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Systems approaches and polypharmacology for drug discovery from herbal medicines: An example using licorice

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ABSTRACT

Ethnopharmacological relevance: Licorice, one of the oldest and most popular herbal medicines in the world, has been widely used in traditional Chinese medicine as a cough reliever, anti-inflammatory, anti-anabrosis, immunomodulatory, anti-platelet, antiviral (hepatitis) and detoxifying agent. Licorice was used as an example to show drug discovery from herbal drugs using systems approaches and polypharmacology.

Aim of the study: Herbal medicines are becoming more mainstream in clinical practice and show value in treating and preventing diseases. However, due to its extreme complexity both in chemical components and mechanisms of action, deep understanding of botanical drugs is still difficult. Thus, a comprehensive systems approach which could identify active ingredients and their targets in the crude drugs and more importantly, understand the biological basis for the pharmacological properties of herbal medicines is necessary.

Materials and methods: In this study, a novel systems pharmacology model that integrates oral bioavailability screening, drug-likeness evaluation, blood–brain barrier permeation, target identification and network analysis has been established to investigate the herbal medicines.

Results: The comprehensive systems approach effectively identified 73 bioactive components from licorice and 91 potential targets for this medicinal herb. These 91 targets are closely associated with a series of diseases of respiratory system, cardiovascular system, and gastrointestinal system, etc. These targets are further mapped to drug–target and drug–target–disease networks to elucidate the mechanism of this herbal medicine.

Conclusion: This work provides a novel *in silico* strategy for investigation of the botanical drugs containing a huge number of components, which has been demonstrated by the well-studied licorice case. This attempt should be helpful for understanding definite mechanisms of action for herbal medicines and discovery of new drugs from plants.

1. Introduction

Chinese herbal medicine, an ancient medical practice system that differs in substance, methodology and philosophy to modern medicine, plays an important role in health maintenance for the people of Asia, and is becoming more frequently used in countries in the West (Cheung, 2011). However, like almost all other ethnopharmacology, herbal medicine also faces severe challenges and suffers from insufficient modern research owing to lack of scientific and technologic approaches. One of the reasons for such failure is that the methodology used in the herbal medicine research follows basically the path of partitioned reductive analysis, which is unable to capture practically the characteristics of traditional herbal medicine scientific system, such as the holistic and dynamic nature of diseases, and the interaction among various biological components. Further, the complexity of herbal ingredients, unknown targets in human body and the mechanism of action underlying the herbal medicine efficiencies increase the difficulties of herbal medicine researches and restrict the development of Traditional Chinese Medicine (TCM) in the world.

Recently, the emergence of network pharmacology is increasingly gaining acceptance as a way to treat polygenic diseases, both from target and drug perspectives (Hopkins, 2008; Gu et al., 2011; Xu et al., 2012a). Polypharmacology is an important area of integration between systems biology and drug discovery, which suggested not only that drugs commonly act on multiple targets but also that drug targets are often involved with multiple diseases (Boran and Iyengar, 2010; Xie et al., 2012). The investigation in genomics, particularly, the findings of multiple targets

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for one drug, has provided considerable support for the polypharmacology development. Mapping the polypharmacology network onto the human disease–gene network would reveal those important drug targets involved with multiple diseases, which will illustrate why the botanical drugs can treat many different diseases (Hopkins, 2007). Therefore, applying this method to TCM pharmacology may open up the possibility to understand the explicit targets of active ingredients and their interactions in the context of molecular networks. To conduct a systematic analysis on human body and diseases under the guidance of holistic view will be an utmost important way for developing herbal medicine towards the future of healthcare. A holistic understanding of the molecular mechanism responsible for the pharmacology effects of the herbal medicines should be extremely important. Therefore, in this work, exemplified by an extensively studied herb, licorice, we will introduce how to develop systems approaches for investigating herbal medicines, and to show how these herbal medicines interact with a human body from a network level.

Licorice (Chinese name Gan-Cao) is the root of three *Glycyrrhiza* species, i.e., *Glycyrrhiza uralensis* Fisch. ex DC., *Glycyrrhiza glabra* L. and *Glycyrrhiza inflata* Batalin used clinically in ancient Egyptian, Greek and China (2nd–3rd century b.c.) (Sun and Pan, 2006). Licorice is almost the most important crude drug in TCM preparations, constituting about 60% of all TCM prescriptions (Wang and Yang, 2007). This botanical drug can be applied for allergic-inflammatory disease, gastrointestinal problems, cardiovascular disease, cancer and bladder and kidney ailments (Hongxia et al., 2004). So far, about 300 diverse compounds have been isolated from licorice, such as triterpene saponins and flavonoids, which are responsible for the antiviral (Fujisawa et al., 2000), anti-inflammatory (Furuhashi et al., 2005) antitumor (Numazaki et al., 1994; Fu et al., 2004), antitussive (Kamei et al., 2005), immunostimulant, antiulcerogenic (Nakamura et al., 2003), anti-oxidant (Vaya et al., 1997), antispasmodic (Sato et al., 2007), metabolic syndrome preventive (Nakagawa et al., 2004) activities. However, how these components exert effects on various diseases and how they interact with each other, synergistically or antagonistically, both on molecular level and systems level, remains unclear.

Our previous studies have developed several ways to enhance our understanding of TCM pharmacology, including the identification of active ingredients and their targets in the context of molecular networks (Li et al., 2012). In the present work, we have proposed an integrated model, combining oral bioavailability (OB) prediction, Blood–Brain Barrier permeation (BBB) prediction, multiple drug targets prediction, network pharmacology techniques, to shed light on the pharmacology and effectiveness of a typical and important herbal drug licorice. To achieve our goal, we have generated and analyzed several drug–target–disease (D–T–D) networks, which extend beyond the incorporation of active ingredients, potential targets and related disease. These attempts offer new opportunities for deep understanding of the biological basis for the pharmacological properties of herbal medicines, and also provide a new methodology for drug discovery form botanical drugs using a network-driven, integrated approach.

2. Material and methods

2.1. Database building and molecular modeling

Two hundred and eighty seven known licorice compounds were obtained from our own Traditional Chinese Medicine Systems Pharmacology Database (TcmSP^{TM}, http://tcmspnw.com/). The latest version of TcmSP^{TM} has 31,871 organic molecules identified from more than 500 herbs in TCM. As a chemically oriented herbal encyclopedia, TcmSP^{TM} is able to provide detailed, up-to-date, quantitative, analytic or molecular-scale information about herbal structural data, oral bioavailability, drug targets and their relationships with diseases, as well as the biological or physiological consequences of drug actions involving drug-likeness, intestinal epithelial permeability and aqueous solubility. For TcmSP^{TM}, the main highlights are as follows: (1) integration of the largest-scale structural data with manually curated information on chemical validation (such database contains 510 effective herbal entries registered in Chinese pharmacopoeia with more than 31,000 ingredients, which spread over 18 different drug classes, and are mapped to 3987 human proteins and 848 associated diseases); (2) incorporation of a wide range of biochemical and pharmacological data from diverse sources; (3) the ability to weight results so as to assemble a ranked list of candidate targets and establish corresponding target–disease networks. Since glycosides might be hydrolyzed to aglycones by intestinal enzymes, the compounds with glycosyl groups are deglycosylated into their products by the rule of glycosidase hydrolysis reaction. In this work, 15 aglycones of licorice were all put into the compound database.

2.2. Drug-likeness prediction

Removing non-drug-like compounds from the drug discovery lifecycle in the early stage can lead to tremendous savings of resources. In this study, the Drug-likeness (DL) index (see Eq. (1)) using the Tanimoto coefficient (Yamanishi et al., 2010) was computed for each compound of licorice:

$$T(x,y) = \frac{x \cdot y}{\|x\|^2 + \|y\|^2 - x \cdot y} \quad (1)$$

where x is the molecular properties of licorice compound based on Dragon soft molecular descriptors, y is the average molecular properties of all compounds in Drugbank database (http://www.drugbank.ca/). A molecule that gives $DL > 0.18$ is considered as "drug-like" compounds and selected as the candidate molecules for the following processes. The threshold of DL is determined upon the fact that the average DL index in the Drugbank is 0.18. The drug-likeness indices of all the licorice compounds are presented in the TcmSP^{TM}.

2.3. Oral bioavailability prediction

Pharmacodynamics can be affected by the absorption, distribution, metabolism, or elimination processes, thus resulting in changes in drug bioavailability. In order to filter out compounds which are not likely to be drugs, the oral bioavailability was calculated using an in-house software OBioavail 1.1 (Xu et al., 2012a, 2012b). This software is based upon a dataset of 805 structurally diverse drug and drug-like molecules that have been critically evaluated for their oral bioavailability (%F) in humans. Three mathematical methods, i.e., multiple linear regression (MLR), partial least square (PLS) and support vector machine (SVM) methods were applied to build various models. The optimal model using SVM method provides excellent performances with $R^2 = 0.80$, SEE = 0.31 for the training set, $Q^2 = 0.72$, SEP = 0.22 for the independent test set. In this work, compounds with $OB \geq 40\%$ were selected as the candidate molecules for further analysis. The OB properties of all the licorice compounds are also presented in the TcmSP^{TM}.

2.4. Blood–brain barrier permeation prediction

Blood–brain barrier can maintain the homeostasis of the central nervous system (CNS) by separating the brain from the

systemic blood circulation, and meanwhile refuses many potentially diagnostic and therapeutic agents from entering the brain. Understanding mechanisms associated with licorice neuroprotection is complicated by the lack of information on their ability to enter the CNS. Hence, we have examined the permeation of licorice compounds across the BBB using a updated model of our previous work (Li et al., 2007). This BBB model is a qualitative model containing 190 related but chemically diverse compounds which are either strong, moderate or non-penetrating cross the blood–brain barrier. Partial least squares discriminant analysis was used to build the statistical model with two significant latent variables. In this model, the compounds with BBB < -0.3 were considered as non-penetrating (BBB−), from -0.3 to $+0.3$ moderate penetrating (BBB±), and > 0.3 strong penetrating (BBB+).

2.5. Target identification and validation

To predict potential drug targets, in-house software (Yu et al., 2012) which efficiently integrates a large scale of chemical, genomic and pharmacological data, based on two powerful methods of Random Forest (RF) and Support Vector Machine (SVM) was used.

The dataset used in building these models included 6511 drugs and 3987 targets with known compound–protein interactions in Drugbank database. In order to obtain the experimental dataset, a set of numerical vectors were constructed for the drug–target pairs (both for positive and negative samples) by concatenating chemical descriptors and protein descriptors. The SVM, one of the most widely used nonlinear algorithms, has been applied to map the input numerical vectors into a higher dimensional feature space to construct a maximal margin hyper-plane that separate the positive from the negative samples by using a kernel function. As a comparison, another relatively new method, RF, which is an ensemble of unpruned classification or regression tree, was also used in building models. Two types of randomness, bootstrap sampling and random selection of input variables, are used in the algorithm to make sure that the classification trees grown in the forest are dissimilar and uncorrelated from each other. The performance of the derived models was evaluated and verified with internally five-fold cross-validation and four external independent validations. The optimal models by SVM show impressive performance of prediction for drug–target interactions, with a concordance of 82.83%, a sensitivity of 81.33% and a specificity of 93.62%, respectively. The consistence of the performances of the RF and SVM models demonstrates the reliability and robustness of the obtained models. In this work, the common targets of a same compound in RF and SVM top 50 ranking targets were selected as potential targets. All target information about the molecules is provided in Table S1.

Molecular docking, which plays an important role in the rational design of drugs, is frequently used to predict the binding orientation of drug candidates to their targets (active sites) and also to predict the binding affinity of the molecules in turn (Kitchen et al., 2004). AutoDock software (http://autodock.scripps.edu/) is one of the most widely used docking programs in computational binding studies. Prior to the docking studies, crystallographic ligands and water molecules were removed. Polar hydrogen atoms were added and Kollman charges were assigned to all atoms. The docking site was defined within a box around residues of the binding site. For each kind of ligand atoms, 60 Å × 60 Å × 60 Å 3D grids centered on the binding site with 0.375 Å spacing were calculated using Autogrid4.2 (Morris et al., 2009). For docking simulations, we selected the Lamarckian genetic algorithm (LGA) for ligand conformational searching because it has enhanced performance relative to simulated annealing or the simple genetic algorithm. The protein structures were obtained from the RCSB protein data bank (www.pdb.org) with their resolutions being carefully checked.

2.6. Network construction and analysis

Construction of the drug–target network and drug–target–disease network for a specific herb could be assisted in the identification of the targets of each compound, understanding of drug effects on diseases, and studying of targets in the context of disease network (Yildirim et al., 2007). Here the candidate compounds and their potential targets were used to generate a bipartite graph of drug–target interactions in which a compound and a protein are connected to each other if the protein is a predicted target (direct or indirect) of the compound, giving rise to a 'drug–target network' (D–T network). The drug–target–disease network (D–T–D network) was produced through two steps: (1) the drug–target connections were generated by linking the candidate compounds with all their potential targets and (2) the potential targets were extracted from the PharmGkb (www.pharmgkb.org) and Therapeutic Targets Database (http://bidd.nus.edu.sg/group/cjttd/TTD.asp) for their diseases information. The D–T network and D–T–D network was generated by Cytoscape 2.8.1 (Shannon et al., 2003), a specialized tool that is able to integrate both molecular interactions and state measurements together in a common framework, and to then bridge these data with a wide assortment of model parameters and other biological attributes. In the resultant network, nodes represent compounds, proteins or diseases and edges represent the compound–target or target–disease interactions.

In order to specify the importance of a node and how this node influences the communication between two nodes, two key topological parameters, degree and betweenness were analyzed on the networks. The degree of a node (biomolecule) is the number of targets that the drug has (respectively, the number of drugs targeting the protein). The betweenness of a node is defined as the ratio of the number of shortest paths passing through a node to the total number of paths passing through the nodes (Gursoy et al., 2008). All the topological properties of these networks were analyzed using Network Analysis plugin and CentiScaPe 1.2 of Cytoscape.

3. Results and discussion

Herbal medicines are becoming popular worldwide, despite their mechanisms of action being generally unknown (Zhou et al., 2007). Investigation of compounds in the herbs might be an essential way for natural origin based drug discovery. Licorice is one of the most widely used herbs in traditional botanical drugs, which has been used to treat bronchitis, coughs, arthritis, gastric ulcer, and spasmodic diseases (Asl and Hosseinzadeh, 2008; Fiore et al., 2005). In this study, we have developed a new systems pharmacology approach to investigate the action mechanism of constituents individually and in combination for this medicinal herb.

3.1. Oral bioavailability prediction and drug-likeness screening

Oral bioavailability is the ratio of how much of an administered drug is absorbed into the systemic circulation when dosed orally compared to the amount in blood upon intravenous dosing. It is an essential pharmacokinetic parameter in drug screening cascades and a good indicator of the efficiency of the drug delivery to the systemic circulation by oral administration. Molecules with high oral bioavailability have the potential to be

Table 1
Chemical information and network parameters of 75 candidate compounds.

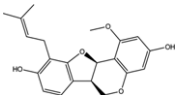
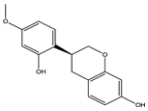
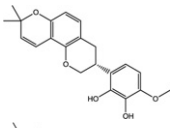
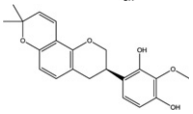
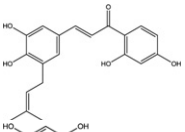
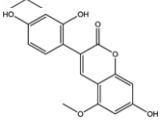
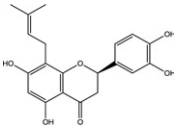
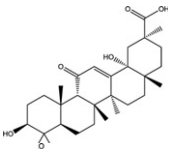
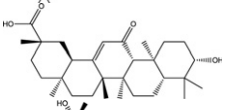
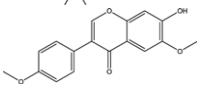
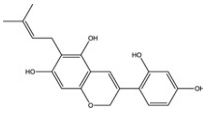
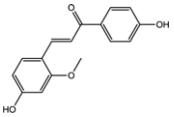
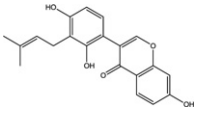
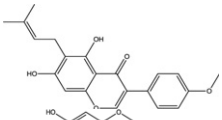
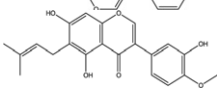
Mol no.	Name	OB (%)	BBB	DL	Degree	Betweenness	Structure
54	1-Methoxyphaseollidin	69	0.32	0.58	32	221.7	
9	3(*R*)-Vestitol	68	0.46	0.24	30	141.8	
47	3′-Hydroxy-4′-*O*-methylglabridin	43	0.75	0.51	34	186.1	
48	3′-Methoxyglabridin	46	0.45	0.52	33	191.5	
62	5′-Prenylbutein	43	−0.49	0.32	33	232.1	
15	7,2′,4′-Trihydroxy-5-methoxy -3-arylcoumarin	83	−0.62	0.31	29	126.3	
68	8-Prenylated eriodictyol	53	−0.44	0.41	30	62.1	
69	18α-Hydroxyglycyrrhetic acid	41	−0.42	0.83	29	1484.0	
75	*18β-Glycyrrhetic acid*	22	−0.50	0.86	29	1666.6	
2	*α-Cadinol*	65	1.31	0.18	27	1961.1	
14	Afromosin	63	0.17	0.30	28	54.8	
22	Dehydroglyasperins C	53	−0.13	0.37	31	79.8	
3	***Echinatin***	66	−0.10	0.19	32	368.7	
26	Eurycarpin A	43	−0.18	0.38	32	101.4	
28	Gancaonin A	51	0.12	0.40	31	117.6	
37	**Gancaonin B**	48	−0.63	0.44	32	100.4	

Table 1 (*continued*)

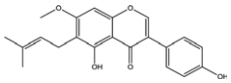
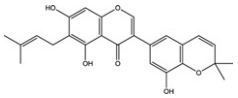
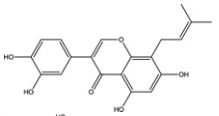
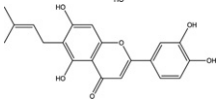
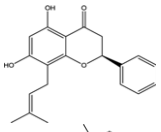
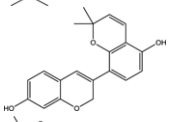
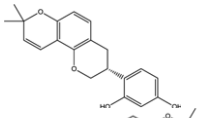
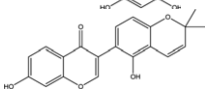
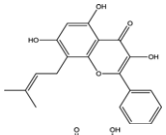
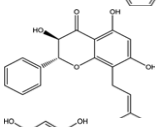
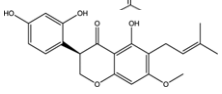
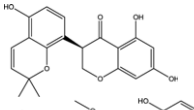
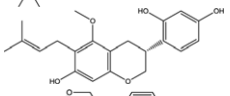
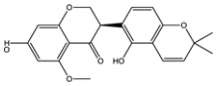
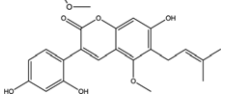
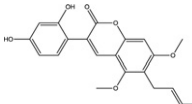
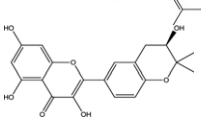
Mol no.	Name	OB (%)	BBB	DL	Degree	Betweenness	Structure
27	Gancaonin G	60	0.25	0.40	31	87.9	
59	Gancaonin H	50	−0.21	0.65	28	232.9	
31	Gancaonin L	66	−0.16	0.42	33	175.9	
30	Gancaonin O	44	−0.42	0.42	30	72.0	
17	Glabranin	52	0.20	0.33	31	181.6	
34	**Glabrene**	46	0.08	0.43	33	168.3	
38	***Glabridin***	53	0.20	0.44	35	272.0	
43	Glabrone	52	−0.08	0.48	30	60.6	
24	Glepidotin A	44	−0.03	0.37	31	67.5	
23	Glepidotin B	64	−0.17	0.37	32	127.2	
36	Glyasperin B	65	−0.18	0.44	32	150.7	
70	Glyasperin F	75	−0.12	0.52	29	97.7	
71	Glyasperins C	45	0.05	0.39	37	401.1	
51	Glyasperins M	72	−0.04	0.55	30	68.7	
35	Glycycoumarin	55	−0.27	0.43	30	78.2	
72	**Glycyrin**	52	−0.20	0.46	32	155.6	
52	Glycyrrhiza flavonol A	41	−0.82	0.56	28	76.7	

Table 1 (*continued*)

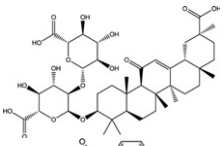
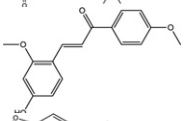
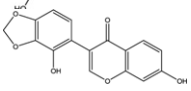
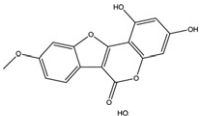
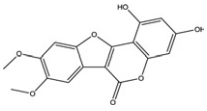
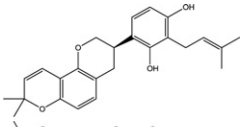
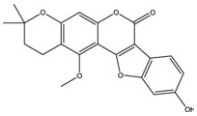
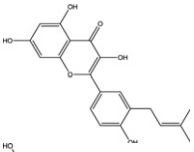
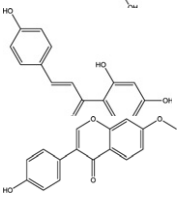
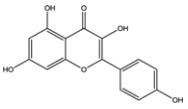
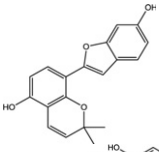
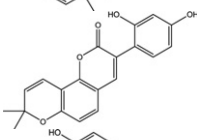
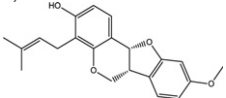
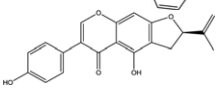
Mol no.	Name	OB (%)	BBB	DL	Degree	Betweenness	Structure
74	*Glycyrrhizic acid*	19	−2.20	0.14	24	2522.3	
4	***Glypallichalcone***	61	0.16	0.21	32	275.9	
21	Glyzaglabrin	61	−0.21	0.36	28	136.9	
33	Hedysarimcoumestan B	48	−0.21	0.42	30	971.3	
45	Hedysarimcoumestan E	62	−0.33	0.49	28	292.0	
57	***Hispaglabridin A***	41	0.49	0.62	34	320.3	
60	Isoglycyrol	44	−0.07	0.80	29	172.1	
29	**Isolicoflavonol**	45	−0.46	0.42	33	127.1	
1	*Isoliquiritigenin*	89	−0.50	0.18	30	645.5	
10	Isoononin_DG[a]	40	0.22	0.24	31	199.1	
13	Kaempferol	42	−0.54	0.29	28	82.2	
64	Kanzonols U	70	0.34	0.39	28	76.4	
44	Kanzonol W	50	0.03	0.48	30	95.0	
63	Licoagrocarpin	55	0.68	0.53	36	389.5	
39	Licoagroisoflavone	57	0.02	0.45	29	69.6	

Table 1 (*continued*)

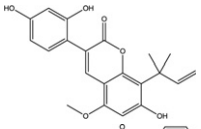
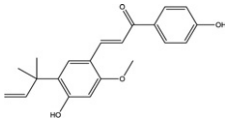
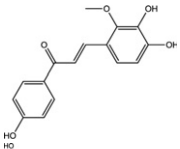
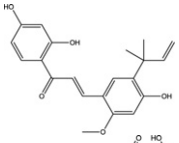
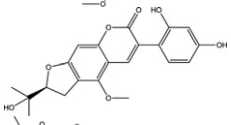
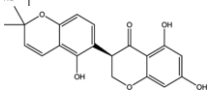
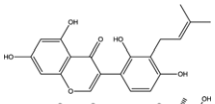
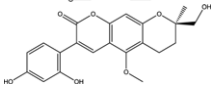
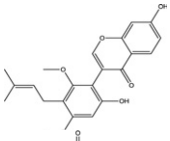
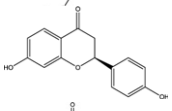
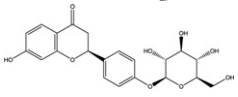
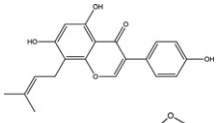
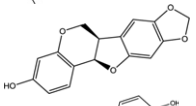
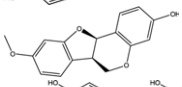
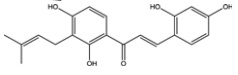
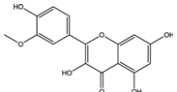
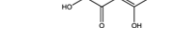
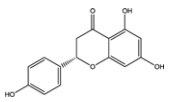
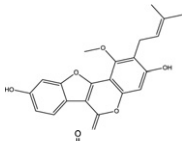
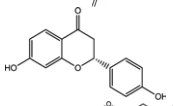
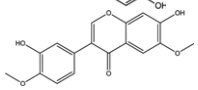
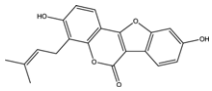
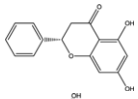
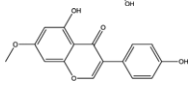
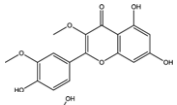
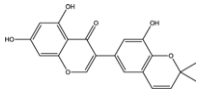
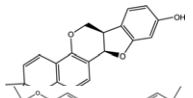
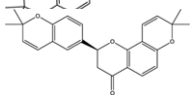
Mol no.	Name	OB (%)	BBB	DL	Degree	Betweenness	Structure
42	Licoarylcoumarin	59	−0.19	0.47	30	99.6	
16	***Licochalcone A***	45	−0.31	0.32	34	197.5	
8	***Licochalcone B***	76	−0.51	0.23	33	249.9	
65	*Licochalcone G*	43	−0.05	0.37	31	263.3	
53	Licofuranocoumarin	60	−0.72	0.56	31	177.4	
49	Licoisoflavanone	52	−0.36	0.52	31	91.3	
32	Licoisoflavone A	41	−0.27	0.42	31	115.0	
55	Licopyranocoumarin	80	−0.67	0.58	30	92.5	
40	**Licoricone**	63	−0.42	0.46	32	144.0	
6	Liquiritigenin	71	−0.26	0.22	30	145.6	
58	***Liquiritin***	65	−2.01	0.63	33	436.3	
25	Lupiwighteone	58	−0.37	0.38	31	97.8	
41	Maackiain	63	0.36	0.46	29	105.1	
19	Medicarpin	71	0.30	0.34	30	111.2	
66	Morachalcone A	40	−0.22	0.32	32	117.9	
20	Narcissin_DG[a]	51	−0.71	0.35	29	76.3	
11	Naringenin	40	−0.60	0.25	28	107.3	

Table 1 (*continued*)

Mol no.	Name	OB (%)	BBB	DL	Degree	Betweenness	Structure
							
56	Neoglycyrol	98	−0.46	0.61	29	175.0	
5	Neoliquiritin_DG[a]	71	−0.24	0.22	30	145.6	
18	Odoratin	49	−0.16	0.34	27	52.1	
50	Phaseol	78	−0.0018	0.53	28	145.7	
7	Pinocembrin	46	0.23	0.22	28	132.8	
12	*Prunetin*	72	−0.20	0.28	29	465.3	
73	Quercetin-3′,3′-dimethylether	46	−0.89	0.37	31	167.2	
46	SemilicoisoflavoneB	48	−0.45	0.51	29	81.1	
67	Shinpterocarpin	80	0.68	0.66	28	178.3	
61	Xambioona	54	0.44	0.80	34	475.2	

Bold figure: active compounds with high degree value in the top 25 compounds. Italic figure: active compounds with high betweenness value in the top 25 compounds.

[a] Aglycones of corresponding molecules.

further developed as therapeutic agents. On the contrary, poor oral bioavailability might result in low plasma exposure and high inter-individual variability, thus limiting their therapeutic usefulness. Therefore, in this work we have carried out a prediction of oral bioavailability for all the chemicals available in licorice to find which one is orally bioavailable.

In addition, the drug-likeness analysis is also performed to evaluate whether a compound is like a drug or not. As we believe only those compounds with favorable oral bioavailability and drug-likeness might exert desirable pharmaceutical activities. The obtained results show that 73 compounds, which account for 25% of all the 287 chemicals in the dataset, have a good oral bioavailability (OB > 40%) and drug-likeness (DL > 0.18).

For example, most licorice flavonoids have both high OB and DL values, such as isoliquiritigenin (OB=89%, DL=0.18), liquiritigenin (71%, 0.22), licochalcone B (76%, 0.23), liquiritin (65%, 0.63), licoricone (63%, 0.46), glycycoumarin (55%, 0.43), glabridin (53%, 0.44), glabrene (46%, 0.43), licochalcone A (45%, 0.32) and hispaglabridin A (41%, 0.62). Particularly, the compound liquiritin is the quality control marker for the licorice water extract which is used in clinical practice (Vaya et al., 1997). Interestingly, among all these compounds, some molecules have been demonstrated to be pharmacologically effective to treat various conditions. Glycycoumarin, for example, acts as a potent antispasmodic agent through inhibition of phosphodiesterase 3 (Sato et al., 2006); glabrene and glabridin are potent anti-oxidants toward low density lipoprotein oxidation (Xu et al., 2006). In addition, some other kinds of ingredients such as licochalcone G, lupiwighteone, glepidotin A, glyzaglabrin, glabranin, maackiain, 4′-*O*-methylglabridin, neoglycyrol and gancaonin L also have good OB (> 40%) as shown in Table 1.

The pentacyclic triterpene saponins, also major components of licorice, have relatively low OB values, such as glycyrrhizic acid (19%, 0.14), licorice-saponin J2 (6%, 0.14), uralsaponin B (17%, 0.14), licorice-saponin G2 (22%, 0.14). This is consistent with the observations that they are poorly orally bioavailable in both rats and humans (Cantelli-Forti et al., 1994; Wang et al., 1994; Gu et al., 2009). The glycyrrhizic acid can be hydrolyzed to 18β-glycyrrhetic acid (OB=22%, 0.86) by endogenous metabolism (biliary or enteric) or bacterial β-glucuronidases in gastrointestinal tracts (Ploeger et al., 2001). Therefore, it is induced that the

hydrolyzed product might be the active form of glycyrrhizic acid in vivo.

Moreover, compounds glycyrrhizic acid and 18β-glycyrrhetic acid are also considered as "candidate compounds" because these molecules are the most abundant constituents in licorice ($\sim > 2\%$) and exhibiting extensive biological activities (Nishino et al., 1984; Sato et al., 1996; Mendes-Silva et al., 2003; Ram et al., 2006; Yuan et al., 2006), although having OB values $< 40\%$. Altogether 75 compounds (Table 1) were obtained for further analysis. The 75 candidate compounds characteristics listed in the TcmSP^{TM} database are provided in Table S2.

3.2. Prediction of blood–brain barrier permeation

BBB permeation is one of the most important pharmacokinetic properties considered in drug discovery and development (Alavijeh et al., 2005). The distribution of potential drugs between the blood and the brain depends on the ability of compounds to penetrate the BBB. An ideal brain-acting drug candidate must be able to penetrate BBB effectively to reach the brain targets, but the peripherally acting drugs must have limited ability to cross BBB to avoid adverse CNS effects (Liu et al., 2001). There are various factors that can influence the BBB penetration e.g. plasma protein binding, large volumes of distribution in the blood, BBB efflux systems, active efflux from the CNS by transporters such as P-glycoprotein, and metabolism.

In this work, it is found that most BBB+ molecules are licorice flavonoids, such as medicarpin (0.30), 7-methoxy-2-methylisoflavone (0.41), xambioona (0.44), 3′-methoxyglabridin (0.45), hispaglabridin A (0.49), 4′-*O*-methylglabridin (0.55) and hispaglabridin B (0.55). Surprisingly, the majority of triterpenoids and saponins exhibit poor BBB permeation, such as oleanolic acid (0.18), glycyrrhetol (−0.35), isoglabrolide (−0.35), isoglabrolide (−0.35), 24-hydroxy-11-deoxyglycyrrhetic acid (−0.46), glabrolide (−0.65) and 24-hydroxyglycyrrhetic acid (−0.80) (see Table 1). Flavonoids are the main bioactive constituents of licorice, which have demonstrated neuroprotective (Qiu and Feng, 2009), cardiovascular (Zhang et al., 2010) and brain function promoting effects (Xu et al., 2006). Moreover, it is also found that certain flavonoids exert neuroprotective actions by modulation of intracellular signaling associated with neuronal survival, death, and differentiation as well as through interactions with mitochondria (Spencer, 2008). All these indicate that, compared with the triterpenoids and saponins, flavonoids in licorice might play a major role in treating CNS diseases especially brain diseases since they more likely penetrate BBB.

3.3. Network analysis

TCM is a complex system consisting of multiple components and targets and it can regulate several biological pathways (Xu, 2011). It is believed that multiple constituents in the herbs could hit multiple targets and might act synergistically to treat diseases (Li et al., 2011). However, the precise mechanisms underlying the herbal function are poorly uncovered, and the modern biological, chemical and computing approaches might provide effective ways to solve the problems in understanding and application of traditional botanical drugs. Network pharmacology has undergone a rapid development in recent years and emerged as an invaluable tool for describing and analyzing complex systems in pharmacology studies (Hopkins, 2007). In this section, the network approach is applied to analyze the effective compounds and their corresponding targets in licorice, and to dissect the molecular mechanism of action for this herbal from a network-modulation point of view.

3.3.1. Topological properties of licorice drug–target interaction network

Identifying the common behavior features from the network would provide important information to understand drug–target interaction mechanisms in the human body (Zhu et al., 2009). As such, topological analysis at the meso-scale (intermediate level between local and global features of networks) may provide global knowledge about the particular properties of compounds and proteins involved in the network. In this work, we use 75 candidate compounds and their potential targets (Table S1) to generate a bipartite graph of drug–potential target interactions in which a drug and a protein are connected to each other if the protein is a potential target of the drug, giving rise to a drug–target association network (D–T network) (Fig. 1). The resultant D–T network contains 166 nodes (75 molecules and 91 potential targets) and 2292 edges, in which blue nodes represent drug compounds and red nodes represent potential drug targets and each edge represents the interaction between them. The centralization and heterogeneity analysis shows the network centralization and heterogeneity are 0.285 and 0.739, respectively, indicating that a few nodes are more central that the other ones in this network, i.e., the drug–target space is biased toward certain compounds and proteins.

Table 1 lists the degree and betweenness of the candidate compounds. Observing the degree distribution of the nodes enables one to detect highly connected molecules or proteins which participate in significant numbers of interactions and play critical roles in the organization of the drug–target interaction network. Molecule **71** (glyasperins C) has the most potential targets (37), following are molecule **63** (licoagrocarpin) and molecule **38** (glabridin) with 36 potential targets. All this indicates that these molecules might play a major role in the pharmacological functions of licorice. In addition, more central proteins in networks are tyrosine-protein phosphatase non-receptor type 1 (PTPN1), histamine H1 receptor (HRH1) and prothrombin (F2), they have the most interacting ligands of 74, 73 and 72, respectively.

It is also interesting to find that, out of the 91 potential targets, 74 have at least two links with other drugs, that is, most proteins share common ligands with other targets. The average number of potential targets per candidate compound is 30. This suggests that the herb might target at biological network-level rather than target one protein in order to reduce the harmful impact in body.

The number of reported active compounds in the top 25 molecules, which have highest degree and largest betweenness in the D–T network, is 12 and 13, respectively (Table 1). The good hit rate ($>$ 50%) indicates the rationality and reliability to find active compounds by using network based analytical methods. Interestingly, glycyrrhizic acid (molecule **74**) exhibits the highest betweenness (2522), following are α-cadinol (molecule **2**, 1961), 18β-glycyrrhetic acid (molecule **75**, 1666) and 18α-hydroxyglycyrrhetic acid (molecule **69**, 1484). Those molecules which have high betweenness might exert important pharmacological effects as they play key roles in the biological network (Jeong et al., 2001; Goñi et al., 2008). For example, 18β-glycyrrhetinic acid exhibits anti-inflammatory effects, and glycyrrhizic acid exhibits detoxification and inhibition of carcinogenic promoters (Mollica et al., 2007; Rahman and Sultana, 2007). Liquiritin, a major flavonoid compound in licorice, has been reported to exert antioxidative and antiviral activities (Hatano et al., 1988; Vaya et al., 1997). Those molecules with unknown bioactivity would be potential active compounds and worth further investigations as they show important properties in the network. For example, compound **33** (hedysarimcoumestan B, betweenness=971.3), compound **61** (xambioona, betweenness=475.2), compound **71**

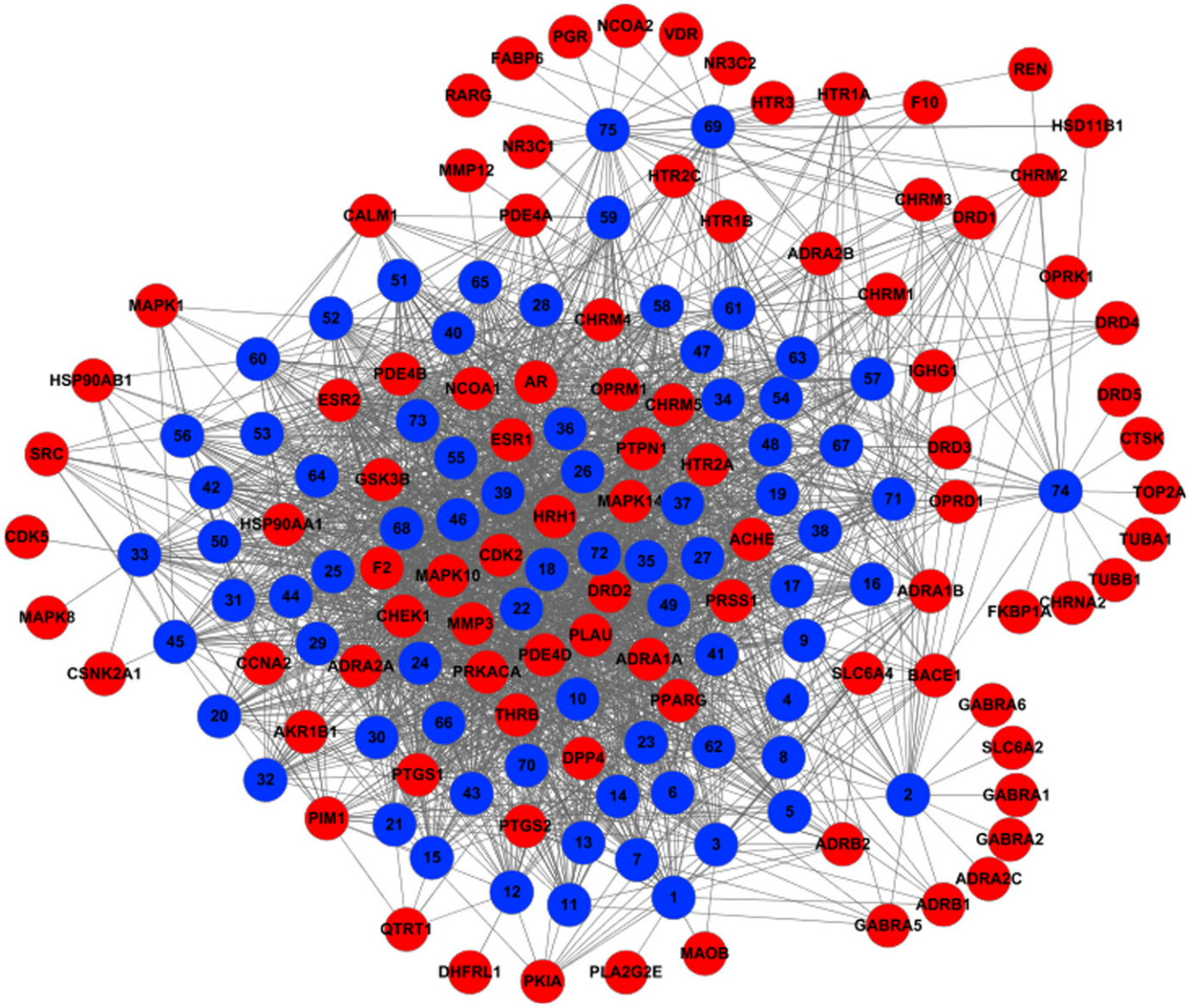


Fig. 1. The drug–target association network of licorice. The red circles represent target proteins, the blue represent compounds. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

(glyasperins C, betweenness = 401.1), and compound **63** (licoagrocarpin, betweenness = 389.5).

The docking analysis using two known targets corticosteroid 11-beta-dehydrogenase isozyme 1 (HSD11B1) and peroxisome proliferator-activated receptor gamma (PPARG) were taken as examples to demonstrate the results from *in silico* approach. HSD11B1 is an approved target for licorice compound 18β-glycyrrhetic acid (molecule **75**) (Gaware et al., 2011), which is also predicted to bind to compounds **69** (18α-hydroxyglycyrrhetic acid) and molecule **74** (glycyrrhizic acid) in our approach. As shown in Table S3, the AutoDock binding energy for molecule **75** within HSD11B1 is −6.58 kcal/mol, while molecules **74** and **69** are −6.64 kcal/mol and −5.85 kcal/mol, respectively. These observations suggest that these compounds bind well to HSD11B1. PPARG is an essential transcription factor and has been predicted as a target for compound kaempferol (molecule **13**) (Fang et al., 2008). The docking study of all candidate compounds for PPARG was carried out, with the resultant binding energies shown in Table S2. Among them, compounds **16** (licochalcone A), **58** (liquiritin), and **62** (5′-Prenylbutein) possess the highest binding affinity in the binding site of PPARG, since their binding energies are −9.63, −10.75 and −10.25 kcal/mol, respectively. All these docking simulations further demonstrate that this approach is effective and reliable for identifying the drug–target associations.

3.3.2. *Unraveling the molecular basis of diseases treated by licorice*

The global understanding of diseases and drug effects based on network relationships between drug targets can also allow repurposing of existing drugs with known targets for use in combination therapies for different diseases. Surprisingly, correlating the drug–target map with a target–disease map suggests that many drugs are not acting on a protein most directly implicated in a disease but rather acting at one or two degrees of separation, at proteins which are linked to the disease genes. Thus, we propose that the drug–target network associated with disease may be an effective strategy to explore the potential therapeutic effect of certain drugs and therefore suggests novel drug targets.

We have constructed the D–T–D network to find novel functions of licorice, based on the assumption that certain compounds act on

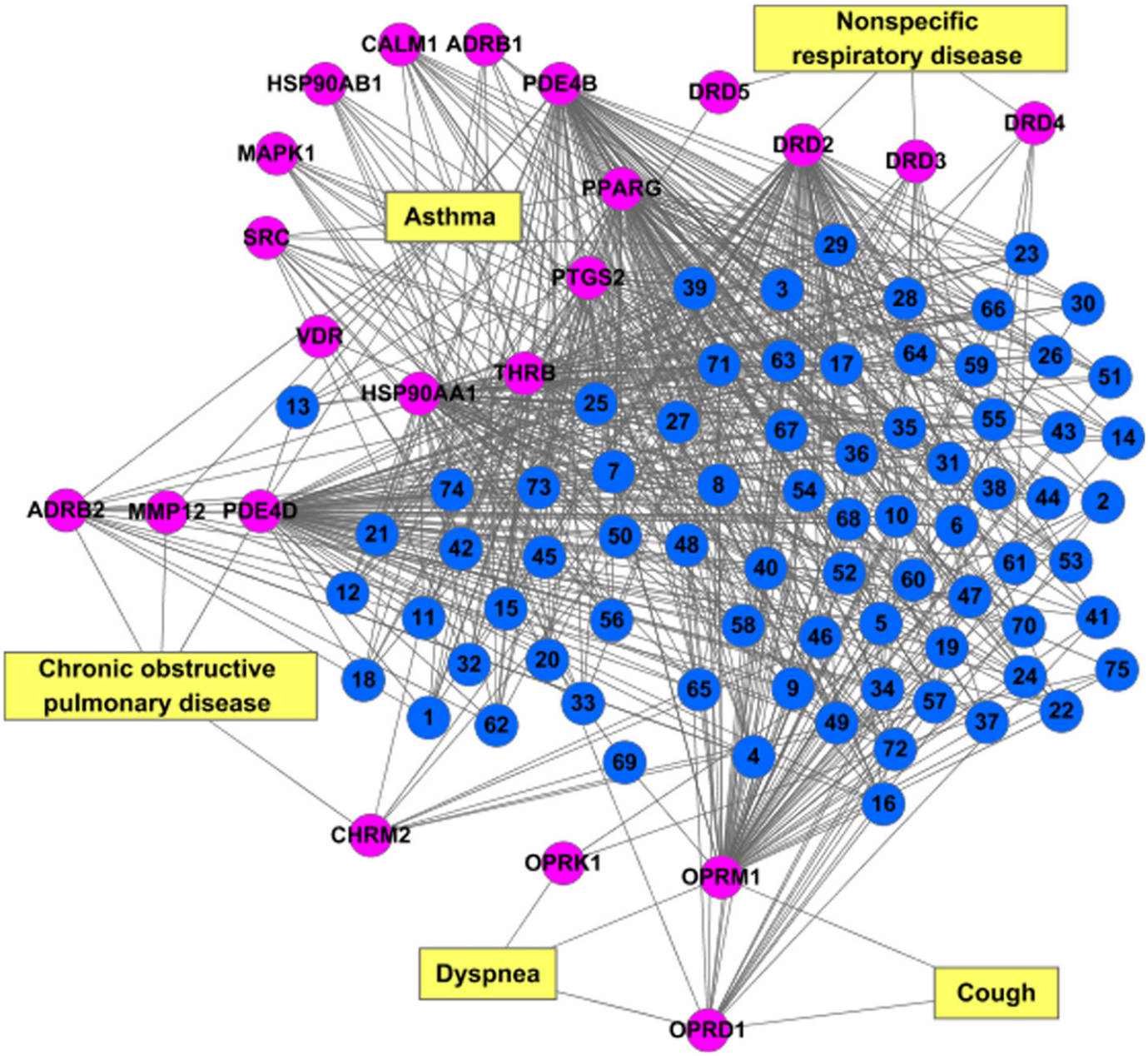


Fig. 2. The drug–target–disease network of respiratory system. The pink circles represent target proteins, the blue represent compounds, the yellow box respects specific respiratory disease. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

the same protein associated with different diseases in a network may cure different diseases. These disease-centered networks provide insights into the specific disease processes and therefore can suggest novel drug targets. In the D–T–D network, the disease is linked to the potential target if the target has relationships with the diseases. In our study, there are 56 potential targets, which have been annotated to have significant relationships with the pharmacologic effects of licorice. Constructing the target protein network for a specific disease or drug could help improve understanding the mechanism of certain drugs on specific diseases.

3.3.2.1. Disease of the respiratory system. Licorice has been traditionally used for treating respiratory system diseases such as asthma, cough and lung diseases for centuries (Fiore et al., 2005). Inspecting of the drug–target–disease network (Fig. 2), there are 22 potential targets related to respiratory system disease and detailed information is given below.

Asthma: It is now one of the most common chronic diseases characterized by airway inflammation and contraction of airway smooth muscle (Barnes, 1996). Current therapies target both the relief of chronic inflammation and improving lung function through bronchodilation (Liu et al., 2008). Among these 22 proteins, proteins concerned with asthma are beta-1 adrenergic receptor (ADRB1), beta-2 adrenergic receptor (ADRB2), calmodulin (CALM1), cAMP-specific 3′, 5′-cyclic phosphodiesterase 4B (PDE4B), cAMP-specific 3′, 5′-cyclic phosphodiesterase 4D (PDE4D), heat shock protein HSP 90-alpha (HSP90AA1), heat shock protein HSP 90-beta (HSP90AB1), peroxisome proliferator-activated receptor gamma (PPARG), thyroid hormone receptor beta-1 (THRB), etc. The control of these proteins will remodel the airway, relax the bronchial smooth muscle and inhibit inflammation in the airway mucosa.

Among these, PDE4B exhibits the highest number of candidate compound interactions (71), followed by PDE4D with 68 compounds, PPARG with 64 compounds, which indicates their key roles played in the asthma therapy by licorice. cAMP-specific 3′,5′-cyclic phosphodiesterase 4 is a useful target in the treatment of severe asthma, inhibition of the protein function can profoundly suppress eosinophil influx and airway hyperreactivity (AHR) (Kanehiro et al., 2001; Kumar et al., 2003; Kuss et al., 2003). Macrophage metalloelastase (MMP12) can regulate the acute airway changes associated with allergic airway inflammation and chronic airway remodeling, which contributes to a more severe clinical asthma phenotype, including a greater risk of exacerbation (Mukhopadhyay et al., 2010). ADRB2 is also an important target by agonists in the treatment of asthma, relaxing airway smooth muscle through a cAMP-dependent mechanism. Molecules binding to the ADRB2 can also result in several targets protein phosphorylated, intracellular Ca^{2+} decreased, the bronchial smooth muscle relaxed, and finally achieve effective bronchoprotection in asthmatics.

An inspection of our network finds that isoliquiritigenin (molecule **1**) has a relative strong interaction with ADRB1, ADRB2, PDE4B, PDE4D, PPARG, PTGS2 (prostaglandin G/H synthase 2), and THRB. Although the possible pharmacological activity of isoliquiritigenin on the respiratory tract has not been evaluated so far, however, it has been demonstrated that isoliquiritigenin induces tracheal smooth muscle relaxations via activation of the sGC/cGMP pathway (Liu et al., 2008). Therefore, we deduce that isoliquiritigenin interacting might modify airway obstruction and airway inflammation, relax smooth muscle and ultimately be active against asthma. It has been reported that glycyrrhizic acid significantly alleviates the asthmatic features such as ovalbumin-induced airway constriction, airway hyperreactivity to methacholine and decreases lung inflammation including marked eosinophil infiltration in the mouse model of asthma (Ram et al., 2006). Our finding for glycyrrhizic acid is that this molecule controls asthma symptoms through regulating the proteins such as CALM1 (calmodulin) as shown in Fig. 2.

Cough: It is found that two types of opioid receptors (mu and delta) airways are involved in the inhibition of cough. The antitussive effects of the μ opioid agonists have also been well recognized (Eddy et al., 1969; Karlsson et al., 1990), and the δ opioid receptor (OPRD1) may counteract the antitussive processes which are mediated by the μ and κ opioid receptor (Kamei et al., 1991). Clearly, the 58 molecules interacting with these opioid receptors associated with cough could eventually help relieve the coughing. It has been reported that the antitussive effect of liquiritin apioside may depend on both peripheral (modulation of ATP-sensitive K^+ channels) and central mechanisms (modulation of serotonergic systems). Liquiritigenin, which is a metabolite of liquiritin apioside and liquiritin, has a BBB ± behavior, so it is reasonable to speculate that the antitussive effect of liquiritigenin might depend on both peripheral and central mechanisms.

Chronic obstructive pulmonary disease: Chronic obstructive pulmonary disease (COPD) is an inflammatory disease characterized by the progressive deterioration of pulmonary function and increasing airway obstruction (Pauwels and Rabe, 2004). Progression of COPD is linked with the accumulation of inflammatory mucous exudates in the airway lumen and infiltration of the wall by innate and adaptive inflammatory immune cells that form lymphoid follicles (Rabe et al., 2007). The current mainstay of treatment is inhaled bronchodilators (β2-adrenoceptor agonists and muscarinic receptor antagonists), which mainly address symptom relief. It was found that 21 compounds connected with beta-2 adrenergic receptor (ADRB2) and muscarinic acetylcholine receptor M2 (CHRM2). So through the modulation to these proteins, these compounds may relieve inflammation activity, decrease airway obstruction and finally slow the progression of the condition and reduce the symptoms. Proteins MMP12 and PDE4D have been confirmed to be closely related to chronic obstructive pulmonary disease. MMP12 could play a predominant role in the pathogenesis of chronic obstructive pulmonary disease, particularly in emphysema (Shapiro, 2000). PDE4 is mainly expressed in inflammatory cells, where it catalyzes the degradation of cAMP to AMP. Inhibiting this process is expected to result in an accumulation of intracellular cAMP, which is known to lead to a broad range of anti-inflammatory effects, making this an attractive target for inter alia respiratory system diseases such as COPD (Boswell-Smith and Spina, 2007). Thus, the compounds interacting with these receptors may be key players in the relief of COPD pathophysiological changes. Interestingly, there are 69 compounds connected with these targets and most of them are licorice flavonoids, such as liquiritigenin (molecule **6**), isoliquiritigenin (molecule **1**), and hispaglabridin A (molecule **57**). It has been suggested that licorice flavonoids effectively attenuated LPS-induced pulmonary inflammation through inhibition of inflammatory cells infiltration, decreases of oxidative stress, and reduction of pro-inflammatory mediator releases in lung, providing with the potential rationale for development of anti-inflammatory compounds from flavonoid extracts of licorice (Xie et al., 2009).

Dyspnea: This may be defined as a sensation of difficult breathing. Evidence, including an adequately powered randomized study and a meta-analysis, demonstrates that opioids reduce the intensity of refractory breathlessness (Jennings et al., 2002; Abernethy et al., 2003). As shown in Fig. 3, 58 compounds were connected with OPRK1 (kappa-type opioid receptor), OPRD1, and OPRM1 (μ-type opioid receptor). Thus, through regulating of these targets, these compounds may achieve the goal of reducing the symptoms of breathing difficulties.

Nonspecific respiratory diseases: Evidence suggests that dopamine receptor agonists may inhibit sensory nerve-mediated responses, which regulates central and local reflexes such as airway plasma leakage, and cough and their function may be enhanced during inflammation (Birrell et al., 2002). There are 72 molecules that have interactions with these dopamine receptors (DRD2, DRD3, DRD4, DRD5) and ultimately against the nonspecific respiratory diseases. All these findings suggest that licorice might regulate the whole respiratory system by a complex protein–protein interaction network, thus affecting some other respiratory system diseases.

As can be seen from Fig. 2, 18 molecules have strong interactions with more than eight proteins. Above all, molecules **63** (licoagrocarpin), **71** (glyasperins C), and **16** (licochalcone A) present highest degrees (10), indicate that these molecules might be important for regulating the functions of targets, finally exerting a pharmacological effect on respiratory system diseases (Table 2).

In search of compounds targeting the CNS, out of 75 molecules analyzed above, only 12 molecules have the BBB+ behavior (see Table 1). These BBB+ molecules have common features that they have higher log *P* (1.6–3.9), lower molecular topological surface area (< 70 Å^2) and molecular weight (< 400) than BBB- molecules. It indicates that molecules with higher lipophilicity and lower molecular weight will be more easily partitioned into the lipid bilayer with more chances to penetrate BBB.

3.3.2.2. Diseases of the cardiovascular system. In traditional medicine, licorice has also been widely used for renovascular and cardiovascular diseases, and hence frequently constitutes a major ingredient of polyherbal formulations for cardiovascular diseases (PRC, 2005). Clinical studies demonstrated that licorice has desirable pharmacological effects on inhibiting inflammatory processes of blood vessels and preventing atherosclerosis, increasing resistance of low-density lipoprotein (LDL) to

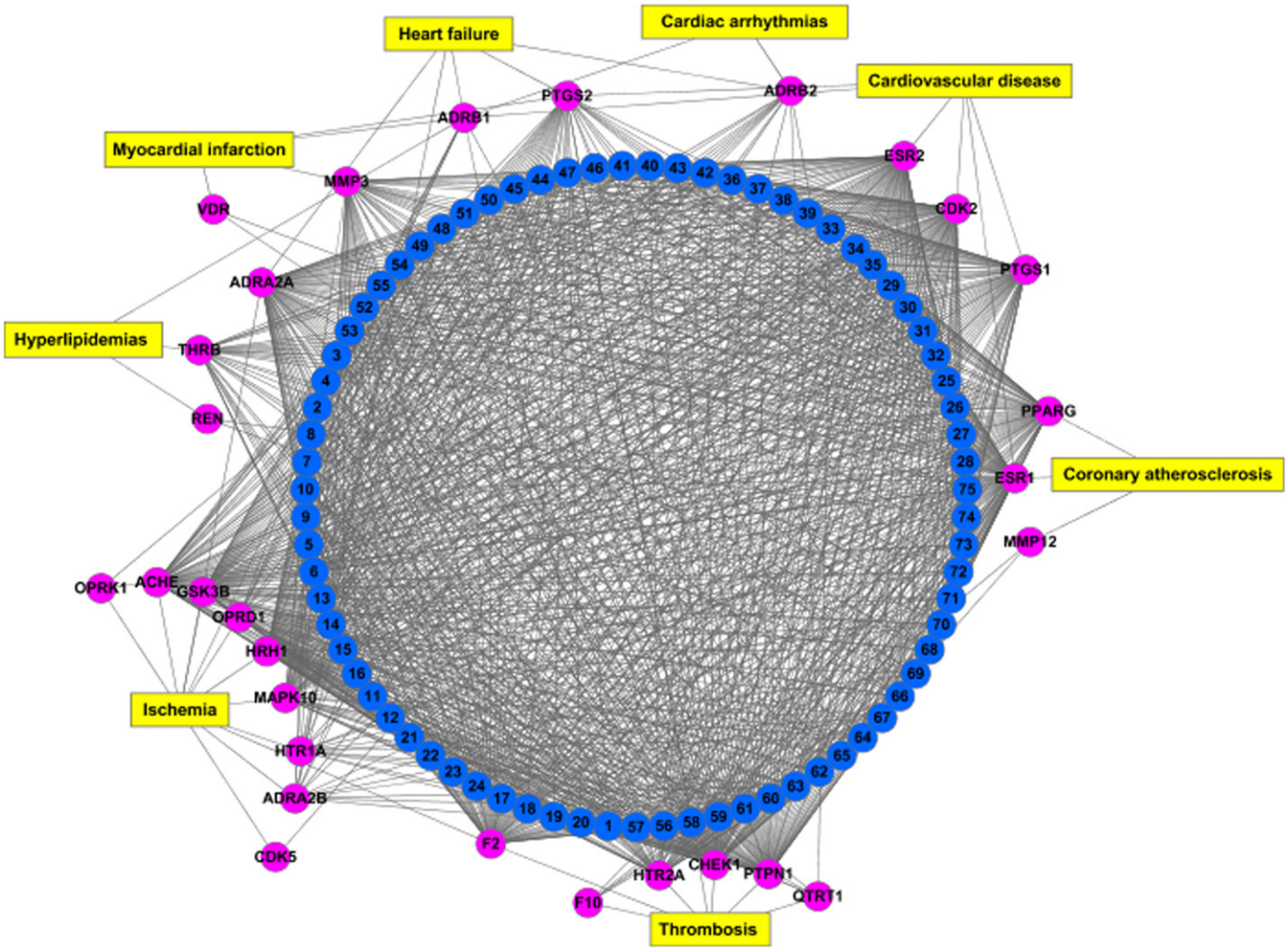


Fig. 3. The drug–target–disease network of cardiovascular system. The pink circles represent target proteins, the blue represent compounds, the yellow box respects specific cardiovascular disease. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

Table 2
The most important compounds in this work and their targets of different diseases.

Disease	Molecule no.	Target
Respiratory system	**16**	PPARG, PTGS2, ADRB2, THRB, DRD2, PDE4D, PDE4B, OPRM1, DRD3, OPRD1
	58	HSP90AA1, CALM1, PPARG, DRD2, OPRM1, MMP12, PDE4B, PTGS2
	63	DRD2, OPRM1, HSP90AA1, OPRD1, PDE4B, CHRM2, PDE4D, PPARG, DRD3, DRD4
	71	DRD2, OPRM1, HSP90AA1, PDE4B, PPARG, CHRM2, OPRD1, PDE4D, THRB, DRD3
Cardiovascular system	**8**	CDK2, F2, PTPN1, PTGS1, PTGS2, PPARG, CHEK1, ADRB2, ADRA2A, ESR1, ESR2, HTR2A, MMP3, ADRB1, HRH1, AChE, THRB
	16	PTPN1, F2, PPARG, ESR2, HTR2A, PTGS2, PTGS1, ADRA2A, CDK2, ESR1, HRH1, CHEK1, THRB, ADRB2, ADRA2B, AChE, OPRD1, MMP3
	38	ESR2, ESR1, HRH1, PTPN1, ADRA2A, HTR2A, F2, CHEK1, CDK2, OPRD1, AChE, PTGS2, ADRA2B, PPARG, PTGS1, HTR1A, GSK3B
	62	F2, PPARG, PTPN1, PTGS1, PTGS2, ESR1, ESR2, CDK2, ADRA2A, CHEK1, HTR2A, THRB, HRH1, ADRB2, MMP3, AChE, GSK3B, ADRB1
Gastrointestinal system	**1**	PTGS2, DRD2, HRH1, DRD2
	11	PTGS2, HRH1, DRD2
	58	DRD2, HRH1, PTGS2, MMP12
	65	PTGS2, HRH1, DRD2, MMP12
Inflammation	**6**	ESR2, PTGS1, PTGS2, ESR1, MAPK14, PPARG, ADRB2, AChE, OPRM1
	8	PTGS1, PTGS2, PPARG, MAPK14, ADRB2, ESR1, ESR2, MMP3, AChE, OPRM1
	16	PPARG, ESR2, PTGS1, PTGS2, ESR1, ADRB2, MMP3, MAPK14, OPRM1, AChE
	66	PPARG, PTGS1, PTGS2, ESR1, ESR2, MAPK14, ADRB2, MMP3, AChE, OPRM1

atherogenic modifications, reducing plasma lipid levels, and decreasing systolic blood pressure (Fuhrman et al., 2002), and even show certain impacts on antiarrhythmic, anti-inflammation, brain ischemia, etc. (Xie et al., 2004; Zhan and Yang, 2006; Zhang and Shen, 2011). Interestingly, inspection of the network identifies 29 proteins, which have significant relationships with the cardiovascular diseases (Fig. 3).

Atherosclerosis: This is a progressive disease characterized by the accumulation of lipids and fibrous elements in the arterial walls and is the primary cause of heart disease and stroke. It has

been suggested that ethanol extract of licorice roots could inhibit the activity of rat liver acyl-coenzyme A: cholesterol acyltransferase (ACAT) enzyme, resulting in cholesterol-lowering and anti-atherosclerotic activity improvement by blocking the intestinal absorption of dietary cholesterol, inhibiting hepatic secretion of very low density lipoproteins (VLDL) and preventing formation of foam cells in the arterial wall (Choi et al., 2007). We found that ESR1 (estrogen receptor), MMP12 (macrophage metalloelastase), and PPARG (peroxisome proliferator-activated receptor gamma) are also responsible for the coronary atherosclerosis. Clearly, the compounds which interact with these receptors may lead to inhibition of the blood coagulation, the platelet aggregation and tackiness, and ultimately against the atherosclerosis (Verma and Szmitko, 2006; Aléssio et al., 2007; Bäck et al., 2010). It is found that compounds such as liquiritigenin (molecule **6**), licochalcone B (molecule **8**), naringenin (molecule **11**), kaempferol (molecule **13**), glabranin (molecule **17**), liquiritin (molecule **58**), and licochalcone G (molecule **65**) have strong interactions with these targets. Among these compounds, some have been demonstrated actively in the atherosclerosis therapy. For instance, glycyrrhizic acid, an anti-inflammatory compound isolated from licorice, exhibits significant antithrombotic activity in vivo (Mendes-Silva et al., 2003). Glabrene, a partial agonist/antagonist of estradiol-17β, is a new agent for modulation of vascular injury and atherogenesis in post-menopausal women (Somjen et al., 2004). All these explain why licorice may achieve the anti-atherosclerotic effects on atherosclerosis disease.

Thrombosis: Proteins HTR2A (5-hydroxytryptamine 2A receptor), F2 (prothrombin), F10 (coagulation factor X), PTGS2 (prostaglandin G/H synthase 2), CHEK1 (serine/threonine-protein kinase Chk1), PTPN1 (tyrosine-protein phosphatase non-receptor type 1), and QTRT1 (queuine tRNA-ribosyltransferase) may play important roles in the thrombosis process, the control of which could lead to the inhibition of the blood coagulation, activation of the fibrinolysis, inhibition of the platelet aggregation and tackiness, decrease of plasma viscosity, and ultimately against the thrombosis (Pawlak et al., 1998; Spencer and Becker, 2000; Van Aken et al., 2001; Cipollone et al., 2008). Clearly, it has been demonstrated that 5-hydroxytryptamine 2A receptor antagonists may inhibit both vasoconstriction and formation of thrombi under certain pathophysiological conditions when serotonin is released from the activated platelets (Pawlak et al., 1998). Thrombin is a trypsin-like serine protease that plays a central role in thrombosis by cleaving fibrinogen to insoluble fibrin in the blood coagulation cascade and also potently activates platelet aggregation by proteolytic activation of the thrombin receptor (Lee et al., 2003). It has also been clarified that the total flavones of licorice have a pharmacological effect on thrombogenesis and coagulation (Yang et al., 2003).

Inspection of the network (Fig. 3) suggests that these effects may be achieved through the regulation of antithrombosis related proteins HTR2A, PTGS2, F2, CHEK1 and PTPN1 targeted by the main flavones such as isoliquiritigenin, liquiritigenin and liquiritin. Glycyrrhizic acid is identified as a new thrombin inhibitor which could prolong plasma recalcification and thrombin and fibrinogen clotting times, and inhibit thrombin-induced, but not collagen-, PAF- or convulxin-induced platelet aggregation (Mauricio et al., 1997).

Hyperlipidemias: The cholesterol-lowering effects of licorice in hypercholesterolaemic rats are related to an increased excretion of cholesterol, neutral sterols, bile acid and an increase in hepatic bile acid content (Visavadiya and Narasimhacharya, 2006). The proteins related to hyperlipidemias are ADRB1 (beta-1 adrenergic receptor), REN (renin), and THRB (thyroid hormone receptor beta-1). The present work finds that many flavonoids are connected to THRB, such as isoliquiritigenin (molecule **1**), echinatin (molecule **3**), licochalcone B (molecule **8**), kaempferol (molecule **13**), licochalcone A (molecule **16**), glabranin (molecule **17**), etc. Flavonoid compounds binding to ADRB1 are isoliquiritigenin, echinatin, glypallichalcone (molecule **4**) and licochalcone B. It has been reported that plant flavonoids can act as potent inhibitors of LDL oxidation via several mechanisms including scavenging of free radicals, chelation of transition metal ions, or preservation of serum paraoxonase (PON1) activity (and as a result, hydrolysis of LDL associated lipid peroxides) (Aviram, 2004). Hence, we deduce that licorice flavonoids may reduce cholesterol levels through modulation of these relevant proteins.

Ischemia: Licorice has been demonstrated to confer protection against ischemic damage of several body organs due to its potent anti-oxidant and free radical scavenging activity (Račková et al., 2007; Lim et al., 2009; Visavadiya et al., 2009). Among these targets, those related to focal ischemia are 5-HTR1A (5-hydroxytryptamine 1A receptor), ADRB1, CDK5 (cell division protein kinase 5), OPRD1 (δ opioid receptor), GSK3B (glycogen synthase kinase-3 beta), HRH1 (histamine H1 receptor), OPRK1 (kappa-type opioid receptor), MAPK10 (mitogen-activated protein kinase 10), F2, ADRA2A (alpha-2A adrenergic receptor), ADRA2B (alpha-2B adrenergic receptor) and AChE (acetylcholinesterase). Target HRH1 has the highest number (73) of interacted compounds, followed by ADRA2A with 70 compounds and AChE with 57 compounds. It has been demonstrated that 5-HTR1A agonists have a potentially marked neuroprotective reaction against cerebral ischemia by both neuroprotective and hypothermic effects (Uchiyama et al., 2001). Gavras et al. (2001) have reported that the clinical effects of central sympathetic suppression with ADRA2A agonist clonidine in patients with ischemic heart disease and/or heart failure.

As can be seen from Fig. 3, the most important compounds treating ischemia are flavonoids from licorice, such as isoliquiritigenin (molecule **1**), licochalcone B (molecule **8**), echinatin (molecule **3**). This series of compounds have protective effects on focal cerebral ischemia reperfusion in rats due to scavenging free radicals (Wu et al., 2003). For example, isoliquiritigenin has the protective potential against cerebral ischemia injury because of its anti-oxidant property toward LDL oxidation and lipid peroxidation (Zhan and Yang, 2006). Inspecting the network finds that the licorice flavonoids such as glabridin, licochalcone A and licoisoflavanone (molecule **49**) mainly target ischemia related proteins HTR1A, OPRD1, GSK3B, HRH1, MAPK10, F2, ADRA2A, AChE, which may be responsible for the anti-ischemia effects of licorice.

This might be proven by the fact from Fig. 3, 18 molecules have strong interactions with more than eight proteins. Above all, molecules **63** (licoagrocarpin), **71** (glyasperins C), **16** (licochalcone A) present highest degree (10), indicating that these molecules binding to several targets might be important for regulating the functions of the relative targets, finally exerting a pharmacological effect on cardiovascular diseases.

Out of 29 potential targets associated with cardiovascular system disease, some proteins have been confirmed closely related to some other cardiovascular diseases. For instance, ADRB1 and ADRB2 (beta-2 adrenergic receptor) are involved in cardiac arrhythmias, ADRA2A, ADRA2B, ADRB1, ADRB2, and PTGS2 play a role in heart failure, ADRB1, PTGS2, MMP3 (stromelysin-1), and VDR (vitamin D3 receptor) have a relationship with myocardial infarction, while CDK2 (cell division protein kinase 2), ESR1, ESR2 (estrogen receptor beta), PTGS1 (prostaglandin G/H synthase 1), PTGS2, and ADRB1 are associated with nonspecific cardiovascular disease. All these findings suggest that licorice might regulate the whole cardiovascular system by a complex protein–protein interaction network, thus affecting the some other cardiovascular diseases.

3.3.2.3. Diseases of the gastrointestinal system. Licorice has been used as an anti-peptic ulcer agent since 1946 (Revers, 1948), and its antiulcer and mucosal protective actions have been confirmed by numerous clinical trials and animal experiments (Van Marle et al., 1981; Kassir, 1985; Aly et al., 2005). It is known that carbenoxolone, a widely used agent treating gastric ulcers, is a synthetic drug derived from the licorice glycyrrhetic acid. The topics in this section describe the potential mechanics of licorice in the treatment of gastrointestinal system diseases.

Gastrointestinal ulcers: MMP12, PPARG, and PTGS2 were found to have a relationship with gastrointestinal ulcers (Gingras et al., 2001; Xiang et al., 2002). MMPs are involved in intestinal injured tissue remodeling such as migration, proliferation and differentiation of intestinal crypt cells, and PPAR gamma inhibits tissue injury associated with immune activation through inhibition NF-KB, while PTGS2 is responsible for the repair of the intestinal mucosal damage (Haworth et al., 2005) and plays a key role in the pathophysiologic processes in ulcerative colitis caused by *Salmonella typhimurium* (Cho and Chae, 2004). Interestingly, these three targets are targeted by 68 compounds, thus explaining why licorice can maintain the small intestinal integrity and inhibit inflammation on the intestinal crypt cells.

Spasmolysis: Although the antispasmodic effect of licorice has been acknowledged both clinically and experimentally (Maeda et al., 1983), the molecular mechanism is still unknown. Previous studies suggested that glycycoumarin acts as a potent antispasmodic agent by means of intracellular accumulation of cAMP through the inhibition of PDEs (Sato et al., 2006). Licochalcone A is shown to exert a relaxant effect on smooth muscle contraction through the inhibition of cAMP PDE (Nagai et al., 2007). As can be seen from the network (Fig. 4), both glycycoumarin and licochalcone A are predicted to target receptors PDEs, together with other 69 molecules in licorice. In addition, some compounds including glycyrrhizic acid and 18β-glycyrrhetinic acid are found to hit muscarinic receptors (Muscarinic acetylcholine receptor M1–M5) which are involved in regulating smooth muscle contraction within the gastrointestinal tract. Therefore, the spasmolysis effect of licorice may be achieved by those compounds interacting with the muscarinic acetylcholine receptor and eventually relieve the spasm pain of gastrointestinal tract.

Moreover, some proteins have been found closely related to some other gastrointestinal diseases. For instance, 5-HTR3 (5-hydroxytryptamine 3 receptor), DRD2 (dopamine D2 receptor), and HRH1 are involved in vomiting (Goiny and Uvnäs-Moberg, 1987; Csáki et al., 1993; Pleuvry, 2006), DRD2 plays a role in gastric emptying disorders (Nagahata et al., 1995), while HTR3 is associated with irritable bowel syndrome (Dunphy and Verne, 2001). All these findings suggest that licorice might regulate the whole gastrointestinal system by a complex protein–protein interaction network, thus affecting gastrointestinal diseases.

3.3.2.4. Anti-inflammation. Inflammation is a beneficial host response to a foreign challenge or tissue injury, which ultimately leads to the restoration of normal tissue structure and function. A normal inflammatory response is self-limiting and involves the down-regulation of pro-inflammatory protein expression, increased expression of anti-inflammatory proteins, and a reversal in the vascular changes that facilitated the initial immune cell recruitment process. Licorice extracts have been known to have strong anti-

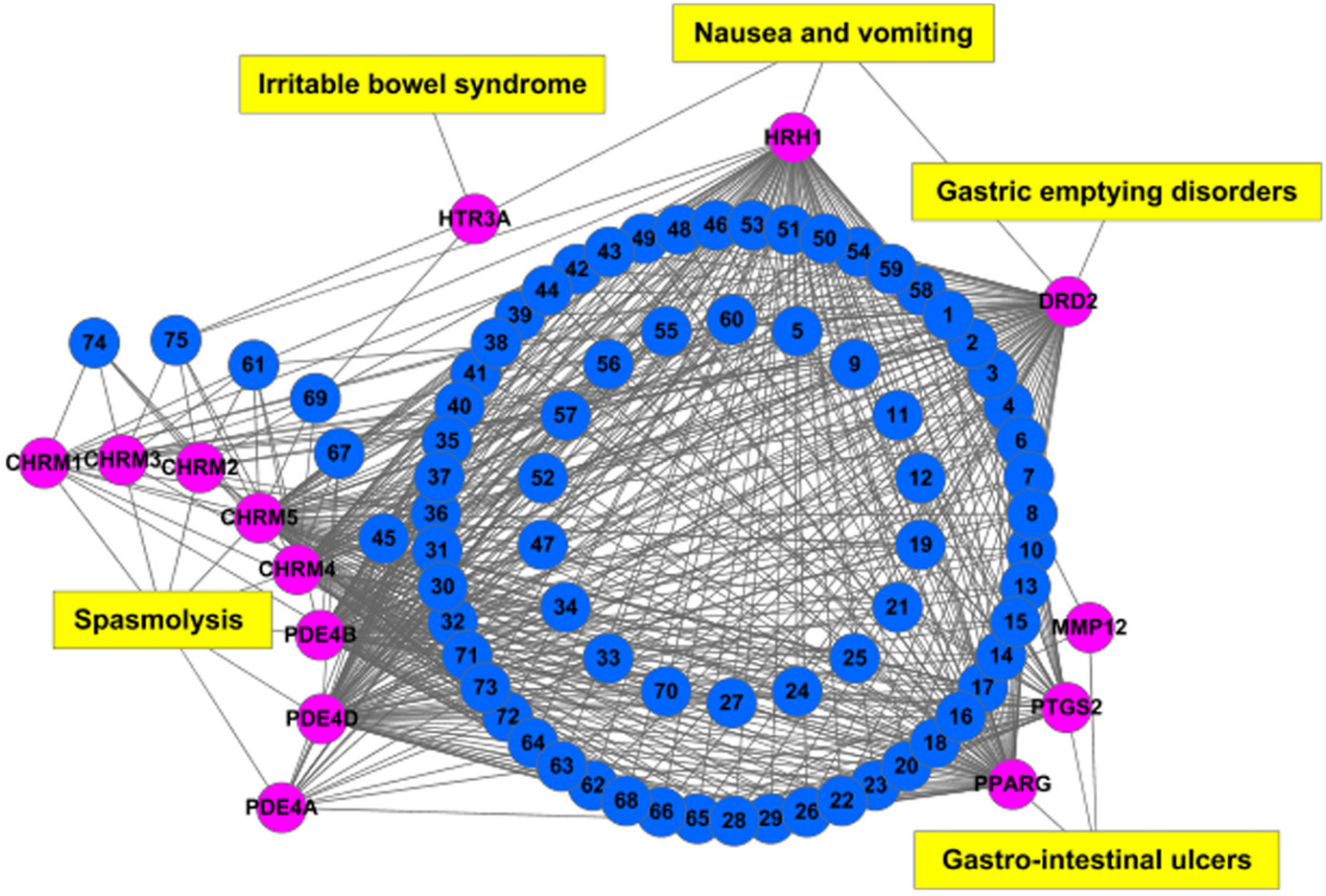


Fig. 4. The drug–target–disease network of gastrointestinal system. The pink circles represent target proteins, the blue represent compounds, the yellow box respects specific gastrointestinal disease. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

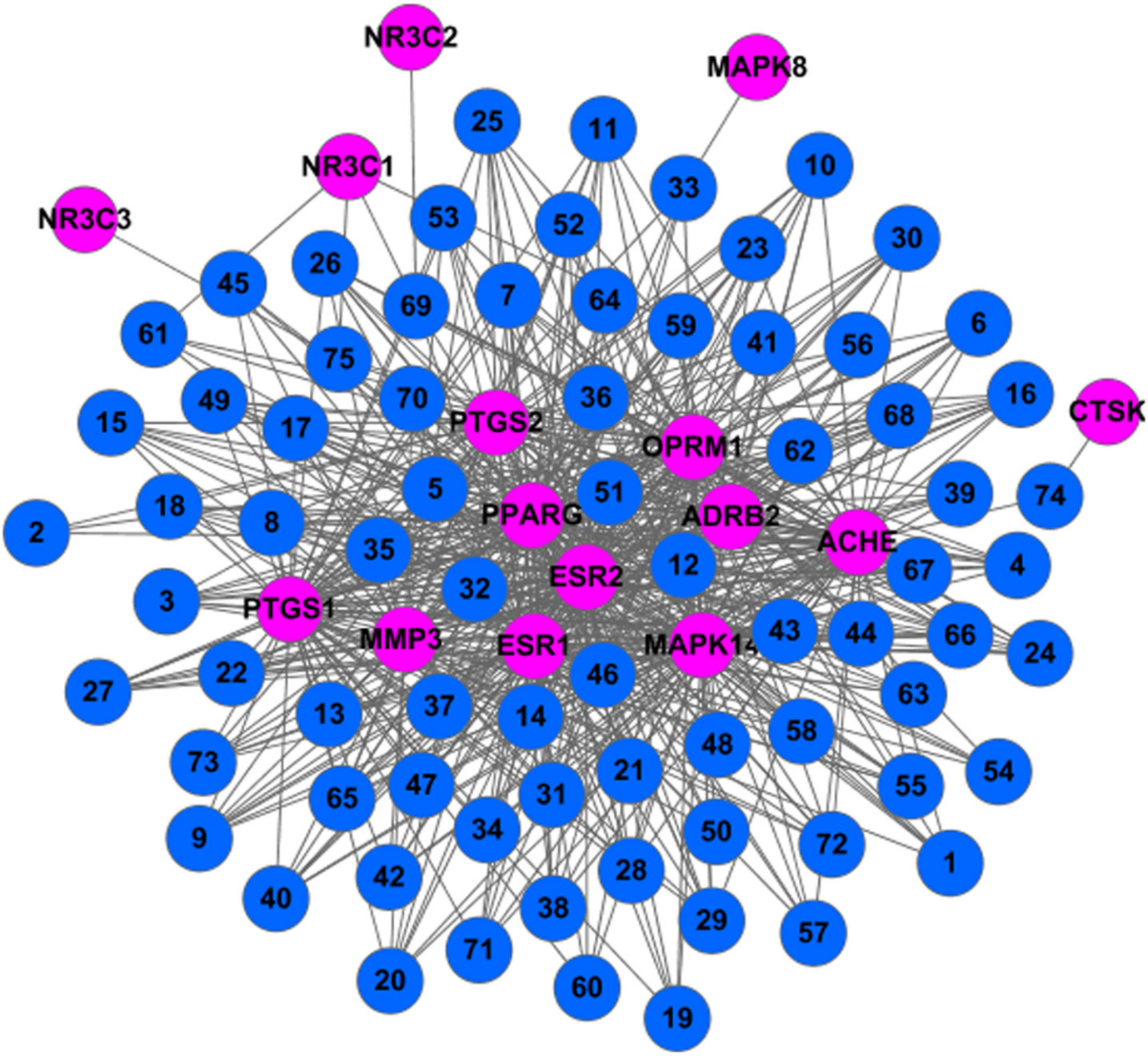


Fig. 5. The drug–target association network of anti-inflammation. The pink circles represent target proteins and the blue represent compounds. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

inflammatory effects by exerting an inhibitory effect on cyclooxygenase and lipoxygenase activities (Inoue et al., 1986).

In this work, 15 potential targets have significant relationships with the inflammatory processes (Fig. 5). For example, PTGS2 (prostaglandin G/H synthase 2) participates in inflammation-mediated cytotoxicity and neuronal death (Xiang et al., 2002), which is hit by 36 compounds. Interestingly, among these 36 compounds, licochalcone A has been reported to inhibit prostaglandin biosynthesis in lipopolysaccharide (LPS)-induced mouse macrophage cells to achieve anti-inflammation effect (Cui et al., 2008). Molecules gancaonin H (molecule **59**), xambioona (molecule **61**), 18α-hydroxyglycyrrhetic acid (molecule **69**) and 18β-glycyrrhetinic acid (molecule **75**) are found to target the nuclear receptors. It is known that the nuclear receptor superfamily are the key regulators of inflammation and lipid homeostasis in macrophages, such as glucocorticoid receptor (NR3C1), which inhibits inflammatory gene expression in response to natural corticosteroids and synthetic anti-inflammatory ligands such as dexamethasone (Valledor and Ricote, 2004).

Furthermore, in response to endogenous eicosanoids and oxysterols, respectively, PPARG regulate transcriptional programs involved in inflammatory responses. The pharmacological effects of ADRB2 agonists in inhibiting acute inflammatory responses in the lung, subcutaneous tissues and the skin are thought primarily due to the inhibition of mast cell mediator release and vascular permeability changes. It has been suggested that licorice flavonoid effectively attenuated LPS-induced pulmonary inflammation through inhibition of inflammatory cells infiltration, decreases of oxidative stress, and reduction of pro-inflammatory mediator releases in lung (Xie et al., 2009). It is reported that 18β-glycyrrhetinic acid could inhibit the passive cutaneous anaphylaxis and skin contact inflammation in a mouse model of contact hypersensitivity (Park et al., 2004). Although the mechanisms of action of glycyrrhizic acid and its metabolite against

inflammation are still not clear, it is believed that the steroid like structure of 18β-glycyrrhetinic acid might produce a cortisol mimicking effect on inflammation process as demonstrated in this work.

3.3.2.5. Antidote. Detoxification is the physiological or medicinal removal of toxic substances from a person's body, or neutralization or mitigation of its effects. Licorice is one of the most important detoxification drug used in traditional Chinese medicine. The compound in licorice, such as glycyrrhizic acid, might react with alkaloids, such as strychnine and atropine to produce precipitate, thus converting toxic into non-toxic compounds. However, the deeper mechanisms of detoxification especially at molecular level are not clear. As a guide to future investigation of potential mechanisms of inter-individual variability of efficacy and toxicity, multiple targets molecules and a simplified depiction of their interactions are elucidated below.

1. *Hormone mimics*: 18β-Glycyrrhetinic acid is structurally similar to adrenal cortex hormones, which can reduce the absorption of poison and improve the body tolerance to the toxic substance. This is well accordance with our network findings that four compounds including this molecule, i.e., 18α-hydroxyglycyrrhetic acid, xambioona and gancaonin H, simultaneously target glucocorticoid receptor. All this indicates that licorice exerts detoxification effect by increasing the adaptability of the body to external stimuli and protecting the human body.
2. *Immune system simulation*: The network analysis shows that eight immune related proteins, i.e., DPP4 (dipeptidyl peptidase 4), PPARG, NR3C1, FKBP1A (FK506-binding protein 1A), GSK3B (glycogen synthase kinase-3 beta), NR3C2 (mineralocorticoid receptor) and VDR are hit by a number of compounds in licorice, including glycyrrhizic acid, 18β-glycyrrhetic acid, lichochalchone A and some analogues. Among these proteins, PPARG has been found recently to regulate macrophage activation in response to mitogens and inflammation (Wang et al., 2001). DPP4 plays a key role in immune function as a T cell activation molecule and a regulator of the functional effect of selected biological factors through its dipeptidyl peptidase 4 enzyme activity (Aytac and Dang, 2004), while GSK3 is active in a number of central intracellular signaling pathways, including cellular proliferation, migration, inflammation and immune responses, glucose regulation, and apoptosis.
3. *Macrophage activation*: Metalloelastase in alveolar macrophage is a leading player in the direct killing of bacteria sequestered in the phagolysosomes (He and van Lookeren Campagne, 2009). Interestingly, this protein has been targeted by compounds liquiritin and licochalcone G and activation of this enzyme might strengthen the performance of the tissue macrophages to defense against exotic invasions.
4. *HSP 90 activation*: A large number of compounds such as neoglycyrol (molecule **56**) and hedysarimcoumestan B (molecule **33**) are found to hit heat shock protein HSP 90-alpha and beta, which is a kind of stress protein with abundant expression when the organism is exposed to adverse conditions like chemical poisoning (De Maio, 1999). Therefore, it is deduced that the detoxification of licorice may be achieved through activating and promoting the expression of such proteins to protect the organism itself.

3.3.2.6. Other diseases. Of all the 91 potential targets, some proteins are found closely related to some other diseases. For instance, HTR2A and AKR1B1 (aldose reductase) are related to diabetic complications. MAOB (monoamine oxidase type B), DRD2, DRD3 (dopamine D3 receptor), and MAPK10 are involved in neurological disease. CDK2, ESR2, PPARG, PTGS2, and CHEK1 play a role in anti-tumor effect, while AKR1B1, PDE4D, OPRD1, OPRK1, CHRM2, CHRM4 (muscarinic acetylcholine receptor M4), OPRM1, and PTGS2 are associated with pain. Clearly, these findings provide important indications for further research to dissect the mechanisms of action of licorice.

3.3.3. Illustrating the relationships between multi-component and drug synergistic effects

Synergistic multitarget effects mean that natural products affect not only one target, but several targets such as enzymes, substrates, metabolites, receptors, ion channels, transport proteins or DNA/RNA and can cooperate in an agonistic and synergistic way. Synergistic interactions are of vital importance in phytomedicines, to explain rational and efficient therapy form of combinational effects to be greater than the sum of the individual effects, and explain the efficacy of apparently low doses of active constituents in a herbal product (Williamson, 2001). In this regard, licorice may have the potential of addressing a relationship between multicomponents and drug synergistic effects, thereby increasing the likelihood of conquering complex diseases, such as cardiovascular diseases, in a synergistic manner. What are the possible mechanisms underlying the synergy effects in licorice? Based on the analysis of the results from the drug–target–disease network of cardiovascular system, the following mechanisms were elucidated:

1. As shown in Fig. 3, many potential targets are targeted by more than one candidate compounds. PPARG, GSK3B, HTR2A, and PTGS2 are examples of highly connected potential targets, whose numbers of candidate compounds are **65**, **54**, **47**, and **36**, respectively. The common targets shared by multi-compounds imply that the licorice might exert synergistic therapeutic effects on cardiovascular diseases, which is probably more effective than single compounds.
2. It has been recognized that cardiovascular disease continuum begins with risk factors that initiate the process, leading to tissue damage. Inhibition of an individual target is insufficient to restore the cardiovascular system to the healthy state. In this case, multiple compounds contained in licorice could hit a series of targets, which have been annotated to have significant relationship with the pathological process of cardiovascular disease, and finally exert synergistic therapeutic efficacies. For example, proteins F2 (prothrombin), F10 (coagulation factor X), and PTGS2 (prostaglandin G/H synthase 2) are closely concerned with thrombosis process, while ADRB1 (beta-1 adrenergic receptor), REN (renin), and THRB (thyroid hormone receptor beta-1) are related to hyperlipidemias. Compounds connected with these targets may ultimately cure thrombosis by modulating the activity of multiple targets. Proteins HTR1A (5-hydroxytryptamine 1A receptor), ADRB1, CDK5 (cell division protein kinase 5), OPRD1 (δ opioid receptor), GSK3B, and HRH1 may play important roles in ischemic heart disease. VDR and REN are concerned with vasoconstriction, the regulation of them may cause hemangiectasis, and then lower blood pressure. Therefore, it can be deduced that the action mechanism of this medical composition is that the licorice systematically controls the cardiovascular disease via potentially synergistic interactions of the active compounds.
3. We have extracted five signal pathways closely associated with CVD, including renin–angiotensin–aldosterone system (RAAS) pathway, anti-arrhythmic pathway, platelet

aggregation inhibitor pathway, glucocorticoid and inflammatory pathway, and VEGF signaling pathway. The RAAS is central to the pathogenesis of cardiovascular disease through vascular inflammation, an increase in reactive oxygen species, endothelial dysfunction, and atherosclerosis with subsequent complications such as myocardial infarction (MI), chronic heart failure (HF), and renal disease (Ferrario and Strawn, 2006). Evidently, angiotensinogen, renin (REN), angiotensin converting enzyme (ACE), NR3C2, and MAPK1 are candidate target involved in the core pathway. The platelet aggregation pathway is related to the platelet activation and coagulation (Sangkuhl et al., 2011) and the anti-arrhythmic pathway is concerned with those targets participate in each cell propagate over the whole heart to generate normal or abnormal rhythms. Glucocorticoid and inflammatory pathway plays an important role in the treatment of inflammatory disease (Smoak and Cidlowski, 2004). VEGF Signaling Pathway is associated with the induction and maintenance of neovasculature (Chou et al., 2002). VEGF comprises several isoforms which bind to different receptors and promote angiogenesis through activation of a kinase cascade that includes MAPK. Thus, compounds interacted with mitogen-activated protein kinase (MAPK1, MAPK8, MAPK10, MAPK14) participates in the modulation of VEGF-induced angiogenesis process. This implies that the candidate drugs in licorice can target different target proteins involved in the same or different signal pathways, and thereby have synergistic effects on the cardiovascular whole signal system.

4. Conclusion

Botanical drug is a multi-component complex system, which might interact with multiple targets and regulate multiple pathways in the whole human body. Licorice is one of the oldest and most popular herbal medicines in the world, and is recorded in the pharmacopoeias of many countries including China, Japan, the United Kingdom and others. In this work, we have constructed a systems pharmacology approach combining the oral bioavailability screening, drug-likeness evaluation, blood–brain barrier permeation, target identification, and network pharmacology analysis to investigate this herbal medicine. Our main findings are as follows:

1. Seventy three components out of 287 ingredients (25%) in licorice are identified as active substances through oral bioavailability and drug-likeness screening. These include many reported active components as liquiritigenin, glabridin, hispaglabridin A, licochalcone B, glabridin, glycycoumarin, isoliquiritigenin, which further validate the reasonability of our screening model. In addition, we also predict some molecules such as licoagrocarpin, glyasperins C, morachalcone A, licopyranocoumarin, neoglycyrol, licochalcone G, and 5′-Prenylbutein as potential bioactive compounds, which might serve to guide our further study of this botanical drug.
2. The identified 91 targets related with different diseases are critical for understanding the pharmacological mechanisms of licorice. The generated drug–target network suggests that compounds **71** (glyasperins C), **63** (Licoagrocarpin), **74** (glycyrrhizic acid) and the target proteins PTPN1, HRH1, F2 with high degree or betweenness are the key components playing important roles in the drug–target interaction network.
3. The drug–target–disease network clearly elucidates the mechanisms of action of licorice that exerts various pharmacological effects against diseases including the respiratory, cardiovascular and gastrointestinal system diseases. For example, the licorice flavonoids mainly target the ischemia-related proteins HTR1A, OPRD1, GSK3B, HRH1, MAPK10, F2, ADRA2A, and AChE to achieve the anti-ischemia effects and curing ischemic heart disease. The resulting network lays the foundation for a more comprehensive visualization of the drug–target–disease interaction landscape.
4. The detoxification mechanism of licorice is also illustrated by the present work. For instance, compounds liquiritin and licochalcone G can destroy bacteria by targeting the metalloelastase and strengthen the tissue macrophages to defense against external invasions.

In summary, the present work has provided a new systems pharmacology framework to study herbal drugs. This method has also been successfully utilized to shed light on the mystery and effectiveness of the botanical drug licorice. The developed system will generate a novel perspective for better understanding of the traditional herbal medicines. Moreover, resources input in TCMSP^{TM} database will also expand the scope and depth of knowledge for investigation of botanical drugs. And these hypotheses predicted with computational tools will be required to further experimentally tested and validated clinically by basic research scientists.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2013.02.004.

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