



ELSEVIER

Contents lists available at ScienceDirect

## Journal of Ethnopharmacology

journal homepage: [www.elsevier.com/locate/jep](http://www.elsevier.com/locate/jep)

# Systems pharmacology strategies for drug discovery and combination with applications to cardiovascular diseases

Peng Li<sup>a,b,1</sup>, Jianxin Chen<sup>c,1</sup>, Jinan Wang<sup>a,b</sup>, Wei Zhou<sup>a,b</sup>, Xia Wang<sup>a,b</sup>, Bohui Li<sup>a,b</sup>, Weiyang Tao<sup>a,b</sup>, Wei Wang<sup>c,\*</sup>, Yonghua Wang<sup>a,b,\*\*</sup>, Ling Yang<sup>d</sup>

<sup>a</sup> Center of Bioinformatics, Northwest A&F University, Yangling 712100, Shaanxi, China

<sup>b</sup> College of Life Sciences, Northwest A&F University, Yangling 712100, Shaanxi, China

<sup>c</sup> Beijing University of Chinese Medicine, Beijing 100029, China

<sup>d</sup> Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, Liaoning, China

## ARTICLE INFO

## Article history:

Received 22 April 2013

Received in revised form

23 June 2013

Accepted 2 July 2013

## Keywords:

Cardiovascular diseases

Drug discovery and combination

Systems pharmacology

Systems biology

## ABSTRACT

**Ethnopharmacological relevance:** Multi-target therapeutics is a promising paradigm for drug discovery which is expected to produce greater levels of efficacy with fewer adverse effects and toxicity than monotherapies. Medical herbs featuring multi-components and multi-targets may serve as valuable resources for network-based multi-target drug discovery.

**Materials and methods:** In this study, we report an integrated systems pharmacology platform for drug discovery and combination, with a typical example applied to herbal medicines in the treatment of cardiovascular diseases.

**Results:** First, a disease-specific drug–target network was constructed and examined at systems level to capture the key disease-relevant biology for discovery of multi-targeted agents. Second, considering an integration of disease complexity and multilevel connectivity, a comprehensive database of literature-reported associations, chemicals and pharmacology for herbal medicines was designed. Third, a large-scale systematic analysis combining pharmacokinetics, chemogenomics, pharmacology and systems biology data through computational methods was performed and validated experimentally, which results in a superior output of information for systematic drug design strategies for complex diseases.

**Conclusions:** This strategy integrating different types of technologies is expected to help create new opportunities for drug discovery and combination.

© 2013 Published by Elsevier Ireland Ltd.

## 1. Introduction

The past decade has seen an intense focus on multi-target drugs and combinatorial therapies that modulate the activities of the targets to achieve therapeutic efficacies, particularly in

complex diseases, such as HIV, cancer and diabetes disease (Home et al., 2009; Lennox et al., 2009; Galsky and Vogelzang, 2010). Compared to drugs that modulate single target, multi-target agents might be more effective due to the synergistic action or negative regulation of resistance (Jia et al., 2009; Chan and Loscalzo, 2012). Indeed, the concept of combinatorial therapies has been practiced in traditional medical treatments for thousands of years, which often use botanical mixtures characterized as multi-component and multi-function to treat disease (Qiu, 2007; Kong et al., 2009; Verpoorte et al., 2009; Cheung, 2011). In development, considerable knowledge has been accumulated concerning clinical efficacy and safety of herbal concoctions in targeting complex diseases. However, how to develop new synergistic combinations against multiple targets which must be based on a rational and systematic drug design strategy is still a big challenge.

The exploration of drug combinations is usually dependent on high-throughput screening procedure which tests large number of combinations in the cell-based assays, following by the investigation of the underlying synergistic details. Some major types of synergistic drug pairs were explored experimentally

**Abbreviations:** ADME, Absorption, distribution, metabolism and elimination; CVD, Cardiovascular diseases; TTD, Therapeutic Target Database; DT network, Drug–target network; TT network, Target–target network; DD network, Drug–drug network; OB, Oral bioavailability; TS, Tanimoto similarity; RSM, *Radix Salviae Miltiorrhizae*; CT, *Carthamus tinctorius*; FC, *Fructus Cartaegi*; DSH, RSM, FC and CT; A5L, Arachidonate 5-lipoxygenase; B1AR, beta-1 adrenergic receptor; CDK2, Cyclin dependent kinase 2; GmpA, cGMP-inhibited 3',5'-cyclic phosphodiesterase A; ERB, Estrogen receptor beta; MAPK14, Mitogen-activated protein kinase14; M2, Muscarinic acetylcholine receptor 2; NOSE, Nitric-oxide synthase endothelial; PPAR, Peroxisome proliferator-activated receptor gamma; PGS, Prostaglandin g/h synthase 1.

\* Corresponding author at: Beijing University of Chinese Medicine, Beijing 100029, China. Tel./fax: +86 10 6428 6283.

\*\* Corresponding author at: Northwest A&F University, College of Life Sciences, Center of Bioinformatics, Yangling, Shaanxi 712100, China. Tel./fax: +86 29 8709 2262.

E-mail addresses: [wangwei@bucm.edu.cn](mailto:wangwei@bucm.edu.cn), [yh\\_wang@nwsuaf.edu.cn](mailto:yh_wang@nwsuaf.edu.cn) (W. Wang).

<sup>1</sup> Contributing equally to this work.

(Cokol et al., 2011). However, this “compound to target” approach is highly inefficient if without a *priori* knowledge of the synergistic effects of the combination drugs. Large-scale experimental drug synergy screens have found that synergistic drug pairs are rare (4–10%) (Borisly et al., 2003; Zhang et al., 2007; Cokol et al., 2011). This is especially difficult for herbal medicines, as their combinations usually contain considerable numbers of chemical compounds, which will make it unfeasible to largely uncover the specific mechanism of action underlying such multicomponent synergy associated with the interacting targets, pathways, and even diseases. Alternatively, a systems strategy follows the “target to compound” direction, which is based on systems pharmacology to explore not only the molecular mechanism of a particular disease but also the underlying relationships among distinct phenotypes, might result in the development of more efficient molecules than the currently favored single-target drugs (Zimmermann et al., 2007; Barabasi et al., 2011).

Systems pharmacology involves the application of omics and systems biology technologies, combined with the pharmacokinetics and pharmacodynamics evaluations, to the study of drugs and their targets and effects (Kohl et al., 2010; Sorger et al., 2011; Uzuner et al., 2012). Systems pharmacology analysis generally counts on profiles of genomics, transcriptomics, proteomics, and metabolites metabolomics/metabonomics to construct networks for evaluating the drug action and understanding the therapeutic mechanisms. As a major tool, the network analysis based on widely existed databases permits us to form an initial understanding of the action mechanisms within the context of systems-level interactions. These technologies allow information-rich and high-throughput molecular observations, expected to link them to biologically and clinically relevant functions using a systems biology approach (Barabasi et al., 2011). For example, genome-scale metabolic networks constructed either in cancer or microbial pathogens have result in the identification of some novel drug targets and their synergies, and consequently effective antimicrobials or anticancer drug combinations (Folger et al., 2011; Kim et al., 2011). Consistently, systems pharmacology approach may be especially suitable for addressing and investigating herbal medicines in a holistic manner (Uzuner et al., 2012). Within this paradigm, we have developed an integrated model, which combines oral bioavailability prediction, multiple drug targets prediction, network pharmacology techniques, to investigate the mechanisms for the well-known herbal recipe Compound Danshen Formula (CDF) (Li et al., 2012b).

In this work, we propose for the first time a systems-pharmacological strategy for systematic pursuit of optimal drug combinations. The proposed methodology can include, but not limited to herbal medicine investigations. This strategy aids to drug discovery from three categories: (1) systematically identify and understand the pharmacological information for relevant diseases; (2) utilize disease associated target space to screen out effective herbal molecules and (3) combine biological network analysis and experimental validation to discover novel drug combinations. Presently, different types of data, such as the physiological, biochemical and genomic information have been collected to build the model which is based on an array of computational and experimental approaches including the machine learning method, network analysis and pharmacological analysis. In the course of this presentation, we will highlight a key study on the CVD, which is an extremely complex system involved in many genes, proteins and pathways with sufficient experimental validations. The accomplishment of this model will demonstrate the power of the combined approach for enhancing our understanding of molecular interconnectedness and its potential regulation in the control of complex diseases.

## 2. Materials and methods

### 2.1. Protocol

In order to find novel efficacious herbs or herbal combinations, we have constructed an integrated systems-pharmacological model by ADME screening, reverse drug targeting and network analysis to revalue various herbs. This tactic is based on the existing pathological and therapeutic information as well as the documented clinical and experimental data for botanical drugs, travelling the path of “target to compound” as follows (Fig. 1):

- (1) a large-scale collection of CVD-associated targets and building of drug–target network;
- (2) building of database for medical herbs and screening of candidate compounds with pharmacokinetic evaluation;
- (3) reverse drug-targeting for potential active compounds;
- (4) network construction for herbs and exploration of novel herbal combinations;
- (5) experimental validation by *in vitro* and *in vivo* model.

### 2.2. Building of CVD-associated drug–target networks

We collect the CVD-associated targets by two ways. First, by data mining and web search from DrugBank database (<http://www.drug.bank.ca/>), a total of 170 small molecule drugs and their 193 targets were extracted. These drugs include 15 antithrombotic agents, 3 antihemorrhagics, 1 antianemic preparations, 5 blood substitutes and perfusion solutions, 35 cardiac therapy, 16 antihypertensives, 16 diuretics, 6 peripheral vasodilators, 16 vasoprotectives, 15 beta blocking agents, 13 calcium channel blockers, 18 agents acting on the renin-angiotensin system and 14 lipid modifying agents. Second, as complement to the dataset, other 233 targets are further obtained by a large literature retrieve (Cases and Mestres, 2009), with 704 CVD relevant drugs from Therapeutic Target Database (TTD) (Zhu et al., 2011). Finally, by deleting the overlapped and abundant targets, a total of 769 drugs and 372 targets associated with CVD were compiled and indexed by DrugBank ID and UniProt ID number, respectively.

The first drug–target network (DT network) was constructed based on the reference set of drug–target interactions connecting the 769 drugs to 372 protein targets. From the bipartite DT network graph, we generate two biologically relevant network projections: target–target network and drug–drug network. In the target–target network (TT network), nodes are proteins, and two proteins are connected if they are both targeted by at least one common drug. In the complementary drug–drug network (DD network), nodes represent drugs, and two drugs are connected to each other if they share at least one target protein. All networks were generated and visualized by Cytoscape 2.8.1.

### 2.3. Building of database for herbs and druglikeness evaluation

#### 2.3.1. Database construction

We manually collected 510 medical herbs registered in Chinese pharmacopoeia with more than 31,000 ingredients and built an integrated herbal database TCM<sup>SP</sup> (<http://tcmspnw.com>). Glycosides in medicinal herbs are usually metabolized to liberate aglycone by intestinal bacteria (Nemeth et al., 2003), thus these metabolites were also added into the database. In order to eliminate the ineffective compounds in herbs, all compounds were evaluated by two druglikeness indices, i.e., oral bioavailability (OB) and Tanimoto similarity (TS).

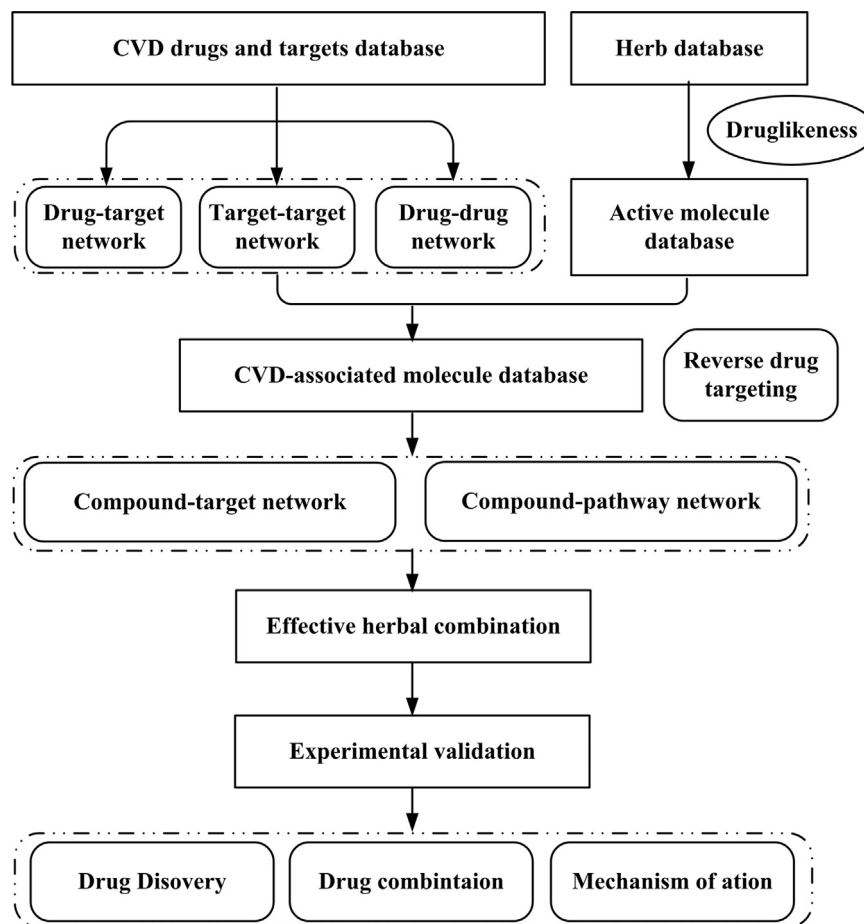


Fig. 1. Systems pharmacology strategy for discovery of herbal combinations.

### 2.3.2. Oral bioavailability

OB represents the percentage of an orally administered dose of unchanged drug that reaches the systemic circulation. In this work, the OB prediction was performed based on a robust in-house system OBioavail 1.1 (Xu et al., 2012). And the compounds with  $OB \geq 40\%$  were selected as the candidate molecules, according to the previous standard (Wang et al., 2013).

### 2.3.3. Tanimoto similarity

To remove compounds deemed to be chemically unsuitable for drugs, TS index is introduced which describes how herb compounds comparable to Western drugs (from DrugBank database). In this work, herb compounds with  $TS \geq 0.2$  were selected as candidate molecules, as the mean TS value for all DrugBank compounds is 0.18.

### 2.4. Reverse drug-targeting

The drug targeting is based on the systematic model that efficiently integrates the chemical, genomic and pharmacological information for drug–target associations, by use of two mathematical tools Random Forest (RF) and Support Vector Machine (SVM) (Yu et al., 2012). This model is supported by a large pharmacological database of 6511 drugs and 3999 targets extracted from DrugBank database. In this study, the herbal ingredients with the RF value  $\geq 0.7$  or SVM  $\geq 0.8$  were chosen as potential active compounds against CVD.

### 2.5. Network construction and screening of new drugs

All active substances in the herbs and their predicted targets were further used to build the compound–target networks in which nodes represent either compounds or proteins, and edges indicate compound–target interactions. In addition, the compound–pathway network was also constructed by linking compound and the targets-involved pathway to examine the systematic effects of herbal compounds on diseases.

To find novel herbal combinations, we hypothesize that if two agents act on the same or related functional pathways, they might act synergistically to promote drug therapy. Based on this, different herbs can be explored and further validated for potential novel drug combinations. As a case study, one optimal herbal combination DSH, which constitutes of three herbs *Radix Salviae Miltiorrhizae* (RSM; Labiatae sp. plant; Chinese name Danshen), *Carthamus tinctorius* (CT; Asteraceae plant; Chinese name Honghua) and *Fructus Cartaegi* (FC; Rosaceae plant; Chinese name Shanzha) was obtained and examined in greater detail.

### 2.6. Experimental validation

In this section, to validate the efficiency of the *in silico* screening protocol, the pharmacological activities of herbal combinations DSH were investigated by molecular biology and animal model.

#### 2.6.1. Preparation of DSH extracts

The dried slices of RSM root, FC fruit and CT flower were acquired from Beijing Tongrentang Drugstore in China. To get the

optimal herbal ratio, DSH extracts with varying weight to weight ratios of RSM:FC:CT (1:1:1, 2:2:1, 2:1:2, 3:2:2, 1:3:2, 3:1:3, 1:2:3, 2:3:3) were prepared using the L9 (3<sup>3</sup>) orthogonal array, and assessed by prevention of myocardial infarction. Each testing mixture was prepared in the corresponding ratio and extracted by water. The raw herbs were first boiled by 5-fold of water (v/w) for 2 h at atmosphere pressure. The decoction was then collected; an additional 2-fold of water was further added and boiled for 1.5 h. The second decoction was separated and mixed with the first, which was then concentrated by heating at low-atmosphere pressure condition. Finally, The RSM, FC and CT herbs in the optimal ratio of 2:2:1 (w/w) was verified and prepared in a large scale for detailed experimental investigations.

### 2.6.2. HPLC analysis

The DSH combination with the RSM, FC and CT herbs in the ratio of 2:2:1 (w/w) was analyzed with a Shimadzu HPLC system, consisting of a LC-10ATvp pump, a SPD-10Avp UV detector, a CTO-10ASvp column oven and a SCL-10Avp system controller. The original data were monitored and processed with a Class-*vp*<sup>TM</sup> software. A C18 column (250 × 4.6 mm, 5 μm) was adopted for the analysis. We qualitatively verify five compounds in the DSH combination including protocatechuic acid, protocatechualdehyde, chlorogenic acid, (–)-epicatechin, (+)-catechin and vitexin as representative of verification of the chemical composition. For protocatechuic acid, protocatechualdehyde, and chlorogenic acid, the mobile phase was comprised of MeOH–0.6% acetic acid (7:93); for (–)-epicatechin and (+)-catechin, the mobile phase was comprised of acetonitrile–water–phosphoric acid (100:900:1); and for vitexin, the mobile phase was comprised of 0.6% acetic acid–acetonitrile (86:14). The flow rate was 1.0 mL/min. Detection wavelength was set at 280, 278 and 340 nm for the above three class compounds. All the compounds were identified by comparison of the retention time.

### 2.6.3. Mice model

All experiments in this study were performed in accordance with the Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985) and Guidelines for Animal Experiments at Beijing University of Chinese Medicine. All efforts were made to minimize the number of the animals used and their suffering. Mice were ventilated with a rodent ventilator (ALC-V8, Shanghai Alcott Biotech CO., LTD.). 60 age-matched male Sprague-Dawley rats (190–220 g) were used in this study. They were fed a standard diet and water and were maintained on a 12-h light and dark cycle. A thoracotomy was performed in the left third intercostal space. A 6-0 polypropylene suture was passed under the left coronary artery (LCA) at the inferior edge of the left atrium and tied with a slipknot produce occlusion. Myocardial ischemia was verified by blanching of the LV and ST elevation in ECGs. In sham operated animals the suture was placed beneath the LCA without ligating. The thoracotomy and skin incision were closed with 3-0 silk. Extubation was performed when spontaneous respiration resumed.

Experiment animals were allocated to a total of 6 groups (N=10/each group): three DSH treated groups from low to high with extracts of 3.75, 7.5 and 15 g/kg/d, respectively; positive groups were lavage by 4.8 mg/kg/d Fosinopril; MI and Sham groups were treated with distilled water as control. All mice were administrated lasting 28 days.

To evaluate global cardiac function of animals, transthoracic echocardiography was performed with a Vevo770<sup>TM</sup> (VisualSonics, Canada) at the indicated time after MI. Mice were lightly anesthetized with 1.5% isoflurane and placed in the supine position on a heating pad. The following parameters were assessed using

M-mode: left ventricular internal dimension diastole (LVIDd), left ventricular internal dimension systole (LVIDs), left ventricular posterior wall diastole (LVPWd), left ventricular posterior wall systole (LVPWs), Left Ventricle Anterior wall diastole (LVAWd) and Left Ventricle Anterior wall systole (LVAWs). The left ventricular ejection fraction (EF) and fractional shortening (FS) were calculated using Vevo 770 (version 2.30) software.

### 2.6.4. RT-PCR analysis

The mRNA expressions of 10 predicated DSH targets, i.e., arachidonate 5-lipoxygenase (A5L), beta-1 adrenergic receptor (B1AR), cyclin dependent kinase 2 (CDK2), cGMP-inhibited 3',5'-cyclic phosphodiesterase A (CgmpA), estrogen receptor beta (ERB), mitogen-activated protein kinase14 (MAPK14), muscarinic acetylcholine receptor 2 (M2), nitric-oxide synthase endothelial (NOSE), peroxisome proliferator-activated receptor gamma (PPAR) and prostaglandin g/h synthase1 (PGS), were validated by RT-PCR analysis. Total RNA was isolated from snap frozen infarcted heart sections (infarct and remote area separated) RNA Isolation Reagent (CWbio.co.Ltd), according to the manufacturer's instruction. The β-actin was used as marker of the mRNA levels. The following primers (oligonucleotide sequences are given in parentheses in the order of forward primer and reverse primer) were designed and used: β-actin (CGTTGACATCCGTAAAGACC and CTAGGAGCCAGAG-CAGTAATC), A5L (CGGCGCGGTGGACTCCTATGA and GGGCA-GATCCTTGTGGCATTTAG), B1AR (CCGCGAGGCCAGAAACAG and GCCAGTGTCTTGAGCGCTTCT), CDK2 (GGCCCTATTCCTGGAGAC-TCTG and TCCGCTTGTGGGGTCATAGTG), CgmpA (CGCGAGGAC CAAGGAAGAGAT and CGGCCAGGAGGTCGGTAATG), ERB (GCCG-GAAGCTGACACACTACTGA and CCGGACCACATTTTTGCACTTC), MAPK14 (GGGGGAGACCTGAACAACATC and GCCGGTCATTCGT-CATCAGTGT), M2 (GGGCCACTCCAGAGATGACAACT and CCGGG-ATGGTGAGGCTTCT), NOSE (GGCGAAGCGTGTGAAGGCGACTATC and GGCCGAGGGGAGCTGTTGTAGG), PPAR (TGCCGAAGCCCTTG-TGAC and TGGCGAACAGCTGGGAGGACTC), and PGS (CGCGAG-CATAACCGTGTGTGAC and AGGCGATGCGGTTGCGGATACTG). The SYBR-GREEN RT-PCR method was used to analyze mRNA levels, and the relative expression of each protein was calculated using the comparative Ct method (2<sup>-ΔCt</sup>) (Schmittgen and Livak, 2008).

### 2.7. Statistical analysis

Variables were analyzed by Student's *t* test and one-way analysis of variance. A value of *p* ≤ 0.05 was deemed statistically significant. Results are reported as mean values ± S.E.

## 3. Results

Herbal medicines are featured as multi-component and multi-target agents, essentially acting in the same way as western drug combinations. In the past decades, molecular and pharmacological studies have accumulated comprehensive information of biological targets and relevant chemical entities (Hopkins and Groom, 2002), which leads to the emergence of systems analysis of drug actions. In this work, we aim at proposing a systems-based platform to drug discovery, particularly applied including, but not limited to medicinal herbs with applications to heart diseases.

### 3.1. CVD-associated drug–target networks

First, 372 cardiovascular targets and their corresponding 769 drugs were carefully collected and the associations between drugs and target proteins are listed Supplementary Table S1. Next, we constructed a bipartite graph consisting of two disjoint sets of nodes, with one set for protein targets and the other for drugs

(Fig. 2A). This network totally contains 1141 nodes and 1773 edges, and notably, more than 100 protein nodes are glabrous, suggesting CVD is of “target-rich and lead-poor” imbalance (Pang, 2007). These cardiovascular targets are composed of 195 enzymes, 72 ion channels, 67G-protein-coupled receptors (GPCRs), 17 nuclear receptors, 10 cytokines, 4 transporters, 4 integrins and 3 calmodulins (Fig. 2A; Supplementary Table S2). This highlights the critical roles of enzymes, ion channels and GPCRs in CVD. For instance, the number of enzyme targets is nearly half of all protein targets. More interestingly, among the enzymes, 85 out of 195 targets are still

not druggable accounting for 81.0% of all 105 undruggable targets. These data suggest that the over-representation of enzyme targets in the current cardiovascular target dataset may be partly historically biased by their tractability and druggability aspects (Cases and Mestres, 2009).

Starting from this graph, we generate two biologically relevant network projections (Fig. 2B; Supplementary Fig. S1). In the “target–target (TT) network” (Fig. 2B), nodes represent targets, and two protein targets are connected to each other if they share at least one drug. In the 372 targets, 247 have at least one link to other targets, that is, they share drugs with other targets. Among them, most targets form a large highly interconnected network with 212 nodes and 1385 edges. Drugs with multiple targets should be responsible for this high interconnectedness of the TT network. Although the TT network layout was generated independently of any knowledge about protein classes, the resulting network is naturally and visibly clustered by major protein classes. The most obvious examples are the large tightly interconnected ion channel and GPCR protein clusters, consistent to its well-ascribed roles in CVD pharmacology (Drake et al., 2006).

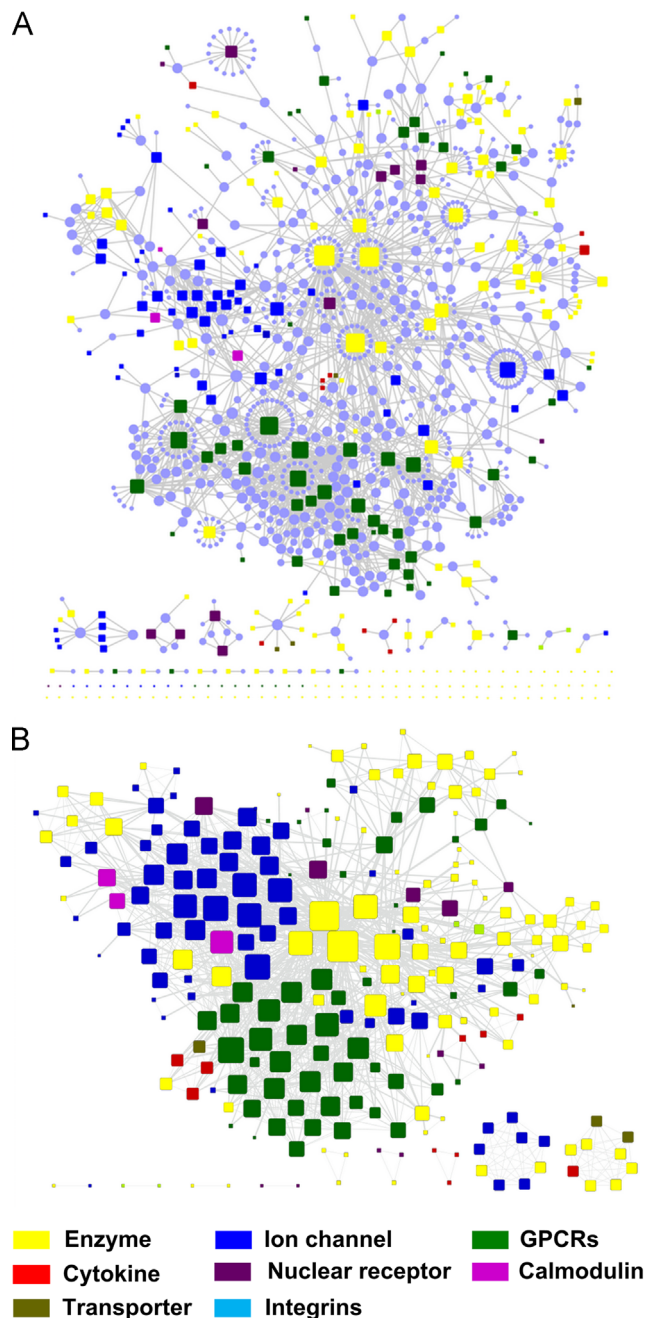
In the “drug–drug (DD) network”, nodes represent drugs, and two drugs are connected if they are associated with the same protein target (Supplementary Fig. S1). 733 out of 770 drugs are connected to other drugs, of which, most drugs (713) are integrated into a large complete network with 20,291 interconnections. Notably, the distribution of CVD drugs is well consistent to that of their corresponding protein targets in the TT network. In addition, those drugs overlap at least two different protein families are blacked. It is observed that most of these nodes are connected between enzyme drugs and other classes, implying enzymes should be relatively more promiscuous and apt to be targeted by multi drugs (Cases and Mestres, 2009).

Next, using this CVD data as an appropriate filter, we will recruit herbal medicines, to explore effective botanical drugs and combinations.

### 3.2. Pharmacologically active compounds in herbal medicines

In order to find effective herbs for CVD, we first build an herb database which includes all the 510 herbs registered in Pharmacopoeia of People's Republic of China and more than 31,000 herbal compounds in total. Now, this database can be found online (TcmSP; <http://tcmspnw.com/>). However, it is known that most herbal compounds may not be drugs due to limitations of efficacy and pharmacokinetics. Before reaching the cellular targets, compounds are usually affected by various *in vivo* factors, such as membrane permeation, plasma protein binding, metabolism, transport into and out of tissues (Li, 2012; Li et al., 2012a), i.e., the so called absorption, distribution, metabolism and elimination (ADME) (Ekins et al., 2005). However, experimental ADME assessments for medicinal herbs are extremely challenging because of the huge numbers of components in each herb. Alternatively, many *in silico* models that allow the early estimation of several ADME properties have been developed to meet this demand (Hou and Xu, 2004).

In this study, the Drug-likeness (DL) analysis was performed to identify the active compounds, which includes two indices, i.e., the human oral bioavailability (OB) and Tanimoto similarity (TS). OB represents the percentage of an orally administered dose of unchanged drug that reaches the systemic circulation. To calculate OB, we have developed a robust in-house system OBioavail 1.1 by integrating the metabolism (cytochrome P4503A4) and transport (*P*-glycoprotein) information in building reliable models (Xu et al., 2012). Here, TS is introduced to describe how herbal compounds are drug like as compared with all Western drugs in DrugBank database. By calculating OB and TS of each herbal



**Fig. 2.** CVD-associated drug–target network (DT network) and target–target network (TT network). Each node size is proportional to its degree number. The target nodes are colored according to their protein families of therapeutic relevance. (A) In the network, circles and round rectangles correspond to drugs and target proteins, respectively. A link represents the interaction between a drug and a target node. (B) In the TT network, each node is a protein target. Two proteins are connected if they are targeted by the same drug. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ingredient, we constructed an active molecule database which contains 425 herbs and 2003 compounds with  $OB \geq 40\%$  and  $TS \geq 0.2$  (Supplementary Table S3). The average number of active compounds per herb is 4.8, and more than 90% (29,868) herbal compounds and 17% (85) herbs have been removed by this screening process.

### 3.3. Reverse drug targeting

The reverse-targeting tactic for screening potential CVD-associated compounds is based on the interaction networks between the ligands and targets, which combines the chemical, genomic and pharmacological information of drugs and targets (Yu et al., 2012). By this technique, all the 273 CVD-associated targets were used to “recognize” the 2003 potential active compounds, resulting in generation of a CVD-associated chemical database. This database totally contains 424 herbs (99.7% of all 425 active herbs) and 1899 compounds (94.8% of all 2003 compounds) (Supplementary Table S4). Detailed analysis suggests that these herbs are mainly divided into three parts: (1) with few targets; (2) with few ingredients but many targets; and (3) with many ingredients and targets. In this work, the last one which is representative for multi-component and multi-target agents was selected for further analysis (Zimmermann et al., 2007).

To get deep pharmacological profiling of these herbal medicines, the corresponding target space was analyzed in the background networks (DT and TT networks). It is found that these herbs totally interact with 76 CVD-associated targets which cover most of functional classes, i.e., 26 enzymes, 11 ion channels, 31 GPCRs, 7 nuclear receptors and 1 calmodulin. Their degrees distribute homogeneously from 0 to 64 and from 1 to 68 over the DT network and TT network, respectively. Interestingly, this distribution highlights the importance of GPCRs for herbal remedy, as this protein family is most frequently targeted by herbal compounds. In addition, these targets include most critical GPCRs such as histamine receptor type 1 (degree=64 in DT network and 31 in TT network), alpha-1A adrenergic receptor (degree=54 in DT network and 50 in TT network) and muscarinic acetylcholine receptor 1 (degree=53 in DT network and 32 in TT network) (Supplementary Table S5). All this indicates that both in traditional and modern medicine, GPCRs play key roles in treatment of diseases.

In this section, the CVD-associated molecule database is built, which contains a wealth of chemical and pharmacological data about herbal medicines. Starting from this, we can obtain a systems level investigation of herbal remedy for designing novel botanical drug combinations.

### 3.4. Physicochemical property analysis

In order to get more knowledge about these compounds, we have considered 8 molecular descriptors including MW (molecular weight), nCIC (number of rings), nHDon (number of hydrogen bond donor), nHAcc (number of hydrogen bond acceptor), RBN (number of rotatable bonds), Hy (hydrophilic factor), TPSA (topological polar surface area) and MlogP (Moriguchi octanol-water partition coefficient), since these parameters can reflect the basic characteristics of a molecule especially its pharmacokinetic properties (Feher and Schmidt, 2003; Grabowski and Schneider, 2007). In this section, the CVD-associated drugs and all DrugBank compounds are also included for comparisons. Fig. 3 shows the distributions of each descriptor for the three groups. Herbal compounds peaks at a similar position as those in CVD-associated drugs and DrugBank drugs. Interestingly, herbal compounds have obviously narrower distributions than other entities, suggesting that herbal compounds are less diverse than those in the other classes.

The average values for these descriptors are shown in Table 1. Except MW, all descriptors of herbal compounds have pronounced differences from those of DrugBank drugs. Similarly, each descriptor of CVD-associated drugs is significantly different from that of DrugBank drugs, except MW and RBN. This is reasonable since both CVD-associated drugs and the obtained herbal compounds are more specific to CVD. Both nCIC and MlogP of CVD-associated drugs ( $2.75 \pm 0.06$  and  $2.07 \pm 0.07$ ) and herbal compounds ( $3.32 \pm 0.02$  and  $2.44 \pm 0.04$ ) are larger than those ( $2.38 \pm 0.02$  and  $1.24 \pm 0.03$ ) of DrugBank drugs. However, nHDon, nHAcc, Hy and TPSA(tot) for CVD-associated drugs ( $1.93 \pm 0.08$ ,  $5.21 \pm 0.14$ ,  $0.45 \pm 0.06$  and  $75.98 \pm 2.31$ ) and herbal compounds ( $2.20 \pm 0.03$ ,  $4.81 \pm 0.04$ ,  $0.56 \pm 0.03$  and  $76.72 \pm 0.77$ ) are smaller than those ( $3.12 \pm 0.04$ ,  $6.46 \pm 0.06$ ,  $1.44 \pm 0.03$  and  $108.46 \pm 1.01$ ) of DrugBank drugs. It is interesting that herbal compounds exhibit similar average patterns to the CVD-associated drugs. However, statistical analysis indicates five out of all eight descriptors between CVD-associated drugs and herbal compounds are significantly different with  $p < 0.001$ , including nCIC ( $2.75 \pm 0.06$  versus  $3.32 \pm 0.02$ ), nHDon ( $1.93 \pm 0.08$  versus  $2.20 \pm 0.03$ ), nHAcc ( $5.21 \pm 0.14$  versus  $4.81 \pm 0.04$ ), RBN ( $5.12 \pm 0.15$  versus  $3.17 \pm 0.06$ ) and MlogP ( $2.07 \pm 0.07$  versus  $2.44 \pm 0.04$ ).

### 3.5. Optimization of herbal combination

Because multiple agents in one combination were usually endowed with different roles (Kong et al., 2009), thus we first try to find the major drug which plays dominant role in treating disease. The hub proteins in the DT and TT networks might play central roles in CVD therapy and should receive more concerns. For example, beta-1 adrenergic receptor (degree=33 in DT network and 41 in TT network) and beta-2 adrenergic receptor (degree=39 in DT network and 29 in TT network) are involved in calcium signaling pathway and neuroactive ligand-receptor interaction, as well as in the maintenance of cardiac homeostasis ([www.genome.jp/kegg](http://www.genome.jp/kegg)). Indeed, these beta adrenergic receptors play well-ascribed roles in cardiovascular biology and have been therapeutic targets for a variety of clinical conditions including hypertension, coronary artery disease, heart failure, and asthma (Devic et al., 2001). Therefore, we hypothesize that those herbs that target more hub proteins should be more important. It is found that many herbs in the CVD-herbs database are of high target connections, such as *Eucommiae Cortex* (43 targets), *Radix Salviae* (37 targets), *Corydalis Rhizoma* (35 targets) and *Evodiae Fructus* (34 targets) (Table S6). Interestingly, many herbs have also been demonstrated to have therapeutic effects on CVD, supporting the reasonability of these screening models. For example, *Corydalis Rhizoma* can reduce infarct size and improves heart function during myocardial ischemia/reperfusion by inhibiting apoptosis in rats (Ling et al., 2006).

RSM, one of the most widely applied medical herbs in TCM, also has cardiovascular protective effects, such as improving microcirculation, causing coronary vasodilatation, suppressing the formation of thromboxane, inhibiting platelet adhesion and aggregation, and protecting against myocardial ischemia (Cheng, 2007; Gao et al., 2012). Consistently, this herb has 37 CVD-associated targets, including most hub proteins such as alpha-1A adrenergic receptor (degree=54 in DT network), muscarinic acetylcholine receptor 1 (degree=53), dopamine receptor type 2 (degree=50) and gamma aminobutyric acid gated-receptor type A (degree=46). In addition, we further find that RSM target many CVD-associated pathways, i.e., neuroactive ligand-receptor interaction, VEGF signaling pathway, regulation of actin cytoskeleton, PI3K-Akt signaling pathway, endocrine and other factor-regulated calcium reabsorption, calcium signaling pathway, complement and coagulation cascades, serotonergic synapse, NF-kappa B signaling

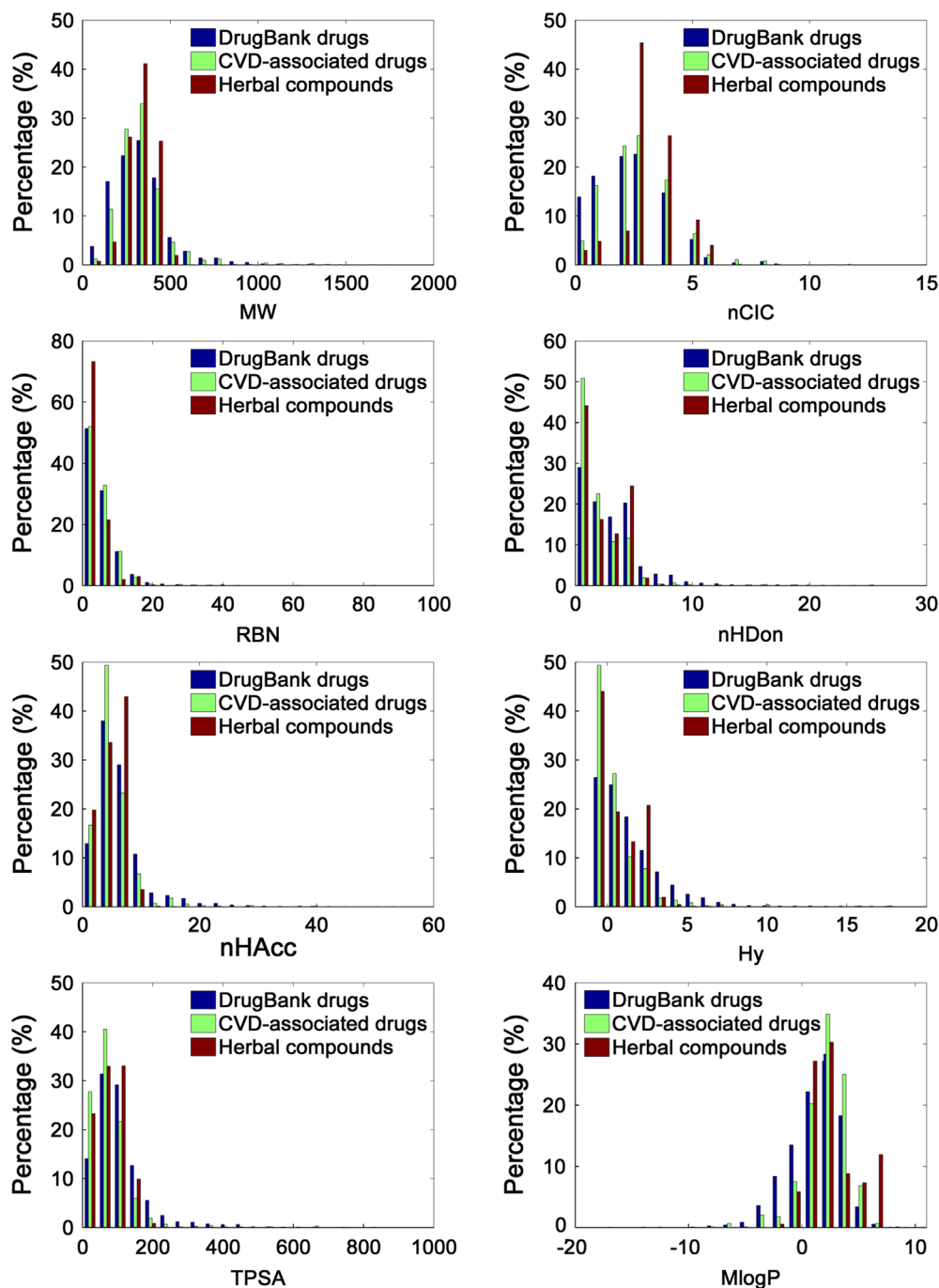


Fig. 3. The profile distributions of eight important molecular properties for DrugBank drugs, CVD-associated drugs and herbal compounds.

Table 1

Comparison of molecular properties between DrugBank drugs, CVD-associated drugs and herbal compounds.

	MW	Ncic	RBN	nHDon	NHAcc	Hy	TPSA(Tot)	MlogP
DrugBank drugs	340.12 ± 2.36	2.38 ± 0.02	5.51 ± 0.07	3.12 ± 0.04	6.46 ± 0.06	1.44 ± 0.03	108.46 ± 1.01	1.24 ± 0.03
CVD-associated drugs	340.25 ± 5.76	2.75 ± 0.06*	5.12 ± 0.15	1.93 ± 0.08*	5.21 ± 0.14*	0.45 ± 0.06*	75.98 ± 2.31*	2.07 ± 0.07*
Herbal compounds	332.91 ± 1.48	3.32 ± 0.02*#	3.17 ± 0.06*#	2.20 ± 0.03*#	4.81 ± 0.04*#	0.56 ± 0.03*	76.72 ± 0.77*	2.44 ± 0.04*#

\*  $p < 0.001$  versus DrugBank drugs.

#  $p < 0.001$  versus CVD-associated drugs.

pathway and HIF-1 signaling pathway. All these demonstrate that RSM is the first choice for a major drug in building drug combinations.

In a drug combination, it is also suggested that those drugs act through similar physiological systems (functional pathways) are thought to enhance drug actions (Spinella, 2002). Indeed, analysis

of those well-known drug combinations indicate that most drug pairs in one combination tend to act on the same/related functional pathways or biological processes (Xu et al., 2010; Zhao et al., 2011; Wang et al., 2012b). And different herbs acting on similar physiological system (functional pathways) might produce pharmacological synergy between drugs (Ma et al., 2009; Wang et al., 2012a).

Therefore, the ten signal pathways most related to RSM were utilized to recruit other herbs in the CVD-associated herbal database. This leads to find many herbs with high pathway overlapping ratios with RSM, such as *Panax Notoginseng* (Araliaceae plant; Chinese name Sanqi), *Radix Astragali* (Leguminosae plant; Chinese name Huangqi), *Caulis Spatholobi* (Leguminosae plant; Chinese name Jixueteng), *Semen Persicae* (Rosaceae plant; Chinese name Taoren), *Carthamus tinctorius* (CT; Asteraceae plant; Chinese name Honghua) and *Fructus Cartaei* (FC; Rosaceae plant; Chinese name Shanzha). Extremely surprising, most of these herbs have been long applied in combination with RSM to treat CVD in clinic practice (State Pharmacopoeia Commission of the PRC, 2005). The most well-known example, i.e., Compound Danshen Formula (CDF), which mainly includes RSM and *Panax Notoginseng*, is officially registered in Chinese Pharmacopoeia and has been widely used to treat CVD in China, Japan, and United States and Europe (Chu et al., 2011). Finally, we select FC and CT as two complementary components to build a combination DSH, since this combination has not been clinically applied. The further efficacy evaluation of this new combination will not only justify our methodology but also possibly generate a new drug formula for treating CVD.

Fig. 4 shows a global view of the bipartite graph of compound-pathway network for DSH compounds (circle) or pathways (square). It is clear that the two herbs interact with all the ten pathways, implying they might coordinate with RSM to produce pharmacological effects. For example, in the neuroactive ligand-receptor interaction pathway, many drug targets such as adrenergic receptor, angiotensin receptor, calcitonin receptor-like, neurotensin receptor are found critical for cardiac function. Further analysis indicates this pathway can be modulated by 35

RSM compounds, 11 FC compounds and 9 CT compounds, suggesting the potential synergistic actions between these molecules (more details in sections “DSH” and “experimental validation”).

### 3.6. DSH combination

Each medical herb usually contains considerable numbers of chemical compounds, and its therapeutic effects mainly depend on the effective substances. In the DSH combination, to the most extent 731 compounds were collected, including 210 in RSM, 312 in FC and 209 in CT, respectively. After ADME and reverse-targeting screening, most compounds have been excluded and only 82 potential active compounds remain for further study, consisting of 40 in RSM, 24 in FC and 18 in CT, respectively (Table 2; Supplementary Table S7). Interestingly, many compounds have been reported as bioactive ingredients for CVD treatment. For example, in RSM, miltirone II presents sedative activity and is a benzodiazepine receptor agonist (Chang et al., 1991); cryptotanshinone and tanshinone VI can protect the myocardium against ischemia-induced derangements by eliciting a significant enhanced recovery of the contractile force upon reoxygenation (Yagi et al., 1989). In FC, icariin produces NO-dependent relaxation in the precontracted coronary artery, due to activation of eNOS protein and NO-cGMP pathway (Xu and Huang, 2007). In CT, Syringaresinol causes vasorelaxation by activating the vascular eNOS/NO/cGMP pathway (Chung et al., 2012). In addition, to verify the chemical composition of the phytocomplex, we have qualitatively verified five compounds in the DSH combination including protocatechuic acid, protocatechualdehyde, chlorogenic acid, (–)-epicatechin, (+)-catechin and vitexin as representative. It was found that all these five compounds were identified in the extract, indicating the reliability of our analysis (Supplementary Fig. S2).

In order to illustrate the molecular basis for DSH, a systematic analysis was performed based on the target profile of DSH. We use DSH potential compounds and their CVD-associated targets generate a bipartite graph of compound-target network (Fig. 5). In this network, it is found that the DSH combination bind to 52 CVD-associated targets, which account for more than half of the 76 CVD-associated targets interacting with all active herbal

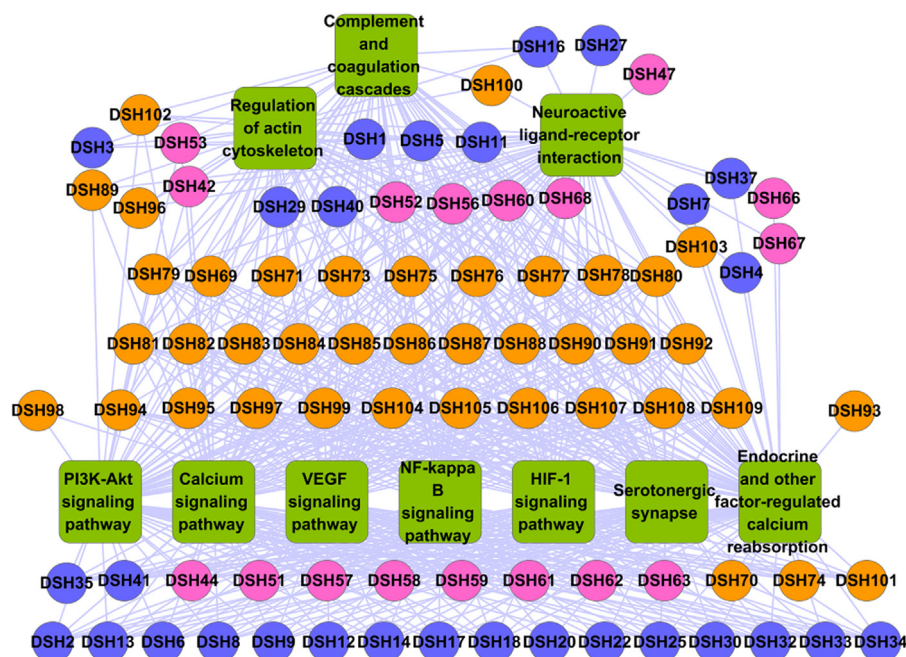
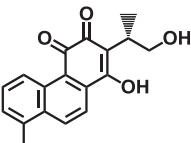
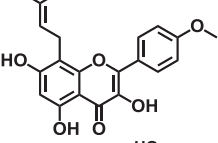
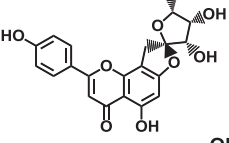
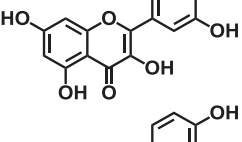
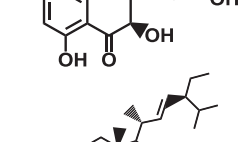
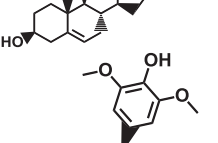
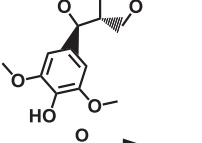
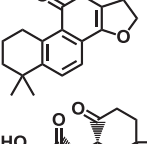
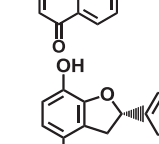
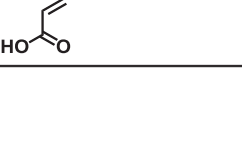


Fig. 4. Compound-pathway network. Circles and round rectangles correspond to DSH compounds and functional pathways, respectively. A link is placed between a drug node and a pathway node if the protein targets of the drug reside in the pathway. nodes (circles) are colored according to their plant origins (purple: FC orange: RSM, pink: CT). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Table 2**

Some representative CVD-associated compounds from DSH combination consisting of RSM, TC and CT, and corresponding calculated oral bioavailability (OB) and Tanimoto similarity (TS).

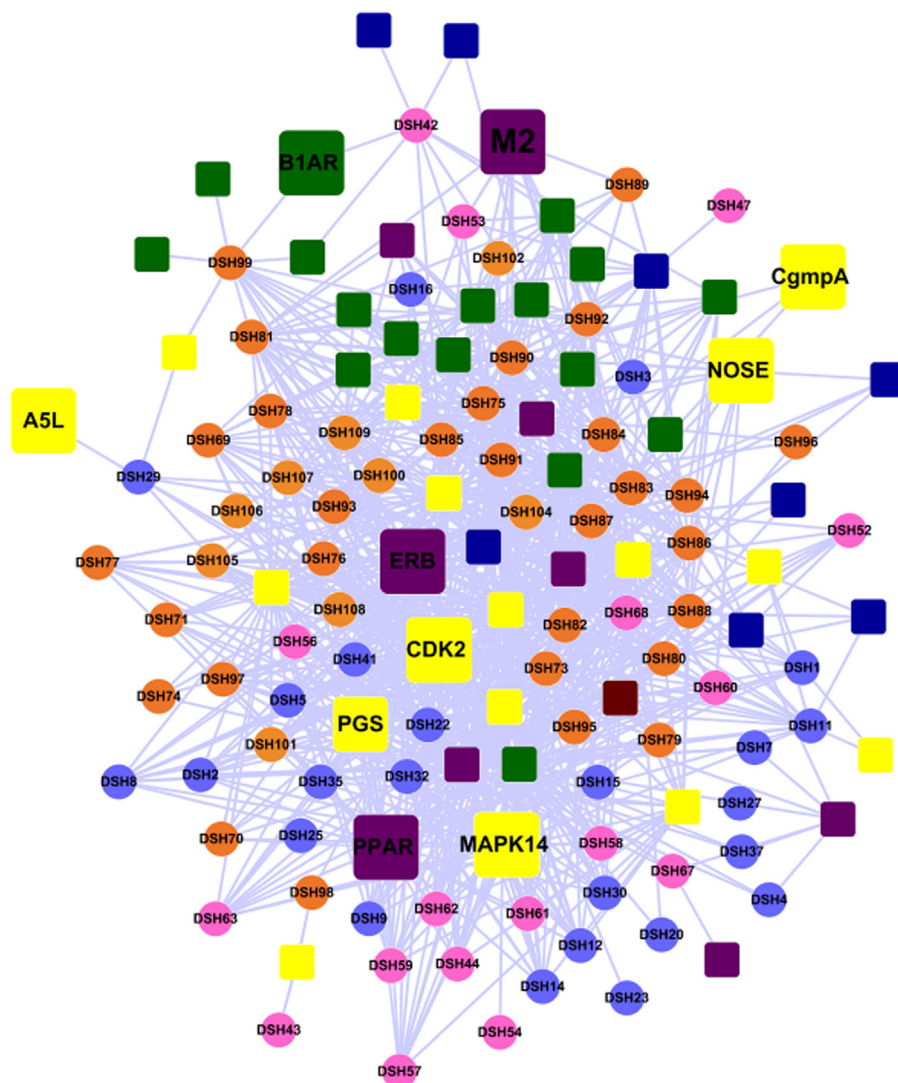
No.	Compound	Structure	OB	TS	Herbs
DSH108	Tanshinone VI		61.70	0.30	RSM
DSH11	Icariin <sub>qt</sub> <sup>a</sup>		41.30	0.44	FC
DSH14	Kaempferol		42.30	0.24	FC CT
DSH32	Rutin <sub>qt</sub> <sup>a</sup>		48.68	0.28	FC
DSH35	Taxifolin		61.97	0.27	FC
DSH67	Stigmasterol		43.83	0.76	CT RSM FC
DSH68	Syringaresinol		71.39	0.72	CT
DSH81	Cryptotanshinone		52.44	0.40	RSM
DSH96	Miltirone II		44.95	0.24	RSM
DSH97	Prolithospermic acid		64.30	0.31	RSM

All DSH compounds are shown in Supplementary Table S7.

<sup>a</sup> qt represents the molecule with deglycosylation.

molecules. These targets consist of 17 enzymes, 19 GPCRs, 8 ion channels, 7 nuclear receptors and 1 calmodulin. To further assess the importance of these compound-target proteins, we overlaid

the compound-target network onto the background maps (DT network and TT network). In the background networks, the target nodes with high degrees generally represent the well-known



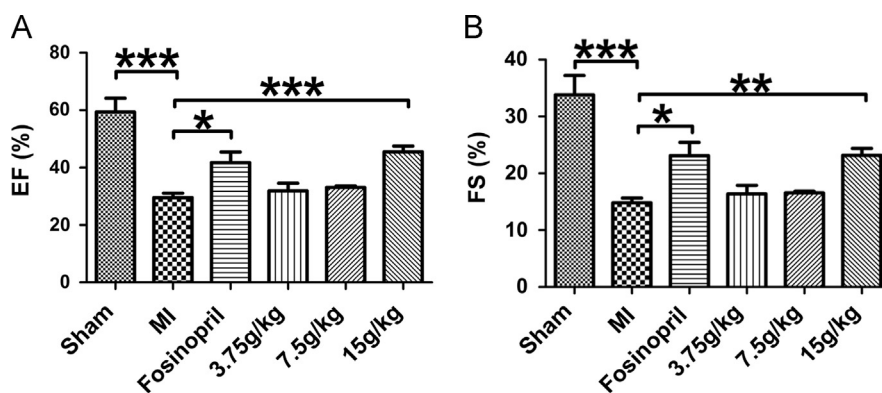
**Fig. 5.** Compound-target network. Circles and round rectangles correspond to DSH compounds and protein targets, respectively. A link is placed between a compound node and a target node if the protein is predicted to be a target of the compound. compound nodes (circles) are colored according to their plant origins (purple: FC orange: RSM, pink: CT), and proteins (round rectangles) are colored according to their protein families of therapeutic relevance. The ten selected proteins, i.e., A5L, B1AR, CDK2, CgmpA, ERB, MAPK14, M2, NOSE, PPAR and PGS are highlighted and labeled. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

targets while those targets with low degrees are newly found or still in exploring state (Yildirim et al., 2007). Exactly, most DSH targets are highly connected proteins, of which, nearly all GPCRs involve in the local communities of the DT and TT networks (Fig. 2; Supplementary Table S8). In addition, some peripheral proteins are also targeted by DSH, such as peroxisome proliferator activated gamma, nitric-oxide synthase endothelial, cyclin dependent kinase 2 and phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit gamma isoform.

However, despite these potentially interesting associations, cautious interpretation is warranted as these findings solely relied on theoretical analysis. To confirm these interactions, 10 representative targets including both the hub and peripheral proteins are determined for further experimental tests (section “experimental validation”). The proteins are arachidonate 5-lipoxygenase (A5L), beta-1 adrenergic receptor (B1AR), cyclin dependent kinase 2 (CDK2), cGMP-inhibited 3',5'-cyclic phosphodiesterase A (CgmpA), estrogen receptor beta (ERB), mitogen-activated protein kinase14 (MAPK14), muscarinic acetylcholine receptor 2 (M2), nitric-oxide synthase endothelial (NOSE), peroxisome proliferator-activated receptor gamma (PPAR) and prostaglandin synthase1 (PGS) (Fig. 5).

### 3.7. Experimental validation

In this section, we investigate the therapeutic effects of DSH using a well-characterized animal model of myocardial infarction (MI). MI mice are treated with oral doses of DSH at 3.75, 7.5 and 15 g/kg each day, and conducted echocardiography after 28 days. As shown in Fig. 6 of echocardiographic assessment of ventricular remodeling, EF and FS are significantly decreased after MI compared with sham ( $29.53 \pm 1.57\%$  EF and  $14 \pm 0.81\%$  FS for MI groups versus  $59 \pm 4.75\%$  EF and  $33.82 \pm 3.34\%$  FS for shams;  $p < 0.001$ ), indicating the occurrence of remodeling in animal models. For groups treated with DSH, EF is better maintained with 15 g/kg treatment as compared to the MI control ( $45.47 \pm 2.04\%$  versus  $29.53 \pm 1.57\%$ ;  $p < 0.001$ ; Fig. 6A). Similarly, FS is preserved in the treated mice ( $23.18 \pm 1.20\%$  versus  $14 \pm 0.81\%$ ;  $p < 0.01$ ; Fig. 6B). Its preserved effect is not different from the positive control treated with Fosinopril ( $45.47 \pm 2.04\%$  EF and  $23.18 \pm 1.20\%$  FS for 15 g/kg treatment groups versus  $41 \pm 3.64\%$  EF and  $23.23 \pm 0.82\%$  FS for Fosinopril groups;  $p > 0.05$ ). However, DSH at 3.75 and 7.5 g/kg do not improve either the EF ( $31 \pm 2.69\%$  and  $33 \pm 0.56\%$ ; Fig. 6A) or FS ( $16.38 \pm 1.49\%$  and



**Fig. 6.** Treatment of DSH improves myocardial function in mice with myocardial infarction. The *in vivo* cardiac function is assessed by echocardiography. Both (A) ejection fraction (EF) and (B) fractional shortening (FS) are improved after 28 treatment of DSH at 15 g/kg, which is not different from the positive control treated with Fosinopril ( $n=10$ ; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ).

$16.55 \pm 0.30\%$ ; Fig. 6B), as compared to MI controls ( $29.53 \pm 1.57\%$  EF and  $14 \pm 0.81\%$  FS;  $p > 0.05$ ).

Furthermore, to evaluate the molecular details of DSH, the expressions of the ten selected targets (Fig. 5) are measured by RT-PCR in the groups of MI, sham and the 15 g/kg treatment (Fig. 7). It is found that the expression levels of mRNAs A5L, B1AR, CDK2, MAPK14, M2, NOSE, PPAR and PGS are unaffected and the expressions of CgmpA and ERB are sharply decreased in MI mouse in comparison to sham group. Comparing to MI groups, Except M2, all the mRNA levels of genes A5L, B1AR, CDK2, MAPK14, NOSE, PPAR and PGS are strongly increased after treating with DSH for 28 days. This result confirms the effectiveness of our predicted model.

To examine the relationships between these changed proteins, a target–pathway network was constructed based on the interactions between these proteins and the pathways they reside (Fig. 8). This network consists of 62 nodes and 64 edges, and all proteins belong to more than one signaling pathways and are connected into a highly interlinked network. MAPK14 exhibits the highest number of pathway connections (degree=26), followed by CDK2 (degree=13), PPAR (degree=6), NOSE (degree=6), B1AR (degree=6), CgmpA (degree=4) and PGS (degree=3). Out of all 55 pathways in this network, 10 pathways are found to be related to CVD, i. e., PI3K-Akt signaling pathway (Shiojima and Walsh, 2002; Tsang et al., 2004), calcium signaling pathway (Drab et al., 2001; Touyz, 2005), VEGF signaling pathway (Cross and Claesson-Welsh, 2001; Olsson et al., 2006), neuroactive ligand-receptor interaction (Liu et al., 2010), serotonergic synapse (Rothman and Baumann, 2009), arachidonic acid metabolism (Hohl and Rösen, 1987), arginine and proline metabolism (Morris, 2005), PPAR signaling pathway (Tontonoz and Mangelsdorf, 2003), p53 signaling pathway (Ho et al., 2007) and HIF-1 signaling pathway (Semenza, 2000). Except CgmpA, all proteins connect to at least one CVD-associated pathway. In addition, it is also found, some target proteins share the same functional pathways with each other. For example, PI3K-Akt signaling pathway is shared by CDK2 and NOSE, and calcium signaling pathway is shared by B1AR and NOSE. These targets residing in the same or related functional pathways might produce more-than-additive effects, suggesting the systematic modulation of DSH at the network level to treat heart disease.

#### 4. Discussion

In this work, based on systems pharmacology, we propose a novel efficient approach combining theoretical and experimental methodologies for drug discovery and combination. CVD associated target and pharmacological space was initially integrated and analyzed by network techniques. Then, the therapeutic

networks were further used to interrogate herbal medicines to explore potential drugs through reverse targeting approach. Finally, the therapeutic effects of the selected herbal combination based on pathway-focused approach were experimentally validated by animal model with myocardial infarction. We hope our work could facilitate the exploration of drug discovery and combination, especially for those complicate systems, such as herbal medicine.

##### 4.1. Overcome the predicament of drug discovery

Past decades has encountered an unprecedented predicament of drug discovery: despite the rapid advances in the knowledge regarding complex biological processes and striking increases in the cost of drug development, pharmaceutical productivity has been continuously declined (Technical report, US Food and Drug Administration, 2010). It has been realized that this declination is mainly attributed to the intrinsically flawed reductionistic approach to drug development focused on identifying “magic bullets” directed against individual molecular target to entirely reverse a given disease phenotype (Chan and Loscalzo, 2012). Actually, human disease rarely results from an abnormality in a single molecular effector. Rather, they are nearly always the net result of multiple pathobiological pathways that interact through an interconnected network. Increasing attention is therefore being given to multi-target therapeutics which depends on shared effectors and pathways in reversing the disease phenotype (Jia et al., 2009).

However, this story has only just begun, as the complexity of multi-target action severely challenges the exploration of drugs or best drug combinations. Indeed, direct screens *in vitro* can identify potential synergistic drugs of a specific bioactivity, but necessitates expensive and massive searches of a vast chemical space by cell-based experiments (Borisy et al., 2003; Zhang et al., 2007). Instead, network-based approaches that address complex interactions in biological system, could, therefore, produce greater levels of efficacy. This is best illustrated in the area of bacterial and human metabolism. The analysis of metabolic network has recently led to the testing and identification of some potential new agents and combinations (Folger et al., 2011; Kim et al., 2011; Spitzer et al., 2011). However, this approach requires high accuracy of metabolic network and validation of novel drug targets, which seriously burden the early phase of this project. Actually, till now, years of efforts from pharmaceutical community have accumulated numerous target data with proven association with pathogenic behavior, especially for those diseases with great concern, just like cardiovascular disease.

Therefore, depending on the existing therapeutic targets, we here build CVD-associated networks including drug–target network as well as deuterogenic protein–target and drug–drug

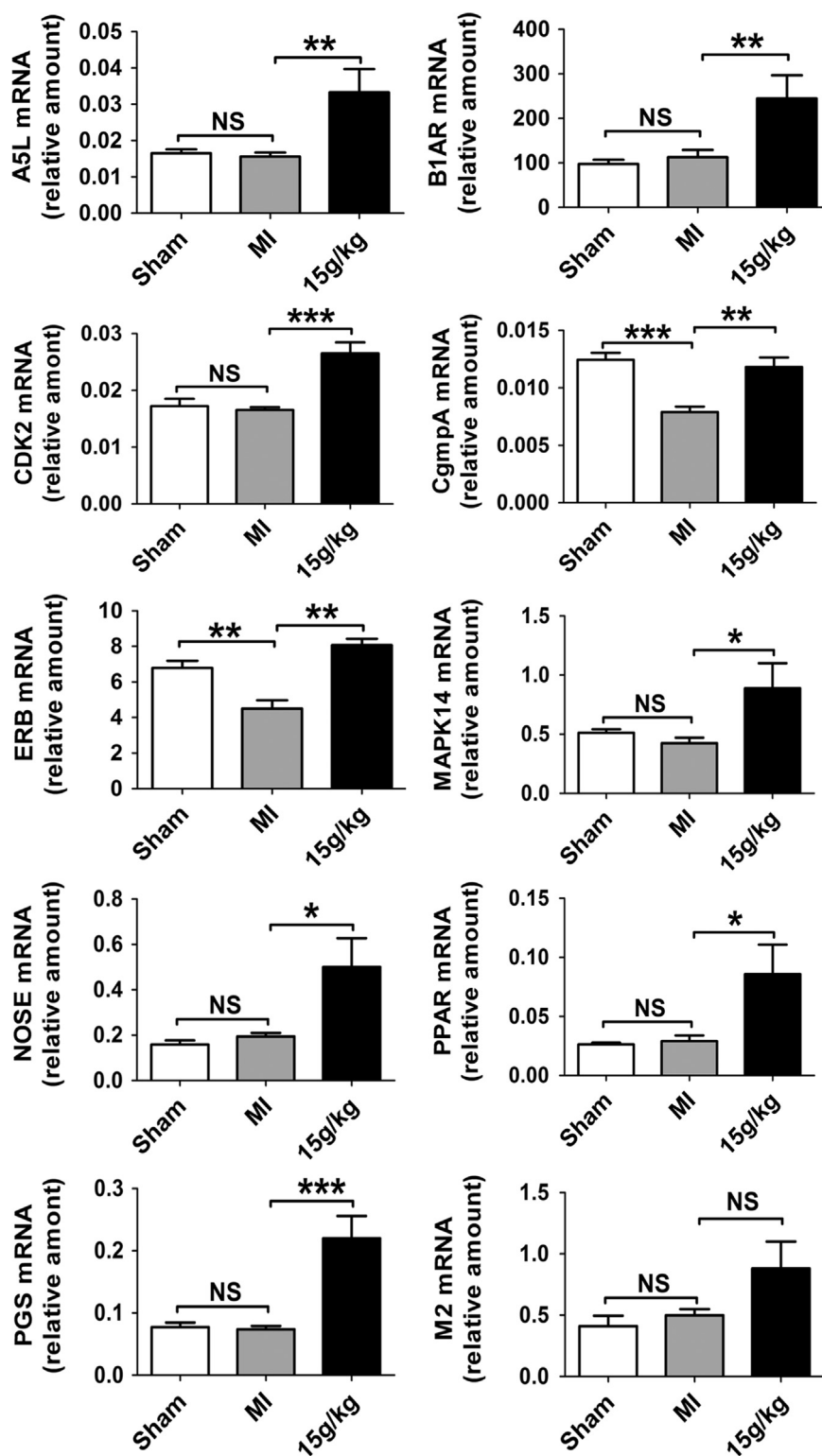
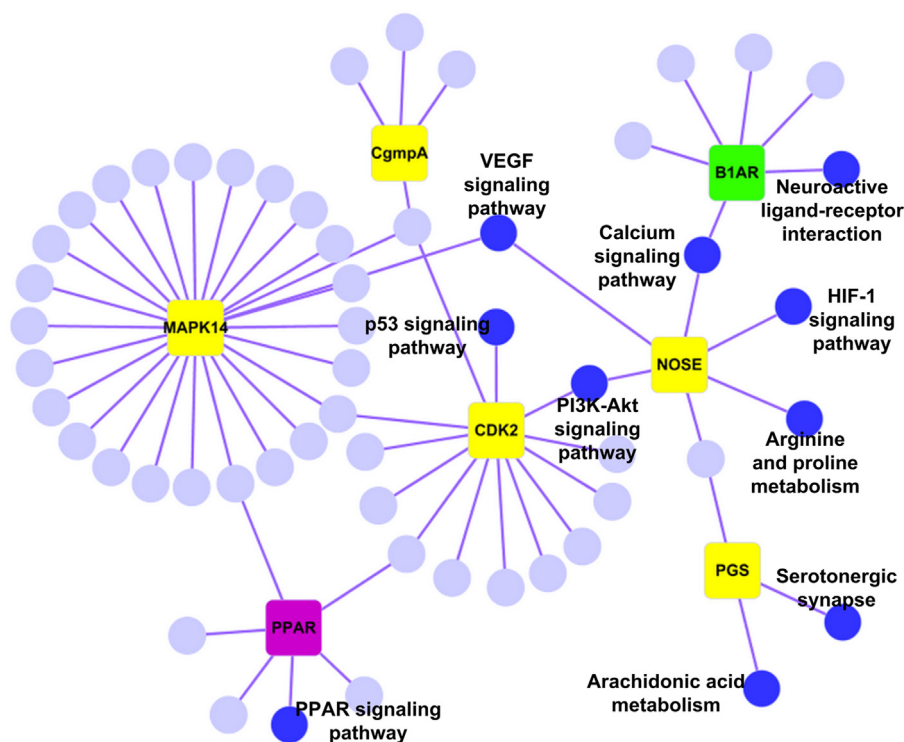


Fig. 7. DSH treatment affects the mRNA expression levels of CVD-associated targets in mice with myocardial infarction. 28 days later, the mRNA expression levels of 10 selected genes (Fig. 5) are measured by RT-PCR. Relative amount of each gene is expressed using  $2^{-\Delta\Delta C_t}$ .  $n=3$ ; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; NS, not significant.

networks. Either CVD-associated targets or corresponding drugs are delicately annotated and prioritized in the network context. These targets can be organized into the main protein families to obtain relatively clearer picture of therapeutic position and relationship between cardiovascular targets (Harris and Stevens, 2006). Knowledge of these therapy-related networks can provide solid basis for the discovery of new drug combinations and

multi-targeted agents, for example, (1) the overall therapeutic targets can be used to screen effective compounds or herbs for certain disease such as CVD; (2) the topology analysis of networks can find the critical targets or target groups, pathways, which can be further applied for rapid drug screening and combinations. In addition, the network analysis also helps identify drug 'communities' that act similarly at the molecular target level. Analysis of



**Fig. 8.** Target-pathway network. Round rectangles and circles correspond to affected targets by DSH and functional pathways, respectively. A link between a target node and a pathway node represents the protein target is included in the pathway. Target nodes (circles) are colored according to their protein families of therapeutic relevance, and CVD-associated pathway nodes are blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

this drug space can deepen our understanding of the CVD pharmacological features (Kong et al., 2009). Clearly all this may be integrated together to further enhance our model for drug discovery.

#### 4.2. Solution to complexity of herbal medicines

Currently, the multi-component and multi-target features make herbal medicines get more and more attentions both in theoretical field and in practical application (Qiu, 2007; Kong et al., 2009; Verpoorte et al., 2009). However, there clearly exists a gap between increasing interests and botanical drug discovery as the mode of action of herbal medicines related to the therapeutic effectiveness is generally not known. Conventionally, drug development from herbs follows a separation, purification, and structure elucidation way to identify discrete valid entities. This approach obviously cannot cope with the complex and considerable compositions in herbs. Hence, some approaches that devote to overcome the complexity of herbal medicines are required. In this work, we try to solve this problem in three aspects:

- (1) *herb information standardization.* Redundant data of herbal medicines including molecular structure, biological relevance, physiological and chemical properties were systemically and hierarchically stored in a well-organized database. In total, an herb molecule database (TcmSP; <http://tcmspnw.com/>) which includes 510 herbs and more than 31,000 herbal compounds, as well as their corresponding properties were built. Despite an initial cumbersome process needed, in this case, the resultant data set can be handled more synchronously and standardly to serve botanical drug discovery and combination.
- (2) *ADME screening.* In herbs, most ingredients have unfavorable pharmacological activities. In some cases, the poor efficacy of herbal agents may be attributed to buffering effects of those non-viable components. To eliminate this unfavorable

interference, we introduce two ADME prediction models including OB and TS to evaluate them. In this step, we calculate OB and TS of each herbal compound and constructed a potential active molecule database with drug-like compounds. In our work, more than 90% (29,868) herbal components are filtered out by this prescreening process. This superhigh filterability suggests the screening criteria might have been rather strict.

- (3) *compound physicochemical property analysis.* To get a deeper understanding of chemical and pharmacological spaces for herbal molecules, we perform a systematic comparison between marketed CVD drugs and natural products. The obtained results suggest that herbal compounds have their unique characteristic in comparison to Western drugs, although both chemical entities have similar pharmacological activities. All these steps are helpful to find novel potential compounds of interest, thus accelerating drug discovery.

#### 4.3. Shortcut to drug combination

The DT network contains many highly connected hub proteins, which are substantially linked by multiple drugs, and typically play central roles in disease therapy. Moreover, most of these proteins also display many connections to other targets in TT network. The most obvious example of clustering is the large tightly interconnected GPCR cluster, which are caused by so called promiscuous drugs with multiple targets. Indeed, promiscuous drugs can enhance the potency of other drugs and have a potentially valuable use in accelerating the search for synergistic drug combinations (Cokol et al., 2011). In addition, the investigation of drug-target protein essentiality indicates that they generally have lesser essentiality compared to those essential proteins (Yildirim et al., 2007), and thus we do not necessarily worry about the consequence of too much toxicity for targeting these hub proteins. All this factors strongly

support the reasonability of using the hub proteins in drug–target networks to recruit the novel CVD-related drugs. Therefore, in this work we speculate that those herbs that target more hub proteins in DT and TT network should be more important for disease treatment (Yildirim et al., 2007; Li et al., 2011). Based on this, many eligible medical herbs can be identified from the herb database, such as *Eucommiae Cortex*, *Radix Salviae*, *Corydalis Rhizoma* and *Evodiae Fructus*. Interestingly, those well-documented clinical data show that most of these herbs have been applied in clinical remedies for CVD (State Pharmacopoeia Commission of the PRC, 2005), corroborating this screening process.

In addition, the compounds acting on the related pathways might have potential synergistic interactions. As we know, the complex diseases are highly relevant with the highly sophisticated, delicate regulatory pathways and feedback loops where certain biochemical protein targets reside. Certain proteins, such as those upstream of a signaling pathway, may directly interact with multiple protein molecules and affect different signaling pathways. Drugs interacting with this protein may result in varied effects, including both beneficial and others detrimental. Perturbation on protein downstream of a signaling pathway may have a smaller impact than interaction with upstream proteins (Zhao and Iyengar, 2012). However, it also usually malfunctions due to the high robustness of biological network, such as the backup mechanisms of isozymes and alternative pathways (Stelling et al., 2002). Accordingly, components simultaneously impact the set of pathways (same or separate) related to the phenotype may be likely to create a better combination effect. It is reasonable as agents in most of existing drug combinations have been found to possess similar therapeutic effects and work in the same or related pathways (Jia et al., 2009; Xu et al., 2010; Wang et al., 2012a). For example, the drug combination Bactrim including sulfamethoxazole and trimethoprim impacts two targets in the folate biosynthesis pathway in bacteria, where sulfamethoxazole targets the upstream DHPS and trimethoprim targets the downstream DHFR. Trimethoprim serves as a backup when sulfamethoxazole becomes less effective (Jia et al., 2009).

Therefore, we here seek combinational drugs based on their corresponding related pathways. As each medical herb usually targets a group of proteins and pathways for the specific function (Zhao et al., 2010; Sun et al., 2012), we hypothesize two herbs that target the common or related pathway groups are likely to work synergistically. Then, we systematically compared the relevant pathway groups of different CVD-associated herbs and discovered that many of them are suitable for combinatorial therapies. Especially, some predicated herbal combinations have been clinically administered for long. For example, the predicated herb pair RSM and *Radix Astragali Mongolic* has been frequently applied in classical prescriptions for CVD (Wu et al., 2008). Our previous work has also revealed that this herb pair can act on different targets in the same pathways (Wang et al., 2012a). In order to further confirm our strategy, in this work, both *in vitro* and *in vivo* experiments are also applied to evaluate the efficiency of a selected herbal combination DSH consisting of RSM, CT and FC. The results further indicate that the treatment at proper dose can affect the gene expression levels of the nine CVD-associated targets, including A5L, B1AR, CDK2, MAPK14, NOSE, PPAR, PGS, CgmpA and ERB. And more importantly, these proteins are also found to compose an integrated functional network with their corresponding pathways, supporting the synergistic actions of this herbal combination based on the drug-targeted pathway discovery theory.

## Acknowledgements

The authors' work is supported by the Fund of Northwest A&F University and is financially supported by the National Natural

Science Foundation of China (Grant No. 31170796), as well as by the China Academy of Chinese Medical Sciences (Grant No. ZZ0608) and a National "973" Program of China (Grant No. 2013CB531805).

## 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.2013.07.001>.

## References

- Barabasi, A.L., Gulbahce, N., Loscalzo, J., 2011. Network medicine: a network-based approach to human disease. *Nature Reviews Genetics* 12, 56–68.
- Borisy, A.A., Elliott, P.J., Hurst, N.W., Lee, M.S., Lehar, J., Price, E.R., Serbedzija, G., Zimmermann, G.R., Foley, M.A., Stockwell, B.R., Keith, C.T., 2003. Systematic discovery of multicomponent therapeutics. *Proceedings of the National Academy of Sciences of the United States of America* 100, 7977–7982.
- Cases, M., Mestres, J., 2009. A chemogenomic approach to drug discovery: focus on CVD. *Drug Discovery Today* 14, 479–485.
- Chan, S.Y., Loscalzo, J., 2012. The emerging paradigm of network medicine in the study of human disease. *Circulation Research* 111, 359–374.
- Chang, H.M., Chui, K.Y., Tan, F.W.L., Yang, Y., Zhong, Z.P., Lee, C.M., Sham, H.L., Wong, H.N.C., 1991. Compounds from Danshen. Part 4. Structure–activity relationship of miltirone, an active central benzodiazepine receptor ligand isolated from *Salvia miltiorrhiza* Bunge (Danshen). *Journal of Medicinal Chemistry* 34, 1675–1692.
- Cheng, T.O., 2007. Cardiovascular effects of Danshen. *International Journal of Cardiology* 121, 9–22.
- Cheung, F., 2011. TCM made in China. *Nature* 480, S82–S83.
- Chu, Y., Zhang, L., Wang, X.-y., Guo, J.-h., Guo, Z.-x., Ma, X.-h., 2011. The effect of compound Danshen dripping pills, a Chinese herb medicine, on the pharmacokinetics and pharmacodynamics of warfarin in rats. *Journal of Ethnopharmacology* 137, 1457–1461.
- Chung, B.H., Kim, S., Kim, J.D., Lee, J.J., Baek, Y.Y., Jeoung, D., Lee, H., Choe, J., Ha, K.S., Won, M.H., Kwon, Y.G., Kim, Y.M., 2012. Syringaresinol causes vasorelaxation by elevating nitric oxide production through the phosphorylation and dimerization of endothelial nitric oxide synthase. *Experimental and Molecular Medicine* 44, 191–201.
- Cokol, M., Chua, H.N., Tasan, M., Mutlu, B., Weinstein, Z.B., Suzuki, Y., Nergiz, M.E., Costanzo, M., Baryshnikova, A., Gjaever, G., Nislow, C., Myers, C.L., Andrews, B.J., Boone, C., Roth, F.P., 2011. Systematic exploration of synergistic drug pairs. *Molecular Systems Biology* 7, 554.
- Cross, M.J., Claesson-Welsh, L., 2001. FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition. *Trends in Pharmacological Sciences* 22, 201–207.
- Devic, E., Xiang, Y., Gould, D., Kobilka, B., 2001.  $\beta$ -Adrenergic receptor subtype-specific signaling in cardiac myocytes from  $\beta$ 1 and  $\beta$ 2 adrenoceptor knockout mice. *Molecular Pharmacology* 60, 577–583.
- Drab, M., Verkade, P., Elger, M., Kasper, M., Lohn, M., Lauterbach, B., Menne, J., Lindschau, C., Mende, F., Luft, F.C., Schedl, A., Haller, H., Kurzchalia, T.V., 2001. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science* 293, 2449–2452.
- Drake, M.T., Shenoy, S.K., Lefkowitz, R.J., 2006. Trafficking of G protein-coupled receptors. *Circulation Research* 99, 570–582.
- Ekins, S., Nikolsky, Y., Nikolskaya, T., 2005. Techniques: application of systems biology to absorption, distribution, metabolism, excretion and toxicity. *Trends in Pharmacological Sciences* 26, 202–209.
- Feher, M., Schmidt, J.M., 2003. Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. *Journal of Chemical Information and Computer Sciences* 43, 218–227.
- Folger, O., Jerby, L., Frezza, C., Gottlieb, E., Ruppin, E., Shlomi, T., 2011. Predicting selective drug targets in cancer through metabolic networks. *Molecular Systems Biology* 7, 501.
- Galsky, M.D., Vogelzang, N.J., 2010. Docetaxel-based combination therapy for castration-resistant prostate cancer. *Annals of Oncology*, 2135–2144.
- Gao, Z.Y., Xu, H., Shi, D.Z., Wen, C., Liu, B.Y., 2012. Analysis on outcome of 5284 patients with coronary artery disease: the role of integrative medicine. *Journal of Ethnopharmacology* 141, 578–583.
- Grabowski, K., Schneider, G., 2007. Properties and architecture of drugs and natural products revisited. *Current Chemical Biology* 1, 115–127.
- Harris, C.J., Stevens, A.P., 2006. Chemogenomics: structuring the drug discovery process to gene families. *Drug Discovery Today* 11, 880–888.
- Ho, T.C., Chen, S.L., Yang, Y.C., Liao, C.L., Cheng, H.C., Tsao, Y.P., 2007. PEDF induces p53-mediated apoptosis through PPAR gamma signaling in human umbilical vein endothelial cells. *Cardiovascular Research* 76, 213–223.
- Hohl, C.M., Rosen, P., 1987. The role of arachidonic acid in rat heart cell metabolism. *Biochimica et Biophysica Acta (BBA)—Lipids and Lipid Metabolism* 921, 356–363.
- Home, P.D., Pocock, S.J., Beck-Nielsen, H., Curtis, P.S., Gomis, R., Hanefeld, M., Jones, N.P., Komajda, M., McMurray, J.J.V., 2009. Rosiglitazone evaluated

- for cardiovascular outcomes in oral agent combination therapy for type 2 diabetes (RECORD): a multicentre, randomised, open-label trial. *The Lancet* 373, 2125–2135.
- Hopkins, A.L., Groom, C.R., 2002. The druggable genome. *Nature Reviews Drug Discovery* 1, 727–730.
- Hou, T.J., Xu, X.J., 2004. Recent development and application of virtual screening in drug discovery: an overview. *Current Pharmaceutical Design* 10, 1011–1033.
- Jia, J., Zhu, F., Ma, X.H., Cao, Z.W.W., Li, Y.X.X., Chen, Y.Z., 2009. Mechanisms of drug combinations: interaction and network perspectives. *Nature Reviews Drug Discovery* 8, 111–128.
- Kim, H.U., Kim, S.Y., Jeong, H., Kim, T.Y., Kim, J.J., Choy, H.E., Yi, K.Y., Rhee, J.H., Lee, S.Y., 2011. Integrative genome-scale metabolic analysis of *Vibrio vulnificus* for drug targeting and discovery. *Molecular Systems Biology* 7, 460.
- Kong, D.-X., Li, X.-J., Zhang, H.-Y., 2009. Where is the hope for drug discovery? Let history tell the future. *Drug Discovery Today* 14, 115–119.
- Kohl, P., Crampin, E.J., Quinn, T.A., Noble, D., 2010. Systems biology: an approach. *Clinical Pharmacology and Therapeutics* 88, 25–33.
- Lennox, J.L., DeJesus, E., Lazzarin, A., Pollard, R.B., Madruga, J.V.R., Berger, D.S., Zhao, J., Xu, X., Williams-Diaz, A., Rodgers, A.J., Barnard, R.J.O., Miller, M.D., DiNubile, M.J., Nguyen, B.-Y., Leavitt, R., Sklar, P., 2009. Safety and efficacy of raltegravir-based versus efavirenz-based combination therapy in treatment-naïve patients with HIV-1 infection: a multicentre, double-blind randomised controlled trial. *The Lancet* 374, 796–806.
- Li, C., 2012. Absorption, disposition, and pharmacokinetics of herbal medicines: what and how? *Current Drug Metabolism* 13, 491–493.
- Li, L., Zhao, Y.S., Du, F.F., Yang, J., Xu, F., Niu, W., Ren, Y.H., Li, C., 2012a. Intestinal absorption and presystemic elimination of various chemical constituents present in GBE50 extract, a standardized extract of *Ginkgo biloba* leaves. *Current Drug Metabolism* 13, 494–509.
- Li, S., Zhang, B., Zhang, N.B., 2011. Network target for screening synergistic drug combinations with application to traditional Chinese medicine. *BMC Systems Biology* 5 (1), S10.
- Li, X.X., Xu, X., Wang, J.N., Yu, H., Wang, X., Yang, H.J., Xu, H.Y., Tang, S.H., Li, Y., Yang, L., Huang, L.Q., Wang, Y.H., Yang, S.L., 2012b. A system-level investigation into the mechanisms of Chinese traditional medicine: compound Danshen formula for cardiovascular disease treatment. *PLoS One* 7, e43918.
- Ling, H.Y., Wu, L.M., Li, L.D., 2006. *Corydalis yanhusuo* rhizoma extract reduces infarct size and improves heart function during myocardial ischemia/reperfusion by inhibiting apoptosis in rats. *Phytotherapy Research* 20, 448–453.
- Liu, G., Ding, M., Wang, H., Huang, J., Jing, Q., Shen, B., 2010. Pathway analysis of microRNAs in mouse heart development. *International Journal of Bioinformatics Research and Applications* 6, 12–20.
- Ma, X.H., Zheng, C.J., Han, L.Y., Xie, B., Jia, J., Cao, Z.W., Li, Y.X., Chen, Y.Z., 2009. Synergistic therapeutic actions of herbal ingredients and their mechanisms from molecular interaction and network perspectives. *Drug Discovery Today* 14, 579–588.
- Morris, S.M., 2005. Arginine metabolism in vascular biology and disease. *Vascular Medicine* 10, S83–S87.
- Nemeth, K., Plumb, G.W., Berrin, J.G., Juge, N., Jacob, R., Naim, H.Y., Williamson, G., Swallow, D.M., Kroon, P.A., 2003. Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *European Journal of Nutrition* 42, 29–42.
- Olsson, A.-K., Dimberg, A., Kreuger, J., Claesson-Welsh, L., 2006. VEGF receptor signalling? in control of vascular function. *Nature Reviews Molecular Cell Biology* 7, 359–371.
- Pang, Y.P., 2007. In silico drug discovery: solving the “target-rich and lead-poor” imbalance using the genome-to-drug-lead paradigm. *Clinical Pharmacology and Therapeutics* 81, 30–34.
- Qiu, J., 2007. ‘Back to the future’ for Chinese herbal medicines. *Nature Reviews Drug Discovery* 6, 506–507.
- Rothman, R.B., Baumann, M.H., 2009. Serotonergic drugs and valvular heart disease. *Expert Opinion on Drug Safety* 8, 317–329.
- Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative CT method. *Nature Protocols* 3, 1101–1108.
- Semenza, G.L., 2000. HIF-1 and human disease: one highly involved factor. *Genes & Development* 14, 1983–1991.
- Shiojima, I., Walsh, K., 2002. Role of Akt signaling in vascular homeostasis and angiogenesis. *Circulation Research* 90, 1243–1250.
- Sorger, P.K., Allerheiligen, S.R.B., Abernethy, D.R., Altman, R.B., Brouwer, K.L.R., Califano A., D’Argenio D.Z., Iyengar R., Jusko W.J., Lalonde R., Lauffenburger D.A., Shoichet B., Stevens J.L., Subramaniam S., Van der Graaf P., Vicini P., 2011. Quantitative and Systems Pharmacology in the Post-genomic Era: New Approaches to Discovering Drugs and Understanding Therapeutic Mechanisms. An NIH White Paper, QSP Workshop Group.
- Spinella, M., 2002. The importance of pharmacological synergy in psychoactive herbal medicines. *Alternative Medicine Review* 7, 130–137.
- Spitzer, M., Griffiths, E., Blakely, K.M., Wildenhain, J., Ejim, L., Rossi, L., De Pascale, G., Curak, J., Brown, E., Tyers, M., Wright, G.D., 2011. Cross-species discovery of synergistic drug combinations that potentiate the antifungal fluconazole. *Molecular Systems Biology* 7, 499.
- State Pharmacopoeia Commission of the PRC, 2005. *Pharmacopoeia of People’s Republic of China*. Chemical Industry Press, Beijing, China.
- Stelling, J., Klamt, S., Bettenbrock, K., Schuster, S., Gilles, E.D., 2002. Metabolic network structure determines key aspects of functionality and regulation. *Nature* 420, 190–193.
- Sun, Y., Zhu, R., Ye, H., Tang, K., Zhao, J., Chen, Y., Liu, Q., Cao, Z., 2012. Towards a bioinformatics analysis of anti-Alzheimer’s herbal medicines from a target network perspective. *Briefings in Bioinformatics*.
- Technical Report, US Food and Drug Administration, (2010). Comparison of NMEs Approved in 2010 to Previous Years.
- Tontonoz, P., Mangelsdorf, D.J., 2003. Liver X receptor signaling pathways in cardiovascular disease. *Molecular Endocrinology* 17, 985–993.
- Touyz, R.M., 2005. Reactive oxygen species as mediators of calcium signaling by angiotensin II: Implications in vascular physiology and pathophysiology. *Antioxidants & Redox Signaling* 7, 1302–1314.
- Tsang, A., Hausenloy, D.J., Mocanu, M.M., Yellon, D.M., 2004. Postconditioning: a form of “modified reperfusion” protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway. *Circulation Research* 95, 230–232.
- Uzuner, H., Bauer, R., Fan, T.P., Guo, D.A., Dias, A., El-Nezami, H., Efferth, T., Williamson, E.M., Heinrich, M., Robinson, N., Hylands, P.J., Hendry, B.M., Cheng, Y.C., Xu, Q., 2012. Traditional Chinese medicine research in the post-genomic era: good practice, priorities, challenges and opportunities. *Journal of Ethnopharmacology* 140, 458–468.
- Verpoorte, R., Crommelin, D., Danhof, M., Gilissen, L.J.W.J., Schuitmaker, H., van der Greef, J., Witkamp, R.F., 2009. Commentary: “A systems view on the future of medicine: inspiration from Chinese medicine?”. *Journal of Ethnopharmacology* 121, 479–481.
- Wang, X., Xu, X., Li, Y., Li, X.X., Tao, W.Y., Li, B.H., Wang, Y.H., Yang, L., 2013. Systems pharmacology uncovers Janus functions of botanical drugs: activation of host defense system and inhibition of influenza virus replication. *Integrative Biology* 5, 351–371.
- Wang, X., Xu, X., Tao, W., Li, Y., Wang, Y., Yang, L., 2012a. A systems biology approach to uncovering pharmacological synergy in herbal medicines with applications to cardiovascular disease. *Evid Based Complement Alternat Medicine* 2012, 519031 519031.
- Wang, Y.Y., Xu, K.J., Song, J.N., Zhao, X.M., 2012b. Exploring drug combinations in genetic interaction network. *BMC Bioinformatics* 13 (7), S7.
- Wu, T.X., Ni, J., Wu, J.F., 2008. Danshen (Chinese medicinal herb) preparations for acute myocardial infarction. *Cochrane Database Systems Reviews*, CD004465.
- Xu, H.B., Huang, Z.Q., 2007. Vasorelaxant effects of icariin on isolated canine coronary artery. *Journal of Cardiovascular Pharmacology* 49, 207–213.
- Xu, K.J., Song, J.N., Zhao, X.M., 2010. A network biology approach to understand combination of drugs. *Computational Systems Biology* 13, 347–354.
- Xu, X., Zhang, W.X., Huang, C., Li, Y., Yu, H., Wang, Y.H., Duan, J.Y., Ling, Y., 2012. A novel chemometric method for the prediction of human oral bioavailability. *International Journal of Molecular Sciences* 13, 6964–6982.
- Yagi, A., Fujimoto, K., Tanonaka, K., Hirai, K., Takeo, S., 1989. Possible active components of Tan-Shen (*Salvia miltiorrhiza*) for protection of the myocardium against ischemia-induced derangements. *Planta Medica* 55, 51–54.
- Yildirim, M.A., Goh, K.-I., Cusick, M.E., Barabasi, A.-L., Vidal, M., 2007. Drug–target network. *Nature Biotechnology* 25, 1119–1126.
- Yu, H., Chen, J.X., Xu, X., Li, Y., Zhao, H.H., Fang, Y.P., Li, X.X., Zhou, W., Wang, W., Wang, Y.H., 2012. A systematic prediction of multiple drug–target interactions from chemical, genomic, and pharmacological data. *PLoS One* 7, e37608.
- Zhang, L., Yan, K., Zhang, Y., Huang, R., Bian, J., Zheng, C., Sun, H., Chen, Z., Sun, N., An, R., Min, F., Zhao, W., Zhuo, Y., You, J., Song, Y., Yu, Z., Liu, Z., Yang, K., Gao, H., Dai, H., Zhang, X., Wang, J., Fu, C., Pei, G., Liu, J., Zhang, S., Goodfellow, M., Jiang, Y., Kuai, J., Zhou, G., Chen, X., 2007. High-throughput synergy screening identifies microbial metabolites as combination agents for the treatment of fungal infections. *Proceedings of the National Academy of Sciences of the United States of America* 104, 4606–4611.
- Zhao, J., Jiang, P., Zhang, W., 2010. Molecular networks for the study of TCM pharmacology. *Briefings in Bioinformatics* 11, 417–430.
- Zhao, S., Iyengar, R., 2012. Systems pharmacology: network analysis to identify multiscale mechanisms of drug action. *Annual Review of Pharmacology and Toxicology* 52, 505–521.
- Zhao, X.M., Iskar, M., Zeller, G., Kuhn, M., van Noort, V., Bork, P., 2011. Prediction of drug combinations by integrating molecular and pharmacological data. *PLoS Computational Biology* 7, e1002323.
- Zhu, F., Shi, Z., Qin, C., Tao, L., Liu, X., Xu, F., Zhang, L., Song, Y., Liu, X., Zhang, J., Han, B., Zhang, P., Chen, Y., 2011. Therapeutic target database update 2012: a resource for facilitating target-oriented drug discovery. *Nucleic Acids Research* 40, D1128–D1136. (Database issue).
- Zimmermann, G.R., Lehar, J., Keith, C.T., 2007. Multi-target therapeutics: when the whole is greater than the sum of the parts. *Drug Discovery Today* 12, 34–42.