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Prediction of material foundation of Ling-Gui-Zhu-Gan decoction for chronic heart failure based on molecular docking

Peng Zhou[1,2*] and Jin-Ling Huang[1,2*]
[1]Department of Integrated Traditional Chinese and Western Medicine, Anhui University of Chinese Medicine, Hefei, China
[2]Research Institute of Integrated Traditional Chinese and Western Medicine, Anhui Academy of Chinese Medicine, Hefei, China

Abstract: Ling-Gui-Zhu-Gan decoction (LGZGD) is a classic Chinese herbal formula, which has been used to prevent and treat chronic heart failure (HF). Reliable therapeutics of LGZGD has also been confirmed in clinical practice. In this study, molecular docking has explored the mechanism of LGZGD as an effective treatment for heart failure. Twenty-one known active compounds of LGZGD in serum were screened based on twelve key receptors involved in myocardial damage. There were fourteen active molecules of LGZGD combined strongly with five or more than five protein targets after molecular docking, only seven active molecules of LGZGD combined strongly with ten or more than ten protein targets. The molecular docking provided a forceful tool for searching material foundation and the mechanism of action of TCM formula.

Keywords: Ling-Gui-Zhu-Gan decoction, receptor, molecular docking.

INTRODUCTION

Chronic heart failure (CHF) is a common cardiovascular disease, is the main cause of death worldwide, and it is still the main life-threatening factor in humans. The clinical diagnosis of HF is mainly left ventricular (LV) systolic and diastolic dysfunctions (Dick *et al.*, 2016). The pathogenesis of CHF is complex and diverse, involving neuroendocrine, inflammatory, metabolic and immune mechanisms (Polsinelli *et al.*, 2017). Many therapeutic targets have been used in the treatment of CHF, but still insufficient.

Ling-Gui-Zhu-Gan decoction (LGZGD) is a traditional Chinese medicine prescription from the medical treatise *Shang Han Lun* in the Eastern Han Dynasty, consisting of four crude herbs: *Poria cocos* (Schw.) Wolf (Fu-Ling), *Cinnamomi Ramulus* (Gui-Zhi), *Rhizoma Atractylodis Macrocephalae* (Bai-Zhu), and *Radix Glycyrrhizae* (Gan-Cao), has also been applied to prevent and treat chronic congestive heart failure (CHF). A meta-analysis concluded that LGZGD increased clinical benefits of inotropes, diuretics and vasodilators therapy in CHF (Qiu *et al.*, 2011). Our previous studies have revealed that LGZGD effectively modulated the structure and function by regulating neuro-endocrine cytokines, such as brain natriuretic peptide (BNP), aldosterone (ALD), angiotensin II (Ang II), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) in rat models of CHF (Huang *et al.*, 2011). However, material foundation and mode of action of LGZGD are not well understood. Consequently, understanding the material foundation and mechanisms of LGZGD are very important.

Molecular docking, as an important means of computer aided drug molecular design, which is mainly used to study the process of virtual docking between protein receptors and drug molecules. The essence of the combination of drugs and targets is the interaction between drugs and receptors to form a drug receptor complex. The interaction between drugs and targets is mainly electrostatic interaction, electrovalent bond, intermolecular hydrogen bond and Van der Waals' force. Molecular docking also provides most detailed possible view of drug-receptor interaction and predicts material foundation of TCM (Mostafa *et al.*, 2015). It is well known that TCM formula contains many kinds of chemical constituents, and chemical compounds exert their biological activities require direct physical binding to one or more receptors or targets. Here, we introduce a new, potentially widely applicable and accurate drug target identification strategy to determine the interactions between active ingredients of TCM and the targets.

In this study, molecular docking of LGZGD was studied twenty-one main constituents of compound in serum based on literature report in each herb including oleanolic acid (1), dehydrotumulosic acid (2), dehydropachymic acid (3), tumulosic acid (4), pachymic acid (5), Polyporenic acid C (6), cinnamic acid (7), taxifolin (8), protocatechuic acid (9), atractylenolide I (10), atractylenolide III (11), glycyrrhetinic acid (12), liquiritigenin (13), isoliquiritigenin (14), liquiritin (15), isoliquiritin (16), glycyrrhizic acid (17), liquiritin apioside (18), isoliquiritin apioside (19), neoliquiritin (20) and schaftoside (21) (Ling *et al.*, 2012; Montero *et al.*, 2016; Wang *et al.*, 2014). The 2D structures of the constituents have shown in fig. 1.

The chemicals were docked to twelve potential targets associated with heart failure including ACE, Bcl-2, GRK2, iNOS, TGF-β, Tie2, TNF-α, thrombin, p38 MAPKγ, p38

**Corresponding author:* e-mail: zp0108@126.com; jinling6181@126.com

MAPKα, p38 MAPKβ and PPAR-γ using the MOE 2008.10 software package (Maier *et al.*, 2014). The study provides a powerful tool for explaining the prevention and treatment of HF mechanism of TCM formula and discovering the material foundation.

MATERIALS AND METHODS

Retrieval of ligands

3D structures of the substrates 1-21 were constructed using MOE-Builder tool, optimized by adding hydrogens, and removing water molecules. MMFF94 Forcefield was also used to 3D protonate and energy minimizes the substates profile.

Preparation of receptor protein

The 3D structures of the ACE, Bcl-2, GRK2, iNOS, TGF-β, Tie2, TNF-α, thrombin, p38 MAPKγ, p38 MAPKα, p38 MAPKβ and PPAR-γ were obtained from the Protein Data Bank (PDB) using PDB ID: 1UZE, 1YSW, 4PNK, 2Y37, 1PY5, 3L8P, 2AZ5, 1YPJ, 1CM8, 1KV1, 3GC9 and 2HFP. Water molecules and ligands were removed from the receptor and the 3D protonation was carried out using the Molecular Operating Environment (MOE, 2008.10) software package. The protein structures were minimized using MMFF94 Forcefield.

Molecular docking

To find the correct conformation of the ligand and obtain the minimum energy structures and the ligand is allowed to be flexible. After finding the location of the ligands, the ligands were removed. Then the predicted drug molecules docked with hydrophobic pocket of the receptor and the value of the combination were given. After protein docking, the resultant complexes were subjected to hydrogen bonding analysis. The LigX feature of MOE was used to find interactions among the ligand and receptor protein.

RESULTS

Except for atractylenolide I and atractylenolide III which were derived from *Rhizoma Atractylodis Macrocephalae* (Bai-Zhu), nineteen constituents of compound in serum combined strongly with protein targets better than self ligand, and fourteen active molecules of LGZGD combined strongly with five or more than five protein targets after molecular docking, which were derived from *Poria cocos (Schw.) Wolf* (Fu-Ling), *Cinnamomi Ramulus* (Gui-Zhi), and *Radix Glycyrrhizae* (Gan-Cao) (table 1). And only seven active molecules of LGZGD combined strongly with ten or more than ten protein targets, which were derived from *Radix Glycyrrhizae* (Gan-Cao) (table 2).

DISCUSSION

Molecular docking studies were used to identify possible interaction modes of these active components of LGZGD on the binding site. In this molecular docking software, the lower the score is, the better the binding ability of ligand and receptors are. Because of Chinese medicine formula has more chemical composition, which is considered as a multi-target. In the results, LGZGD has 21 characteristic chemical constituents, 14 active molecules of LGZGD combined strongly with five or more than five protein targets, which can also called multi-target.

The 2D pictures of the docked conformations of most active compounds have shown in fig. 2. The binding mode of glycyrrhizic acid (17) revealed the formation of two hydrogen bonds with the residues Arg 104 and Asp 108 of Bcl-2, the direct formation of five hydrogen bonds with the residues Asn A115, Glu A371, Arg A382 and Arg 260 of iNOS, and the formation of one hydrogen bonds with the residues Ser 289 of PPAR-γ. Liquiritin apioside (18) docked with TNF-α via the formation of six hydrogen bonds with the residues Lys A98, Lys B98, Tyr B151, Glu A116. Isoliquiritin apioside (19) docked with ACE via the direct formation of six hydrogen bonds with the residues Lys 511, His 383, Glu384, His 353, and Ser 284. Isoliquiritin apioside also revealed the formation of three hydrogen bonds with the residues Tyr 282, Glu 284 and Ser280 of TGF-β, the direct formation of four hydrogen bonds with the residues Asp912, Asn 909 and Pro 906 of Tie2, and the direct formation of five hydrogen bonds with the residues Glu192, Gly193, Ser195, Gly219 and Lys60F of thrombin. The binding mode of schaftoside (21) revealed the formation of six hydrogen bonds with the residues Lys A319, Asp A278, Arg A199, Met A274, Lys A220 and Asp A335 of GRK2, and also strongly docked with different subtypes of p38 MAPK. Radix Glycyrrhizae may play a major role in LGZGD, which has been used to prevent and treat HF.

CONCLUSION

Fourteen potential active molecules in LGZGD against heart failure were predicted by serum pharmacochemistry, and only seven active molecules combined strongly with ten or more than ten protein targets, which were derived from Radix Glycyrrhizae (Gan-Cao). Molecular docking is helpful to reveal the possible interactions between the chemical compounds and receptors prompt the search for mechanism of LGZGD in prevention and treatment of cardiovascular diseases. The material foundation and pharmacological mechanism of LGZGD need to be further verified.

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Table 1: Docking scores of protein targets and their active ligands - Docking score is less than its own ligand.

Chemical compounds	ACE	Bcl-2	GRK 2	iNOS	TGF-β	Tie2	TNF-α	thrombin	p38 MAPKγ	p38 MAPKα	p38 MAPKβ	PPAR-γ
ligand	-16.9724	-12.0050	-12.7410	-9.9908	-11.0672	-12.9927	-11.2386	-13.2310	-19.0830	-10.9744	-13.3340	-12.3404
1	-	-	-	-13.1775	-11.3743	-	-12.8935	-	-	-11.1220	-	-
2	-	-	-	-11.6149	-12.1094	-	-12.3455	-13.5442	-	-11.2907	-13.6898	-
3	-	-	-13.1673	-16.2225	-11.8724	-	-	-14.5461	-	-	-	-
4	-	-	-	-13.2000	-13.0491	-13.3635	-	-	-	-	-13.4857	-14.5381
5	-	-	-13.5528	-12.8656	-12.7410	-13.1832	-11.5383	-14.2554	-	-11.0343	-13.6006	-
6	-	-	-13.7341	-12.4191	-12.5212	-	-12.1756	-	-	-11.0362	-	-
7	-	-	-	-11.1151	-12.6972	-	-	-	-	-11.0507	-	-
8	-25.5888	-	-	-11.6717	-12.5050	-	-12.0109	-	-	-11.9475	-	-
9	-19.2362	-	-13.0625	-13,9759	-13.7637	-	-14.3710	-	-	-12.5616	-13.8187	-13.1814
10	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-13.4047	-13.3412	-11.2085	-	-	-	-	-	-	-
13	-18.1081	-	-13.5919	-13.7185	-14.2621	-	-12.1992	-15.2989	-	-12.6051	-15.1865	-12.4118
14	-22.5051	-	-13.8185	-16.8180	-16.8470	-13.5172	-	-16.2554	-	-12.9478	-13.7672	-14.1418
15	-23.7279	-12.7893	-16.0364	-17.3437	-16.3995	-16.2860	-14.0614	-15.9887	-	-14.8218	-15.8531	-15.4940
16	-28.7416	-	-15.0478	-19.5103	-16.0252	-19.4158	-15.3315	-16.7882	-	-15.2365	-14.5679	-14.1436
17	-28.1493	-14.2343	-13.7209	-24.3443	-13.2793	-	-14.8040	-18.9465	-20.5308	-	-17.7441	-16.2536
18	-23.9831	-12.6345	-14.8661	-21.7881	-15.2770	-15.3954	-16.3112	-18.7367	-	-13.0018	-14.6149	-14.4987
19	-30.9320	-13.5775	-15.3273	-19.5208	-20.0834	-19.4768	-15.3494	-19.2330	-21.1478	-13.5859	-17.4308	-15.8558
20	-26.1131	-	-15.8386	-15.3477	-18.1758	-15.2534	-15.5456	-15.8521	-	-15.3286	-15.6078	-14.0923
21	-28.8298	-12.4377	-16.7405	-20.8390	-20.0593	-15.3951	-15.0159	-18.8305	-23.8598	-18.1604	-18.1896	-15.9439

Table 2: Docking scores of chemical compounds docked with 10 or more than 10 protein targets

	liquiritin	isoliquiritin	glycyrrhizic acid	liquiritin apioside	isoliquiritin apioside	neoliquiritin	schaftoside
ACE	-23.7279	-28.7416	-28.1493	-23.9831	-30.9320	-26.1131	-28.8298
Bcl-2	-12.7893	-	-14.2343	-12.6345	-13.5775	-	-12.4377
GRK2	-16.0364	-15.0478	-13.7209	-14.8661	-15.3273	-15.8386	-16.7405
iNOS	-17.3437	-19.5103	-24.3443	-21.7881	-19.5208	-15.3477	-20.8390
TGF-β	-16.3995	-16.0252	-13.2793	-15.2770	-20.0834	-18.1758	-20.0593
Tie2	-16.2860	-19.4158	-	-15.3954	-19.4768	-15.2534	-15.3951
TNF-α	-14.0614	-15.3315	-14.8040	-16.3112	-15.3494	-15.5456	-15.0159
thrombin	-15.9887	-16.7882	-18.9465	-18.7367	-19.2330	-15.8521	-18.8305

Continue…..

p38 MAPKγ		-	-20.5308	-	-21.1478	-	-23.8598
p38 MAPKα	-14.8218	-15.2365	-	-13.0018	-13.5859	-15.3286	-18.1604
p38 MAPKβ	-15.8531	-14.5679	-17.7441	-14.6149	-17.4308	-15.6078	-18.1896
PPAR-γ	-15.4940	-14.1436	-16.2536	-14.4987	-15.8558	-14.0923	-15.9439

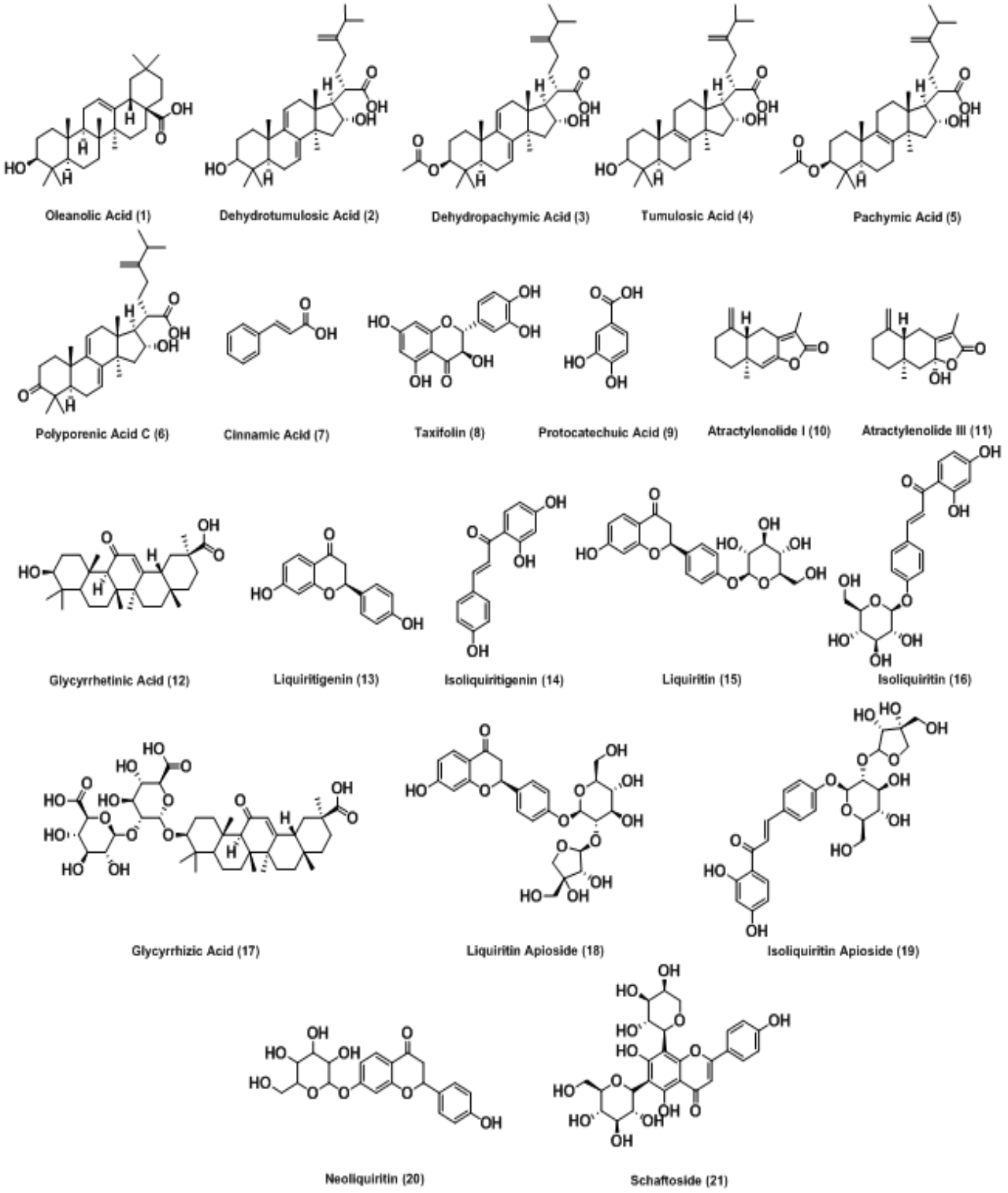

Fig. 1: The 2D structures of main constituents of compound

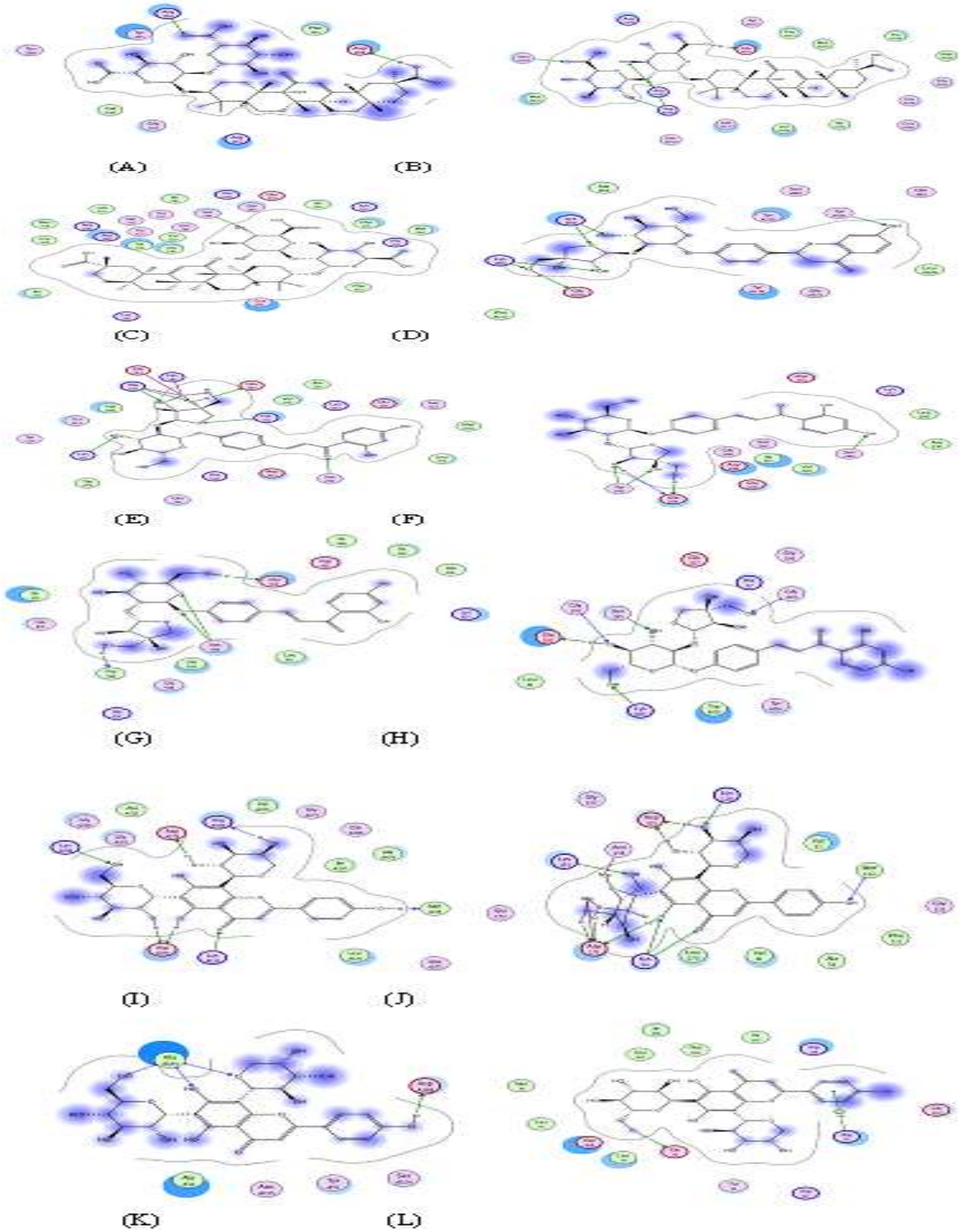

Fig. 2: The 2D pictures of the docked conformations of most active compounds, (A) glycyrrhizic acid docked with Bcl-2, (B) glycyrrhizic acid docked with iNOS, (C) glycyrrhizic acid docked with PPAR-γ, (D) liquiritin apioside docked with TNF-α, (E) isoliquiritin apioside docked with ACE, (F) isoliquiritin apioside docked with TGF-β, (G) isoliquiritin apioside docked with Tie2, (H) isoliquiritin apioside docked with thrombin, (I) schaftoside docked with GRK2, (J) schaftoside docked with p38 MAPKγ, (K) schaftoside docked with p38 MAPKβ , (L) schaftoside docked with p38 MAPKα

REFERENCES

Dick SA and Epelman S (2016). Chronic heart failure and inflammation: What do we really know? *Circ. Res.*, **119**(1): 159-176.

Huang JL, Wang L, Shi H and Hou XY (2011). Effect of Lingguizhugan decoction on myocardial nuclear factor kappa B protein expression in rats with chronic heart failure. *J. Tradit. Chin. Med.*, **33**(3): 343-348.

Ling Y, Chen M, Wang K, Sun Z, Li Z, Wu B and Huang C (2012). Systematic screening and characterization of the major bioactive components of Poria cocos and their metabolites in rats by LC-ESI-MS^n. *Biomed. Chromatogr.*, **26**(9): 1109-1117.

Maier JK and Labute P (2014). Assessment of fully automated antibody homology modeling protocols in molecular operating environment. *Proteins*, **82**(8): 1599-1610.

Montero L, Ibáñez E, Russo M, di Sanzo R, Rastrelli L, Piccinelli AL, Celano R, Cifuentes A and Herrero M (2016). Metabolite profiling of licorice (*Glycyrrhiza glabra*) from different locations using comprehensive two-dimensional liquid chromatography coupled to diode array and tandem mass spectrometry detection. *Anal. Chim. Acta.*, **913**: 145-159.

Mostafa NM, Ashour ML, Eldahshan OA and Singab AN (2015). Cytotoxic activity and molecular docking of a novel biflavonoid isolated from *Jacaranda acutifolia* (Bignoniaceae). *Nat. Prod. Res.*, **30**(18): 2093-3000.

Polsinelli VB and Shah SJ (2017). Advances in the pharmacotherapy of chronic heart failure with preserved ejection fraction: an ideal opportunity for precision medicine. *Expert. Opin. Pharmacother.*, **18**(4): 399-409.

Qiu ML, Mao JY, Wang JY, Wang XL and Hou YZ (2011). A meta-analysis: effect of Linggui Zhugan or added formula on chronic heart failure. *Chin. J. Exp. Tradit. Med. Form.*, **17**(16): 243-247.

Wang Q, Jiang P, Ye FY, Shi R, Ma YM, Zhong J, Wu JS, Liu P, Liu CH and Jia YQ (2014). Identification and pharmacokinetics of multiple constituents in rat plasma after oral administration of Yinchenzhufu decoction. *J. Ethnopharmacol.*, **153**(3): 714-724.