中图分类号 R28, Q947

DOI: 10.16476/j.pibb.2021.0388

* 国家自然科学基金 (81503353) 和国家科技部仪器专项 (2012YQ040140) 资助项目。

** 通讯联系人。

Tel: 010-68949331, E-mail: lvfangbeijing@bit.edu.cn

收稿日期: 2021-12-13, 接受日期: 2022-01-25